Example

TOXICITY IDENTIFICATION EVALUATION

Introduction

Company X has conducted a series of eight definitive whole effluent toxicity tests using two aquatic species. The test results have shown that, after mixing, the effluent is not chronically toxic to either species at concentrations allowed in the company's NPDES permit. However significant acute toxicity (defined as 48 or 96-hr LC50s less than 100% effluent) is consistently observed for both Ceriodaphnia dubia and fathead minnows. A summary of the acute toxicity results is shown below (expressed as 48 or 96-hr LC50 in percent effluent):

Ceriodaphnia tests	42	45	16	33
Fathead minnow tests	94	94	67	28

Concurrent chemical sampling done indicated that much of the toxicity observed to date has been associated with chloride, sulfate, and ammonia in the effluent. At least one other yet-to-be-identified toxicant was also thought to be present in this test.

A Phase I Toxicity Identification Evaluation (TIE) to further evaluate potential toxicants in the effluent was initiated. This TIE used fathead minnows as the test animal, since the latest round of tests indicated that fish were the more sensitive of the two species and that all of the toxicity to fish could not yet be accounted for. Only acute toxicity was evaluated, since meeting chronic toxicity limits does not appear to be a problem for the company. The TIE procedures used in the study were adapted from those recommended by U.S.EPA (EPA/600/6-91/003).

Methods

The TIE was initiated using a single sample collected from outfall 001. Initial testing of this sample indicated that mortality to fathead minnow larvae occurred very rapidly (100% mortality within 3 hours). This sample had the following chemical characteristics:

pH = 9.3
conductivity = 8600 uS
total ammonia = 6.0 mg/l
sodium = 1500 mg/l
sulfate = 3750 mg/l
chloride = 550 mg/l
hardness = 30 mg/l as $CaCO_3$
alkalinity = 450 mg/l as $CaCO_3$

The effluent sample was treated in the following ways:

1. No treatment
2. Exposure to zeolite for 60 minutes
3. Fine-bubble aeration for 60 minutes
4. Sulfuric acid addition to lower pH to 7.0
5. Exposure to powdered activated carbon for 30 minutes
6. Addition of EDTA chelator (50 mg/l)
7. Addition of sodium thiosulfate (15 mg/l)
8. Removal of surface active agents (see below)
9. Filtration through a 47u cellulose filter

Seven larval fathead minnows (less than 24 hours old) were exposed to each treatment and examined hourly for mortality. For removal of surface active agents (surfactant treatment), the effluent was aerated vigorously in a slender vial for 60 minutes. Once aeration was completed, the water was quickly siphoned off and replaced with moderately hard reconstituted water. Deposits of solids associated with this "sparging" process (generally defined as surface active agents) were usually present on the sides of the vial. The vial was then stoppered and shaken so the vial sides were completely rinsed free of all potential surface active agents. This sample was then tested for toxicity. A positive response would indicate that surfactants removed in the treatment process could be recovered in the dilution water. The technique is described in EPA/600/6-91/003.

Results

No treatment -	14% mortality the first hour 57% mortality within 2 hours 100% mortality within 3 hours
Zeolite treatment -	no mortality the first 17 hours 28% mortality after 72 hours Toxicity was significantly reduced. Indicates that ammonia was responsible for at least some of the observed toxicity. Ammonia was reduced from 6 mg/l to 2.5 mg/l. Conductivity was also reduced, indicating that zeolite may have also removed other ions.
Aeration -	no mortality the first 2 hours 43% mortality within 4 hours 100% mortality within 17 hours Toxicity was reduced somewhat. The effect of the treatment was relatively minor. A slight degree of ammonia removal could have occurred. Volatile substances were probably not responsible for toxicity.
Lowered pH -	no mortality after 4 hours 57% mortality after 72 hours Toxicity was significantly reduced. The results are consistent with ammonia as a toxicant. Little or no ammonia was removed in the treatment process, but the most toxic un-ionized form was reduced 4-100 times during the exposure period.
Activated Carbon - Treatment	71% mortality during the first hour 86% mortality after 2 hours 100% mortality after 3 hours The treatment had no effect on toxicity. The toxicant is not adsorbed by activated carbon.
EDTA chelation -	No mortality after the first hour 43% mortality after 2 hours

	100% mortality after 3 hours EDTA at 50 mg/l had no effect on toxicity. The toxicant was probably not a commonly encountered metal.
Thiosulfate - Treatment	No mortality after 2 hours 86% mortality after 3 hours 100% mortality after 4 hours The treatment had no effect on toxicity. The toxicant is probably not an oxidant.
Surfactant - Recovery	No mortality after 72 hours. The toxicant was probably not a surface active agent.
Filtration -	86% mortality within 75 minutes 100% mortality within 3 hours Filtration did not affect toxicity. The toxicant is probably in a "dissolved" form and not associated with suspended solids.

The effluent fractionation procedure continued to support previous evidence that ammonia is a potentially significant toxicant in the effluent. When total ammonia was reduced or converted to a less toxic form in these treatments, toxicity of the effluent was also reduced. Two treatments (zeolite additions and lowering pH) were capable of reducing the effluent LC50 for fish to values which would come close to passing acute toxicity limits in the company's NPDES permit (LC50 less than 100% effluent).

IDENTIFICATION OF ADDITIONAL TOXICANTS

To determine whether ammonia was the only toxicant of concern in this test, we made up an artificial effluent containing 6 mg/l total ammonia in moderately hard reconstituted water. We adjusted the pH to 9.0 to closely match conditions observed in the whole effluent sample, then exposed six newly hatched fathead minnow larvae to the water.

After 3 hours of exposure, only 17% mortality had occurred in the artificial effluent. After 48 hours, only 66% mortality was observed. These results showed that the artificial effluent was less toxic than the whole effluent tested previously (100% mortality in 3 hours), and suggested that an additional toxicant which had not yet been accounted for was probably present in the effluent.

The company submitted an MSDS sheet for a chemical cleaner (Parco 305) used in the manufacturing process. We reviewed the data on the MSDS sheet and noted that the cleaner contained 17 to 22% potassium hydroxide. Records of cleaner use maintained by company personnel indicated that potassium concentrations in the effluent (derived from KOH in Parco 305) could exceed 1000 mg/l at times. The effluent sample contained 430 mg/l, as measured by the ICP analytical technique.

Commonwealth reviewed all available data on potassium toxicity to fathead minnow larvae. Testing commissioned by EPA as part of a Quality Assurance/Quality Control program used potassium chloride as a reference toxicant. Potassium chloride samples were tested for acute toxicity to fathead minnows by 123 different laboratories. The results showed that the mean 48-hr

LC50 for potassium (from KCl) was 470 mg/l. The 95% confidence intervals for this value ranged from 170 to 750 mg/l.

Commonwealth tested our own lab-raised fish for toxicity to KCl. This purpose of this test was to determine whether our animals had similar sensitivity to KCl. The measured 48-hr LC50 for potassium to larval fathead minnows from our lab was 715 mg/l (95% C.I. = 624 to 840 mg/l). Since Commonwealth's LC50 value fell within EPA's acceptability range as determined above, the fish appeared to have normal sensitivity to potassium.

The potassium concentration in the company's effluent sample (430 mg/l) fell very close the 48-hr LC50 determined by EPA (470 mg/l) and was well within the 95% confidence interval for this chemical. These results provide strong evidence that potassium could have contributed to the observed whole effluent toxicity.

Earlier testing had shown that ammonia alone could probably not have caused the rapid mortality observed to minnows exposed to effluent. To help determine whether potassium also contributed to the toxicity of the effluent sample, Commonwealth initiated an artificial effluent test. The artificial effluent, made from moderately hard reconstituted water, was designed to match the ammonia, potassium, and pH conditions observed in the actual effluent sample (total ammonia = 6 mg/l, potassium (from KCl) = 450 mg/l, pH = 9.3).

Eight newly hatched fathead minnow larvae were exposed to the artificial effluent and mortalities were counted hourly during the first four hours of the test. The following results were obtained:

	1 hr	2 hr	3 hr	4 hr
Cumulative % mortality	13%	38%	63%	88%

Mortality in this test with 450 mg/l potassium was much more rapid than in the test with ammonia alone (17% mortality after 3 hours, as reported from p. 5 above) and matched fairly closely with the rapid mortality observed in the whole effluent test (100% within 3 hours). These results add additional evidence that potassium probably acounted for much of the additional toxicity observed in the whole effluent sample.

SUMMARY AND RECOMMENDATIONS

1. The final effluent exhibits consistent acute toxicity which does not meet the company's NPDES toxicity limit.

2. Toxicants which are suspected of contributing to the previously observed acute toxicity to <u>Ceriodaphnia</u> include ammonia, sulfate (and perhaps chloride). These results are based on literature values for toxicity and the predicted additive effects of each chemical.

3. Toxicants which have been identified in this Phase I effluent characterization procedure as contributing to the observed acute toxicity to fathead minnows include both ammonia and potassium.

4. Reducing pH and treating the effluent with zeolite both show promise in their ability to reduce effluent toxicity to acceptable levels for fathead minnows.

5. Future approaches to consider in reducing whole effluent toxicity include the following:

A. Consider modifying wastewater treatment to include a zeolite resin process or reducing pH prior to discharge.

B. Consider using alternative cleaners which do not contain such high concentrations of potassium.