PROCEEDINGS OF A WORKSHOP ON CHRONIC TOXICITY IDENTIFICATION EVALUATIONS IN THE SAN FRANCISCO BAY REGION

March 16-17, 1992
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Richmond, CA

Sponsored by: Bay Area Dischargers' Association (BADA)

San Francisco Bay Regional Water Quality Control Board

Convened by: Aquatic Habitat Institute

PREFACE

Early in 1991, the Aquatic Habitat Institute (AHI) was asked by the Bay Area Dischargers Association (BADA) and the San Francisco Bay Regional Water Quality Control Board (RWQCB) to convene a workshop on chronic toxicity evaluation. The idea for the workshop was developed in discussions between the San Francisco Bay Regional Water Quality Control Board and bay area dischargers during the adoption of Basin Plan amendments which moved toxicity testing from a study-based approach to a regulatory approach. The purpose of the workshop was the transfer of technical information concerning the conduct and use of chronic toxicity testing.

This proceedings is the result of that workshop which was held on March 16 and 17, 1992, at the University of California at Berkeley Field Station in Richmond, California. On day one of the workshop, five technical experts with considerable experience in the field of chronic toxicity testing were asked to present case studies in which they had participated. The purpose of the presentations was to illustrate both the effectiveness of the procedures and the issues that regulators and permitted dischargers must consider in order to apply these techniques in a regulatory setting. On day two, representatives of both the regulatory agencies and the regulated dischargers responded to the information presented from their perspective. They laid before the experts an agenda of specific questions which they hoped could be answered in the process of the workshop. The workshop concluded that toxicity identification evaluation can be sucessful and highly valuable, but considerable flexibility is desirable in the application of the procedure in a regulatory framework. The workshop ended with a call for improved information sharing as more and more dischargers engage in chronic toxicity work.

On behalf of the Aquatic Habitat Institute, I wish to thank the Bay Area Dischargers Association for providing the substantial finanical support, and the San Francisco Bay Regional Water Quality Control Board for providing the invaluable staff support that was required to hold this workshop. I also wish to thank all of the presenters and participants who contributed their efforts to the success of this effort. Mr. Chuck Batts of BADA and Mr. Michael Carlin and Dr. Lynn Suer of the Regional Board were instrumental in making the workshop a success.

I also wish to thank all of the AHI staff without whose effort this workshop would not have been possible. Dr. Joseph O'Connor organized and convened the workshop, Ms. Elizabeth Hartman and Ms. Gabriele Marek provided logistical support, Mr. Michael May recorded the proceedings, and Mr. Jay Davis edited the proceedings.

Margaret R. Johnston, Executive Director
Aquatic Habitat Institute

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DAY ONE

OPENING REMARKS

Steve Ritchie
San Francisco Bay Regional Water Quality Control Board

The effluent toxicity characterization program began with the 1986 Basin Plan. Since these brand new techniques had not previously been used in a regulatory setting, the initial program followed a "study-based" approach, rather than a regulatory enforcement approach. This initial part of the program was extremely successful, resulting in the generation of a significant body of information on toxicity testing techniques and the presence of toxicity in effluents. The next step for the program, as outlined in the new Basin Plan amendments, is the establishment of chronic toxicity effluent limits. The existence of these limits will demand that dischargers have techniques for identifying toxic components of effluents and reducing toxicity. When toxicity is found in an effluent, the Regional Board will expect that efforts begin immediately to reduce the toxicity. The Regional Board wants to take a positive, cooperative approach to implementing these regulations. Any enforcement actions relating to the chronic toxicity limits would be entered into deliberately, taking into account efforts on the part of the discharger to identify and reduce toxicity. Studies have shown that toxicity identification and reduction can be successful. The goal of this workshop is to provide a forum for sharing information on the practice of conducting successful TIEs and TREs.

Chuck Batts
Bay Area Dischargers' Association (BADA)

I have been asked to make some opening remarks on the dischargers' views on implementation of chronic TIEs. I am encouraged to see so many faces out there and such a great deal of interest in chronic TIEs. I was asked to set the stage for the workshop by making a "problem statement". That term seems too broad and too negative. These remarks will be more of a statement of concerns that the dischargers have.

In the 1986 Basin Plan the Regional Board, again ahead of its time, initiated the effluent characterization program. Susan Anderson of the Regional Board did an excellent job of putting together a detailed program that incorporated input from the dischargers. The program started in the summer of 1987 with one-time screening of several effluents. These tests, performed by Don Mount, showed that several discharges were toxic, some at low dilution levels. This initial work, along with later tests, indicated that there was toxicity and it had to be dealt with.

One of the questions that arose even in the early screening work was how to handle the large amount of variability found in effluent toxicity tests. This variability poses a problem in the testing phase, and also the identification and verification phases that we are

into now. State Board and Regional Board policies call for no chronic toxicity outside the mixing zone in ambient waters. The variability of the tests makes it difficult to define what is meant by "no". Statistical procedures must be used to determine what levels of toxicity are significantly greater than zero. The meaning of "chronic" is clearer, but sometimes defining the endpoint is difficult. The main question that we want to resolve is how to cope with variable test results in saltwater chronic TIEs. Another concern is sample preservation. Several studies have found "toxicity decay", where samples can lose toxicity over very short periods of time. Organism sensitivity is another complicating factor; many studies have shown that there can be significant variability in organism sensitivity.

Dischargers are also concerned about what management actions may result from TIEs. The initial screening studies showed that diazinon caused toxicity, and still may be a matter of concern. Would a regional sales ban be implemented to reduce this toxicity? Another area of concern is interpretation of effluent toxicity when significant ambient toxicity has been found in the Bay. The occurrence of ambient toxicity suggests that there may be a problem with the test organisms rather than a toxicity problem. If there is a toxicity problem in the Bay (e.g., copper concentrations in the Bay exceed EPA criteria) how do we determine an acceptable amount of toxicity in effluents?

From this workshop the dischargers hope to gain a starting point toward obtaining meaningful results from chronic TIEs. From the broad sieve of the identification phase to the variability and testing phases, dischargers are concerned about how enforcement will be carried out and what meaningful results will be obtained. The mission of the workshop, from our perspective, is to examine the methods and results of successful chronic TIEs, especially the statistical approaches used, the validation of those approaches, and the endpoints employed.

TECHNICAL PRESENTATIONS

Teresa Norberg-King
US EPA
Environmental Research Laboratory - Duluth

It is hard to decide how to organize this presentation since I had envisioned a smaller meeting with about 30 people and a great deal of interaction. With a group of this size some of the interaction will have to be omitted. Since the emphasis of this workshop is on testing effluents discharged into marine waters I will be speaking for the first time on the marine TIE work being done by EPA. We have been working with our sister laboratory at Narragansett, which specializes in toxicity testing of effluents discharged into marine waters, and have both performed testing on samples from the same effluent.

TIEs usually involve sample simplifications to enable analytical results to be interpretable. TIEs can be performed on other media in addition to effluents, including ambient waters, sediment pore waters and elutriates. Aquatic organisms are used to detect and track the toxicity of the components of the sample medium.

There are three major parts of a TIE: Characterization (Phase I); Identification (Phase II); and Confirmation (Phase III). We have rewritten the acute manual for Phase I and are rewriting the acute manual for Phases II and III. The Phase II and III portions of the acute manual will address concerns relating to chronic TIEs. There will not be separate Phase II and III manuals for chronic TIEs. We feel that general guidance on Phases II and III apply equally well to both acute and chronic TIEs.

Phase I procedures serve to identify the physical and chemicals nature of the constituents causing toxicity. Characteristics examined include solubility, volatility (although not many toxicants are volatile), decomposability, complexability, filterability, and sorbability. The acute Phase I manipulations include pH adjustment, filtration, aeration, solid phase extraction (SPE) of nonpolar organics on a C_{18} column, EDTA chelation, sodium thiosulfate addition, and graduated pH change. The acute TIE manual calls for a pH 3 adjustment and a pH 11 adjustment, followed by the other manipulations, a return to initial pH, and finally toxicity tests. Toxicity tests are performed after each manipulation to determine whether any change in toxicity occurs. Any manipulations that are performed must have predictable and comprehensible effects to allow for valid interpretation of results.

Our core philosophy in performing TIEs is to concentrate on characterization steps that have the clearest effects on the unidentified toxicant(s). Several other principles are also discussed in the revised acute TIE manual. Performing several manipulations in tandem can be helpful in characterizing the cause of toxicity in effluents. An example might be the addition of EDTA to a post-SPE effluent. Several manipulations can be useful for single and multiple toxicants. It is sometimes easy to be mislead into suspecting multiple toxicants when only one is actually causing toxicity. It is also important to use patterns in identifying

and confirming toxicity observed in Phase I. We may actually immediately begin with Phase II and Phase III with the first toxic sample we see. As the identity of the toxicant becomes clearer, these patterns provide additional evidence.

Often more than one toxicant is present at toxic concentrations in an effluent. When multiple toxicants are present, if one can be identified then identification of additional toxicants is easier for several reasons, including:

1) the contribution of the identified toxicant to overall toxicity can be established;
2) the number of Phase I manipulations that affect toxicity of the known toxicant can be determined;
3) interactions of identified and unidentified toxicants can be evaluated; and
4) information on the relative contributions of identified and unidentified toxicants can be used to elucidate additional physical and chemical characteristics of the unidentified toxicant.

This approach applies to both acute and chronic TIEs. Currently the Duluth EPA lab is directing all of its efforts toward chronic TIE work.

An important term to be familiar with is "matrix effect". Toxicants can interact with other effluent constituents in ways that alter their toxicity. For example, some toxicants (e.g., ammonia, HCN) change chemical form depending on the characteristics of the effluent. Manipulations of the sample can also change the characteristics of the matrix such that observed toxicity may not be an accurate reflection of the original effluent.

I will now review some of the guidance from the document on Phase I of the acute TIE. Several classes of compounds can be implicated in Phase I. A non-polar toxicant would be suspected if toxicity is removed by a C_{18} column and if toxicity is recovered in the methanol eluate from the column. If the methanol eluate is toxic, addition of piperonyl butoxide (PBO) can be used to remove toxicity due to acetylcholinesterase inhibitors. If the methanol eluate is not toxic it is still possible that the toxicant could be nonpolar. Several 100% methanol elutions may be needed to recover the toxicant. Sometimes it may also be appropriate to proceed directly to the methanol/water fractionation scheme of Phase II.

Toxicity due to non-polar toxicants cannot be automatically assumed whenever the post-C_{18} column effluent is nontoxic. Evidence shows that the C_{18} column also removes nonpolar toxicants such as zinc, nickel, silver, copper (sometimes) and aluminum. It is best to see if results of the SPE are consistent with results of the other manipulations.

I also thought I would give some results from our testing with SPE eluate and PBO. We have found that for *Ceriodaphnia dubia* PBO inhibits the toxicity of parathion, methyl parathion, diazinon, and malathion, but does not inhibit the toxicity of dichlorvos, chlorfenvinphos, or mevinphos. PBO addition does not work for all acetylcholinesterase

inhibitors, but if it does remove toxicity then it is likely that an acetylcholinesterase inhibitor is contributing to the toxicity of the effluent.

We have also started using PBO for chronic TIEs. A potential problem in using PBO in chronic TIEs is that it may only inhibit toxicity for 24 hours. Consequently, using *C. dubia* we thought it might be necessary to add more PBO at 48 hours and every day thereafter. Adding PBO to a POTW effluent (Table 1) improved survival in the whole effluent and in the 85% methanol eluate. In this case we added slightly too much PBO, causing a decrease in reproduction, but percent survival greatly improved. We have since successfully tried using lower levels of PBO. PBO does appear to be a another tool that can be used in establishing the identity of unknown toxicants.

Several findings might indicate the presence of toxicity due to cationic metals, including:

1) toxicity reduced by EDTA additions;
2) toxicity reduced by sodium thiosulfate (STS) additions;

POTW 2

RESULTS OF PBO ADDITION ON CHRONIC TOXICITY

Test	% Survival w/o PBO	% Survival w/ PBO
Whole effluent	60	100
Methanol eluate		
80%	80	100
85%	0	80
90%	100	80
Control (HRW)	100	80

Table 1. Results of PBO addition on chronic toxicity

3) toxicity reduced by C_{18} SPE column and toxicity not recovered in methanol eluate;
4) erratic dose response (the dilution water may have different hardness or pH than the effluent causing the toxicity to vary at different dilutions);
5) graduated pH test shows toxicity differences;
6) toxicity removed by filtration and recovered in pH 3 dilution water extract; and
7) toxicity removed by cation exchange resin (e.g., zeolite).

The sensitivity of test organisms can vary with pH as shown for *C. dubia* in Table 2. At higher pH *C. dubia* is more sensitive to cadmium, nickel, and zinc. On the other hand, at higher pH *C. dubia* is less sensitive to copper and lead. This kind of information is currently available from our lab, and I am sure other labs are finding similar patterns.

The sensitivity of fathead minnows also seems to vary somewhat with pH (Table 3). Zinc was generally more toxic at higher pH. No trend is clear from the data for cadmium and lead.

We have also investigated the amount of EDTA needed for chelation of metals. The acute TIE manual offers several methods for determining the amount, including one based on the LC50 of EDTA and another on the hardness of the effluent. In this case we tried to establish the amount actually needed to chelate cadmium, copper, and zinc, and whether the EDTA had to chelate the calcium and magnesium prior to the metals. When we spiked these metals at a 1:1 molar ratio with EDTA the toxicity was always reduced, demonstrating that we could use much less EDTA than indicated by the other methods.

The ENSR group in Colorado has done some work comparing the effects of EDTA and STS additions (Table 4). Both EDTA and STS remove cadmium, copper, and mercury, but STS does not remove lead, manganese, nickel, or zinc. These differences can be used in identifying toxicants. Note that there were no anionic toxicants isolated by these procedures. Chromium in particular is a real problem; no Phase I procedures remove chromium.

We have been finding that surfactants are a problem in several discharges. Findings implicating surfactants include:

1) toxicity reduced by filtration;
2) toxicity changed by aeration;
3) toxicity may or may not be recovered from aeration vessel;
4) toxicity removed by C_{18} SPE column;
5) toxicity may or may not be recovered in SPE eluate; and
6) toxicity degrades over time.

Ammonia is my least favorite toxicant. Ammonia toxicity is reduced by lowering the pH. Ammonia toxicity is greater to fathead minnows than to *Ceriodaphnia* in both acute and

Toxicity of Various Metals at Different pHs to *Ceriodaphnia*

Chemical	*pH*	LC50 (µg/L) 24 h	48 h
Cadmium	*6.5*	>1460	563
	7.3	908	350
	8.6	417	121
Lead	*6.4*	410	280
	7.2	>2700	>2700
	7.9	>2700	>2700
Copper	*6.5*	22	10
	7.3	69	28
	8.7	201	201
Nickel	*6.5*	>200	>200
	7.3	>190	171
	8.7	>230	48
Zinc	*6.6*	1170	610
	7.2	880	770
	8.2	140	130

Table 2. Toxicity of Various Metals at Different pHs to *Ceriodaphnia*

Toxicity of Various Metals at Different pHs to Fathead Minnows

Chemical	*pH*	LC50 (µg/L) 24 h	48 h	96 h
Cadmium	*6.5*	72	73	73
	7.2	64	64	60
	8.6-8.7	65	65	65
Lead	*6.6*	>2700	>2700	810
	7.1	>2700	>2700	>2700
	7.6	>2700	>2700	>2700
Zinc	*6.6-6.7*	883	883	780
	7.0-7.1	884	508	330
	7.5-7.8	1767	1894	502

Table 3. Toxicity of various metals at different pHs to fathead minnows.

EDTA Removes Toxicity

Thiosulfate Removes Toxicity

	YES	*NO*
YES	Cd Cu Hg	Ag Se (selenate)
NO	Zn Mn Pb Ni	Fe Cr (III) Cr (VI) As (ite or ate) Se (selenite) Al

Table 4. Effects of Sodium thiosulfate (STS) and EDTA additions

chronic tests. A zeolite column will remove ammonia toxicity, but since zeolite also removes other constituents (e.g., cationic metals and probably some anions too) this alone is not conclusive evidence of ammonia toxicity. Large surface/volume air stripping can also reduce ammonia toxicity. Ammonia toxicity would be expected if total ammonia concentrations exceed 10 mg/l.

We have done some work to gain a better understanding of how pH is controlled in the graduated pH test. This is not as critical in Phase I, but in confirmation it is important to know that pH is not fluctuating over time. Several methods are available. One is simple acid/base adjustments using NaOH or HCl. Another technique is to control the CO_2 concentration in the headspace of the test vessel. H^+ ion buffers can also be used in freshwaters, but they probably will not work in marine waters. Different types of buffers are being investigated by the Narragansett Laboratory. Another point regarding pH is that we have found that high pH (above 8) causes volatilization of NH_4. This might be misleading if ammonia concentrations are not measured in confirmation if the test runs over 48 hr.

Three buffers that I will be referring to are MES (2-(N-morpholino)ethan-sulfonic acid, pK_a=6.15), MOPS (3-(N-morpholino)propane-sulfonic acid, pK_a=7.15), and POPSO (piperazine-N,N'-bis(2-hydroxypropane)-sulfonic acid, pK_a=7.8). These buffers have been shown to be non-toxic at much higher concentrations than those used in TIEs (we use 4 mM even on a chronic basis). These buffers allow us to do a pH adjustment test without using an environmental chamber. We have found that the pH's hold well even in small volumes.

In the CO_2/air mixture procedure, trays of cups containing the test organisms are placed in the test chamber. Concentrations of CO_2 should not exceed 5% to avoid CO_2 toxicity. The test cups are left open and the pH is allowed to drift normally. This technique has been very successful even in chronic tests.

PHASE II

After identifying the general characteristics of the compound causing toxicity in Phase I, the specific identity is investigated in Phase II (Table 5). The most commonly encountered toxicants are nonpolar organics, cationic and anionic metals, and ammonia. We are also starting to see toxicity due to resins, polymers. Filterable toxicity is difficult to identify because many substances are recovered off the filter.

The presence of toxic concentrations of a chemical does not prove it is a cause of toxicity. For example, all metals measured as "totals" may not be bioavailable. Phase III must be performed to confirm the actual presence of toxicity.

Techniques to identify toxicant(s):

Non-polar organics	SPE, HPLC, GC/MS, MS-MS
Cationic metals	AA and ICP
Volatiles	Purge and Trap GC/MS
Polar organics	LC-MS
Ammonia, cyanide, sulfide	Specific ion electrodes/ colorimetric techniques
Anionic metals	AA and ICP

Table 5. Phase II: Toxicity Identification Procedures. Methods to specifically identify suspect chemicals in toxic samples.

PHASE III

Phase III procedures are used to confirm the presence of toxicity due to the suspect toxicant (Table 6). EPA is currently rewriting the Phase III document, as I mentioned earlier. These confirmation steps have been developed based on our experience over the last few years.

One standard Phase III procedure is evaluating the correlation between the toxic units of the suspect toxicants with effluent toxic units. Additivity must be taken into account if there is more than one toxicant. Looking at the variability seen in sample manipulations can be useful in correlation, especially for ammonia and cationic metals.

The use of one sample in correlation is important, even in the chronic test. Measured concentrations in effluent cannot be correlated with toxicity if multiple samples are used. The toxicant may not be present in all samples, or other toxicants may appear.

The chronic TIE approach is similar to the acute TIE scheme. The chronic TIE procedures were developed from a combination of the methods in short-term chronic toxicity testing and acute TIEs (Figure 1).

Some modifications to the short-term chronic testing methods were required. Fewer replicates are used in chronic TIEs because it is difficult to handle ten replicates with five concentrations for every sample manipulation. We wanted to look at whether tests needed to be performed to the full duration specified in the short-term manual, and also whether the solution renewal frequencies and test volumes were appropriate. As I just mentioned, chronic TIEs should be performed on one sample. The short-term chronic manual, in contrast, calls for daily samples or a minimum of three samples. In chronic TIEs we opt for renewal frequencies of every day for fathead minnows and every other day for *Ceriodaphnia.* Appropriate storage of whole effluent and characterization samples is an issue that I expect we will discuss thoroughly later in the workshop.

Use of appropriate dilution water is also important in chronic TIEs. The dilution water must provide for adequate performance of the test organisms, must not change responses to toxicants, and must not contain trace levels of toxicants that can be concentrated and cause toxicity.

A point I alluded to earlier is thAT depending on the concentrations of metals present in a particular sample, the characteristics of the test matrix may be influenced by the amount of dilution water used. At low metal concentrations the test matrix will be approximately 100% effluent. At high metal concentrations, however, the test matrix will be diluted and the dilution water may begin to affect toxicant behavior.

We split the chronic Phase I procedures into two tiers (Figure 2). Extreme pH adjustment is not required for chronic work. We are confident that most toxicants will be

Correlation

Symptoms

Species Sensitivity

Spiking

Mass Balance

Deletion

Miscellaneous Approaches

Hidden Toxicants

When Treatability Approach Has Been Used

Table 6. Phase III procedures

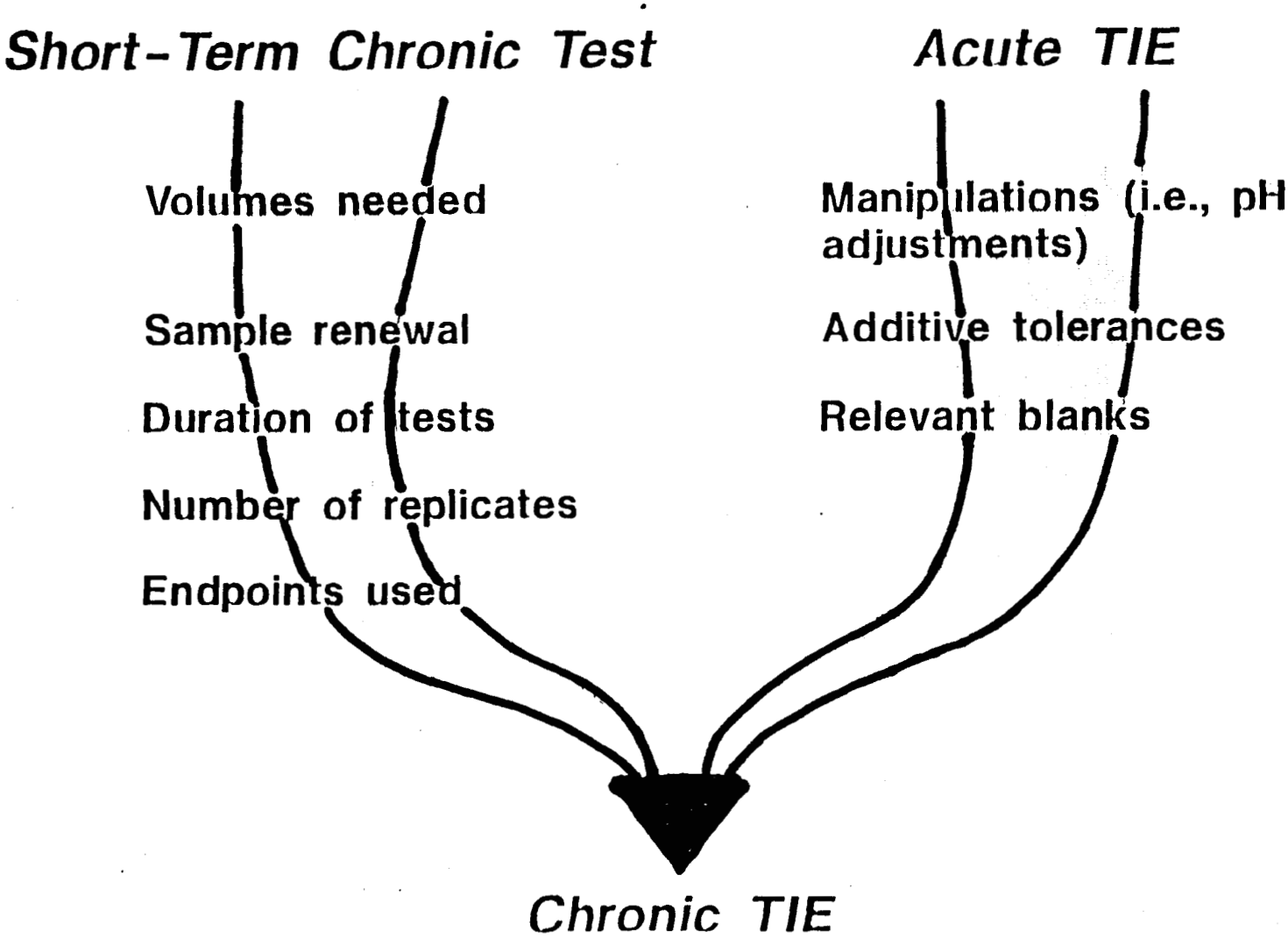


Figure 1. Development of Chronic TIE Procedures

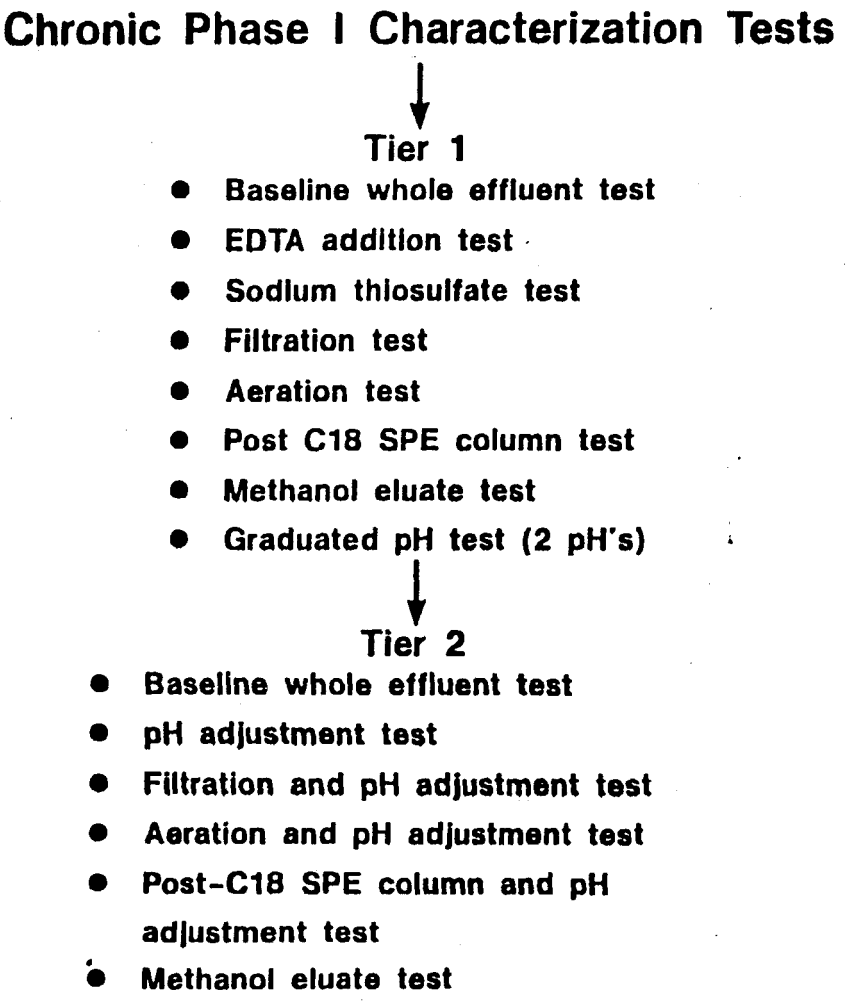


Figure 2. Chronic Phase I Characterization Tests.

characterized without pH 3 and pH 11 tests. The methanol eluate test is a new addition to Phase I. The latest rewrite of the chronic TIE manual (which was final in May) requires testing of several methanol fractions. In the graduated pH test we recommend doing two pH's to see if the toxicant is pH sensitive. If Phase I does not yield definitive results or if further confirmation of Tier 1 findings is needed, Tier 2 procedures can be employed.

The first thing we did in development of the chronic TIE procedure was to determine threshold levels for exposure of *Ceriodaphnia* and *Pimephales* to EDTA, sodium thiosulfate, methanol, acid/base additions, and ammonia. These findings are all discussed in the chronic TIE manual. Tables 7 and 8 show acceptable ranges of concentrations for EDTA and sodium thiosulfate.

The chronic TIE procedures for *Ceriodaphnia* and fathead minnows are outlined in Tables 9 and 10. For *Ceriodaphnia* the 4 day test should be used if possible in Phase I. The 4 day *Ceriodaphnia* test starts with 72 hour old animals and the endpoint is three broods. This test is sensitive enough to detect toxicity and provides a relatively quick result. The sensitivity of the 4 day *Ceriodaphnia* test compares favorably with the 7 day test (Table 11).

In determining the effect concentration it is better to use point estimation (ICp) than a hypothesis testing approach.

MARINE TIES

EPA is also working on developing TIE procedures for effluents discharged to marine or estuarine environments, saline ambient waters, and marine interstitial waters. This work is being performed by George Morrison and his coworkers at the Narragansett lab. The major difficulty they face is the adaptation of the freshwater manipulative techniques to saline samples. The approach being taken is to test to what extent existing freshwater TIE methods can be employed with saline samples and saltwater organisms.

Organisms that hold potential as saltwater test species include *Mysidopsis bahia* and *M. berylina* for acute endpoints, and *Champia parvula* and *Arbacia punctulata* for chronic endpoints. TIE developmental work on *C. variegatus* was discontinued because of the relative insensitivity of this species. Some test parameters for these species are given in Table 12.

Studies are being conducted to determine the effectiveness of TIE manipulations on saline samples. Studies on additions of EDTA, sodium thiosulfate, and methanol and C_{18} column extraction have been completed. Work is still in progress on the use of CO_2, organic buffers, and inorganic buffers in the graduated pH test, C_{18} column elution, and extraction and elution of cation exchange columns. Data on species sensitivities to sample manipulations are shown in Table 13. Sensitivity to EDTA addition was similar for all species. *A. punctulata* was very insensitive to thiosulfate. Sensitivity to methanol ranged from extreme tolerance

Concentrations of EDTA for chronic TIEs. Values given are the final water concentration in mg/L.

Species	Water Type	Final Concentration (mg/L)		
C. dubia and Fathead minnow	SRW, MHRW, HRW, VHRW	0.5	3.0	8.0

Note: **SRW = soft reconstituted water; MHRW = moderately hard reconstituted water; HRW = hard reconstituted water; VHRW = very hard reconstituted water.**

Table 7. Concentrations of EDTA for chronic TIEs. Values given are the final water concentration in mg/L.

Concentrations of sodium thiosulfate for chronic TIEs. Values given are the final water concentration in mg/L.

Species	Water Type	Final Concentrations (mg/L)		
C. dubia and Fathead minnow	SRW, MHRW, HRW, VHRW	1	5	10

Note: SRW = soft reconstituted water; MHRW = moderately hard reconstituted water; HRW = hard reconstituted water; VHRW = very hard reconstituted water.

Table 8. Concentrations of sodium thiosulfate for chronic TIEs. Values given are the final water concentration in mg/L.

C. dubia Chronic TIE Tests

- 5 organisms/treatment
- 1 organism per cup or beaker
- ≤ 12 h old animals (window of age)
- 10 mL/cup or beaker
- Either daily renewal or alternate day renewal (1 or 2 renewal)
- Use 4-d test when possible in Phase I
- Use 7-d test in Phase III
- Endpoints: reproduction, survival, trends of response

Table 9. *C. dubia* Chronic TIE tests.

P. promelas Chronic TIE Tests

- 20 fish/treatment
- 10 fish/replicate
- ≤ 48 h old fish (24 h window)
- 50 mL/4 oz plastic cups or 100 mL/400 mL beaker
- daily renewal required
- *Artemia* fed 2x/day
- Endpoints: growth, survival, trends of response

Table 10. *P. promelas* Chronic TIE tests.

Comparison of 4-d and 7-d *C. dubia* Tests

Effluent	IC50 (%) 4-d Test	7-d Test
POTW - A	71	63
POTW - B	79	55
Industry - A	40	46

Table 11. Comparison of 4-day and 7-day *C. dubia* tests.

Toxicity Test Exposure Conditions

Species	Test Volume	Exposure Duration (h)	Endpoint
M. bahia	20 mL	48	Survival
M. beryllina	20 mL	28	Survival
C. parvula	--*	48	Reproduction
A. punctulata	5 mL	1.3	Fertilization
C. variegatus	--	--	

* In progress.

Table 12. Toxicity test exposure conditions.

Species Sensitivity to Additives

Species	Mean LC50s EDTA mg/L	Thio-sulfate mg/L	Methanol (%)	Aer.	Filt.	C_{18}
M. bahia	304	116	2.13	NP	NP	NP
M. beryllina	293	7870	3.04	NP	NP	NP
C. parvula	198	255	0.13	--*	--*	--*
A. punctulata	281	>15000	8.65	NP	NP	NP
C. variegatus	--	--	--			

* Difficulties related to test volume.
NP - No problems encountered.

Table 13. Species sensitivity to additives.

(*A. punctulata*) to extreme sensitivity (*C. parvula*). No problems have been encountered relating to aeration, filtration, or C_{18} extraction.

One problem that remains to be resolved is ammonia toxicity. There is a need for development of procedures to adjust and hold pH at constant levels for the desired exposure periods. Complicating matters is the observation that pH toxicity occurs at pH < 7.

Modifications to the chronic test procedures for mysids are needed to prevent cannibalism as the volumes are reduced. The minimum volume for *Menidia* is also being investigated. I am hoping that we can use the same volume that is used for fathead minnows (10 per 50 ml); the fish may be similar enough in size to do so. Endpoints for the mysid tests include survival, growth, and trends in response if possible.

I will now present some data, starting with data from some of our early work on an industrial effluent. Results of tests using mysids, *Menidia*, and *Ceriodaphnia* are shown in Tables 14-16. Each sample manipulation was not performed on every sample because some procedures were still under development at the time.

Each sample was toxic to the mysid. EDTA removed the toxicity somewhat in September and October. Thiosulfate never removed the toxicity. The C_{18} column removed the toxicity in each sample.

Each sample tested with *Ceriodaphnia dubia* was found to be toxic. EDTA and the C_{18} extraction removed the toxicity in each case, and thiosulfate removed the toxicity in all but one sample. In examining recovery from the C_{18} column, as we have been doing for years, we were able to recover the toxicity in the October sample. Multiple manipulations on the post-C_{18} effluent are indicative of copper toxicity. The toxicity is removed by EDTA and sodium thiosulfate, and a graduated pH test on the post-column effluent results in much greater toxicity than in the post-column effluent without pH adjustment.

In tests of these samples with *Menidia* only two of four were toxic. In the samples that were toxic, both EDTA and sodium thiosulfate removed the toxicity, again indicating that copper could be causing toxicity.

FUTURE EFFORTS

Table 17 shows an anticipated timetable for future TIE method development. The Phase II and III Marine Methods may not differ much from the freshwater methods.

We are also working on a municipal wastewater treatment plant with chronic tests. Using four species (*Champia, Arbacia, Pimephales,* and *Ceriodaphnia*), the C_{18} column removed the toxicity for all species. The toxicity was recovered from some of the fractions. In a short

Freshwater/Marine TIE

Mysid

	7/29	8/5	9/11	10/8	11/19
Whole Effluent	toxic	toxic	toxic	toxic	toxic
EDTA	no	no	yes	partial (?)	no
Thiosulfate	no	no	no	?	no
Post-C_{18}	--	yes	yes	yes	yes
Filtration	--	--	no	?	no
Aeration	--	--	no	?	no

Table 14. Freshwater/Marine TIE - *Mysid.*

Freshwater/Marine TIE

Menidia

	7/29	8/5	9/11	10/8	11/19
Whole Effluent	NT	toxic	--	toxic	--
EDTA	NT	yes	--	yes (?)	NT
Thiosulfate	--	yes	--	yes (?)	NT
Post-C_{18}	--	--	--	--	NT
Filtration	--	--	--	no	NT
Aeration	--	--	--	yes	NT

NT - Not toxic

Table 15. Freshwater/Marine TIE - *Menidia*.

Freshwater/Marine TIE

C. dubia

	7/29	8/5	9/11	10/8	11/19
Whole Effluent	--	toxic	toxic	toxic	toxic
EDTA	--	yes	yes (partial)	yes (partial)	yes
Thiosulfate	--	yes	yes	no	yes
Post-C_{18}	--	yes	yes	yes	yes
Filtration	--	--	--	no	no
Aeration	--	--	--	--	--
C_{18} Recovery	--	--	--	yes	no

Table 16. Freshwater/Marine TIE - *C. dubia.*

Future Efforts and Expected Time Frames

• Draft Phase I Methods Manual	92 (Oct.)
• *M. bahia* and *M. beryllina* Chronic Methods	93
• Phase II and III Marine Methods	94
• Marine Sediment TIEs	94

Table 17. Future efforts and expected time frames.

time we should have an idea of whether the freshwater and marine TIE methods are interchangeable.

QUESTIONS

Q: Did you see any change in TIE response when doing renewals with *Ceriodaphnia* on daily or every other day basis in terms of perhaps feeding influence and how toxicity was expressed?

A: We haven't. We have seen instances where feeding itself has reduced or changed toxicity.

Q: Do you have any information on the success of treatability of identified toxicants?

A: I don't have much information on treatability.

DON MOUNT
AScI Corporation

Dr. Mount was not able to allow reproduction of the overheads he used in his presentation.

I have decided to present some essentially raw data rather than prepare slides, since I thought it would be instructive to see where some of the holes in the data are and what real data sets look like. I would like to say that there is far more fear of chronic TIE methods than there needs to be. A year ago I didn't think I would say that myself. At the AScI Environmental Testing Division we now take on new effluents for chronic TIE work with no more hesitation than for acute TIEs. In fact, most of the work at AScI is chronic TIE work.

This is not to say that every chronic TIE will be successful or that all people will be equally successful at performing them. Experience is extremely important in successful completion of this work, as my analysis of these case studies will show. The reasoning required in analysis of TIE data is analogous to the reasoning applied by physicians to the interpretation of the parameters they measure to assess the health of a patient. It is rare that any one piece of information from a TIE is definitive. A "weight of the evidence" approach, as the attorneys would call it, is often required. To borrow a quote from my son, who is also in this line of work, there are many times when Phase I data have been completely confusing, but upon completion of the TIE the Phase I data always make sense in retrospect.

As suggested by the conveners of this workshop, I am going to provide for discussion of details of chronic TIEs.

I would like to make a comment to the regulators in the audience. Across the country there is a move toward state certification, or perhaps better termed "licensing", of laboratories. This is probably good for routine biomonitoring. However, the states are about to shoot themselves in the foot by requiring certification for TIE work. As I mentioned earlier, success in TIE work depends upon experience in analyzing the data. Certification will greatly increase the number of labs doing TIEs, and that will diminish the success rate because the experience base will be diluted. There is a need for more labs to perform TIEs, but the work should be concentrated for best results. Furthermore, it is important that people performing TIEs work jointly with chemists, engineers, and biologists; only larger organizations can provide this type of support.

The data I am presenting are from interim reports. The titles have been removed to protect my clients.

CASE 1

The data I am going to present for this first effluent are from Phase I. This effluent was only toxic to *Ceriodaphnia*; it was essentially non-toxic to fathead minnows. One very peculiar characteristic of this effluent is that it is not lethal to test organisms, but only at

about a 5% dilution do you see normal reproduction. This suggests that either the toxicants have a very large acute/chronic ratio or that there is an equilibrium with a non toxic reservoir involved. Phase I results using number of offspring as the endpoint show that filtration removed the toxicity. EDTA, aeration, and sodium thiosulfate had no effect. Effluent from the C_{18} column was also nontoxic, but the sample had already been filtered. Eluates from the C_{18} column were not toxic. In summary, the toxicity in this sample was filterable but was not affected by the other treatments.

I would like to make a comment on elution. In Phase I it is possible to misinterpret the results of the single 100% methanol elution of the C_{18} column when comparing it to the results from Phase II where a graded series of methanol solutions is used. For one effluent that we are working on presently, toxicity does not appear until the fourth 100% elution, and it is still being recovered at six elutions. On the other hand, in Phase II elution scheme, starting with a lower percentage of methanol, the toxicant is recovered in the 85% and 90% fractions. The chromatography of the elutions in Phase I and Phase II are therefore quite different, and if elution is performed in Phase I it should be done with more than one elution volume. Don't just increase the volume of a single elution - use several separate elutions.

Now let's look at another sample; this was actually the first sample that we collected. Again looking at reproduction, EDTA, thiosulfate, aeration, pH adjustment, and C_{18} extraction (this sample was not filtered prior to C_{18} extraction) did not remove the toxicity. Filtration removed the toxicity completely. Some indication of toxicity was observed in the blank. This was not a major problem, but the data should be interpreted with this in mind.

In chronic TIE work it is not appropriate to rely entirely on statistical analysis of the

We had a suspicion of what was causing toxicity in this effluent. This plant had trouble with solids control for a long time. The fact that all of the toxicity was associated with solids implicated polymers, though this was certainly not the only possibility. In talking with the plant operators, we knew that they used a lot of polymers (approximately 10 ppm). Polymers pose a real analytical problem. There really are no decent methods for polymer analysis. The plant operators were surprised to hear that polymers were the probable cause of toxicity.

We sampled the primary clarifier overflow and found that filtration had no effect on the toxicity, indicating that there was an important difference between the primary clarifier overflow and the final effluent. This is a complex, somewhat unconventional plant, though it is basically an activated sludge plant. Paper mills generate a relatively small amount of sludge. The sludge generated is sent back to the head of the plant to come back through again. This meant that there were polymers in the primary clarifiers. However, the sludge and the polymers were only added to one of two primary clarifiers. This is somewhat complicated, but provided us with reference points for the presence of polymers. After discovering that the toxicity was filterable in the final effluent but not in the primary clarifier overflow, the next question we asked was whether the toxicity was coming into the plant or introduced within the plant. On a visit to the plant I noticed barrels of defoamer and biocide. It turned out that more chemicals were being used in the plant than the operators had initially thought.

The next step was to determine if the amount of polymers used was sufficient to cause the toxicity. We found that the effect level for the polymers they were using was about 1 ppm. Therefore, the 10 ppm that they add could certainly be causing the toxicity we have observed.

Given the probable role of polymers in toxicity we then considered the question of the flat dose/response curve. Other toxicity tests on polymers that we performed prior to this study showed only a small difference between acute and chronic toxicity. I speculate that polymers are toxic when they are in solution, but not when they become associated with solids. As the effluent becomes diluted, enough polymer may be released from solids to maintain dissolved concentrations until the effluent is very diluted.

Having found that the polymers are toxic enough to be causing the problem we are now moving on to treatability studies. We will simulate the treatment plant operation so that we can look at overflow from both primary clarifiers, set up activated sludge mimicking nutrient loads, and run secondary bench scale treatment with and without polymers to see if the polymers are toxic. Of course we expect that it will be toxic with polymers. If the secondary effluent without polymers is not toxic this will become an engineering or substitution study. This treatability work will begin in a couple of weeks.

CASE 2

My second example is of a POTW effluent. The effluent is not toxic to fathead minnows, and is often not toxic to *Ceriodaphnia.* We started work on this effluent last summer. It took three or four months before we had a toxic sample.

This sample killed all of the *Ceriodaphnia* at 100% effluent. Filtration and aeration seemed to improve survival. Young production followed the same pattern. The C_{18} column removed toxicity and the toxicity was recovered in the first fraction. This combination of results suggests toxicity due to surfactants, as Ms. Norberg-King stated earlier. This toxicity was not due to polymers, which are not used by the POTW.

After learning that this effluent was toxic, the manager of this plant decided to perform a TIE before the permit required it. We are finding that many medium-sized cities do this, and we advise them to, because generally regulators are more lenient if efforts are being made to solve the problem.

Q: At the 2.5 EDTA addition there was 40% survival?

A: Yes. I don't understand that. A point I would like to make is not to get derailed because there is something that you don't understand. It is possible that it was metal toxicity. The plant manager, in fact, did suspect that a metal finisher was a cause of the toxicity of the POTW effluent.

Clearly, the way to proceed with this effluent is to look more closely at the possibility of surfactant toxicity. In TIE work it is advisable to first work on what you understand best. Don't worry about understanding all of the details at once. Suppose the EDTA effect was real and there is metal toxicity. Many times it would not be important in the overall toxicity of the final effluent, and once the major toxicant was removed the effluent would be non-toxic. That is surely the case here because the effluent is not often toxic and not very toxic. Chances are they will meet their effluent limits if they control surfactants.

Q: Did you do any pH adjustment on this sample?

A: Yes. It had no effect.

I wanted to show another slide for this effluent where the sample lost its toxicity. One of the problems faced in performing chronic TIEs is knowing whether the sample is toxic. If you test the sample first it will be up to seven days old by the time the TIE starts. The toxicity could be lost during this period. On the other hand, if you begin TIE manipulations and the sample is not toxic the effort can also be wasted. We set up an initial test immediately after the sample comes in. As soon as we see visual evidence of toxicity we start TIE manipulations. This often allows us to start the TIE when the sample is only two or three days old instead of seven days old.

CASE 3

Even though I am showing some industrial effluents here, there is really no difference from municipal effluents in the approach used. Some industrial effluents can be simpler, but by and large they are all fairly complex.

This next dataset is from a metal fabrication facility. The effluent is toxic to both *Ceriodaphnia* and fathead minnows. The effluent from this plant is the only inflow to the receiving water - the stream starts at their outfall. The permit requirement is for no chronic effects on both species at 100% effluent. The effluent is almost 100% cooling water which presumably does not come into contact with any toxic materials. The plant operators do admit, however, that they have pinhole leaks in some of their condensers. The plant uses well water pumped up from wells at the plant. This water is very soft (8 mg/l hardness). Fathead minnows don't do well in soft water, so the client suspected that the fathead minnow toxicity may have been due to the low mineral content of the water.

We have certainly seen problems with fathead minnows in soft water. Unless the fish are in very good condition you can get random mortality. With fathead minnows I would much rather work with an effluent that is 2 ppt salinity than I would with this one. Anything the sample contacts is likely to cause contamination.

In testing with fathead minnows, the whole effluent did not reduce the growth rate to an unacceptable level, but did produce less than 40% survival. EDTA at both levels of addition removed the toxicity, as did the C_{18} extraction. Interpretation of these data requires some subjective judgement, but this is the best approach. Thiosulfate, filtration, aeration, and pH adjustment had little or no effect. The blanks looked good. At pH 6 the survival was poor (35% after 24 hr) so the growth rates could not be assessed. This raises another point: end the test when you have got the answer. There is no need to run the test for seven days in this situation. Nothing was recovered in the methanol eluate from the C_{18} column. This pattern (EDTA and C_{18} removal) suggests metal toxicity.

A decrease in toxicity with pH helps narrow the list of metals that might be causing the toxicity. As we saw in Ms. Norberg-King's presentation (Table 2), copper and lead decrease in toxicity at higher pH. The data seemed to suggest copper toxicity, but, as I am about to show, fathead minnows were more sensitive to this effluent than *Ceriodaphnia*. Since *Ceriodaphnia* is more sensitive to metals than fathead minnows, this seemed to contradict the possibility of metal toxicity.

In testing another sample with fathead minnows, we found about half the growth of the control. Note that we did not see very high growth in the control because we were mimicking the characteristics of the dilution water, which was extremely soft. In this case about 5% DMW was appropriate. In this sample, aeration and filtration had no effect on either growth or survival. EDTA addition resulted in good growth and survival. Thiosulfate improved growth and survival, especially at the 50 mg/l level. The C_{18} column had no effect

this time. This finding was problematical; copper toxicity should have been removed by this treatment. Adjustment to pH 6 using the CO_2 technique killed everything.

This prompts me to make a point about cautious use of buffers. The use of buffers is fairly new, and we don't know everything they do to an effluent. If the cause of toxicity is not known, you cannot assume that CO_2 and a buffer would yield the same results because they act differently. The EPA lab has clearly shown that the buffers can work for ammonia, but if the toxicant is unknown buffers must be used with caution.

On this sample pH adjustment gave a decent graded response. At pH 7 the toxicity was about the same effect on growth as in the whole effluent, but the survival was not very good. At pH 8 the toxicity disappeared, again suggesting copper toxicity.

You are probably wondering why we didn't measure metals up to this point. We knew this effluent was high in zinc due to high concentrations in the well water. Chemists often like to measure things right away. The problem is that there is no analytical technique that measures the toxic forms of metals. This is a particular problem with metals in POTW effluent. There is probably not a POTW effluent in existence that doesn't have enough metal of one kind or another, especially zinc, in it to be toxic. The beauty of the TIE procedure is that it does sort out the toxic forms. One of the worst problems in interpretation of TIE results are prior assumptions.

Q: What was the pH of this effluent?

A: I believe it was in the mid-sevens.

Let's look at some *Ceriodaphnia* data from this effluent. With the whole effluent there was poor survival and poor young production. Aeration caused some improvement in both survival and reproduction. Filtration also caused some improvement. EDTA and thiosulfate did not have a clear effect. If this effluent had copper in it, then *Ceriodaphnia* should be sensitive. It is possible that we are just seeing a small amount of toxicity reduction by removing copper and that something else is also causing toxicity. Aeration and filtration should not affect copper at neutral pH. C_{18} extraction caused some improvement. Eluate one was not toxic. Eluate 2, however, was toxic. Adjustment to pH 8 caused improvement in survival and limited improvement in reproduction. pH adjustment with the CO_2 method yielded similar results as with the buffer. To summarize, we saw improvement with aeration, filtration, and C_{18} extraction (as well as toxicity recovery in the eluate): a pattern implicating surfactants. The pH adjustment procedure indicates that copper is probably also causing toxicity.

We are now in Phase III with fathead minnows on this effluent. There is strong evidence that copper is causing toxicity to the fathead minnows. For confirmation we are doing regression, pH adjustment, thiosulfate, and EDTA. As Ms. Norberg-King showed in a matrix, EDTA removes toxicity of both copper and zinc, while thiosulfate removes toxicity

for copper but not zinc. Using these manipulations in combination allows for the separation of copper and zinc toxicity. Plenty of zinc is present in this effluent, but it apparently is not biologically active.

With *Ceriodaphnia* we are still in Phase II. Since copper does not come off of a C_{18} column we know that some other toxicant is also acting on *Ceriodaphnia.*

Q: If this is non-contact cooling water where are the copper and surfactants coming from?

A: There are copper heat exchangers throughout the plant. The only treatment they do, since this is non-contact cooling water, is to adjust the pH to 8, run the effluent through an equalization basin, and then release it. As soon as copper was identified as a problem, I suggested that, since this water has very low solids content, they move the pH adjustment to the well head and circulate higher pH water to reduce copper loss. I think they are going to test this on a bench scale. It is fairly certain, however, that copper will sometimes be toxic to *Ceriodaphnia* even at the observed pH. The pH doesn't stay at 8, it decreases, as is very typical of pH-adjusted water.

Regarding surfactants, I was initially told that this effluent was purely non-contact cooling water. It turned out that the plant uses a number of biocides to control bacterial growth. They also use some of this water to cool metal molds. Staff at the plant were not trying to withhold information; they just didn't think about it. This is why site visits can be very valuable. Some operations are so complex that site visits are a waste of time, but in some cases they can be very valuable.

I then found out that the coolant used on some surfaces in the plant are routed to the heat exchanger. This coolant is a kerosene-type material. The plant did run about 10 ppm oil and grease in their routine monitoring. In fact a sheen on the surface was visible at the discharge point. The source of this material is pinhole leaks in the heat exchangers.

We have enough data to suspect surfactants of some kind, and I don't necessarily mean detergents. We are beginning to use a procedure similar to that used for the paper mill, looking at the toxicity of different components. Again in this case we are hindered by the lack of analytical methods, as frequently is true at this stage of a TIE (especially with polymers).

As I mentioned, we are now in confirmation with copper and fathead minnows. There is no evidence of surfactant toxicity to fathead minnows, even though there is ample evidence that surfactants are affecting *Ceriodaphnia.* Copper is probably also affecting *Ceriodaphnia,* depending on the prevailing pH.

I just found the slide that I wanted to show earlier illustrating toxicity decay in the POTW effluent. When the sample first came in we found it to be toxic. By the time we began Phase I, however, the toxicity was gone. Toxicity decay is another characteristic of surfactants. This decay may be due to decomposition, sorption, or some other process. This can be used as another piece of evidence to identify surfactant toxicity.

Polymers are a big problem. Bill (Goodfellow), can you verify that?

Bill Goodfellow: Yes. We are finding that even within the same polymer type there can be orders of magnitude differences in toxicity and persistence.

Another important point is that the toxicity of polymers depends upon the matrix. If high concentrations of solids are present the polymers are going to be much less toxic. Have you solved the problem of the lack of analytical methods for polymers?

Bill Goodfellow: No. We have suggested going through a process of polymer substitution in these situations.

Q: **Couldn't you do a sorption isotherm study where you vary solids concentration versus a nominal polymer dose to get equilibria and then pick the one with the best sorption?**

A: I haven't done it but it seems quite reasonable to me. We will probably be forced to it one of these days.

With POTWs the problem is different. It is probably not too difficult to determine whether the polymers used in treatment are causing toxicity. However, not all polymers are added within the plant. The various kinds of surfactants in POTW effluents have a wide range of toxicity, varying by at least a factor of 40 for some common ones. Surfactant breakdown products can also be toxic in the ug/l range. Again, the analytical methods for surfactants are inadequate. If you only have 3-4 toxic units and there is a range of 40 toxic units for different surfactants, there is no way to identify the surfactant causing toxicity. Furthermore, surfactant analyses, as poor as they are, are also expensive. They can be sorted into different groups, but even that is not good enough. Consequently, intuitive reasoning is required in identifying surfactant toxicity in TIEs instead of relying on analytical work.

We had a representative of a big polymer manufacturer at a workshop I gave last fall and he left in tremors. Those of you involved in engineering and treatment should insist that the companies selling surfactants do the testing. A couple of refineries have done so - we did the testing.

I know this presentation appeared disorganized, but I have demonstrated the type of thinking that goes into analysis of TIE data. There are a couple of other points that I wanted to mention.

First, you cannot use multiple samples in Phase I of a chronic TIE. This comment is directed especially at regulators. If the toxicants switch, or even merely their ratios switch, this will cause complete confusion. Composite samples should be used, and one sample should be carried through the entire scheme. Phase III is a different story. In Phase III grab samples are appropriate, though it is still important to work with a single sample, because many of the confirmation techniques are aided by effluent variability.

I also would like to comment on the treatability approach. There is a strong tendency to only perform treatability studies once or twice. Treatability studies are subject to the same errors as TIEs if multiple samples are not analyzed. In the case of the metal fabrication facility we would have missed the copper toxicity to fathead minnows if we had not analyzed more samples in Phase I. The composition of effluents changes, so successful treatment of one problem may leave other problems unresolved.

I also wanted to comment on the importance of randomization in toxicity tests. In chronic tests it is very important to randomize position. Large differences in physical conditions, such as evaporation rate and/or temperature, can exist on test boards. When you are attempting to establish the significance of test results it is very important to minimize extraneous sources of variability. It can be easy to put your EDTA test on the side of the board and conclude that it did nothing when evaporation caused a 50% concentration of the sample. The need for quality control of this type cannot be stressed enough.

Q: **Have you seen any differences in the effects of glass versus plastic containers on loss of toxicity due to polymers?**

A: In the two cases discussed today involving surfactants and polymers we tested for this by shipping in both glass and plastic containers and we saw no difference.

I would also like to make a point about the behavior of surfactants and polymers in C_{18} extraction. Recovery of these compounds from the column depends on the rate at which they adsorb. Gerry Ankley of Duluth EPA showed in the Boston Metro work that if surfactants were added to municipal waste, immediate filtration could not remove them from the sample because not enough time had elapsed to allow them to sorb to solids. In performing chronic tests it is important to consider equilibrium time. This will affect decisions such as whether to make post-C_{18} effluent daily or once every seven days, or whether to add EDTA in one batch or in several aliquots. There is no clear best answer to these questions, but it is important to be consistent and to understand the pitfalls of the chosen method. This is another example of the importance of professional judgement in TIE work.

I would like to make a comment about costs. We break TIEs into two tiers, as shown by Ms. Norberg-King (Figure 2). We have yet to perform the procedures in Tier 2. We

charge the same price for a Tier 1 chronic TIE as we do for an acute TIE including the radical pH adjustment.

Another comment I wanted to make had to do with a philosophy that I put into the EPA manual, although with the Phase III this is changing somewhat. The manual gives the impression that the three phases of a TIE should be performed in sequence. There are several problems with this approach, especially since regulatory agencies have put so much emphasis on single samples. The abnormal sample is as important as the average sample. This is in spite of the fact that TIE methods were not designed to handle episodic events, as stated in the manuals. At our laboratory we do Phase I and in some cases start right in on Phase II or Phase III. We have found that in some cases conclusions can be reached based on one sample. When a pattern begins to emerge (e.g., indicating detergents or metals) you can start on Phases II and III while still completing Phase I. It is still necessary to run several samples in Phase I, however, because in many cases other toxicants appear. This approach also allows you to complete the TIE quicker, which is always a concern of clients.

Q: Are you successful in identifying which polymers or surfactants are causing toxicity in Phase II?

A: In the case of the paper mill we have solid evidence that the one and only polymer used in the plant is the one causing toxicity. In cases where there are more than one polymer in use you need to do some tracking. You can't track toxicity in untreated waste without doing something, because some toxicity will be removed by the treatment process. There are essentially two choices. If, for example, you are tracking filterable toxicity, filtration can be used as a sorting tool. If filtration does not remove the toxicity from untreated effluent then the toxicant is different from the toxicant that is affecting the final effluent. If it is removed this also narrows down the possibilities. Perhaps the better approach is start the batch reactor treatability studies. These are quite successful. In these studies the influent is fortified with material from the interceptor or collection system that is of concern. If the toxicity of the fortified treated waste is greater than the unfortified treated waste, this is evidence of pass-through toxicity in the plant. Some situations may arise in which there are multiple sources and you can't go any farther. We haven't encountered this yet but I think there are ways to deal with such a situation.

Q: Would these methods be considered Phase II?

A: Yes. Phase II is identification, however you go about it. When the guidance for Phase II was written, we didn't realize that there would be so many areas where analysis was lacking. Chlorine compounds is another group that poses a problem analytically. These compounds have widely varying toxicities, and the methods are so prone to interferences in effluents that they don't work very well. Furthermore, you can't add chlorine to an effluent and duplicate the chlorination that happens in the

treatment plant. One of the reasons for choosing the examples that I gave was to show that you can proceed with a TIE even if analytical methods are lacking.

Q: **Have you considered the effect of EDTA on the osmolarity of the samples?**

A: Osmolarity is not a problem at any of these concentrations -they are far too low. Even with freshwater organisms and total dissolved solids it is not an osmoregulatory problem.

Q: **Have you ever been asked to use *Selenastrum* as a TIE organism?**

A: No, but others have done TIEs with *Selenastrum.*

Q: **Have they had success?**

A: My impression was that they did. You have the same problem with *Selenastrum* as with the other marine organisms - their tolerance levels are not known. There may also be methodological problems with some procedures such as pH changes. Manipulation without additives, however, can be performed, such as C_{18} extraction or ion exchange. Don't be afraid to try the techniques. If you can run the right blanks you can at least tell whether an observed effect is real.

Q: **In interpretation of TIE data you recommend throwing away statistics and ignoring contradictory data. Is this really good science?**

A: I didn't recommend throwing away statistics. I said that statistics alone are not enough and that there is a lot more information in the data than would be indicated by hypothesis tests. I also said that statistics have a more important role in Phase III, where definitive data are being generated. You will see confusing data and you should pursue the clear points. As Ms. Norberg-King showed, once one toxicant is identified it becomes easier to identify another.

The conflicting results with EDTA and the metal waste pointed out earlier may have been due different chemical conditions in the test chambers changing the relative toxicities of the two toxicants. In that case you don't know what to control. At this early stage when you don't know what parameters are important you simply make your best effort at interpretation and proceed with further experimentation until a clear pattern emerges.

The single biggest problem in TIE work is the pH meter. We review TIE data generated by others, and have found that in a third of the studies ammonia problems have gone unrecognized. The reason for this is a lack of good pH measurements. This is especially important for ammonia. When pH changes from 7.6 to 7.8 the LC50 of a given amount of total ammonia changes by 40%. The pH of almost all

effluents will drift during a toxicity test. POTW effluents usually drift up. A typical POTW effluent with pH 7.2-7.4 will drift to pH 8.5-8.8 at equilibrium in the test chamber. This shift changes the toxicity of a given amount of total ammonia by five to six times. If EDTA is added it will lower the pH a little and may cause the appearance that EDTA removed toxicity. C_{18} extraction also tends to lower pH. Radical pH adjustments also tend to induce pH shifts after the sample is returned to pH_i. A shift of one or two tenths in pH can be very confusing in a TIE, especially in chronic tests.

Q: If the pH of the sample tends to rise after it is collected, what do you call pH_i?

A: That is a relatively unimportant question. It is important that pH_i always be the same number and that you know what it is. Ammonia is probably the most pH-sensitive toxicant commonly encountered. Enough is known about ammonia toxicity that you can correct for pH if the pH is known. If you are doing TIE work get the ammonia criteria document and the tables of dissociation, which are published separately.

Stephen Hansen
S. R. Hansen and Associates

I must admit that when I was first asked to participate in this workshop I was surprised that there was so much controversy over whether chronic TIEs could be performed successfully. I hope that with my talk and the other talks that some of this controversy will be put to rest. At S.R. Hansen and Associates we have performed hundreds of acute TIE procedures (not whole studies but individual manipulations). We have also worked on quite a few treatability studies which answer the important question of how to reduce toxicity once it is identified. Over the past two or three years we have primarily concentrated our efforts on development of chronic TIE methods at the same time that EPA was developing their chronic TIE methods. We have been successful both at developing methods and using the methods to identify causes of toxicity in municipal and industrial effluents.

I would like to point out that the work I will describe was a group effort. People responsible for much of the work were Gary Wortham, Scott Ogle, Marcus Cole, Mary Helen Garcia and Jeffrey Cotsifas.

My talk will consist of four parts. First I will give a brief overview of the strategy of performing chronic TIEs. Then I will present three case studies of chronic TIEs. I will present three case studies because it provides an illustration of the versatility and the robustness of the approach.

PERFORMING CHRONIC TIES

The objective of a chronic TIE is to identify the chemical or chemicals causing exceedance of a chronic toxicity limit for an effluent. Usually chronic toxicity limits take initial dilution into account, so a limit might be no chronic toxicity at a 10% concentration of effluent. The strategy in toxicity identification is to fractionate the sample by removing or isolating chemicals (either into individual chemicals or groups of chemicals) and then test the fractions for chronic toxicity. A third step which is very important is to select and validate methods for fractionation and detection of chronic toxicity. Many separation schemes have been developed, including the EPA scheme and several others. Additional schemes will certainly be developed as more is learned. The principal methods that appear in the literature are those of Walsh and Garnas, Jop *et al.* (REF), Doi and Grothe, and the EPA manuals for Phases I and II. All of the chemical techniques used to manipulate the sample are standard techniques. For a number of the procedures the efficiency is described in the literature. However, for other procedures, such as those for removing metals on ion exchange columns, the techniques were not designed for the low concentrations of interest in chronic TIEs. For example, in the Walsh and Garnas procedure XAD resin is used to remove organics, but different types of XAD resin perform differently. XAD-2 resin has been shown to remove 45% of the phenol in a sample, while XAD-7 removed 86%. In the EPA Phase I graduated pH procedure a change in pH from 6 to 8 can drastically alter the toxicity of ammonia (Table 18). The relationship between pH and ammonia toxicity is well worked out. Other

procedures are not selective. Clinoptilolyte removal of ammonia, for example, reacts with chemicals in the following sequence of decreasing reactivity: Cs > K > Ag > Rb > NH_3 > Pb > Na > Ba > Sr > Ca. This is the kinetic reactivity series. There is also a thermodynamic reactivity series that should be taken into consideration. Another example is copper removal on Sep-Pak cartridges, which is being done at the Narragansett lab. They have been successful at bringing concentrations on the order of 850 µg/l down to 50 µg/l. However, copper can be toxic at concentrations of 5 to 20 µg/l, depending on the binding capacity of the water, so the Sep-Pak may not remove a toxicologically significant amount of copper. In summary, some of these procedures are well understood and some are not, and further research is needed on certain aspects of them.

Selection and validation of the chronic toxicity detector poses a larger problem because the use of the chronic bioassay as a detector is not standard. There are several major areas of uncertainty.

1) Will the animals suffer artifactual toxicity when subjected to fractionation and chronic exposure periods?
2) If artifactual toxicity does occur, are there ways to modify the fractionation procedures?
3) Is it better to renew with the original sample or with new samples?
4) Is it better to concentrate the toxicity and use an acute bioassay as the detector?

I will address the last issue first. Two conceptual alternatives for assessing chronic toxicity are 1) to concentrate the toxicants and use acute bioassays, and 2) to use the unaltered effluent and employ chronic bioassays. Both of these approaches have advantages and disadvantages. The advantage of the pre-concentration method is that the validated acute toxicity TIE methods can be used. There are two major disadvantages of the pre-concentration method. One is that you do not know what to concentrate. There is also a potential for false positives. For example, the fathead minnow NOEC for cadmium is 15 ppb for chronic toxicity and the NOEC for acute toxicity is 31 ppb. If cadmium is present in the effluent and shows no chronic effect at 10 ppb, acute toxicity may be observed if the sample is concentrated five times.

Advantages of using unaltered effluent samples are that responses causing regulatory action are measured directly and there is little chance of false positives. The major disadvantage is that acute TIE methods are not directly applicable to chronic TIEs, and therefore chronic TIE methods must be developed. I am pursuing this second option, mainly to avoid the problem of false positives.

There are two basic options for renewal of samples in chronic TIEs. One is to fractionate a large volume of the original sample and refrigerate it to use for renewals. The other is to collect new samples on each renewal day, fractionate them, and use them for

SELECTION & VALIDATION OF SEPARATION TECHNIQUES

MANY SEPARATION SCHEMES ARE CURRENTLY AVAILABLE

EPA Phase I & II	Walsh & Garnas
Jop et al.	Doi & Grothe

ALL CHEMICAL TECHNIQUES IN SCHEMES ARE STANDARD

Efficiency Info in the Chemical Literature

Some Techniques being Pushed to New Limits & Need to be Quantified

FOR EXAMPLE:

1. Walsh & Garnas Procedure

Sorption of phenol onto XAD-2	= 45%
Sorption of phenol onto XAD-7	= 86%

2. EPA Phase I - Graduated pH Test

pH	% Unionized NH_3	Total NH_3 Chronically Toxic to Fatheads
6.0	0.057	439
8.0	5.7	4.6

3. Other Procedures

Clinoptilolyte Removal of NH_3 - Not Selective

Cs > K > Ag > Rb > NH_3 > Pb > Na > Ba > Sr > Ca

Copper removal on Sep-Pak Plus CM

Efficiency: 856 ug/l —> 50 ug/l
Toxicity to oysters at 5 - 20 ug/l

Table 18. Selection & validation of separation techniques.

renewal. Renewal with the original sample may allow the toxicity to degrade. Renewal with new samples may avoid the problem of toxicity decay, but may result in dilution of the toxicity in the original sample if later samples have lower toxicant concentrations. A major point in favor of renewal with the original sample is the cost of fractionation, which is high for renewal with the original sample but is very high for renewal with new samples.

CASE STUDY 1

This first case study is of the Palo Alto wastewater treatment plant. They began evaluating toxicity as part of the effluent variability study of the Effluent Toxicity Characterization Program. They ran three chronic tests: the 4-day *Selenastrum* population growth test; the 7-day mysid survival, growth and reproduction test; and the 7-day silverside survival and growth test. Their compliance requirement, since they are not given any dilution credit, was no chronic toxicity in 100% effluent (i.e., < 1 TUc).

Biomonitoring results over the course of 12 months showed a violation of the effluent limit in every sample (Table 19). The level of toxicity was highly variable and the variability was random. *Selenastrum* was the only sensitive species. Chronic TIEs had not previously been performed with *Selenastrum* so we had to develop and validate a chronic TIE method with *Selenastrum,* and then design a study to try to explain the observed variability.

Table 20 shows one of the validation steps for the use of *Selenastrum* in Phase I testing. None of the fractionation procedures had a negative effect on survival compared to survival in the untreated effluent.

Usually EDTA and STS additions are slightly toxic to the test organisms (Table 21). As more of each reagent is added the population size becomes smaller. If a metal is present initially, the solution may be toxic at first, but when EDTA is added the metal and the chelator bind to each other, resulting in lower toxicity due to both the metal and the chelator until the amount of chelator becomes an excess. At that point the toxicity will again begin to increase. With STS addition, biostimulation occurs up to a point. This is an artifact of the procedure. These patterns seen in controls must be kept in mind to avoid misinterpretation of results of tests on effluent samples.

We also use more than one procedure in our TIEs. The Walsh and Garnas procedure is a conservative fractionation procedure in which classes of compounds are separated and then tested to see whether they are toxic (Table 22). This is in contrast to the EPA procedure which removes toxicants from the sample. Both procedures yield satisfactory results.

Results from Phase I work on this effluent (Figure 3) show that the adjustment to pH 11 almost always removed the toxicity. This suggests either that something was precipitating out or being transformed at high pH, such as a metal forming a metal hydroxide that does not revert to its orginal state when the sample is returned to pH_i. The filtration step also showed removal at pH 11. Much of this was due to the pH adjustment, but filtration enhanced the

RESULTS OF PALO ALTO BIOMONITORING

SAMPLE DATE	CHRONIC TOXICITY UNITS (TUc)
5/26/90	1.6 *
6/25/90	2.8 *
7/14/90	>10 *
8/04/90	10 *
9/01/90	2.8 *
9/08/90	1.6 *
9/25/90	1.6 *
1/27/91	2.8 *
2/07/91	5.6 *
4/15/91	11.2 *

* - EXCEEDS CHRONIC TOXICITY LIMIT

RESULTS:

1. OVER 12 MONTHS ONLY SELENASTRUM WAS AFFECTED
2. LEVEL OF TOXICITY HIGHLY & RANDOMLY VARIABLE

CONCLUSIONS:

1. MUST DO CHRONIC TIE WITH SELENASTRUM
2. SHOULD DESIGN STUDY TO CAPTURE VARIABILITY

Table 19. Results of Palo Alto Biomonitoring.

CONTROL RESULTS FOR "PHASE I" CHRONIC TIE USING SELENASTRUM AS DETECTOR

FRACTION	Cells/ ml (X 10^5)	% of Baseline
BASELINE (UNTREATED)	2.41	100
pH-ADJUSTMENT		
I-3-I	2.27	94
I-11-I	2.95	122
FILTRATION		
pH 3	3.22	134
pH I	2.77	115
pH 11	2.26	94
AERATION		
pH 3	2.55	106
pH I	2.61	108
pH 11	1.64	68
C-18 ADSORPTION		
pH 3	3.26	135
pH I	2.92	121
pH 9	2.29	95

Table 20. Control results for "Phase I" chronic TIE using *Selenastrum* as detector.

CONTROL RESULTS FOR EDTA & STS TESTS IN "PHASE I" USING SELENASTRUM AS DETECTOR

	POPL'N SIZE (cells/ ml X 10^5)	
ml ADDED	EDTA	STS
0.0	1.03	1.8
0.0125	0.12	4.8
0.025	0.15	6.2
0.05	0.11	4.9
0.1	0.15	2.6
0.2		0.26
0.4		0.13
0.6		0.11
0.8		0.10
1.0		0.09

Table 21. Control results for EDTA & STS tests in "Phase I" using *Selenastrum* as detector.

CONTROL RESULTS FOR "WALSH & GARNAS" CHRONIC TIE USING SELENASTRUM AS DETECTOR

FRACTION	Cells/ ml ($X 10^5$)	% of Baseline
XAD 4 RESIN		
BASELINE	2.41	100
NON-POLAR	2.91	121
POLAR	2.10	87
ANION XCHGE	1.49	62
CATION XCHGE	3.06	127

Table 22. Control results for "Walsh & Garnas" chronic TIE using *Selenastrum* as detector.

SUMMARY OF RESULTS - PALO ALTO PHASE I CHRONIC TIEs

Characterization Test	TIE #1	TIE #2	TIE #3	TIE #4	TIE #5
% Reduction	92	51	84	89	97
pH Adjustment					
I-3-I	-	+	-	-	-
I-11-I	-	+	+	+	+
Filtration					
3	-	+	-	-	-
I	-	-	-	-	-
11	-	++	+	++	++
Aeration					
3	-	+	S+	+	+
I	+	-	-	-	-
11	+	+	S+	S+	++
C-18 Adsorption					
3	-	+	S+	S+	-
I	+	-	S+	-	-
9	-	++	++	++	++
EDTA Chelation	++	+	+	S+	S+
Thiosulfate Addition	-	-	-	++	-

Figure 3. Summary of results - Palo Alto Phase I Chronic TIEs (- no effect, + removes toxicity, ++ biostimulation, S+ slight removal).

population growth even further. Aeration had no effect beyond that caused by the pH adjustment. C_{18} adsorption was preceded by filtration, and yielded similar results as the filtration step. EDTA chelation reduced toxicity in each sample, suggesting metal toxicity. STS only had an effect on one sample. Overall, these data suggest that we are dealing with an EDTA-chelatable metal that forms an insoluble hydroxide at high pH.

Table 23 is a reproduction of a matrix shown earlier by Teresa that shows the differing effects of EDTA and STS on metals. Since we found that EDTA had an effect and STS did not, the candidate toxicants are zinc, manganese, lead, and nickel. Note, however, that not all of the elements are included in this matrix. Also, the matrix is different for different species, especially for algae, which have binding sites on the cell wall that can strip metals off of the EDTA and the STS.

Comparison of metal concentrations in these samples with LOECs for *Selenastrum* indicate that zinc and silver are prime suspects as the cause of the observed toxicity (Table 24). As is often the case, we had to generate the LOECs for silver and zinc in our own laboratory. It is surprising how often holes are encountered in the database on toxicity to even common test organisms. These results, combined with the EDTA and STS results implicate zinc. Silver may also have had a role in the toxicity of sample #4 that was removed by STS. We found a strong correlation between zinc concentration in these samples and reduction in *Selenastrum* growth (Figure 4).

We also think we had a hardness problem in this effluent (Table 25). The hardness in the effluent was around 300, a level that is known to reduce *Selenastrum* growth. We found that increasing the hardness from 15 mg/l to 300 mg/l reduced growth by an average of 55%. This suggests that even if Palo Alto removed zinc from their effluent they would not meet their toxicity limit for *Selenastrum* because of this effect of hardness. Since the discharge is released into San Francisco Bay, this degree of hardness would not be expected to have an effect in ambient waters. We also noticed that the composition of the hardness varied over time. Altering the calcium:magnesium ratio from 50:50 to 57:43 changed the effect on *Selenastrum* entirely, causing stimulation (Table 25). We think the magnesium is causing these results. Regression of hardness (50:50 mixture of calcium:magnesium) and reduction in algal growth also showed a strong correlation (Figure 5).

In summary, this effluent appeared to show a zinc problem, and even if the zinc problem was solved a hardness problem would still be present. This is something that regulatory agencies will have to be aware of in implementing effluent toxicity limitations, especially for freshwater effluents discharged to marine systems.

CASE STUDY 2

A second example is the El Paso Haskell Street wastewater treatment plant. We were working on this effluent and developing procedures at the same time that EPA in Duluth was

EDTA\STS METALS REMOVAL MATRIX

		EDTA	
		YES	NO
STS	YES	Cu, Cd, Hg	Ag, Se(6)
	NO	Zn, Mn, Pb, Ni	Fe, Cr, Al, As(4), Se(4),

Table 23. EDTA\STS metals removal matrix.

COMPARISON OF CHEMICAL CONCENTRATIONS IN PALO ALTO'S EFFLUENT & KNOWN MINIMUM EFFECT CONCENTRATIONS

PARAMETER	TIE #1	TIE #2	TIE #3	TIE #4	TIE #5	LOEC
% Reduction in Algal Growth	92	51	84	89	97	
Metals (ug/l)						
Arsenic	1.9	2.1	1.5	1.9	1.6	690
Cadmium	1.4	<1	<1	<1	<1	50
Chromium	<5	<5	<0.5	<5	1.0	397
Copper	19.4	26.7	17.2	12.7	14.4	50
Lead	4.0	2.7	4.4	<1	1.9	500
Mercury	<0.1	<0.1	<0.1	0.1	<0.1	59
Nickel	2.9	2.3	6.0	3.8	4.8	—
Selenium	1.0	1.1	<1	1.2	<1	199
Silver	0.5	1.5	0.4	1.6	0.2	1*
Zinc	92	52	82	88	101	30*
Hardness (mg/l $CaCO_3$)	272	208	280	276	300	<300
Conductivity (umhos)	1700	1200	1500	1100	1600	?

Table 24. Comparison of chemical concentrations in Palo Alto's effluent & known minimum effect concentrations.
(Growth reduction in 100% effluent. LOEC for *Selenastrum.*)

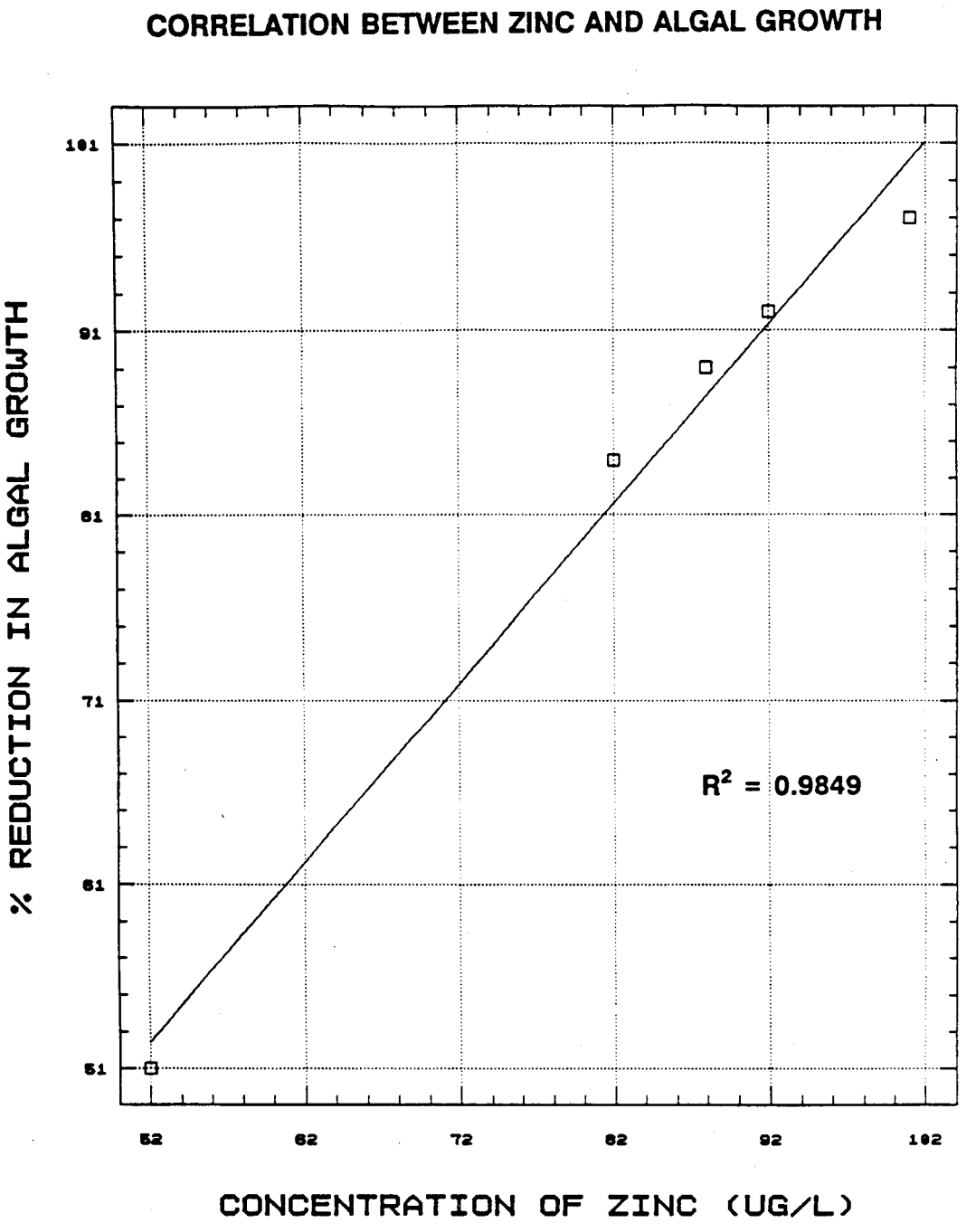


Figure 4. Correlation between zinc and algal growth.

EFFECT OF HARDNESS ON SELENASTRUM GROWTH

	CELLS/ ML X 10^5		
TEST #	LOW HARDNESS (15 MG/ L)	HIGH HARDNESS (300 MG/ L) (50% Ca - 50% Mg)	% REDUCTION
1	2.83	1.26	55
2	1.23	0.38	69
3	1.77	1.03	42
			AVG = 55%

	CELLS/ ML X 10^5		
TEST #	LOW HARDNESS (15 MG/ L)	HIGH HARDNESS (300 MG/ L) (57% Ca - 43% Mg)	% REDUCTION
4	2.61	3.75	-44 (stimulation)

Table 25. Effect of hardness on *Selenastrum* growth.

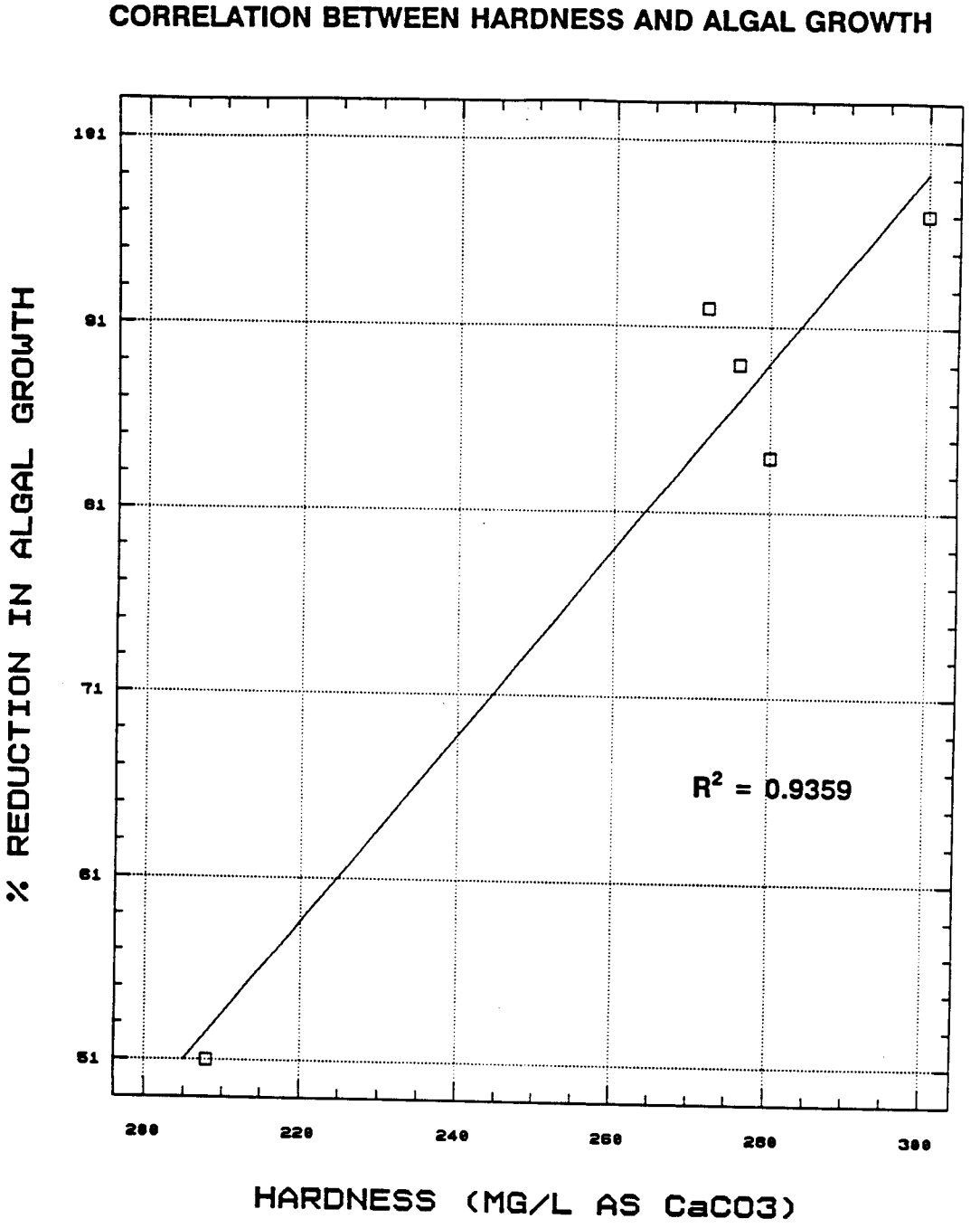


Figure 5. Correlation between hardness and algal growth.

developing theirs. This plant was running the 7-day fathead minnow growth and survival test and the 7-day *Ceriodaphnia* reproduction and survival test. Their compliance requirement was no chronic toxicity in 48% effluent (i.e., < 2.1 toxic units [TUc]).

Table 26 shows biomonitoring data for seven months. Effluent limits were almost always violated in the *Ceriodaphnia* test, and were sometimes violated in the fathead minnow test. No acute toxicity was observed for either species. As with the Palo Alto effluent, the level of toxicity varied randomly and widely. In fathead minnow test, for example, the chronic toxicity went from greater than 4 TUc on the first sampling date to less than 1 TUc in the next (Table 26). The observation that *Ceriodaphnia* was usually, but not always, more sensitive to the effluent suggested that more than one toxicant could be present in the effluent.

Based on these data, we concluded that we had to perform a chronic TIE, the study design should allow characterization of the variability in toxicity, and the use of both species was necessary because of the possible presence of more than one toxicant.

We developed a chronic TIE procedure by modifying the acute TIE procedures. The modifications we made closely matched those made by EPA-Duluth in development of their chronic TIE methods. These included:

1) Expose test organisms to each fraction for a chronic duration (i.e., 7 days);
2) Use chronic endpoints to evaluate impacts (survival and growth for fathead minnnows and survival and reproduction for *Ceriodaphnia*);
3) Renew exposure solutions on days 2, 4, and 6;
4) Use larger volumes of sample for exposures (100 mL rather than 10 mL); and
5) Feed organisms daily throughout exposures.

The modified acute TIE procedures worked well. Artifactual chronic toxicity was not observed in controls. The volume of effluent required for the chronic TIE was acceptable, though quite large (a total of 25 L for each species for initial fractionation and renewals).

Table 27 shows results for Phase I controls for *Ceriodaphnia* and fathead minnows. Ideally the results on each fraction should approximate results for the baseline, and generally these results looked very good. We have run these tests a number of times and gotten similar results. Tests on controls for the Walsh and Garnas procedure also yielded very satisfactory results (Table 28).

After performing about ten groups of TIE procedures (including the Walsh and Garnas procedure, Phase I, and some Phase II procedures) we began to suspect that more than one toxicant was present. One of these was silver. Passing the effluent through the XAD resin (part of the Walsh and Garnas procedure) did not alter the toxicity, suggesting the presence of polar toxicants. Subjecting the post-XAD effluent to cation exchange partially removed the toxicity, suggesting cationic metal toxicity. Chelation with EDTA (Phase I) had no effect,

RESULTS OF HASKELL STREET BIOMONITORING

Date	Chronic Toxic Units (TUc)	
	Fatheads	Ceriodaphnia
09/25/89	>4 *	>4 *
10/09/89	<1	2.9 *
10/23/89	2.9 *	2.9 *
11/26/89	2.1	2.9 *
12/10/89	2.1	2.9 *
01/07/90	2.1	>4 *
02/13/90	2.1	2.1
03/20/90	4 *	2.9 *

* - EXCEEDS CHRONIC TOXICITY LIMIT

RESULTS:

1. OVER 7 MONTHS BOTH SPECIES EXCEEDED TOXICITY LIMIT
2. NO ACUTE TOXICITY PRESENT FOR EITHER SPECIES
3. LEVEL OF TOXICITY VARIES RANDOMLY
4. SPECIES MAY BE RESPONDING TO DIFFERENT TOXICANTS

CONCLUSIONS:

1. MUST DO CHRONIC TI/RE
2. SHOULD DESIGN STUDY TO CAPTURE VARIABILITY
3. SHOULD USE BOTH SPECIES AS DETECTORS

Table 26. Results of Haskell Street biomonitoring.

CONTROL RESULTS FOR "PHASE I" CHRONIC TIE USING CERIODAPHNIA & FATHEAD MINNOW DETECTORS

FRACTION	CERIODAPHNIA		FATHEADS	
	% SURV	YOUNG/ FEMALE	% SURV	GROWTH (MG/ IND)
BASELINE	100	27.2	80	0.28
pH ADJUSTMENT				
I-3-I	100	27.0	100	0.25
I-11-I	100	33.0	100	0.26
FILTRATION				
pH 3	100	32.0	80	0.20
pH I	100	28.6	100	0.28
pH 11	100	33.8	80	0.28
AERATION				
pH 3	100	34.8	100	0.34
pH I	100	26.6	100	0.28
pH 11	100	32.2	80	0.33
C-18 ADSORPTION				
pH 3	100	36.4	80	0.30
pH I	100	25.6	100	0.22
pH 9	100	20.8	80	0.25

Table 27. Control results for "Phase I" Chronic TIE using *Ceriodaphnia* & Fathead minnow detectors.

CONTROL RESULTS FOR "WALSH & GARNAS" CHRONIC TIE USING CERIODAPHNIA & FATHEAD MINNOW DETECTORS

FRACTION	CERIODAPHNIA		FATHEADS	
	% SURV	YOUNG/ FEMALE	% SURV	GROWTH (MG/ IND)
BASELINE	100	27.2	80	0.38
XAD-2 RESIN				
NON-POLAR	100	19.0	100	0.36
POLAR	100	31.4	100	0.26
ANION XCHGE	100	21.4	80	0.40
CATION XCHGE	100	25.2	80	0.30
XAD-8 RESIN				
NON-POLAR	100	20.6	80	0.33
POLAR	100	31.2	100	0.26
ANION XCHGE	100	18.8	100	0.16
CATION XCHGE	100	25.2	80	0.28

Table 28. Control results for "Walsh & Garnas" Chronic TIE using *Ceriodaphnia* & Fathead minnow detectors.

precluding the possibility of toxicity due to copper, cadmium, mercury, manganese, nickel, lead, or zinc, but not the possibility of toxicity due to other metals such as arsenic, chromium, selenium, and silver. A pH adjustment to pH 3 with HCl removed much of the toxicity (Table 29). We suspected that this was due to Ag^+ combining with Cl^- to form an insoluble, nontoxic AgCl precipitate. Acidification with HNO_3 had no effect, supporting this hypothesis (Table 29).

We then measured concentrations of suspected toxicants in effluents and compared them with the LOECs for the two species (Table 30). LOECs for silver (which we developed ourselves) were quite close to the concentrations observed in effluent samples.

We also think that ammonia is another cause of toxicity in this effluent. Since ammonia toxicity is due to the unionized form (NH_3), ammonia toxicity increases at higher pH. The graduated pH test showed this pattern in the effluent. However, adjustment to pH 6 did not remove all of the toxicity, indicating that ammonia was not the only toxicant present. Ammonia concentrations in the effluent average 18.7 mg L^{-1}, with a range of 7.6 - 27.1 mg L^{-1} (at pH 7.5). For both species the chronic LOEC is about 0.25 mg L^{-1} (unionized ammonia) and acute effects occur at about 2 mg L^{-1}. Using the equilbrium constant for the conversion between total and unionized ammonia it is possible to determine the amount of total ammonia required to cause acute and chronic toxicity at various pHs (Table 31). At pH 7 about 40 mg L^{-1} is enough to cause chronic toxicity. At pH 8 about 5 mg L^{-1} is enough. Since most toxicity tests to measure compliance are done without controlled headspace pH will almost always rise to about 8. The concentrations in this effluent clearly suggested an ammonia problem.

Similar to the problem with *Selenastrum* sensitivity to hardness, *Ceriodaphnia* are sensitive to high conductivity. Conductivity is a general measure of the abundance of dissolved ions. *Ceriodaphnia*'s is probably sensitive to particular ions, not dissolved ions in general. Reference toxicant tests with NaCl showed reduced reproduction at conductivities as low as 900 umhos and reduced survival at conductivities as low as 2,000 umhos. The Haskell Street effluent had conductivities ranging from 1200 to 1900 umhos in 1990 and 1991. This indicates that "conductivity" could be causing toxicity. In some samples, however, we have seen conductivities as high as 4000 have no effect. The toxicity is entirely dependent on the composition of the conductivity. In effluents with high background conductivity it may be inappropriate to use *Ceriodaphnia* as a test organism because of the potential for conductivity-related toxicity.

In summary, toxicity to *Ceriodaphnia* in this effluent was probably due to silver, ammonia, conductivity, and non-polar organics. Toxicity to fathead minnows was probably due to silver, ammonia, and polar organics. Confirmation procedures are still being performed on this effluent.

CASE FOR SILVER TOXICITY

WALSH & GARNAS TEST RESULTS:

MOST TOXICITY NOT SORBED BY XAD - POLAR TOXICANTS

CATION EXCHGE PARTIALLY REMOVED TOXICITY - METALS

PHASE I TEST RESULTS:

EDTA CHELATION TEST - NEGATIVE

Not - Cu, Cd, Hg, Mn, Ni, Pb, or Zn

Does not preclude other metals - e.g., As, Cr, Se, & Ag

pH ADJUSTMENT TEST - TOXICITY REDUCED BY pH 3 SHIFT

	% SURVIVAL			REPROD (TOT #)		
TREATMENT	100	50	Cont	100	50	Cont
Baseline	0	100	100	0	75	73
pH Adjustment						
I-3-I (HCl)	80	100	100	22	81	86
I-3-I (HNO_3)	0	100	100	0	51	86
I-11-I (NaOH)	0	100	100	0	74	90

$Ag^+ + Cl^- \longrightarrow AgCl$

Table 29. Case for silver toxicity.

METAL CONCENTRATIONS OBSERVED IN WWTP EFFLUENT:

	LOEC (ug/ l)		EFFLUENT CONC (ug/ l)			
METAL	Fathead	Cerio	#6	#7	#8	#9
Arsenic	3,026	<1,800	13	8	6	6
Chromium	1,987	40	<5	<5	<5	<5
Selenium	113	<604	<2	3	2	<2
Silver	<10	1.4	1.4	2	1.6	1.2

Table 30. Metal concentrations observed in WWTP Effluent
< sign indicates acute LOEC.

CASE FOR AMMONIA TOXICITY

GRADUATED pH TEST RESULTS:

GREATER TOXICITY AT HIGHER pH - NH_3 TOXICITY

$$\underset{\text{toxic}}{NH_3} + H^+ \longrightarrow \underset{\text{non-toxic}}{NH_4^+}$$

TOXICITY PARTIALLY REMOVED AT pH 6 - NOT ONLY NH_3

OBSERVED AND EFFECT CONCENTRATIONS:

HASKELL STREET EFFLUENT 1990-91 (AS TOTAL NH_3):

RANGE = 7.6 - 27.1 MG/L
MEAN = 18.7 MG/L
(pH = 7.5)

SENSITIVITY OF FATHEADS & CERIOS (AS UN-IONIZED NH_3)

CHRONIC EFFECTS AT 0.25 MG/L

ACUTE EFFECTS AT 2 MG/L

SENSITIVITY CONVERTED TO TOTAL AMMONIA

pH	Total NH_3 (mg/l) for Acute Toxicity	Total NH_3 (mg/l) for Chronic Toxicity
6.0	3515	439
7.0	353	44.2
7.5	113	14.1
8.0	37.2	4.64
8.5	13.1	1.63

Table 31. Case for ammonia toxicity.

CASE STUDY 3

A third case study is the Tosco Avon petroleum refinery. Tosco Avon has been performing TIEs for a long time in an effort to keep ahead of the regulatory curve. The latest test being used is an acute 4 day flow-through rainbow trout survival test. The anticipated compliance endpoint is 90% survival in 100% effluent (i.e., < 1 TUa).

Preliminary biomonitoring showed that the effluent was not meeting the anticipated effluent limit most of the time (failing on 11 of 14 samples) (Table 32). The toxicity was highly and randomly variable. The facility decided to perform a TIE to determine what could be done to control this toxicity. A problem to overcome was that this was acute toxicity, but the toxicity could not be detected in static tests. We decided to develop a chronic rainbow trout test (a more sensitive detector) to be used in identifying the cause of this toxicity.

We used early life stage rainbow trout. We developed methods for holding them and exposing them to the various fractionation procedures. Phase I tests run on controls yielded excellent results (Table 33). Phase I tests on effluent samples showed that the only manipulation to affect toxicity was C_{18} adsorption (Table 33). C_{18} adsorption at pH 3 removed all of the toxicity; C_{18} adsorption at pH i removed 40% of the toxicity. This pattern suggests acidic organics. This example illustrates why I favor doing all of the pH adjustments on all of the tests. Complete removal of toxicity after the pH 3 provided a critical piece of evidence as to the identity of the toxicant in this effluent. Results from Walsh and Garnas manipulations confirmed the Phase I results (Table 34). Anion exchange completely removed the toxicity, as would occur with acidic organics, which are anions under basic conditions.

Future efforts for this facility include examination of the treatability of the final effluent (possibilities are granular activated carbon or enhanced biodegradation) and source identification and upstream treatment (Phase II fingerprinting and microcosm for biodegradability).

CONCLUSIONS

1) Chronic TIEs can be done, but they are not necessarily routine.
2) Any TIE requires extensive chemical insight to be properly interpreted.
3) Chronic TIEs and use of chronic detectors are extremely useful tools for evaluating treatment options.
4) Chemical separation schemes evolve during the course of a TIE or TRE and are site-specific.
5) Simultaneous use of multiple separation schemes is better than using just one. Multiple schemes provide both validation and additional information. XAD removes different compounds than C_{18} columns. Furthermore, there are several kinds of XADs.
6) The toxicological database is usually limited for the detector species and therefore reference toxicant test data often must be generated during the TIE.

RESULTS OF TOSCO'S PRELIMINARY BIOMONITORING

SAMPLE WEEK	% SURVIVAL IN 100% EFFLUENT
6/23/91	40 *
6/30/91	70 *
7/07/91	100
7/14/91	90 *
7/21/91	100
7/28/91	100
8/04/91	40 *
8/11/91	45 *
8/18/91	45 *
8/25/91	60 *
9/01/91	55 *
9/08/91	65 *
9/15/91	0 *
9/22/91	80 *

* - EXCEEDS ANTICIPATED ACUTE TOXICITY LIMIT

RESULTS:

1. FAILED ANTICIPATED LIMIT IN 11 OUT OF 14 TESTS
2. LEVEL OF TOXICITY HIGHLY & RANDOMLY VARIABLE
3. TOXICITY NOT DETECTED IN STATIC TESTS

CONCLUSIONS:

1. MUST USE MORE SENSITIVE DETECTOR IN TIE
2. CHOSE "CHRONIC" EARLY LIFE STAGE TROUT DETECTOR

Table 32. Results of Tosco's preliminary biomonitoring.

CONTROL & EFFLUENT RESULTS FOR "PHASE I"
CHRONIC TIE USING RAINBOW TROUT AS DETECTOR

	CONTROL	EFFLUENT
FRACTION	% SURV	% SURV
BASELINE	100	0
pH-ADJUSTMENT		
I-3-I	80	0
I-11-I	80	0
FILTRATION		
pH 3	100	0
pH I	80	0
pH 11	100	0
AERATION		
pH 3	80	0
pH I	100	0
pH 11	100	0
C-18 ADSORB		
pH 3	100	100
pH I	100	40
pH 9	100	0
EDTA	100	NEG
STS	100	NEG
GRADUATED pH	6=7=8	6=7=8

Table 33. Control & effluent results for "Phase I" Chronic TIE using Rainbow Trout as detector.

CONTROL & EFFLUENT RESULTS FOR "WALSH & GARNAS" CHRONIC TIE USING RAINBOW TROUT AS DETECTOR

	CONTROL	EFFLUENT
FRACTION	% SURV	% SURV
XAD 4 RESIN		
BASELINE	100	0
NON-POLAR	100	40
POLAR	100	40
ANION XCHGE	100	100
CATION XCHGE	100	20

Table 34. Control & effluent results for "Walsh & Garnas" Chronic TIE using Rainbow Trout as detector.

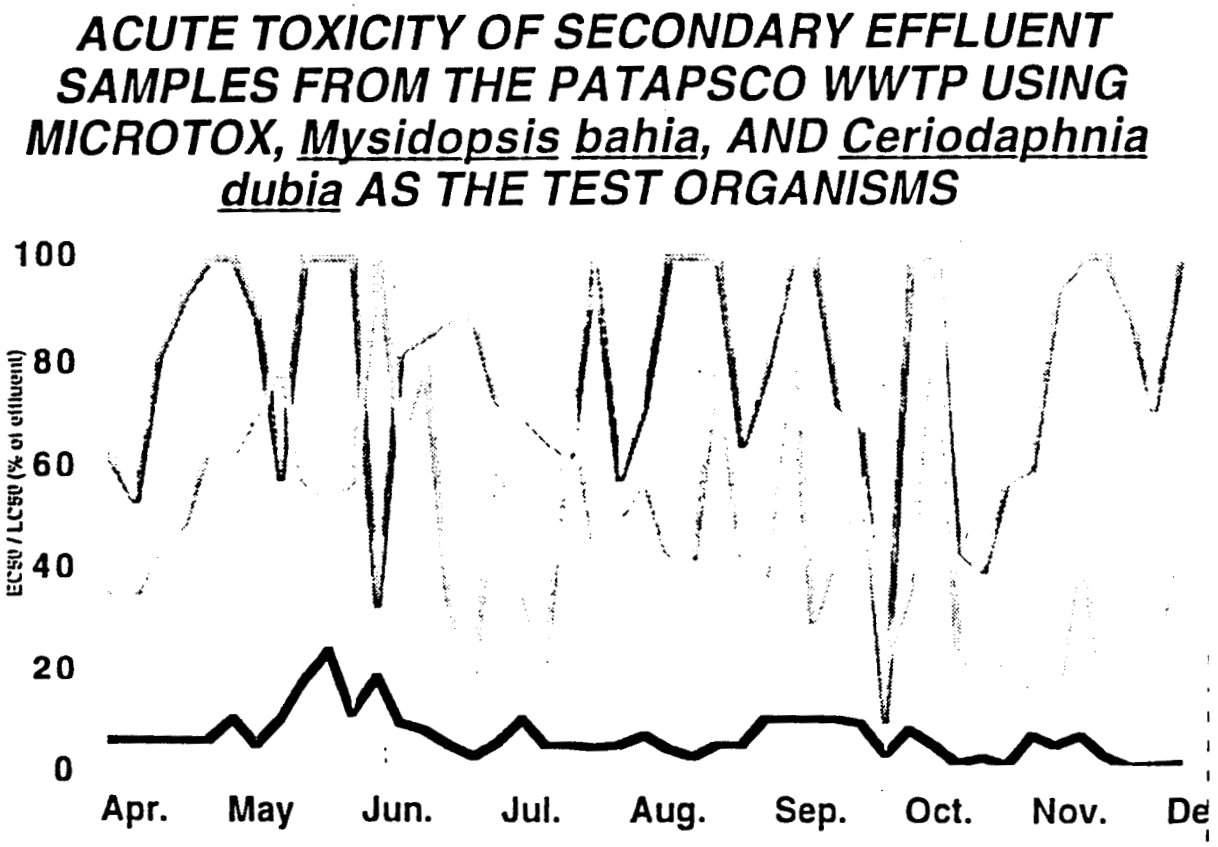


Figure 6. Acute toxicity of secondary effluent samples from the Patapsco WWTP using Microtox *Mysidopsis bahia*, and *Ceriodaphnia dubia* as the test organisms.

Bill Goodfellow
EA Science and Engineering

Much of what I will be presenting has already been touched upon by the previous speakers. In marine TIEs we find a lack of established procedures and an even greater lack of data for comparative purposes in Phases II and III.

Wastewaters exhibit both quantitative variability and qualitative variability. Quantitative variability is the variability observed over time, as shown for three species in Figure 6. Qualitative variability is the presence of different toxicants at different times (e.g., seasons, weeks, or days). In designing a wastewater treatment plant qualitative variability must be taken into consideration. In a hypothetical example (Figure 7), a plant might be faced with loads of solids and nonpolar organics in the spring. In the fall the plant might not be nitrifying very well, and ammonia and volatiles might be causing a problem. A plant designed to treat nonpolar organics might not provide adequate treatment throughout the year.

TIE techniques require toxicity. The long duration of chronic tests may allow for degradation or alteration of toxicity. We like to have LC50 or IC25 values of less than 80% effluent (i.e., > 1.25 TUa or TUc) to be confident of identification of toxicants. Less toxic effluents generally yield lower confidence in TIE results.

Influent toxicity does not always translate into effluent toxicity. If influent toxicity is not altered by the treatment process then the plant is either not functioning properly or the toxicant is recalcitrant. Influent treatability studies may be just as important as effluent treatability.

Salinity poses a problem in performing TIEs for discharges into marine waters. Typically, freshwater effluents discharged to marine waters must be augmented with artificial sea salts or a hypersaline brine to provide a suitable medium for the test organisms. In other situations low salinity effluents are discharged into saltwater and high salinity effluents are discharged into freshwater.

For freshwater discharges into saltwater we favor the following approach. First, quantify the toxicity to saltwater species by augmenting the salinity of the effluent. Then use freshwater species for the TIE. This avoids the problem of complexing toxicants in the effluent by the addition of large quantities of salt. Next, determine an effective way to remove the toxicity. Finally, confirm the effectiveness of the toxicity reduction using saltwater species.

For high salinity discharges into freshwater, the first step is to quantify the toxicity due to salinity alone. Even waters of the same overall salinity may have very different

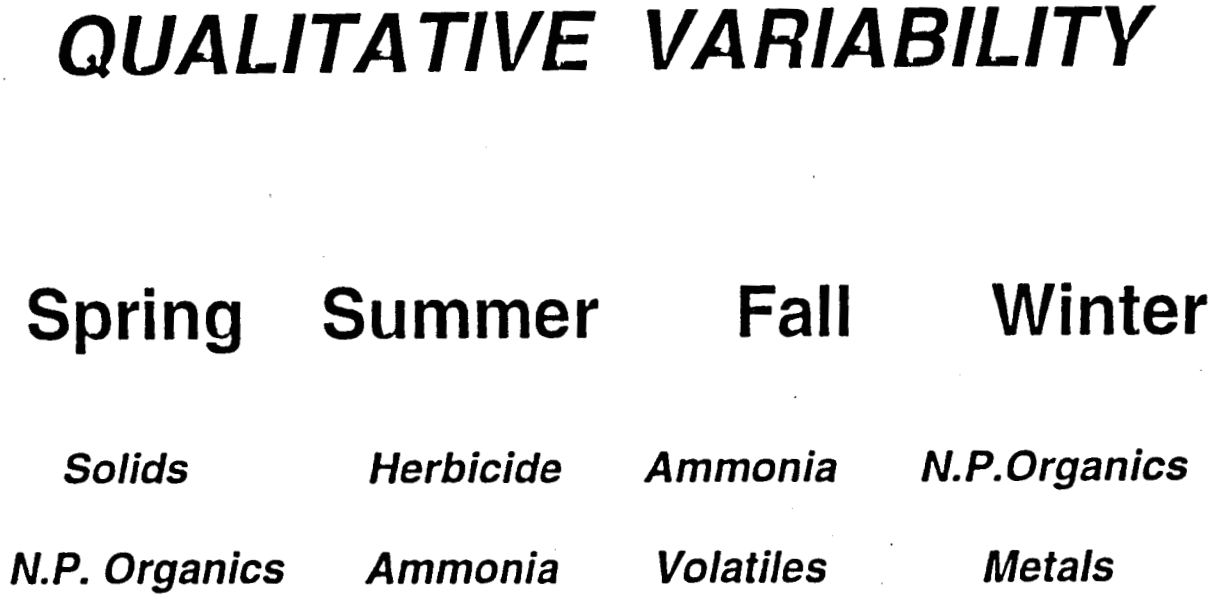


Figure 7. Qualitative variability.

chemical composition and toxicity. For example, the toxicity of $CaCl_2$ is different from that of $CaSO_4$. Second, use salinity tolerant species to quantify residual toxicity. Then determine effective treatment and confirm using appropriate species.

I will now present some results from a case study that we are currently working on. This is a refinery that discharges to an estuary. Their test species are *Mysidopsis bahia* (opossum shrimp) and *Cyprinodon variegatus* (sheepshead minnow). The permit limit for chronic toxicity is for no effects at 4% effluent. Violations occur for the mysid but not *Cyprinodon.* We did not want to run a chronic TIE with mysids, and consequently looked at *Daphnia magna* and *Ceriodaphnia dubia* as possible surrogates. We found a large range in responses to the effluent (Table 35). For the mysid the 48 hr LC50 ranged from 8% to >100% effluent, and the chronic NOEC ranged from 0.26% to 26% effluent. With *Cyprinodon*, the effluent was never acutely toxic, and was chronically toxic (at 50% effluent) in one of six samples. Neither *Daphnia* nor *Ceriodaphnia* were sensitive enough to be used as surrogates for *M. bahia.*

We therefore tried to adapt freshwater chronic TIE procedures for use with *M. bahia.* In order to prevent cannibalism we increased the test volumes to 100 mL per replicate. In the first round of the TIE ("sample A") no toxicity was removed by filtration, aeration, C_{18} extraction, or powdered activated carbon (Table 36). Since this refinery had a historical oil and grease problem we had suspected organic constituents, but sample A made us reconsider this suspicion. For sample B we added anion and cation exchange. The C_{18} column and anion exhange resin gave minimal removal, and the other manipulations had no effect. At the time we did not have enough of a database to support use of EDTA and STS. For sample C we added a C_{18} treatment. Again, the C_{18} column removed some of the toxicity, as did the PAC (this sample had a petroleum component). The observation that the effluent was very toxic to a mysid but not to sheepshead minnows or daphnids led us to suspect fluoride. This example illustrates that sometimes looking at the database before a study can be very helpful. At first the plant personnel stated that they did not have fluoride in their system. After obtaining the data, we asked again and a different person said that they generate 20,000 pounds a day! Even though the plant had a chronic limit, we had enough acute toxicity to begin evaluating it. At this time we also encountered a two week period in which the effluent was consistently toxic. Toxicity due to fluoride was found to correlate well with effluent toxicity (Figure 8).

We then reviewed the literature for techniques for fluoride removal, and tested several of them on a bench-scale (Figure 9). Activated aluminum provided the most effective fluoride removal. As fluoride concentrations declined so did the toxicity of the effluent. I should point out that the LC50 for fluoride is about 5 mg L^{-1}. We are currently trying to determine the chronic LC50, which is probably below 1 mg L^{-1}. We were able to duplicate these results this week using the chronic TIE procedures with mysids. For many years researchers have struggled to get mysids to consistently reach their fecundity endpoints within

CASE STUDY #5

Species Correlation Study Evaluating the Ranges in Acute and Chronic Toxicity of Effluent Samples from 1989 to 1991

	48-hour LC50	*7-day NOEC*
• Mysidopsis bahia	8->100 (22)	0.26-26 (15)
• Cyprinodon variegatus	>100 (8)	50->100 (6)
• Daphnia magna	>100 (6)	---
• Ceridaphnia dubia	90->100 (2)	---

Values expressed as percent effluent

Table 35. Case Study #5 - Species correlation study evaluating the ranges in acute and chronic toxicity of effluent samples from 1989 to 1991. ( ) = numbers of samples.

CASE STUDY #5

Chronic Toxicity Evaluation Using Mysidopsis bahia

	Sample A	*Sample B*	*Sample C*
• Filtration/pH3	NR		
• Filtration/pHi	NR	NR	NR
• Filtration/pH11	NR		
• Aeration/pHi	NR		
• C_{18} SPE/pH3			NR
• C_{18} SPE/pHi	NR	SR	SR
• C_{18} SPE/pH9			NR
• C_8 SPE/pHi			NR
• Anion Exchange		SR	
• Cation Exchange		NR	
• PAC	NR	NR	SR

NR = No chronic toxicity removal *SR = Slight chronic toxicity removal*

Table 36. Case Study #5 - Chronic toxicity evaluation using *Mysidopsis bahia*.

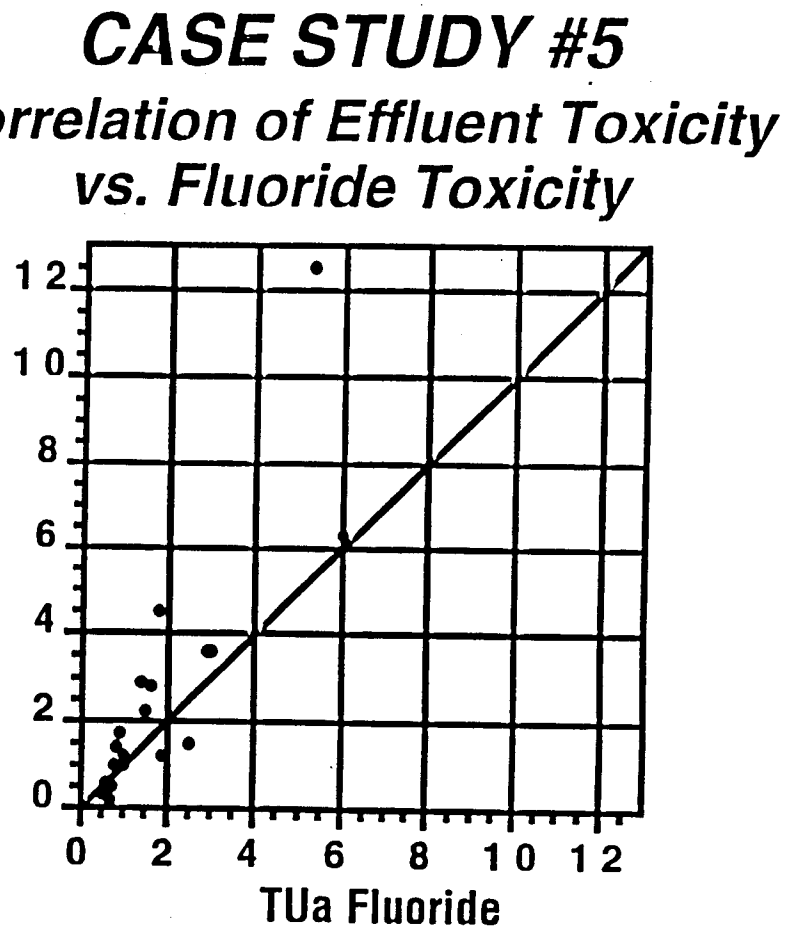


Figure 8. Case Study #5 - Correlation of effluent toxicity vs. fluoride toxicity.

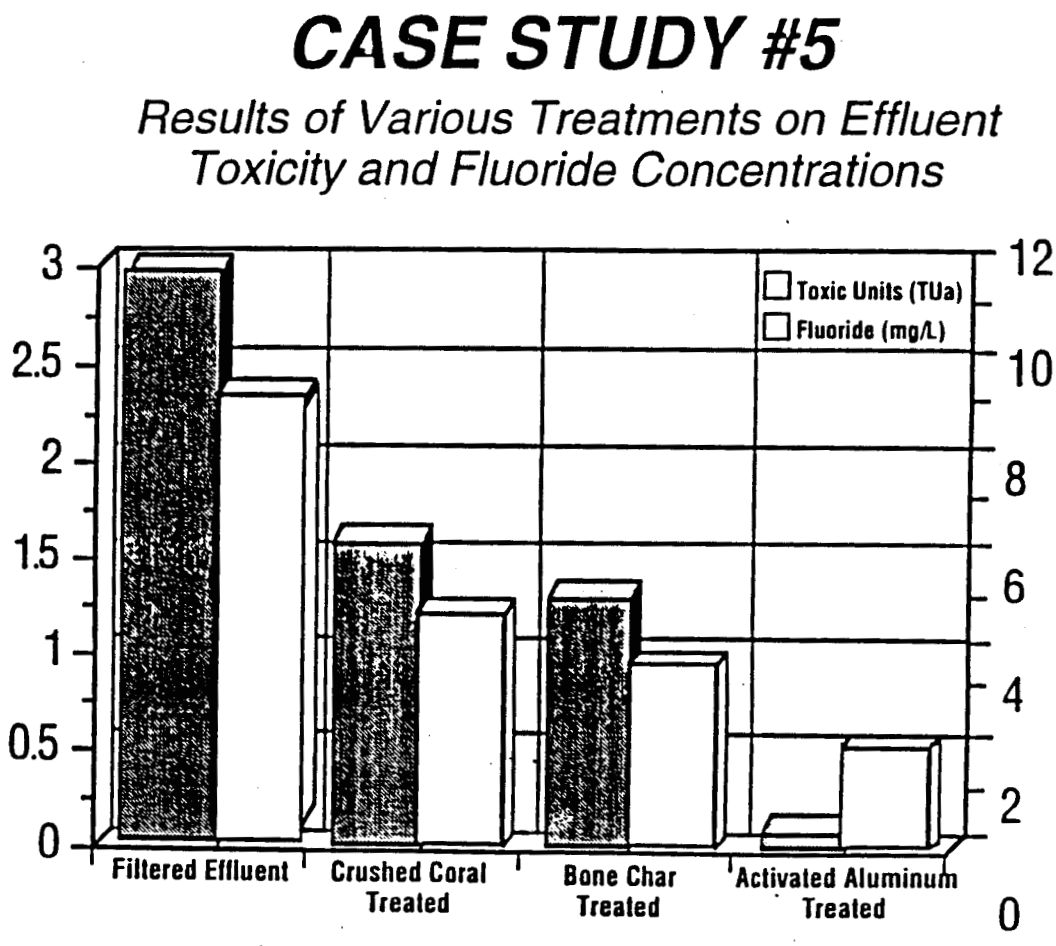


Figure 9. Case Study #5 - Results of various treatments on effluent toxicity and fluoride concentrations.

7 days in marine toxicity tests. The endpoint affected by fluoride is the fecundity endpoint. We noticed that at low exposure concentrations the organisms would show a whitening of the oviducts. We have since found verification of this observation in the literature.

I would also like to talk about two other kinds of chronic TIEs that we have done for discharges into freshwater. These examples show that procedures outside of the EPA scheme can be useful in toxicity identification and reduction.

One of these was a study performed for the City of Durham in North Carolina. Their permit limit for chronic toxicity was a NOEC of >100%. In two years the City is changing to a different treatment system, with two regional plants instead of five smaller plants. The state water quality agency allowed us, in cooperation with Engineering Science, to develop a simulated treatment system for testing compliance with effluent limits for chronic toxicity. Numerous trials showed that the effluent from the new plant would most likely provide effective treatment of the toxicity that has been identified in the existing effluent. In effect, toxicity reduction was achieved by switching to the new treatment system.

A second example is work we are performing for an auto parts company in Arkansas. This effluent showed consistent chronic toxicity to fathead minnows and *Ceriodaphnia* due to surfactants. We found, as Dr. Mount did, that chemical analysis of surfactants is very expensive and not very informative. It is not possible to identify individual surfactants such that they can be tracked through the treatment system. We identified a surfactant and then examined surfactant use in the plant. We found that surfactants were often present as byproducts from the use of oils for lubrication. For each oil and polymer in heavy use (15 compounds) we asked the vendor for toxicity information; in each case no information was available, even though each compound was intended for use in treatment systems. We decided to develop our own database for exposure of fathead minnows and *Ceriodaphnia* to these compounds. One of the compounds, a biocide used to stabilize the surfactant, was extremely toxic (in the parts per billion range). Substitution of two other compounds for this compound reduced the toxicity of the effluent. In this case, even though we did not establish a weight of evidence, we were able to render the effluent nontoxic.

Kurt Kline
MEC Analytical

One thing I would like to talk about today is the use of alternative species. Most TIE work has been done on *Ceriodaphnia* and fathead minnows. In the Bay region, however, the sensitive species are often marine or estuarine species such as *Mysidopsis* and *Menidia*. I will discuss some of the other species that might be used in TIEs.

One of the major drawbacks of looking at other species is a lack of information on how to deal with TIEs using marine species. Attempting to correlate toxicity to freshwater species with toxicity to marine or estuarine species may not be appropriate in the Bay region. In testing effluents in the Bay region we have used 14 different species, including a number of marine species. In cases such as bivalve larvae or the echinoderm fertilization test, correlation with freshwater species is not possible. Leading alternatives to *Ceriodaphnia* and *Mysidopsis* include kelp (a test developed by the Marine Bioassay Project of the California Department of Fish and Game), bivalve larvae, echinoderm fertilization, abalone, *Mysidopsis*, *Menidia*, Skeletonema/Thalassiosira (marine diatoms), and *Selenastrum*. For many of these tests one sample is enough to run a successful test. Some of these chronic tests also can be completed in only 48 hours, avoiding potential problems with archive time for samples. The echinoderm test takes only one hour. In the bivalve larvae test, *Crassostrea gigas* is used in the summer and *Mytilus edulis* is used in the winter, but neither species is reliable in testing during the spring and the fall.

As Dr. Mount emphasized, successful completion of bioassays requires experience and technically competent staff, especially when unusual species are used.

Marine bioassays have several positive aspects: 1) most require a single sample pickup; 2) toxicity is generally quickly determined; 3) marine species have very sensitive life stages; 4) they are less labor-intensive and therefore cheaper than freshwater bioassays; 5) they only require a single TIE manipulation, reducing artifactual toxicity; and 6) in some cases such as the echinoderm test it is possible to switch species (i.e., from sea urchins to sand dollars) at different times of the year.

We have run a number of TIEs using saltwater species and have encountered some problems. These problems have already been mentioned by other speakers. pH is difficult to monitor and buffer in seawater. Ammonia toxicity is reduced in seawater relative to freshwater. Chelation in seawater is also a problem. EDTA will not chelate in saltwater. Saltwater chelators must be used instead; we used a compound called Chelex.

As a case example, we conducted a TIE on an industrial effluent. The test required radical pH adjustments. The effect of pH shift was minimized because of the short duration of the echinoderm test. A saltwater chelator was used. Thresholds for additives were determined readily because of the short term of the test. This entire Phase I procedure took only three days.

When a discharger is required to perform a TIE, as most in the Bay region will at one time or another, the Regional Board has a set of requirements that trigger a TIE. EPA has procedures for performing TIEs. The Basin Plan for the Bay states a TIE can be triggered either through NPDES monitoring or the Variability Testing Program. The question then becomes whether sufficient toxicity is present to run a TIE. There are four questions that need to be answered. First, what level of toxicity is required to effectively run a TIE? If survival is 100% in controls and 85% in samples, and significant chronic effects are observed, you may not have enough toxicity to perform a TIE. For instance, if you have 16 neonates in controls in the *Ceriodaphnia* test and a significant difference at 6.25% concentration, you have four animals to work with. Often when TIEs are conducted based on such small increments they yield strange results.

We did a TIE for a discharger with a trigger of six out of 54 chronic tests. Our results with *Ceriodaphnia* showed chronic toxicity in eight of 19 samples with NOECs of less than 1%. In only four of the 19 tests were LC50s less than 100%. Chronic toxicity was statistically significant but mortality was not. We ran four TIE procedures using methods based on the acute manual because the chronic manual was not yet available. None of the procedures had an effect. We should have stopped this effort as soon as we realized there was not enough toxicity to work with. The chronic rules now specify that if you have no initial toxicity a TIE should not be performed.

The rules specify that the Regional Board expects to hear from the discharger within 30 days after a TIE is triggered. The discharger is required to begin testing and to submit a workplan to the Regional Board. I offer the following as a modest workplan. The workplan should begin with routine biomonitoring performed every other week, with samples collected every day and daily renewals just like in routine testing. One difference is that each individual daily sample should be archived. Storage times will be different for each sample but there is no way to get around that. These samples can be used to determine which individual daily samples contributed to the toxicity. I would rather see an IC25 rather than an IC50 used as the standard. The IC25 triggering a TIE should be less than 80%. Sufficient toxicity in this case may be different from the level of toxicity that triggered the TIE. If a toxic sample is found in the seven samples then you begin with Phase I, Tier 1. If no LC50 or IC25 is observed over time but the NOEC remains low. At that point a TIE should be performed, but the potential for failure to identify the toxicant would be high. The biweekly intensive monitoring should continue either until the toxicity is identified or until 12 samples have been analyzed, after which the discharger should return to routine biomonitoring. This proposed framework would achieve the goals of toxicity identification and reduction in a cost-effective manner. Multispecies toxicity tests and variability tests would only be performed for species showing toxic responses, and tests would not be run on samples that are not sufficiently toxic for a successful TIE.

Comment by Lynn Suer (San Francisco Bay Regional Water Quality Control Board):
The trigger for requiring a TIE has changed. We are now using an 11 sample median. This applies to the Effluent Characterization Program and to NPDES permits. Second, I think that the use of hypothesis testing alone to trigger a TIE can be inappropriate, especially for shallow water discharges with no dilution credits. We are considering the use of point estimates for determining compliance with effluent limitations.

PANEL DISCUSSION

The speakers were given the opportunity to make opening comments.

Teresa Norberg-King

I would like to note that the manuals for chronic freshwater TIEs and the second edition of the manual for Phase I are available. I also would like to show a few more slides.

After running the sample through the C_{18} column, methanol solutions should be used to elute the toxicant. For the chronic procedure we added a step in addition to testing the post column effluent for toxicity, and also a 100% methanol ... as Don Mount described. We get a 333 times original effluent concentration. We either test that or concentrate the sample further (since recovery off of the C_{18} column is not always 100%) by nitrogen evaporation, keeping the methanol concentration at 60%.

In our lab we feel that we can drop the 25% methanol fraction and use the 50% instead, resulting in 7 fractions. I think that this should be the first cut in a chronic TIE instead of just the 100% methanol elutions. If three 100% methanol elutions are performed, you may end up with all of the constituents in one 100% fraction; better separations might be obtained by using a graded series of eluants.

The volume of sample needed for fractionation depends on several factors, including the toxicity present, the type of test, the frequency of renewals, the volume of effluent passed over the column to cover some additional short-cuts that make chronic TIEs even more cost effective. We have recently begun to use a larger C_{18} column made by Baker (a 5 g or 10 g column), which allows us to run the *Ceriodaphnia* and fathead minnow tests using the eluates from one column. Often you need to work in reverse to determine the amount of sample needed to perform a TIE.

Bill Goodfellow

I think that marine TIEs should not be a problem procedurally. There may be some quirks that require subtle ways of thinking. There may be some freshwater procedures that do not work in marine water, but conversely there may be procedures that do not work in freshwater but do work in marine water. A major limitation at present with chronic TIEs for marine waters, especially Phases II and III, is the weakness of the toxicity database. Often the database must be generated in each case. This is not necessarily very difficult, but it does increase the cost of the TIE. Once we begin performing marine chronic TIEs I think they will prove to be less frightening than they now seem.

Don Mount

I agree with Bill Goodfellow's comments on marine TIEs. I think that we would make better progress at solving water quality problems quickly if the number of species used in marine TIEs is narrowed down initially. Use of additional species could be postponed until a later stage. A much greater amount of uncertainty surrounds selection of dilution values, critical flow for flowing water, and other factors than variation between species. We would liked to have used more species in freshwater TIEs also, but it was not practical.

In my opinion the biggest obstacle in performing TIEs in either freshwater or marine water is unclear results from Phase I. This is where experience is often critical to proper interpretation.

Stephen Hansen

I think that treatment chemicals are a very important problem. Almost every facility that we work with uses a tremendous number of coagulants, flocculants, and other chemicals with very little toxicity data. The few data that do exist are usually not for the species of interest. Some of these compounds, especially the polyquaternary ammonium compounds, are extremely toxic (often around 10 to 100 ppb in chronic tests). If these chemicals are not screened before they are used they can cause major problems in TIEs. The few analytical techniques that are available do not reveal the form taken by the polymer (i.e., bound vs. unbound). We have been suggesting that our clients screen these compounds. Plant operators often resist this because it is very difficult to find coagulants and flocculants that work well. A number of our clients have used substitute treatment polymers, and the toxicity problems have been solved.

Kurt Kline

While the comments I made in my talk indicated that a number of marine species can be used in toxicity tests, I would agree to some extent with Don Mount that there is a limited experience-base with use of most of these species in toxicity testing and TIEs. It currently looks like the species that will be used for marine chronic TIEs are *Menidia, Mysidopsis, Champia,* and *Arbacia.* Only two of these species are available in California. I think that the Regional Board and other locations in California are heading in a different direction.

I think it is possible the regulators have begun applying the TIE concept a little too quickly. We really don't know enough about some of the procedures and all of the sensitive species.

Stephen Hansen

I have one more comment. All of our successful TIEs have relied upon close cooperation with plant personnel, including management and operators. The presence of the toxicity

depends on how the plant is operated and chemicals used in the plant in addition to what comes into the plant.

The workshop conveners and sponsors developed the following list of questions they hoped would be answered during the proceedings. These questions were posed to the technical experts:

QUESTIONS

Q: **Is anybody setting up a system for data storage and retrieval?**

Teresa Norberg-King: The Duluth lab has a system called AQUIRE (Aquatic Information Retrieval System). This system is available to state, federal, and contract labs. For information call (218) 720-5602. AQUIRE contains published toxicity data. In addition, data that we have generated are stored in dBase and data from that database can be provided upon request.

Bill Goodfellow: Generally, if our data are not published they are the property of the clients. The Water Environment Federation publishes a good annual review. The EPA criteria documents also contain good compilations of toxicity information for priority pollutants, but chemicals causing toxicity in effluents are often not priority pollutants. The Handbook of Environmental Data on Organic Chemicals, Verschueren, Carel, 1983. 2nd Ed. Van Norstrand Reinhold, NYC. 1310 p., is good, but very expensive. The Wisconsin series for fatheads is also good.

Teresa Norberg-King: Those sources are covered in the AQUIRE database.

Joe O'Connor: It would seem that if many of these TIEs are being performed for government agencies that the information should be available through those agencies.

Q: **What is a typical amount of time to conduct a TIE?**

Stephen Hansen: That is totally dependent on the amount of variability, but probably a year.

Bill Goodfellow: We suggest at least a year of sampling to observe annual variability.

Q: **What is the frequency of failure and what criteria are used to determine when failure occurs?**

Teresa Norberg-King: We have never failed to learn at least something about the characteristics of the toxicant, which is enough to guide treatment. I don't think complete failure would be a problem.

Q: Since we have a "due diligence" clause in the Basin Plan we need to establish a way to define due diligence on the part of the dischargers.

Bill Goodfellow: Cooperation between the discharger, the regulators, and the laboratory is important. In some cases practical solutions may not exist. In these situations consensus should be reached among all parties. It is easier to handle these situations if the regulators are involved from the beginning.

Q: Can identification of the toxicant class really be considered a success?

Bill Goodfellow: There are situations where success in terms of elimination of toxicity may not be possible. It may simply be impossible to remove the toxicant from the water.

Q: When can we expect peer-reviewed publications from EPA describing the data they are generating?

Teresa Norberg-King: They are on our agenda. A higher priority in the near term was to finish the methods documents. There are five or six papers describing aspects of our work with diazinon and the Colusa Basin Drain in California. We would like to publish more.

(References listed below):

Amato, J.R., D.I. Mount, E.J. Durhan, M.T. Lukasewycz, G.T.Ankley, and E.D. Robert. 1991. An Example of the Identification of Diazinon as a Primary Toxicant in an Effluent. Environ. Toxicol. Chem. 11:209-216.

Norberg-King, T.J., E.J. Durhan, G.T. Ankley, and E. Robert. 1991. Application of Toxicity Identification Evaluation Procedures to the Ambient Waters of the Colusa Basin Drain, California. Environ. Toxicol. Chem. 10:891-900.

Durhan, E.J. and E.A. Makynen. 1990. Analysis of Diazinon in Effluents at ng/l Levels. Presented: SETAC, November, Arlington, VA.

Norberg-King, T.J., M. Lukasewycz, and J.J. Jenson. 1989. Results of Diazinon levels of POTW effluents in the United States. NETAC Technical Report 02-90. U.S. Environmental Research Laboratory, Duluth, MN.

Q: Regarding the diazinon paper, I think the data support different conclusions than those drawn by the authors.

Teresa Norberg-King: Even though a paper has been peer-reviewed it may still be possible to draw different conclusions.

Joe O'Connor: I think that a gathering such as this provides a forum for peer review that is just as valuable as the review of a paper.

Q: **How can we have confidence in the extrapolation of toxicity test results on effluent samples to predicted effects in the receiving water when changes occur in the matrix and the characteristics of the toxicant over time?**

Lynn Suer: The ambient toxicity program is addressing that. In studies of ambient toxicity along gradients from discharges we have seen toxicity consistent with results from effluent toxicity tests.

Q: **The lack of published, peer-reviewed case studies is a cause for skepticism. Plants often have a different view than consultants regarding the success of TIEs.**
Stephen Hansen: A problem in publication of TIE results is that clients are interested in treatability, not publishable science. In the course of a TIE there may not be enough data generated for a publishable report. For example, identifying a toxicant as an acidic organics is good enough for a treatment engineer, but might be considered too trite for publication.

Bill Goodfellow: The ASTM books published over the last five years usually contain case studies on TIEs. There have also been several SETAC publications in the last two years coming out of Duluth, including a couple on POTWs.

Kurt Kline: A lot of the data are in obscure locations. TIEs are not research efforts, and don't produce publishable data. The databases created in performing TIEs may be publishable, however. We perform 300-500 bioassays per year.

Q: **How can we properly account for the variability in the response of organisms to toxicant exposure? The use of reference toxicants has not been discussed. In 1987 when we began testing with echinoderms, 90% fertilization in controls was considered acceptable and samples with a reduction to 50% or less were considered to be toxic. Now, fertilization in controls can be as low as 70%, and as small as a 4% difference from the control can be considered statistically significant. It seems that biological variability should be carefully considered.**

Don Mount: There are really two aspects to the question of variability. One relates to whether a permit limit is being met and in this context the question of accounting for biological variability is a valid concern. The other relates to the TIE, and in this context precise quantitation of variability is less critical. In a TIE the question is whether the problem goes away after the suspected cause of toxicity has been removed. In the early stages of TIEs the toxicity test is used as a rough analytical tool. It is unfortunate that more TIE results have not been published, but it is even more unfortunate that the number of problems that have already been resolved is not widely known. Reference toxicant testing is a bigger issue in the regulatory realm. The variability in TIE work

will be greater because the test conditions can barely be tolerated by the test organisms. Clients generally don't want to pay for Phase III where the variability would be accounted for.

Stephen Hansen: We have never completely finished Phase III for any of our clients.

Q: Biological variability is also important in determining which test species to use. Since different species vary in sensitivity, how should we determine which species to use?

Bill Goodfellow: The regulatory community will make sure that the most sensitive species will be used. Studies comparing the sensitivities of different species are very important. In the example I gave where fluoride was the toxicant, if we had not looked at multiple species the problem would have not been detected.

Teresa Norberg-King: A lot of information on variability is available. Susan Anderson studied variability within tests. Lynn Suer and Susan Anderson just prepared another document discussing variability. In addition, interpretation of the significance of these tests depend on qualitative biological judgment. Hypothesis testing will be a sufficient tool only with a high number of replicates and extremely low variability. Experience with the test organisms, the sample manipulations, and the expected outcomes is essential to proper interpretation of the significance of test results.

Bill Goodfellow: I would like to emphasize that it is not appropriate to proceed directly from a TIE result to an engineering solution. I recently encountered an example of this in which a facility built a $40,000 pilot plant based on aeration at pH 3 eliminating toxicity. Benchtop treatability studies should precede expenditures on engineering solutions.

Q: It seems that people would have greater confidence in chronic TIE procedures if they saw data from toxicity reductions. At one time EPA was publishing draft abstracts on some of their acute toxicity reductions. Will similar summaries be made available for chronic toxicity reductions?

Teresa Norberg-King: Yes. There is currently a cooperative agreement to evaluate chronic TIEs and TREs work, and that evaluation is in progress. If anybody has success stories that they want to share I would be glad to pass them on.

Bill Goodfellow: There is a revision of the municipal TRE protocol manual that includes about a dozen case studies. I think production of it is stuck while they wait for printing funds. For information call Fred Bishop at the EPA Environmental Risk Reduction lab in Cincinnati. The report is probably available in draft form.

Q: Identification of the chemical class of a toxicant may not be adequate for a POTW. If the toxicity is not treatable, we may have to rely on pretreatment, which requires that the identity of the toxicant be known.

Dount Mount: Some of the examples presented today have stopped at the point where the toxicant class is identified, but many studies have identified specific chemicals. I would like to make an additional comment about availability of data. The last issue of Environmental Toxicology and Chemistry did contain an article and I was one of the authors, but I don't think publication necessarily solves the problem. Just because research appears in a peer-reviewed journal does not mean that it is right. Success in TIEs depends on whether or not the toxicity can be eliminated, and that is the information that needs to be disseminated. There was a TRE abstracts summary maintained by Claudia O'Brien that had 20 or 30 case examples in it. She is no longer doing this; Jim Pendergast may know where it is. Most study results, however, are buried in contractual reports and unavailable.

Stephen Hansen: If municipal dischargers want to use their pretreatment program to reduce toxicity, they will have to spend more money for more precise identification. The chemical techniques are sophisticated and expensive, but they are available.

Teresa Norberg-King: I have a lot more data on acute TIEs for POTWs where toxicants were identified, such as organophosphates and nickel. We have a one page flyer called ETAC Communique at our lab that describes some of these successes. We send this to a mailing list. Contact me if you are interested.

Q: Regarding the diazinon paper, could we discuss that article at this workshop?

Don Mount: I have data from a similar case with me.

Joe O'Connor: That is one vote for a subject to discuss tomorrow.

Q: Can we develop a list of "priority pollutants" based on outcomes of TIEs?

Bill Goodfellow: Ammonia would be at the top of my list, both on acute and chronic bases. Ammonia will probably affect 98% of all municipal dischargers on a chronic basis.

Teresa Norberg-King: As a result of TIE work EPA is developing or revising criteria documents for diazinon and ammonia. For classes of compounds like the surfactants that are showing up it is difficult to develop a list of individual compounds of concern. Nonylphenol surfactants are being studied at our lab, so we may be able to identify individual members of this class that are of concern.

Don Mount: I have a list of pollutants identified in TIEs that we developed at EPA about two years ago. This was based on about 36 effluents. Much of this work was done in the southeast, explaining the prevalence of organophosphates on the list. Recent additions would include hexavalent chromium, surfactants and polymers, and fluoride.

Q: Is there any information on how diazinon gets into POTWs?

Teresa Norberg-King: The source is probably public use spraying (e.g., in hotels and other parts of the private sector). Reregistration of diazinon is under consideration by EPA.

Don Mount: Several studies have been performed and no particular input stream has been identified.

Q: How can a pollutant of this type be controlled?

Don Mount: Several municipalities in the southeast have started public education campaigns.

Q: The initiation of the NPDES stormwater program will include much public education, which should help with this problem.

Don Mount: We have seen herbicides in many effluents, but not at toxic amounts. Of course, if algae was used as the test organism toxicity may have occurred.

Margaret Johnston
Aquatic Habitat Institute

Joe O'Connor asked me to summarize today's proceedings. It seemed to me that dischargers received alot of information, but not as much comfort as they might have wanted. We have heard that TIE/TREs have been successful in reducing toxicity, though not always in identifying the toxicant. The importance of experience and intuition have been stressed. We have also heard differences of opinion arising from application of these experimental procedures in a regulatory setting. It appears that more information is available on TIEs that have been conducted than people may be aware of. These are all issues that need further consideration.

We asked the sponsors of the workshop to provide lists of questions that they wanted addressed at the workshop. I think all of them have already been touched on to some degree, though for many questions consensus on the answers does not always exist. These questions will be the focus of discussion tomorrow. Tomorrow we will have a discussion between the sponsors and the panel of experts to try to resolve some of these questions.

DAY TWO

Debra McKeown
State Water Resources Control Board

Proposal for Development of Marine Chronic TIE Proceedings

I work in the Ocean Waters Unit, Division of Water Quality, at the State Water Resources Control Board (State Board). I would like to discuss a State Board staff proposal on development of marine chronic TIE procedures using west coast organisms. TREs are required in the Ocean Plan adopted in March 1990 and the Enclosed Bays and Estuaries Plan adopted in April 1991. At present no marine chronic TIE procedures are available. EPA-Narragansett is currently developing TIE procedures for four east coast organisms: *Champia, Arbacia, Mysidopsis,* and *Menidia.* Three of these are not indigenous California species, and the California Department of Fish and Game (DFG) has expressed concern over the use of nonnative species in toxicity testing. Therefore, it is important that California have TIE procedures for species that are used in effluent toxicity testing. However, California can build on EPA's work by adapting the EPA procedures for west coast organisms. The proposal (Appendix 1) I will describe today will enable dischargers to have State Board-approved procedures for TIEs with native organisms.

Under this proposal the State Board would initiate a program to develop marine TIE procedures through a cooperative effort with NPDES dischargers. We are giving the dischargers an opportunity to participate from the beginning in this process. The total projected cost for developing procedures for four west coast organisms is $450,000, based on the cost estimates provided by EPA-Narragansett. We propose to develop procedures for *Macrocystis, Haliotis,* purple sea urchin, and topsmelt (a topsmelt protocol should be finished by the end of 1992). The topsmelt test will be examined during the Ocean Plan triennial review fro potential use in California and may replace the *Menidia* test.

We think it would be reasonable for the State Board to provide $100,000 in seed money to initiate the work. The State Board has also submitted a proposal to EPA Region IX for $100,000 from their near coastal waters funds for development of chronic marine TIE procedures. If this grant is received it would be applied to the total cost of $450,000. The remaining $250,000 would have to come from sources yet to be identified. State Board staff is currently discussing this proposal with NPDES dischargers. One possible obstacle is the lack of a mechanism allowing dischargers to funnel money to the State Board for this project. A potential solution to this problem would be use of an outside research institute, such as the Southern California Coastal Waters Research Project (SCCWRP). We will be discussing this possibility with SCCWRP in the next two weeks.

A State Board representative would manage the project and a technical advisory committee would be formed to ensure success of the project. Members of the committee would include staff scientists from the State and Regional Boards, SCCWRP, NPDES

dischargers, and independent scientists. The committee would guide management of the project, providing input on tasks, products, and time schedules. This cooperative approach would enable State Board staff to gain experience needed to evaluate TIE results. Dischargers would have approved procedures for determining compliance with toxicity limitations.

Staff of the Division of Water Quality has recommended that the State Board approve funding of the initial work for FY 1992/1993 contingent upon obtaining funds from the dischargers and EPA for continued support of the program. State Board staff would also work on establishing a mechanism for channeling discharger funds. Our management will submit this proposal to various discharger associations in the coming weeks. If you have any questions call me at (916) 657-0894.

STATEMENTS FROM THE PANEL

Lynn Suer
San Francisco Bay Regional Water Quality Control Board

The Regional Board was asked to discuss implementation issues relating to the toxicity section of the Basin Plan and then comment on the outlook for implementation over the next two years. The most important implementation issue concerns the "diligence" clause. This clause was a primary reason for having this workshop. It seems that there are two ingredients needed to implement this clause. One is a better understanding of the technical issues on the part of the dischargers and the regulators, and that was one function of this workshop. Second, we need further development of technical resources both at the state and regional level.

The presentations yesterday showed that chronic TIEs can be successful, but they are an art, and dischargers and regulators may not necessarily be able to become the artists since they will not personally be performing the tests. However, the regulators will do a better job if they are better educated. We would like to encourage the flow of information from the technical community to the dischargers and regulators, and would like to hear concrete suggestions on how this might be accomplished. This workshop is a start, perhaps we should have more workshops. We should take advantage of the data that are available. Perhaps the regulators should take the lead in this since we have the most at stake. A certain amount of information is submitted to us in discharger reports and could be made available.

In developing technical resources, we first need to determine our specific needs in this region. I passed around a summary of the results of our Effluent Characterization Program that discussed cases where TIEs were triggered. This kind of information should be used to establish priorities for further technical development.

I gathered from the information presented yesterday that there are two possible reasons for a discharger failing to reduce toxicity once it is identified. One would be toxicity that is not strong or persistent enough. This also relates to the issue of the trigger for TIEs. We got some indication yesterday of the minimum amount of toxicity needed for a successful TIE. That sort of information will help us determine when a reasonable amount of effort is being expended in TIEs. A second reason for failure would be that the cost of treatment might be inordinately expensive. These situations would have to be evaluated on a case-by-case basis.

Terry Oda
U.S. EPA

EPA supports the efforts of the Regional Board and State Board. The definition of diligence is a primary issue that we are concerned about.

I would like to make some observations on yesterday's proceedings. The amount of success that has been achieved in chronic TIEs is surprising. A substantial amount of information is being generated on the toxicity of specific compounds. Dischargers may be well-advised to stay ahead of the regulatory curve by taking note of problems being encountered in chronic TIEs. Several types of pollutants have been shown to consistently cause toxicity, including metals, surfactants, and polymers. For POTWs, reduction of this toxicity will require aggressive pretreatment, pollution prevention and source reduction, and inventories of chemicals used in the treatment plant. Enough data are available to indicate that some of these steps can be taken immediately.

I drew three main conclusions from yesterday's discussions. One is that there is a need for more effective dissemination of case results. A newsletter may be one way of doing this. Second, there is a need for more test species, especially on the west coast and in the Pacific Islands. Perhaps a procedure should be determined for more rapid adaptation of existing protocols to indigenous species. As an example, in Hawaii they are encountering great difficulty in adapting the sea urchin test to local species. Finally, I challenge the dischargers to make more routine use of biomonitoring. Greater familiarity with the techniques will remove some of the mystique associated with them.

Michael Carlin
San Francisco Bay Regional Water Quality Control Board

During the revision of the Basin Plan there was concern expressed that chronic TIEs could not be successful. The presentations at this workshop have shown that they can be successful. I also want to point out that TIEs are just one part of an overall strategy for toxicity reduction. We have been asked whether TIEs are necessary in situations where treatment systems are put in that remove the toxicity. The answer is yes, the removal must be demonstrated after the system is installed. Another point is that dischargers should always be in a mode of toxicity identification and reduction, even if effluent toxicity is slightly below the effluent limit.

Chuck Batts
Bay Area Dischargers Association (BADA)

Our goal is to define what aspects of TIEs are workable, with the goal of elimination of toxicity. The mandate from the State Board in their control plan is very broad: no chronic toxicity. The Regional Board initiated the effluent toxicity testing program in 1986, and since then the dischargers have developed a lot of experience and comfort with TIE work. The

Regional Board has followed a prudent process to get us where we are now. There are still concerns about triggers, species sensitivity, and definition of due diligence, but it is evident that TIEs do work and will be broadly applied in the future. It is also clear that TIE procedures and regulatory application of TIEs will improve. TIEs are rigorous and time-consuming, and I think we need latitude in establishing the endpoints of TIEs. I also think it is important to realize that sufficient toxicity is needed to perform a TIE. Bill Goodfellow said that 1.25 TU is probably a minimum. Kurt Kline also discussed this issue. Variability and statistics are important issues in enforcement that need further attention. TIEs can always be performed, but dischargers must consider costs. For example, a chronic TIE for a marine species with a highly variable complex effluent can be arduous. In such cases the definition of due diligence will be important. In some cases the cost of control will be very high, and I agree with Lynn Suer that diligence in these situations should be determined on a case-by-case basis.

The Regional Board has been very cooperative in planning this workshop. We are discussing following up this workshop with a demonstration project involving dischargers, the Regional Board, EPA, and perhaps research foundations and contractors.

Bob Berger
Bay Area Dischargers Association (BADA)

TIEs have been successful and are successful. Something that is apparent from yesterday's discussions is that more information is needed for application of TIEs in saline water bodies and having confidence that effects on beneficial uses are really being addressed. There will be people with permit limits for chronic toxicity by July, but procedures are still being developed. One of my initial questions to the panel is what should we be doing in the interim? Do we have enough information to be routinely conducting marine chronic TIEs in a regulatory setting? All parties agree that we must make chronic TIEs work. There is a question, however, of how the toxicity tests in use relate to protection of beneficial uses. This is really an issue that is beyond the scope of this workshop. One thing that is needed is a means to review and revise, in a timely fashion, procedures that are being developed. A better effort must be made at dissemination, evaluation, and incorporation of information into improved procedures.

QUESTIONS

The workshop sponsors had a list of questions they wanted to see addressed at the workshop. Discussion of these questions is summarized below.

1) What specific parts of the chronic TIE guidance for freshwater must be modified for marine or estuarine waters?

Stephen Hansen: Metals removal is a major problem for marine waters. It will be difficult to remove metals without affecting the sample matrix. EDTA does not work. Ion exchange columns seem like they could work. This is a major question we are investigating right now. Other column exchanges also remove many constituents from the matrix, which should be altered as little as possible. For marine waters determining the effects of these treatments on the sample matrix is more complex than it is for freshwaters, although I think it can be done.

Teresa Norberg-King: I think that a lot of the procedures we have been developing for marine discharges are immediately applicable. I am curious to know how Stephen Hansen came to the conclusion that EDTA won't work in saline samples; we have found that EDTA has reduced toxicity when added to brine waters. The mechanism may by unclear but the result was toxicity reduction. If EDTA is not working then we need to figure out why. For marine work more information on thresholds (e.g., for EDTA and STS) is needed. EPA Narragansett has some of this information, but I am not sure how we will disseminate it before we publish a manual. Since the freshwater chronic manual is not a cookbook, much of it will apply to marine discharges.

Stephen Hansen: An important distinction should be made here. Freshwater effluents discharged into marine waters are relatively easy to work with, but real saline samples are a bit more complicated.

Chuck Batts: For freshwater discharges into saline water, is there a way to determine whether the toxicity carries over into the ambient environment?

Teresa Norberg-King: Marine species should be used in testing the freshwater discharge. I don't think that ambient toxicity testing will be required as part of the demonstration of toxicity reduction.

Lynn Suer: Our ambient toxicity testing program assesses ambient toxicity in certain portions of the Estuary, but is not intended to provide evidence of toxicity reduction near outfalls.

Don Mount: Tolerance levels for all of the additives used must be determined. This will be much easier to do if it is done cooperatively. Problems such as determining the tolerance of marine species to pH adjustment and the difficulty of pH adjustment in

seawater can be worked out if the effort is made. I don't believe that marine waters are more complex. In freshwater TIEs we work primarily with effluents, and effluents are every bit as complex as marine waters. A number of procedural issues must be resolved. For example, when should salt be added to the matrix? This would depend on the fractionation procedure (i.e., C_{18} column or ion exchange column). Chuck Batt's suggestion of getting a group together is a good one. I also would like to repeat my earlier statement that it would be most cost-effective to narrow down the number of species used. Species that would most often trigger a TIE are the ones that should be looked at first.

Lynn Suer: Interestingly, in our region effluents are often toxic to *Selenastrum*, but *Selenastrum* has not been targeted for methods development by EPA. *Mysidopsis* would also be a high priority. Echinoderms would be the next highest priority below *Selenastrum* and *Mysidopsis*.

Bob Berger: Regarding the question of whether to use brine or dry sea salts in salinity adjustment, we have found that dry sea salts do not give the proper response in the echinoderm test. The problem with brine is that you are limited to a certain concentration of effluent that can be adjusted to the proper salinity. This is one example of the type of procedural issues that must be resolved before TIEs can be routinely performed on discharges to San Francisco Bay.

Don Mount: There was a time when we thought it was impossible to use *Ceriodaphnia* in freshwater TIEs, mostly because we did not know how to feed them properly. With more information the problem went away. The same will be true with marine tests and use of the proper salt. I would advise you to avoid the use of brines, because of possible problems with contamination. It is important to use materials of known quality.

Lynn Suer: In this region we have found that commercial salts vary in toxicity, even within single brands. A first step may be to do a TIE on these salts.

Don Mount: In freshwater we had a very similar problem, and the cause turned out to be the diet of *Ceriodaphnia*.

2) What are the scientific and bureaucratic steps to be taken in EPA developing and distributing marine chronic TIE guidance?

Teresa Norberg-King: There are several ways in which this could be done. We could prepare an interim document containing some of the data that I presented yesterday. The newsletter from EPA Duluth is one informal outlet for some of this information. We will distribute a draft marine TIE document in the fall that will be handled in the same way as past documents. Copies will be distributed to the regional offices, who

will distribute the document within their regions. Interested parties are always welcome to call and ask about progress on this work.

3) ***What specific research is EPA performing on marine chronic TIEs? Give examples of laboratory procedures under development.***

Teresa Norberg-King:I think we covered that under question #1.

Bob Berger: How will the revision of the short term chronic test procedures influence marine chronic TIE procedures?

Teresa Norberg-King: The revised short term chronic testing manual should be available soon. There have not been many changes from the 1988 edition, so I don't think it will have much of an effect on marine chronic TIEs. EPA Narragansett is largely responsible for both the short term methods and marine TIEs, so any changes will certainly be incorporated.

4) ***What is the minimum level of persistent chronic toxicity that must be present for TIEs to be successful? The minimum level is defined by the frequency and degree of toxicity.***

Don Mount: I think it is inappropriate to latch onto Bill Goodfellow's rule of thumb that was not intended to be taken as firm guidance. There is no clear answer on that question. The only real answer is that there must be a measurable difference. This will be laboratory-specific. In freshwater TIEs careful laboratories have been able to work with a difference as small as 10%. Other labs have needed a 50% difference. The minimum level of toxicity also depends on how the toxicity is expressed. To use 1.5 TU implies that above that there is no impact. I have seen effluents that at 95% effluent cause approximately 50% mortality; a TIE can be performed on this effluent. If it is true, as someone pointed out yesterday, that marine tests are more variable, then that will also affect this answer. It is not wise to try to identify a numerical answer to this question at this time.

Bill Goodfellow: That number was taken out of context. At 1.25 TU we feel confident that we can identify the problem. Below that our level of confidence diminishes to the point where we would not try to work with that sample and would instead collect another sample.

Don Mount: Regarding the frequency of toxicity, that depends on how long regulators are willing to wait. Sometimes toxicity is observed in long cycles. For example, in winter in the eastern U.S. ammonia concentrations are higher because denitrification is slower. For this reason deadlines for compliance are not appropriate; it is better to set milestones for performing tests and completing certain steps. Both sides can suffer if compliance deadlines are too rigid. For difficult problems dischargers could be faced

with requirements that can't be met, and if a problem is readily solved regulators can be faced with dischargers dragging their feet on an easily remedied problem.

Bill Goodfellow: Another issue relating to persistence of toxicity is toxicity decay. In cases where the toxicity diminishes over time it may be necessary to perform the manipulations before the sample is screened for toxicity. There is a risk that some nontoxic samples will be processed, but sometimes this approach must be taken.

Don Mount: It is important to clearly define terms. Persistent toxicity is toxicity that does not decay during the TIE. Consistency of toxicity, which I think is what is meant by frequency in this question, is a separate characteristic. The degree of toxicity is how much toxicity is present in the effluent.

Terry Oda: Are you suggesting that we should trigger a TIE at each finding of toxicity that might vary on a seasonal basis?

Don Mount: No. I think there should be one trigger for initiating TIE/TREs, and a properly run TIE must include that variability.

Stephen Hansen: In dealing with the persistence issue, it is important to determine whether the toxicity is degradable in preliminary testing. If the toxicity is degradable it may be necessary to do a number of false runs.

Kurt Kline: Most of the examples given yesterday did show large differences in toxicity between controls and tests. I still think we need a better definition of sufficient toxicity.

Don Mount: For the metal fabrication plant that I discussed yesterday only about one-third of the samples have been toxic. The IC_{25}s in some of the samples were approximately 80%. We don't consider that to be a very toxic sample.

Stephen Hansen: Similarly, for the El Paso study there was no mortality in *Ceriodaphnia* and reduced reproduction of around 25-40%.

Teresa Norberg-King: It is important to remember not to overemphasize the use of statistics in TIEs. Experience and judgment are essential in carrying out these procedures.

Don Mount: I would like to offer an example of this. Using statistics you would make individual comparisons between each adjustment and the baseline. However, if results from a group of manipulations (e.g., pH adjustment, pH adjustment followed by filtration, pH adjustment followed by aeration, and pH adjustment followed by C_{18} extraction) are all lower than the baseline, then you might conclude that toxicity was reduced even if the individual statistical comparisons were not significant.

Teresa Norberg-King: In many TIE procedures there are not enough replicates to show statistically significant differences. In Phase I of a TIE it is not practical to do four replicates for each step. In Phase III more rigorous testing and statistical analysis can be done.

Bob Berger: Although statistics may not have a large role in interpretation of Phase I results, it would be desirable to have some kind of general rules of thumb. A large amount of TIE work will happen very soon and will be performed by labs without experience because of the large volume of work to be done. The reduction portion of TIE/TRE is a grave concern of dischargers, who want to have confidence that they are not making expensive mistakes.

Don Mount: One mechanism would be to convene a small advisory panel of people who are experienced to review results.

Lynn Suer: That is a fabulous idea, but I wonder who would pay for it.

Bob Berger: Dischargers want to have confidence in the results of these studies, and I think it would be money well spent.

Don Mount: It would not be necessary to consult the advisory panel for each case. A lot of TIEs are relatively easily interpreted.

Stephen Hansen: TIEs are only one part of this process, and probably not the most important part. Treatability studies will prevent expensive mistakes from being made.

*5) **Cite criteria for abandoning TIEs. Give examples of the application of the criteria.***

Teresa Norberg-King: Some information will always come out of a TIE, so in that sense we have never abandoned one.

Kurt Kline: TIEs can always be performed on a toxic effluent. However, the degree of toxicity needed for a successful TIE is an unresolved question. How long should intensive sampling be conducted before concluding that an effluent is not toxic? Biweekly sampling would be excessive for an effluent that is not toxic. The monitoring program proposed by the Regional Board will detect toxicity. Intensive sampling should be performed after the toxicity is detected, not continuously. I don't think TIEs should be abandoned. A lack of toxicity should simply signal a return to less intensive sampling.

Bill Goodfellow: In industrial plants especially, once a TRE is initiated the plant management often solves the problem by improved housekeeping. This would be a successful TRE, even though the toxicant was not necessarily identified.

Chuck Batts: We have talked about awareness of toxicity. I think there is an awareness out there. We recently made a bid for polymers in which we asked 15 salespeople for toxicity information. Only three were able to provide the data. There is an awareness that products used in treatment can sometimes cause toxicity, and that these products can be avoided.

6) What site specific information should be included in a TIE study plan?

Stephen Hansen: All of it! The biomonitoring data are obviously the most important. Sampling and testing to characterize variability is important. In that sense it is nice to have at least a year's worth of biomonitoring data. Chemistry data are useful if they are available. Familiarity with these data can prejudice interpretation of TIE results, but on the other hand can also indicate obvious possibilities. An inventory of chemical usage and plant performance is also an important first step.

Don Mount: I think that study plans should include a clear statement of the sequence of steps to be performed. One would be gathering the data that already exist. Characterization should be performed before a TIE or potentially expensive treatability studies. Study plans should include a description of the characterization to be done. Also, the regulator is obliged to state the objectives of the study, including what and when goals are to be met. Moving targets are often a problem in TIE/TREs. The qualifications of the laboratories performing the study should also be carefully considered by regulators.

Lynn Suer: How can we encourage other laboratories to become capable of performing TIEs? We will clearly have a need for increased capacity for TIEs in our region.

Don Mount: I think you should require that a consultant provide review of the work. We need to have a requirement for residency in this business similar to that for physicians.

Lynn Suer: How would we evaluate the adequacy of this residency?

Don Mount: By their track record.

Teresa Norberg-King: It would be important to evaluate their routine testing skills, whether they are cognizant of the intricacies of the tests and how they would interpret the results.

Bill Goodfellow: Many dischargers will do this type of evaluation when they select the laboratory. It is in the best interest of the discharger to make sure that they are not wasting their money on a poorly run TIE. Ultimately it should be the responsibility of the discharger.

Terry Oda: How should regulators deal with quality assurance information on these complicated procedures?

Bill Goodfellow: It is fairly easy to tell if the individual procedures comprising the TIE are run properly. It is more difficult to be sure that a correct interpretation of the results has been made. I don't see any recourse for ensuring this on a lab-by-lab basis.

Terry Oda: Would the use of reference toxicants help in this respect?

Bill Goodfellow: Yes. That is probably already being done.

Chuck Batts: The problem with that approach is that high interlaboratory variability (40%) is observed in tests with reference toxicants. Actually visiting the lab and seeing how samples are handled is more indicative of competence than testing with reference toxicants.

Lynn Suer: A lab's ability to run toxicity tests is different than the ability to run a TIE.

Teresa Norberg-King: Testing of reference toxicants will not indicate whether a TIE is being performed properly or is giving the right answer.

Don Mount: The pros and cons of laboratory certification should be carefully considered. Two states have recently established certification programs for routine toxicity testing that have been expanded to TIEs. In one of those states labs from outside the state cannot be certified, meaning that labs outside the state cannot be hired for TIEs. That poses a number of problems. Large companies with offices in different states will have to hire a different lab in each state.

Teresa Norberg-King: Several more states are considering certification as well. This will be done on a state-by-state basis because federal agencies are not going to institute a national certification program.

Michael Carlin: Labs must be state-certified in California, but labs from outside California can be certified. I would like to return to the issue of the elements of a study plan. Some dischargers in the audience may soon have a permit requirement to prepare a study plan. A target number of samples to examine in Phase 1 would be a good idea. Prescreening of nontoxic samples might provide useful background information. Biomonitoring data should be reviewed at the start of the TIE. Some sort of schedule for Phase 1 should be prepared. Seasonality should be taken into consideration. Dischargers with chronic toxicity limits are supposed to have these plans on the shelf.

*7) **Should local labs develop chronic procedures for marine species or use freshwater species until EPA publishes a procedure?***

Lynn Suer: The Regional Board wants to begin solving toxicity problems in marine waters. Labs have been developing TIE methods for marine species and seem to be doing an adequate job. Waiting for EPA guidance does not seem necessary.

Chuck Batts: Now that EPA has mandated that their Gold Book almost become a national standard there is a new national interest in meeting Gold Book standards and problems associated with meeting those standards. A lot of information is being developed nationwide concerning these specific issues. The problem with developing a regional standard using marine species is that the database will not be as extensive. Most marine work has been done on the east coast. For enforcement I think we need to work with species that are well understood.

Lynn Suer: That would mean that chronic TIEs could only be performed with *Ceriodaphnia* and fathead minnows, and that would only cover half of the discharges that are known to be toxic. In some cases the toxicity observed with marine species might also affect *Ceriodaphnia*, but not in every case. Other species must also be used to solve the problems with the remaining discharges.

Chuck Batts: I think there are two leaps of faith there. One is that effluent toxicity is not necessarily equivalent to an effect on beneficial uses. Unless ambient toxicity is assessed, or the fate of a chemical in the complex environment of the Bay, effects on beneficial uses have not been demonstrated. Second, there is a problem in finding a sensitive freshwater species to mimic the response of marine species.

Lynn Suer: How frequently can we find surrogate species?

Teresa Norberg-King: We don't have that information at present, but we are now seeing how well the east coast marine species correspond with the freshwater species. I am not sure that the use of surrogates is a good approach.

Stephen Hansen: Whether a surrogate can be found is a site-specific question. We have tried substituting species. In one case where we succeeded we used Microtox instead of fish after doing a correlation study to show that they were responding in a similar way.

Don Mount: For some effluents it will not be possible to find an available sensitive freshwater organism. It would be easier to develop tolerance data for a couple of marine species than to establish correlations with freshwater species.

Lynn Suer: Would EPA consider tackling the problem with commercial salts causing toxicity?

Teresa Norberg-King: I will discuss that with others at EPA.

Chuck Batts: There is also the problem with brines diluting toxicity and concern over the behavior of marine salts when combined with chelating agents or run through columns.

Lynn Suer: These are problems that are common to all marine tests.

Chuck Batts: These are problems that should be solved nationally.

Bob Berger: In comparison to the implementation of chemical limits, for toxicity limits dischargers are being asked to perform a great amount of research and method development themselves. A far more substantial database was available before implementation of chemical limits. I think this may set a bad precedent for reasonable regulatory control.

Lynn Suer: We don't think that an inordinate burden is being placed on dischargers. Waiting for guidance that is not a cookbook anyway will just delay the process of solving water quality problems.

Bob Berger: I don't think we have a clear idea of where we are going. From the information presented by Ms. Norberg-King, I don't think enough information is available to support relatively standard guidance, and it will not be available by the time permits are required in July.

Lynn Suer: TIEs are solving water quality problems. Standard guidance may or may not help solve such problems any better.

*8) **Describe follow up activities once toxicants have been identified. How often do these result in toxicity reductions?***

Don Mount: Not always. For example, diazinon problems still have not been resolved. Some problems require more time for Phases II and III.

Chuck Batts: Once the toxic agent is identified other questions must be answered. Is it desirable to eliminate the toxicity? For example, with copper in the Bay, high background concentrations may mean that reduction of the toxicity may not have much effect. Other questions are whether the technology is available or affordable.

*9) **How does chronic testing account for variability in the tests (dose/response), especially with regard to sample preservation and species sensitivity?***

Chuck Batts: I would like to clarify the question. In some cases no dose/response relationship is evident even though a TIE would be triggered. The issue with sample preservation is toxicity decay.

Don Mount: Decay of toxicity is expected. If it decays very rapidly it may not be a problem in the environment. Toxicity decay can affect the cost of a TIE but doesn't prohibit it. A dose/response relationship is needed to evaluate a toxicity test. Use of ICp will prevent one kind of dose/response problem.

10) How does chronic testing account for quantification of biomonitoring results with respect to test variability and interlaboratory variability?

Chuck Batts: This was a biological question. Since we are dealing with chemical questions we can move on to the next one.

11) How are Phase III confirmation procedures (correlation, mass balance, and synergistic effects) applied in chronic testing?

Don Mount: The revised guidance for Phases II and III will apply to both acute and chronic TIEs. Phase III procedures are not seriously affected by extension to chronic testing. I might comment that I have never seen a synergistic effect. Often a total lack of additivity is observed, which can make it very difficult to identify either toxicant because one toxicant can mask the other. There is a discussion, which applies equally well to both acute and chronic TIEs, of these problems in the manual.

Bill Goodfellow: I agree. A greater emphasis on quality control is important in the chronic procedures due to the length of the tests and the greater possibility of the introduction of artifacts during Phases II and III.

Teresa Norberg-King: A cookbook does not exist for Phase III. Often it is obvious from Phase I results what needs to be done in Phase III. This is true for chronic TIEs as well as acute TIEs.

Bill Goodfellow: Often Phase III really consists of Phase I techniques with subtle twists to fill in holes left from Phase I.

Don Mount: The distinction between Phases I, II, and III is often blurred. As soon as a sample is successfully processed through Phase I, Phase II should begin immediately, and Phase III should begin as soon as the likely identity of the toxicant becomes apparent.

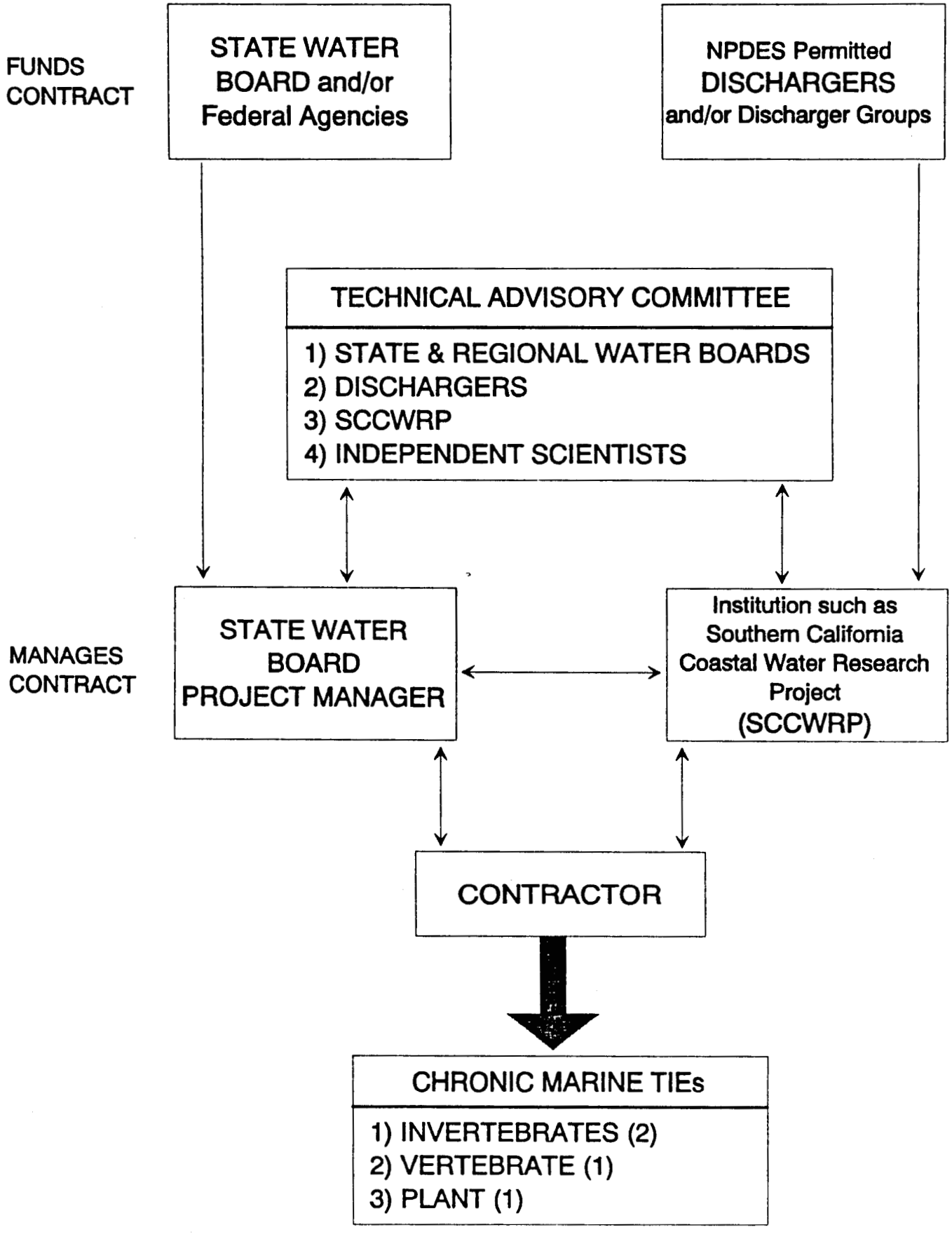
CLOSING COMMENTS

Lynn Suer

There has been a tremendous amount of useful information presented here today. One of the things that has become clear is that during the course of conducting TIEs, dischargers will be producing a great deal of technical data regarding the toxicity of various chemicals and the characteristics of test organisms. Without an efficient means of sharing this information among the regulatory agencies and the dischargers, we could duplicate a lot of effort. We should plan to develop a good data sharing mechanism in the near future. In the meantime, dischargers seeking information in the course of designing or conducting TIEs should contact both EPA (Teresa Norberg-King's office) and the Regional Board.

APPENDIX I

DEVELOPMENT OF CHRONIC MARINE TIES FOR CALIFORNIA USE



STAFF PROPOSAL
BY THE
DIVISION OF WATER QUALITY
STATE WATER RESOURCES CONTROL BOARD

TITLE: DEVELOPMENT OF MARINE TOXICITY IDENTIFICATION EVALUATION (TIE) PROCEDURES USING WEST COAST ORGANISMS FOR DETERMINING SOURCES OF TOXICITY IN DISCHARGES TO MARINE WATERS

ISSUE: At present, there are no chronic marine TIE procedures available. Should State Water Board manage and partially fund the development of marine TIEs, or should this responsibility and cost be borne by marine dischargers?

PROPOSAL: TIEs rely on the use of aquatic toxicity tests to detect the constituents causing toxicity. A TIE is most successful when the TIE test organism used is the same as the species that identified the presence of chronic toxicity and subsequently "triggered" the Toxicity Reduction Evaluation (TRE). The State Water Board proposal is to develop TIE procedures for marine waters using west coast organisms (a vertebrate, a plant, and two invertebrates). Total projected costs are $450,000; these cost estimates are based on current work to develop chronic TIE procedures using east coast organisms at U.S. EPA's Environmental Research Laboratory in Narragansett, Rhode Island.

Under this proposal, the State Water Board would initiate a program to develop marine TIE procedures through a cooperative effort with NPDES dischargers. The program would focus on test organisms approved in the statewide water quality control plans (Ocean Plan and Enclosed Bays and Estuaries Plan [Plans]). A reasonable approach would be for the State Water Board to provide funding at $100,000 as seed money to initiate marine TIE work. State Water Board funds would be contingent upon obtaining external funds to complete the project. State Water board staff recently submitted a proposal to the U.S. EPA for $100,000 in Near Coastal Waters Funds for chronic marine TIE procedures development. If the grant is received, it would be used as part of the total projected cost of $450,000. The remaining $250,000 still requires that additional fund sources be identified. Currently State Water Board staff is discussing with NPDES dischargers the potential for obtaining such funding.

One possible obstacle is lack of a mechanism to enable dischargers to funnel funds to the State Water Board Project Manager. Under the current State contracting process, combining State Water board and discharger funds into one contract is a difficult and time-consuming process. Each discharger must first contract with the State Water Board in order to encumber funds. Then the State Water Board must prepare a request for proposal, identify a contractor and, finally, prepare a contract. One

possible alternative mechanism is to utilize an outside research institute such as the Southern California Coastal Water Research Project (SCCWRP). The discharger funds could be funneled through SCCWRP to the same contractor used by the State Water Board. The State Water Board Project Manager would manage the contract to develop the TIE procedures.

This cooperative effort enables State Water Board staff to gain the expertise necessary to evaluate TIE procedures. In return, NPDES dischargers would have approved State Water Board TIE procedures to use when required to perform a TRE. The effort would also demonstrate to dischargers and the public that the State Water Board is committed to ensuring that TIEs will be fully developed so that TREs can be successfully conducted.

A Technical Advisory Committee (TAC) will be established to ensure the success of the project. The composition of the committee will include scientists from State and Regional Water Board staff, SCCWRP, and NPDES dischargers, as well as independent scientists. The TAC will be responsible for providing sound scientific information on which project management decisions will be made. TAC input will also be important in outlining and refining project tasks, products, and time schedules.

DISCUSSION: TIE procedures to address toxicity are performed in three phases: Phase I - characterization, Phase II - identification, and Phase III - confirmation. In each phase, toxicity tests are necessary to confirm toxicity. Implementation of the Plans requires the use of TIEs to determine the cause of toxicity in complex effluents.

Rapid advances are occurring in development of methods for conducting TIEs. U.S. EPA-Duluth researchers have released a TIE document: "Characterization of Chronically Toxic Effluents, Phase I" (EPA 600/6-91/005) using two freshwater species: Pimephales promelas (a fish) and Ceriodaphnia dubia (a crustacean). This manual describes procedures for characterizing the physical and chemical nature of toxicants in effluents that exhibit chronic toxicity.

Fundamental characterization procedures such as quality assurance, effluent handling, facilities and equipment, health and safety, dilution water, and principles of the chronic TIE testing have been designed for freshwater TIE testing. These fundamental procedures can be and will need to be modified for use in marine TIE procedures.

U.S. EPA-Narragnasett researchers are currently developing chronic TIE procedures using East Coast marine species: Champia parvula (red algae), Menidia beryllina (a fish), Mysidopsis bahia (mysid),

Arbacia punctulata (sea urchin). Three of these four organisms are not indigenous California species, and the Department of Fish and Game has expressed concern over the use of non-native species for toxicity testing. Therefore, it is important that California have TIE procedures that use species currently required by the Plans. However, California can build upon EPA's work by adapting these East Coast TIE procedures for West Coast species.

RECOMMENDATION: The Division of Water Quality recommends the following: 1) that the State Water Board approve funding of the initial work on marine TIE development for FY 1992-1993 at $100,000, contingent upon obtaining funds from dischargers and possible EPA funds for continued development, and 2) that State Water Board staff develop a mechanism to channel discharger funds through SCCWRP or a similar institution in order to streamline commencement of the project.

TIME SCHEDULES TO DEVELOP CHRONIC MARINE TIE PROCEDURES

	1992									1993												1994											
YEARS	----------1----------																					----------2----------											
MONTHS	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
A.ARRANGE FUNDING SOURCES																																		
1 STATE BOARD																																		
State budget approved				xxxx																														
Funds available					xxxx																													
2 EPA PROPOSAL																																		
Proposal due	xxxx																																	
Initial selection		xxxx																																
Workplans due			xxxx																															
Award funds					xxxx																													
3 DISCHARGERS &/or ASSOCIATIONS																																		
Present proposal	xxxxxxxx																																	
Dischargers commitment				xxxx																														
B.DEVELOP TRANSFER MECHANISM																																		
1 SCCWRP or other Insitution																																		
Mail proposal to SCCWRP	xxxx																																	
Present proposal to CTAG\SCCWRP		xxxx																																
C.SELECT CONTRACTOR & PREPARE CONTRACT																																		
1 Solicts State Agencies		xxxx																																
2 Solicts State Universities		xxxx																																
3 Prepares bid\advertisemt package			xxxx																															
4 Select contractor				xxxx																														
5 Prepare contract package			xxxxxxxx																															
6 Contract in place																																		
D.WORK SCHEDULE																																		
1 Select test organisms										xxxxxxxxxx																								
2 Develop two invertebrate TIE procedures											xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																							
3 Develop plant TIE procedures																							xxxxxxxxxxxxxxxxxxxxxx											
4 Develop vertebrate TIE procedures																																		
5 Prepare final report & TIE manual																																		