Appendix A
Original Case Histories: Lessons Learned

Since the USEPA research studies in the 1980s and the first TREs performed to meet permit requirements, there have been significant advances in the development and refinement of TRE procedures. These advancements become apparent upon review of the original case histories published in the first edition of the TRE manual. The case histories have been revisited in this manual to note the lessons learned and new approaches that can be taken to conduct TREs.

Many lessons have been learned in applying TIE/TRE procedures to different types of effluents using a variety of freshwater and saltwater test species. Perhaps the most significant improvements in the methods since the original case histories were performed have been the development and application of methods to:

- Identify causes of short-term chronic toxicity to both freshwater and estuarine/marine species.
- Track sources of chronic toxicity that can not be readily characterized in the TIE.
- Characterize, identify, and confirm organophosphate insecticide toxicity.
- Characterize toxic metals using improved EDTA and sodium thiosulfate tests.
- Characterize surfactant toxicity using multiple TIE manipulations.
- Confirm toxicants by the correlation approach.

The use of some of these updated methods is described below using the original case histories as examples. The following summaries are intended to show how similar TREs can be performed more quickly, cost-effectively, and accurately using the current procedures. These summaries also portray the steps taken over the last 10 years to improve the TRE procedures.

Baltimore, Maryland

In January 1986, USEPA, in cooperation with the City of Baltimore, began the first research study to develop a pragmatic approach and methods for conducting TREs at WWTPs (Botts et al., 1987). The City's Patapsco WWTP was selected for this study because of evidence of acute and chronic effluent toxicity. In addition, USEPA was interested in conducting a TRE at an urban WWTP, like the Patapsco WWTP, which receives its influent from a wide range of industrial discharges. The objectives of the TRE were to characterize the WWTP's capability for treatment of toxicity, evaluate techniques to identify the specific components of toxicity, and assess methods to trace toxicity to its source(s).

The study results showed that the WWTP influent had significant acute and chronic toxicity as measured by *C. dubia* [(mean 48-hour LC50=2.6% and mean 7-day chronic value (ChV)=1.2%], *M. bahia* (mean 96-hour LC50=23%), and Microtox® (EC50=8%). Although significant toxicity reduction occurred through treatment, substantial toxicity remained. The 48-hour LC50 for *C. dubia* averaged 6.3% effluent. An evaluation of the WWTP operations indicated that treatment performance was not the major cause of effluent toxicity.

Results of the TIE showed that acute effluent toxicity was removed by passing effluent samples through a C18 SPE column. Recovery of toxicity in the 75 to 95% methanol/water eluates from the C18 column suggested that the toxicants were non-polar organic compounds with relatively high octanol-to-water partition coefficients. However, GC/MS analysis of the toxic non-polar organic fractions was not successful in identifying the specific nonpolar organic toxicants. Additional testing showed that the toxicants sorbed onto suspended solids in the effluent. Solids greater than 0.2 µm were found to be the major toxic fraction.

TIE Procedure Update

Since this study, USEPA developed procedures for identifying non-polar organic toxicants (1993a). If non-polar organic toxicity is indicated in the Phase I of the TIE, the toxicant(s) can be isolated and concentrated to improve the chances of identification using GC/MS analysis. This approach has been helpful in identifying organophosphate insecticides as causes of effluent toxicity at some POTWs (see examples below and Appendix F).

An evaluation of wastewater samples from selected candidate industries was performed to determine the major contributors of refractory toxicity to the WWTP. An important goal of this study was to develop and evaluate methods for tracking sources of toxicity in POTWs. A protocol was designed to measure the toxicity remaining after treatment at the WWTP, which is the toxicity that passes through in the final effluent. The residual or “refractory” toxicity of five major industrial users of the WWTP was evaluated by treating wastewater samples in a bench-scale batch simulation of the WWTP activated sludge process. Microtox® results indicated that two of the five industries were contributing refractory toxicity to the WWTP. Results of *C. dubia* tests were inconclusive due to an interference in the treatment simulation. This interference appeared to be caused by residual toxicity in the RAS used in testing.

RTA Procedure Update

Biomass toxicity may be reduced by washing the RAS with buffer solutions or laboratory water. Alternatively, a surrogate biomass from a POTW with a similar type of biological treatment process may be obtained for testing. Details are presented in Section 5.

Hollywood, California

In the late 1980s and early 1990s, the USEPA laboratory in Duluth, Minnesota, tested several POTW effluents in the process of developing TIE procedures. One of these effluents was the City of Hollywood POTW, which exhibited acute toxicity to *C. dubia* (Amato et al., 1992).

TIE Phase I tests showed that treatment with a C18 SPE column was the only step that reduced effluent toxicity. Acute toxicity was recovered from the C18 column by eluting the column with methanol. Additional C18 SPE column tests performed on 16 effluent samples showed that toxicity was consistently eluted in the 80 and 85% methanol fractions, which suggested that the cause of toxicity was the same among the various samples. These results provided evidence that the toxicant(s) was a non-polar organic compound(s). Further concentration and separation of the toxic fractions was done, followed by confirmation GC/MS analyses of the fractions. Analysis of selected 80 and 85% methanol fractions by GC/MS found sufficient concentrations of the insecticide diazinon to account for the observed acute toxicity to *C. dubia.*

TIE Procedure Update

In recent TIE guidance, USEPA (1991 and 1993a) recommends adding a metabolic blocker, PBO, to toxic effluent samples or methanol eluates as a subsequent test for the presence of metabolically activated toxicants like organophosphate insecticides. PBO has been shown to block the acute toxicity of diazinon, parathion, methyl parathion, and malathion to cladocerans, but does not affect acute sensitivity to dichlorvos, chlorfenvinphos, and mevinphos (Ankley et al., 1991). A reduction in acute or chronic toxicity by the PBO addition together with toxicity removal by the C18 SPE column and concentration data can provide strong evidence for the presence of selected organophosphate insecticides.

In the confirmation step (USEPA, 1989b), three Phase III confirmation steps were used to confirm diazinon as a cause of effluent toxicity: toxicant correlation, mass balance, and additional species testing.

Toxicant correlation was evaluated by plotting effluent diazinon concentrations and effluent LC50 values as shown in Figure A-1. The correlation coefficient (r value) was significant and confirmed that, from sample to sample, diazinon was consistently the cause of acute effluent toxicity. Also, the intercept of the regression line at 100% effluent (0.325) was near the diazinon LC50 of 0.35 µg/L, which indicated that diazinon accounted for nearly all of the observed acute effluent toxicity.

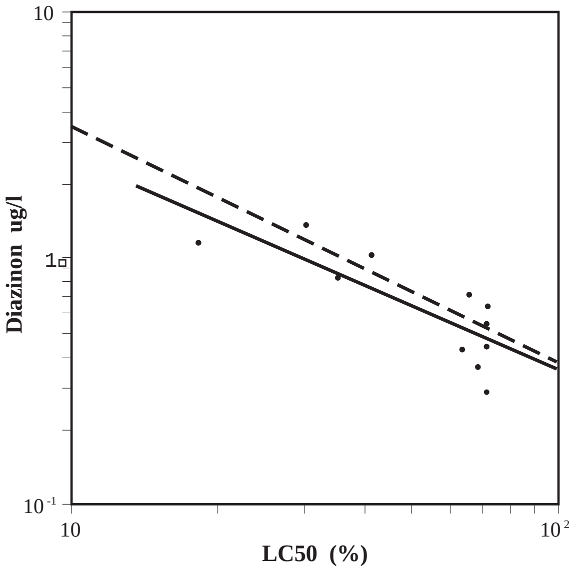

Figure A-1. Acute LC50 of Hollywood effluent versus diazinon concentration (actual correlation shown by solid line; predicted 1:1 correlation by dashed line) (Source: USEPA, 1988).

TIE Procedure Update

USEPA (1993b) recommends a straight-forward correlation approach to determine if a consistent relationship exists between the concentration of the toxicant(s) and effluent toxicity. This approach involves comparing the toxic units of the toxicant to whole effluent toxic units. Toxicant concentrations are converted to toxic units (i.e., measured concentration divided by the toxicant's acute or chronic endpoint) and the resulting values are plotted versus whole effluent toxic units. Since this study, additional acute toxicity data for diazinon and other organophosphate insecticides have become available for calculating toxic units for these toxicants (Ankley et al., 1991; Amato et al., 1992; and Bailey et al., 1997). The correlation approach is useful for determining the extent to which the identified toxicants contribute to effluent toxicity. Using the above example, diazinon would be confirmed as the primary toxicant if the slope is 1 and the intercept is 0 for a plot of diazinon toxic units versus effluent toxic units. In some cases, additional toxicants may be indicated using this technique (see the City of Largo, Florida, example below).

The mass balance confirmation approach involved passing samples through a C18 SPE column, eluting the column with a series of eight methanol concentrations, and testing the toxicity of the methanol fractions. The combined toxic, combined nontoxic, and all fractions were combined and tested at whole effluent concentrations. The results showed that the toxicity of the combined toxic fractions was similar to the toxicity of all fractions together and the toxicity of the original effluent samples. These results provided further confirmation that effluent toxicity was associated with non-polar organic toxicants.

The final confirmation step involved testing effluent samples with *P. promelas*, which are at least 100 times less acutely sensitive to diazinon than *C. dubia* (USEPA 1987, 1988). Test results showed only slight acute toxicity to the minnows as compared to the average acute LC50 of about 60% for *C. dubia*. Acute toxicity to *P. promelas* was interpreted as evidence that a toxicant other than diazinon was present in the samples. However, this additional toxicant(s) was not a significant contributor to toxicity and its identity was not evaluated. In summary, the Phase III testing confirmed that diazinon was the principal effluent toxicant.

Largo, Florida

The USEPA Duluth Laboratory also evaluated effluent samples from the City of Largo POTW. A TIE was performed to identify the causes of acute effluent toxicity (USEPA, 1987).

TIE Phase I tests showed that C18 SPE column treatment removed acute effluent toxicity. Toxicity was not reduced by the other Phase I treatments, including filtration, EDTA addition, or sodium thiosulfate addition.

An additional 18 effluent samples were passed through C18 SPE columns in Phase II. Elution of the columns with methanol showed that acute toxicity was consistently isolated in the 75 and 80% methanol concentrations, although occasional toxicity was also observed in the 70 and 85% methanol concentrations. GC/MS analysis of the toxic fractions identified diazinon as a cause of acute effluent toxicity.

In Phase III, five confirmation steps were used to verify that diazinon was the cause of effluent toxicity: toxicant correlation, toxicant spiking, mass balance, additional species testing, and test species symptoms.

Acute effluent toxicity and diazinon concentrations were converted to TUs and were plotted to determine the toxicant correlation to whole effluent toxicity (USEPA, 1989b). As shown in Figure A-2, more acute toxicity was present than would be explained by diazinon alone; the slope of the linear regression was less than 1 and all of the plotted data points are below the expected 1:1 relationship for diazinon and effluent toxicity. Spiking experiments also showed that doubling the concentration of diazinon in effluent samples did not result in a doubling of effluent toxicity. These results suggested that diazinon was not the sole cause of acute effluent toxicity.

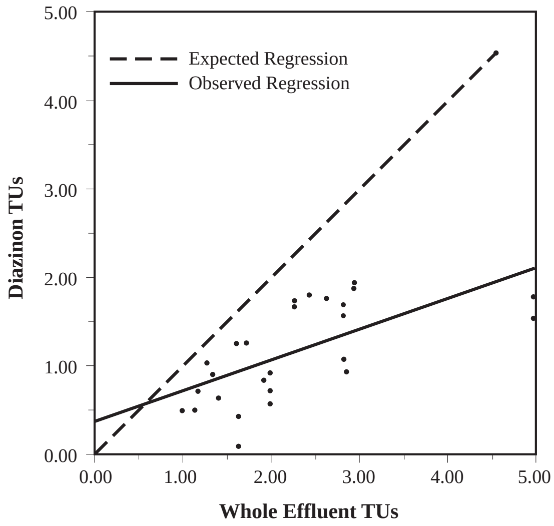

Figure A-2. Correlation of diazinon TUs versus whole effluent TUs (Source: USEPA, 1988).

TIE Procedure Update

The current approach (USEPA, 1993b) is to plot effluent TUs on the Y-axis (dependent variable) and toxicant TUs on the x-axis (independent variable). See Figure A-3.

Follow-up GC/MS analyses identified chlorfenvinphos (CVP) and malathion in effluent samples. Malathion did not appear in concentrations high enough to cause acute toxicity to *C. dubia*, although CVP concentrations were sufficient to contribute to effluent toxicity (48-hour LC50s of 1.4 and 0.35 µg/L, respectively, according to D. Mount, personal communication, USEPA, Duluth, Minnesota, 1989).

The correlation analysis was repeated using the summed toxic units for both diazinon and CVP versus whole effluent toxic units (USEPA, 1993b). As shown in Figure A-3, the slope of the regression line was close to 1, the y-intercept was nearly zero, and the r-value indicated a good correlation (r = 0.73). These results show that diazinon and CVP accounted for nearly all of the acute effluent toxicity.

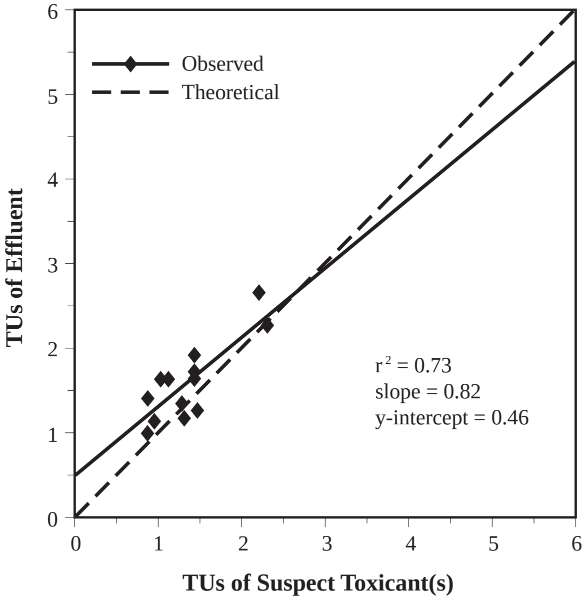

Figure A-3. Correlation of diazinon and CVP TUs versus whole effluent TUs (Source: USEPA, 1993b).

Additional confirmation testing involved analyzing 13 effluent samples using the C18 SPE column mass balance approach. As shown in Table A-1, in 12 of the 13 tests, the toxicity of all methanol fractions combined was slightly greater than the toxic fractions combined. Various mixtures of the three identified insecticides were tested to determine if interactive effects (i.e., antagonistic or synergistic) could account for the difference in toxicity. These tests showed that the toxicity of the insecticides was strictly additive. These results indicated that the higher toxicity of "all fractions" compared to the toxicity of the "toxic fractions" may be due to another unidentified toxicant, rather than interaction among the identified toxicants.

The additional toxicity observed in the "all fraction" test was attributed to 70% methanol/water fraction, which exhibited slight and intermittent toxicity. This fraction was initially included in the "nontoxic fraction" test; however, the mass balance approach

Table A-1. Comparison of Whole Effluent TUs and Methanol Fraction TUs

Sample	Acute Toxic Units (TUa)		
	Whole Effluent	All-Fractions	Toxic-Fractions
A	1.18	1.64	1.43
B	2.00	2.94	3.13
C	1.93	2.86	2.53
D*	<1.00	1.15	<1.00
E	2.00	1.75	1.64
F*	1.15	1.06	<1.00
G	1.33	1.52	1.13
H	3.70	3.03	2.86
I	2.86	2.86	2.44
J	2.27	1.72	1.64
K	2.27	2.04	2.00
L	2.27	1.67	1.59
Mean	2.13	2.18	2.00

* Values excluded from mean calculations due to less-than values.

indicated it to be a slightly toxic fraction. When the toxic units of the 70% fraction are added to the "toxic fraction" result, nearly all of the toxicity is accounted for. Due to the intermittent toxicity of this fraction, additional testing to identify the toxicant was not performed.

Additional species testing with *P. promelas* provided further evidence that the toxicants were organophosphate insecticides. No acute toxicity was observed with *P. promelas*, which are known to be orders of magnitude less sensitive to diazinon than *C. dubia* (USEPA 1987, 1988).

As a final confirmation step, the same symptoms to *C. dubia* were observed after exposure to effluent samples, toxic methanol fractions, and laboratory water spiked with near lethal levels of diazinon, CVP, and malathion. Similar symptoms were observed for all test solutions, which suggested that the same toxicant was responsible in each case.

Lawton, Oklahoma

The City of Lawton was required by USEPA Region 6 to initiate a TRE study in 1991, based on evidence of chronic effluent toxicity at its POTW (Engineering Science, Inc., 1991). The permit limit of no chronic lethality at the critical instream dilution of 96% (i.e., NOEC >96% effluent) was exceeded. Toxicity test results showed that the effluent was toxic to *C. dubia*, but not *P. promelas*.

TIE Phase I tests were conducted in 1991 to characterize the chronic effluent toxicants (Engineering Science, Inc., 1991). The permit limit was based on lethality to *C. dubia* and *P. promelas* in chronic toxicity tests; therefore, the TIE tests focused on lethality instead of reproduction or growth effects. The Phase I tests evaluated percent survival of *C. dubia*, the most sensitive organism, over 5 to 7 days in 100% effluent. In addition, acute lethality results (48- to 72-hour exposure) also were collected to assist in the evaluation.

The results indicated a consistent reduction in effluent toxicity by passing samples through the C18 SPE column. Chronic lethality data showed that no other treatment consistently removed toxicity. Toxicity was recovered by eluting the C18 SPE column with methanol, which indicated the presence of nonpolar organic toxicants. Sample adjustment to pH 3 and pH 11 also reduced toxicity in all but two samples, which suggested that the toxicants could be denatured under acidic or basic conditions.

TIE Procedure Update

As noted above, PBO, a metabolic blocker, can be added to toxic effluent samples, C18 SPE fractions, or HPLC fractions to test for the presence of metabolically activated toxicants such as organophosphate insecticides.

Reproduction data for C. dubia, although not required as part of compliance testing for the Lawton POTW, may have been useful in characterizing the effluent toxicants. These data may provide a more sensitive endpoint than survival in 100% effluent when comparing the effects of the various TIE treatments.

TIE Phase II tests were performed on three samples evaluated in the Phase I characterization and involved the following steps as described by USEPA (1989a):

- C18 SPE columns were eluted with a series of increasing methanol concentrations (25, 50, 75, 80, 85, 90, 95, and 100%) to isolate the toxicants.
- The acute toxicity of each eluted fraction was determined and the fractions found to be toxic were combined. The combined toxic fractions were then reconcentrated using a second C18 SPE

column. Acute toxicity tests were used instead of chronic toxicity tests because the methanol elution concentrated the toxicants to acutely toxic levels.
- The concentrated sample was separated into 30 fractions using HPLC and the toxicity of each fraction was measured. Again, the toxic fractions were combined and reconcentrated on another C18 SPE column.
- The combined toxic sample was then analyzed by GC/MS.

As shown in Table A-2, toxicity was consistently isolated in the 75 and 80% methanol fractions, although toxicity was also recovered in the 50% methanol fraction of one sample. Further separation of the toxicants by HPLC recovered toxicity in a relatively narrow band of fractions (fractions 22 to 28).

Table A-2. Summary of TIE Phase II Results

Sample	Sample Collection Data (1992)		
	4/28	**6/11**	**7/16**
C. dubia percent survival in 100% sample			
Original effluent	50	0	0
Post C18 SPE	100	100	80
SPE eluate (1× effluent)	0	0	0
Toxic methanol fractions (>20% mortality)			
Methanol/water (1× effluent conc.)	50% 75% 80%	75% 80%	75% 80%
HPLC fraction no. (1× effluent conc.)	15 22–25 30	25 28	22 24
Organophosphate insecticides in effluent (µg/L)			
Diazinon	0.22	0.42	0.71
Diazinon oxon	0.1	<0.1	1.45

GC/MS analysis of the toxic HPLC fractions identified several potentially toxic compounds, including the organophosphate insecticide, diazinon, and its metabolite, diazinon oxon (Table A-2). The 48-hour LC50 of diazinon to *C. dubia* is reported to range from 0.35 to 0.61 µg/L (Amato et al., 1992; Ankley et al. 1991). Based on the low end of this range, the diazinon concentrations in the Lawton effluent were high enough to cause acute toxicity to *C. dubia* in two of the three samples tested (0.42 and 0.71 µg/L for the June and July samples, respectively). *C. dubia* acute toxicity tests were conducted to evaluate the potential contribution of diazinon oxon to effluent toxicity. The 48-hour LC50 for diazinon oxon was determined to be 1 µg/L. These data indicate that the diazinon oxon concentration in the July effluent sample (1.45 µg/L) was high enough to contribute to the observed acute toxicity.

TIE Procedure Update

Data on the chronic toxicity of organophosphate insecticides is limited. Unpublished data (TRAC Laboratories, 1992) suggest that *C. dubia* may be chronically sensitive to 0.12 to 0.38 µg/L diazinon (see also Section 2). Chronic data would have been useful in defining the potential for diazinon to contribute to chronic toxicity at the Lawton POTW.

Further testing focused on confirming the contribution of diazinon and diazinon oxon to effluent toxicity. A partial Phase III confirmation was performed using the following steps (USEPA, 1989b):

- Assessing diazinon's physical/chemical properties in relation to the TIE results.
- Determining the contribution of diazinon and diazinon oxon to whole effluent toxicity based on measured effluent concentrations.
- Reviewing effluent toxicity data for a 3-year period to determine if the occurrence of effluent toxicity matched seasonal insecticide use (Engineering Science, Inc., 1992).

Diazinon matches the general toxicant profile developed as part of the TIE. Removal of diazinon on the C18 SPE column and its elution at high methanol concentrations is consistent with diazinon's characteristic as an organic chemical of low polarity. The observed reduction in toxicity by pH adjustment also is indicative of diazinon's tendency to break down under acidic and alkaline conditions.

Concentrations of diazinon and diazinon oxon were measured in 13 effluent samples collected from April 1 through August 21, 1992. Chronic toxicity data for the insecticides were not available at the time; therefore, it was not possible to apply the correlation approach. However, in seven cases, diazinon exceeded the 0.35 µg/L acute toxicity value for *C. dubia*. In two of these cases, diazinon oxon concentrations also exceeded the acute toxicity value of 1.45 µg/L. These data suggested that diazinon and diazinon oxon were likely to cause mortality equal to or greater than that found in the effluent samples.

A review of effluent toxicity data from 1989 to 1992 indicated a greater incidence of toxicity in the spring and summer of each year when insecticides are most often used. Effluent toxicity decreased in late summer and fall and generally disappeared in the winter months. These data support the evidence that toxicity is associated with insecticides.

TIE Procedure Update

Confirmation of the role of diazinon and other toxicants would have been more definitive if the current Phase III procedures (USEPA, 1993b) for chronic toxicants had been applied. Useful procedures for confirming organophosphate insecticide toxicity include the correlation, mass balance, and species symptoms approaches. An example of the use of these procedures is presented in Appendix F.

Based on previous studies (City of Greenville, 1991; C. Kubula, personal communication, City of Greenville, Texas, Public Works Department, 1992), the City of Lawton decided to implement a public awareness program in 1993 to control the discharge of insecticides to the POTW. Information on the proper use and disposal of insecticides was printed in newspaper articles and on monthly water bills (Engineering-Science, Inc., 1993). An electronic message sign with insecticide information was also located at major intersections. Since August 1993, the POTW effluent has met the toxicity permit limit (NOEC >96% effluent) with the exception of 2 months in 1994 and several months in 1995 (as of September 1997). Although diazinon was not confirmed as an effluent toxicant, the City's ongoing insecticide control effort appears to have been successful in achieving compliance with the chronic toxicity limit.

Akron, Ohio

A survey of six Ohio municipal wastewater treatment plants was conducted to determine the level of toxicity reduction that can occur in POTWs (Neiheisel et al., 1988). Of the six WWTPs, the City of Akron's Botzum WWTP received the most toxic influent wastewater. Significant toxicity reduction was achieved through treatment; however, the effluent had an impact on the Cuyahoga River. A bioassessment study of the river in 1984 revealed a severe impairment to aquatic communities downstream of the WWTP discharge. A review of the WWTP's operating records showed a history of intermittent bypasses of raw wastewater during storm events.

Based on the survey results, the Botzum WWTP was selected by USEPA as a site for a TRE research study. The research study focused on conducting toxicity tests of the effluent and the bypassed wastewater and characterization of the variability and sources of the impairment to the receiving water (Mosure et al., 1987). In addition, TIE tests were performed to try to identify the effluent toxicants.

Toxicity test results indicated that although CSOs may contribute intermittently to poor river quality, the continuous effluent discharge was probably the major cause of the observed impact (Mount and Norberg-King, 1985).

The TIE testing isolated toxicity on the C18 SPE column and the toxicity was eluted in the 85% methanol/water fraction (Mosure et al., 1989). These results suggested that non-polar organic compounds were a principal cause of effluent toxicity. Metals also were implicated as effluent toxicants. However, before toxicant identification and confirmation could be performed, effluent toxicity abated.

The cause of this abatement is not known, although the following events may have contributed to the improved effluent quality. These events include:

- Increasing MLSS concentrations in the WWTP aeration basins.
- The shutdown of a large chemical manufacturing plant that discharged to the WWTP.
- Overall improvements in WWTP operation and the pretreatment program (Mosure et al., 1987).

Biological surveys of the Cuyahoga River in 1986 continued to show poor water quality despite the decrease in effluent toxicity (Mosure et al., 1987). It is possible that other dischargers to the river were contributing to the impairment or the recovery rate of the river was slower than anticipated.

Billerica, Massachusetts

A study was conducted at the City of Billerica's WWTP to evaluate sources of toxicity in the facility's

Toxicity Control Evaluation Update

Abatement of effluent toxicity during the course of TREs is not uncommon. However, efforts to ensure ongoing compliance can be difficult when the original causes and sources of toxicity are not known. These situations dramatize the importance of documenting industrial pretreatment activities and POTW operations in the early stages of the TRE. Weekly or daily reports of production and waste discharge activities by industrial users can provide a useful history of events that can be used to indicate potential sources. This information is also helpful in subsequent pretreatment control studies if an industrial user is identified as a source of toxicity (Botts et al., 1994).

RTA Procedure Update

Toxicity screening tools such as Microtox® have been used to identify sources of toxicity in POTW collection systems. It is necessary to first determine if a correlation exists between the compliance test and the screening test to ensure that the toxicity measured by the surrogate tool is the same toxicity indicated by the species used for compliance testing. This correlation can be performed using POTW effluent; however, it is important to note that correlation results may be different for individual industrial discharges. As a result, the screening test may yield false positive or false negative results.

The advantage of screening tests is that a large number of samples can be processed at relatively low cost. As an alternative to these tools, POTW staff may consider using the permit test species in an abbreviated test procedure such as that used in the TIE (USEPA 1991). The cost of these tests can be comparable to commercially available screening tests if the number of replicates or sample concentrations is reduced or the exposure time is decreased.

Although this study indicated a potential source of toxicity, a final determination of the source(s) of toxicity would require first treating the sewer samples in a simulation of the POTW to provide an accurate estimate of the refractory toxicity of the waste stream. Otherwise, as discussed in Section 5, the toxicity results may overestimate the toxicity of the discharge because some toxicity removal generally occurs in the POTW. A description of the updated RTA protocol is given in Section 5.

collection system (Durkin et al., 1987). A purpose of the study was to evaluate the usefulness of Microtox® as a tool for tracing sources of toxicity.

The Billerica study was conducted in five stages:

- Screening for WWTP influent toxicity.
- Testing samples from pump stations in the collection system.
- In-depth testing to determine the time of day when toxicity was observed at the pump stations.
- Testing of the main sewer lines above the pump stations where toxicity was indicated.
- Final testing of tributary sewers.

Of the 11 pump stations tested, 2 were found to have highly toxic wastewaters. In one of these pump stations, high levels of toxicity occurred only during the 8 a.m. to 2 p.m. time period. Further investigation of the intermittently toxic pump station provided evidence that the principal source of toxicity was an industrial park.

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Appendix B

TRE Case Study:
Central Contra Costa Sanitary District, Martinez, California

Abstract

TRE Goal:	NOEC ≥ 10%
Test Organisms:	Echinoderms (*S. purpuratus* and *D. excentricus*)
TRE Elements:	TIE
Toxicant Identified:	Copper
Toxicity Controls:	Pretreatment requirements

Summary

Chronic toxicity was detected in a municipal effluent with the echinoderm fertilization assay. *D. excentricus* (sand dollar) appeared more sensitive to the effluent than did *S. purpuratus* (purple urchin). A Phase I TIE was conducted using procedures described by USEPA (1988a) that were adapted to the echinoderm fertilization toxicity test. The Phase I TIE implicated cationic metals as the cause of chronic toxicity, and follow-up investigations suggested that Cu was the primary cation responsible. As part of the TIE, toxicity tests were conducted on ammonia and several cations. No observable effect concentrations for *D. excentricus* were >13.4 µg/L silver (Ag), >9.4 µg/L Cd, 3.8 to 13.1 µg/L Cu, >0.7 µg/L mercury (Hg), and 10 mg/L nitrogen as total ammonia. The data also suggested that inter-specific differences in sensitivity to Cu and ammonia exist between *D. excentricus* and *S. purpuratus*.

Key Elements

1. TIE procedures for freshwater organisms can be successfully modified to apply with the echinoderm fertilization toxicity test.
2. This study demonstrated that Cu could have accounted for the intermittent effluent toxicity observed.
3. Echinoderms exhibited comparatively high sensitivity to Cu with EC50s for both species of approximately 25 µg/L.
4. Source control measures were successful in reducing Cu concentrations by approximately 25%.

Introduction

Permit Requirements

The Central Contra Costa Sanitary District (CCCSD, Martinez, California) was required by the State Water Quality Control Board, San Francisco Bay Region, to conduct a TRE to identify the chemical constituents in their final effluent that were responsible for observed chronic toxicity in the echinoderm fertilization toxicity tests. Results of monthly compliance tests showed frequent exceedance of the discharge permit limit (NOEC ≥ 10% effluent).

Description of the Treatment Plant

The CCCSD WWTP provides secondary level treatment for combined domestic, commercial, and industrial wastewater from a 126-square mile area with a population of approximately 400,000. The treatment plant has an average dry weather design capacity of 45 mgd and currently discharges an annual average flow of 38.7 mgd into upper San Francisco Bay. Treatment facilities consist of screening, primary sedimentation, activated sludge, and secondary clarification followed by chlorination in contact basins. In the treatment process, waste-activated sludge is thickened via flotation thickeners, and lime is added to assist in dewatering with centrifuges. The combined primary and waste-activated sludge is dewatered and incinerated in multiple-hearth furnaces. The effluent TSS and BOD concentrations average <10 mg/L. Total ammonia concentrations range from 10-35 mg/L with an average of 25 mg/L.

Toxicity Identification Evaluation

General Procedures

The echinoderm fertilization toxicity tests were conducted on the final effluent according to published procedures (Dinnel, et al., 1982, as modified by S. Anderson, 1989) using the West Coast species *S. purpuratus* and *D. excentricus*. The purpose of the test is to determine the concentration of a test substance that reduces egg fertilization by exposed sperm relative to fertilization in a control solution. Two species were used in this test because the echinoderms are obtained from feral populations which are gravid at different times during the year. Effluent samples were 24-hour flow-proportional composites. Samples were screened for toxicity within 36 hours of collection. The effluent salinity was adjusted to 30% using hypersaline brine (90%), and the pH was adjusted to 8.0 ± 0.05.

Phase I TIE Studies

The results of this TIE have been published elsewhere (Bailey, et al., 1995). The Phase I TIE included the procedures described by USEPA (1988a). After completing the TIE manipulations, the effluent was salinity and pH adjusted as previously noted.

Table B-1. Summary of Results of Phase I TIE Conducted on Two Effluent Samples with *D. excentricus*

Treatment	Sample 1	Sample 2
pH 3	No effect on toxicity	Increased toxicity
pH 11	Eliminated toxicity	No effect on toxicity
Filtration	No effect on toxicity	No effect on toxicity
Aeration	No effect on toxicity	No effect on toxicity
EDTA	Eliminated toxicity	Eliminated toxicity
Sodium thiosulfate	Eliminated toxicity	Eliminated toxicity
Post C18 SPE column	No effect on toxicity	No effect on toxicity
Methanol eluate add-back	No toxicity	No toxicity

Phase I TIEs were conducted on two effluent samples. The data for both samples (Table B-1) suggested that EDTA and sodium thiosulfate were consistently the most effective treatments in reducing toxicity. Extraction of the sample with SPE columns did not reduce toxicity, suggesting that non-polar organics and weak organic acids and bases were not causes of toxicity. This conclusion is supported by the fact that elution of the columns with methanol did not yield toxicity. The effectiveness of EDTA in eliminating toxicity suggested that a divalent cation(s) was responsible for toxicity in the samples tested. The concurrent effectiveness of sodium thiosulfate in reducing toxicity suggested that the potential suite of cations was limited to Cd, Cu, and Hg (USEPA, 1991). In one case, toxicity also appeared to be increased by temporarily reducing the sample pH to 3; greater toxicity at lower pHs has been associated with Cu (Schubauer-Berigan et al., 1993).

Because the effluent samples contained moderate levels of ammonia (20–25 mg/L total ammonia), the potential contribution of ammonia to effluent toxicity was determined by comparison with ammonia toxicity tests. This approach was taken because the TIE guidelines evaluate ammonia toxicity by adjusting the pH of the test solution and preliminary data indicated that these pH adjustments adversely affected fertilization success.

Contribution of Ammonia to Toxicity

Ammonia toxicity tests were conducted in natural seawater spiked with ammonia chloride; fertilization success was evaluated using logarithmically spaced concentrations across a range of 1.0 to 100.0 mg/L N as total ammonia. Test solutions were adjusted to pH 8.0 ± 0.05 prior to exposure.

The NOECs for *D. excentricus* and *S. purpuratus* were both 10 mg/L N as total ammonia. Based on the unionized fraction, the NOECs were 0.21 and 0.17 mg/L N for *D. excentricus* and *S. purpuratus*, respectively (calculated per USEPA, 1988a). However, large differences existed between the response of the two species at concentrations higher than the NOEC. For *S. purpuratus*, the IC25 was greater than 100 mg/L N as total ammonia (1.69 mg/L N as unionized ammonia) compared with an IC25 estimate of 16.5 mg/L N (0.34 mg/L N as unionized ammonia) for *D. excentricus*. Because the upper limit of ammonia concentrations in the effluent was 25 mg/L N as total ammonia, these results suggested that ammonia alone could not account for NOECs that were $\leq$33% effluent, a concentration that would correspond to a maximum of 8.25 mg/L N as total ammonia.

Identification and Confirmation of the Role of Cationic Metals

Sensitivity of echinoderms to cationic metals

Once it appeared that a divalent cation was responsible for the effluent's toxicity, candidate metal ions (Cd, Cu, and Hg) and Ag were evaluated for toxicity with

D. excentricus and *S. purpuratus*. Metal solutions were prepared in moderately hard freshwater (USEPA, 1991) using reagent grade salts of Cu, Cd, and Hg. The CCCSD also was concerned about the potential for Ag to contribute to effluent toxicity; therefore, tests were performed with silver salts. Stock concentrations of metals were confirmed by either graphite furnace (Ag, Cu, and Cd) or cold vapor (Hg) AA spectroscopy (APHA, 1989). Hypersaline brine was then added (1/3 brine:2/3 metal solution) to bring the salinity to 30%, and the pHs of the solutions were adjusted to 8.0 ± 0.05 prior to exposure. This procedure was analogous to the preparation of the effluent samples prior to testing. Serial dilutions that incorporated a 50% dilution factor were made from the stock solutions to achieve exposure concentrations that bracketed those found in the effluent. The NOECs from multiple toxicity tests on Ag, Cd, Cu, and Hg with *D. excentricus* and *S. purpuratus* are summarized in Table B-2. Side-by-side comparisons between the two species are shown by the paired values in the table.

Table B-2. NOECs Obtained for *D. excentricus* and *S. purpuratus* Exposed to Different Metals*

Metal	NOECS (µg/L)	
	D. excentricus	***S. purpuratus***
Ag	>13.4	>13.4
Cd	>9.4 >67.0	Not tested >67.0
Cu	10.0 13.1 5.4 3.8 8.0	20.0 19.7 Not tested Not tested Not tested
Hg	>0.7 >2.2	>0.7 Not tested

* When seasonally available, concurrent tests were conducted with both species. Values given as µg metal/L (Bailey et al., 1995).

In some cases, seasonal spawning constraints precluded conducting concurrent tests with *S. purpuratus*. One comparison was conducted with Ag; the NOECs for both species were >13.4 µg/L. Two tests were conducted with Cd; in both cases the highest concentrations tested (9.4 and 67.0 µg/L) failed to produce any measurable effects on fertilization success. Five tests were performed on Cu with *D. excentricus*. The NOECs ranged between 3.8 and 13.1 µg/L with an average of 8.1 µg/L. In two of three concurrent tests with *S. purpuratus*, the NOECs were 1.5 to 2 times greater than those obtained with *D. excentricus*. In two tests with Hg, no effects on fertilization success were found at concentrations up to 0.7 and 2.2 µg/L, respectively.

Comparison of toxic concentrations of metals with concentrations found in the effluent

The NOECs for each of the metals were compared with the discharger's analytical records to determine which metals were present individually in the effluent at concentrations high enough to inhibit fertilization success. Toxicity ratios were calculated for each metal [metal concentration in effluent (µg/L) ÷ NOEC (µg/L)]. A ratio greater than 1 suggested that the metal(s) was present in the effluent at concentrations high enough to produce toxicity. Conversely, a ratio of 1, or less, suggested that the concentration of metal was ≤NOEC and, therefore, probably not directly responsible for toxicity, although some additive effects could possibly exist in combination with the other metals present.

Toxicity ratios calculated for each metal are presented in Table B-3 for *D. excentricus*. The comparatively small ratios associated with Ag, Cd, and Hg suggest that effluent concentrations of these metals were not high enough to produce the intermittent toxicity associated with the effluent. Cu was the most promising of the metals to be identified in this analysis as effluent/toxicity ratios frequently exceeded 1.

Table B-3. Comparison of Effluent Concentration of Selected Metals with NOECs Derived from Laboratory Studies with *D. excentricus*

Metal	Effluent concentration*	NOEC*	Ratio
Ag	<0.2 – 4.0	>13.4	≤0.3
Cd	<0.2	>9.4; >67	≤0.2
Cu	5.0 – 20.0	3.8 – 13.1	0.4 – 5.3
Hg	<0.2 – 0.4	>0.7; >2.2	≤0.6

* Values given as 7.5 (80.0 ± 2.0)µg metal/L.

Confirmation of the role of Cu in effluent toxicity

The next confirmation step compared fertilization success in an effluent sample against that in seawater spiked with copper sulfate ($CuSO_4$) to the same concentration found in the effluent. These exposures were conducted simultaneously using the same gametes from *D. excentricus.* Fertilization success also was evaluated in an effluent sample spiked with different concentrations of Cu, such that subsamples of the effluent contained 1, 2, and 3 times the amount of Cu (measured concentrations) as the original sample. Serial dilutions, which incorporated a 50% dilution factor, were then prepared from the unspiked and 2x spiked samples and fertilization success evaluated with *D. excentricus*. Depending on the results, it could be determined whether Cu was responsible for toxicity in the effluent. The reasoning was if Cu was the primary factor controlling toxicity, then the LOECs and NOECs obtained for the spiked and unspiked samples should be the same, based on Cu concentration. Similarly, based on percent effluent, the NOEC and LOEC associated with the spiked sample should be one dose level lower than in the unspiked sample.

The results of parallel toxicity tests with *D. excentricus* on effluent and seawater spiked with Cu at concentrations found in the effluent are summarized in Table B-4. Based on Cu concentration, the NOECs and LOECs were the same between the effluent sample and the concurrent toxicity test with seawater spiked with Cu. Furthermore, the percent fertilization was similar at corresponding Cu concentrations in both toxicity tests. These data suggested that Cu accounted for the reduction in fertilization success associated with this effluent sample. Fertilization success in an effluent sample and the same sample spiked with Cu is shown in Table B-5.

Table B-4. Comparison of NOECs, LOECs, and Percent Fertilization Obtained with *D. excentricus* Exposed to Effluent and Seawater Spiked with Cu

Treatment	NOEC*	LOEC*
Effluent	3.8 (89.3 ± 3.0)	7.5 (73.3 ± 6.1)
Seawater Cu spike	7.5 (80.0 ± 2.0)	7.5 (80.0 ± 2.0)

* Percent fertilization given in parentheses (mean ± SD).

Discussion

The data demonstrated that procedures for conducting TIEs with freshwater organisms can be successfully applied to the echinoderm fertilization toxicity test. The results of this study suggest that Cu could have accounted for the intermittent toxicity demonstrated by the echinoderm fertilization test. Of the four metals identified in the Phase TIE, Cu was the only one that occurred in the effluent at concentrations that overlapped the toxic range. Confirmatory studies conducted with two different effluent samples also showed that Cu could account for the adverse effects observed with the whole effluent. Paired tests also suggested that Cu exhibited greater toxicity to *D. excentricus* than to *S. purpuratus*. This is important because *S. purpuratus* generally exhibited less sensitivity to the effluent.

Source control measures implemented by the CCCSD successfully reduced Cu concentrations in the effluent by 25%. This reduction made it difficult to obtain samples with sufficient toxicity to fully complete the confirmatory phase of the TIE. In fact, nearly all the samples tested at the end of the TIE failed to produce a measurable response with *S. purpuratus*.

Acknowledgments

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Table B-5. Percent Fertilization Obtained with *D. excentricus* Exposed to Effluent and Effluent Spiked with Cu*

Unspiked effluent			Effluent spiked with Cu		
Effluent (%)	µg/L Cu	Fertility (%)	Effluent (%)	µg/L Cu	Fertility (%)
0.0	0.0	96.0 ± 2.5	0.0	0.0	96.0 ± 2.5
8.4	0.8	96.7 ± 3.1	8.4	1.6	90.7 ± 2.3
16.8	1.6	97.3 ± 1.2	16.8	3.3	90.3 ± 2.3
33.5	3.3	91.3 ± 1.2	33.5 (1×Cu)	6.6	83.3 ± 2.7†
67.0 (1×Cu)	6.6	82.0 ± 4.7†	67.0 (2×Cu)	13.2	74.8 ± 2.2†
			67.0 (3×Cu)	19.8	71.7 ± 12.9†

* Fertilization data are the means and standard deviations of three replicates.
† Significantly less than controls; $p < 0.05$.

Bhupinder Dhaliwal, and Jim Kelly of the CCCSD managed the various aspects of this project. The TRE studies were conducted at AQUA-Science, Davis, California, under the direction of Jeffrey L. Miller, Ph.D., and Michael J. Miller.

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Appendix C

TRE Case Study:
City of Reidsville, North Carolina

Abstract

TRE Goal:	NOEC >90%
Test Organism:	*C. dubia*
TRE Elements:	TIE and Toxicity Tracking Assessment (RTA)
Toxicant Identified:	Surfactants
Toxicity Controls:	Pretreatment requirements

Summary

The TRE study used a novel approach to identify the sources of POTW effluent toxicity. Subsequent modifications in chemical usage by industrial contributors successfully reduced effluent toxicity to the NOEC limit in 1994. Further studies are in progress to ensure consistent compliance with the toxicity limit.

Key Elements

1. Other TRE procedures can be used if the TIE cannot identify the effluent toxicants. One such procedure uses a toxicity-based tracking approach to locate the sources of toxicity in municipal collection systems.
2. The toxicity-based tracking approach, referred to as the RTA procedure, can be adapted to fit the site-specific conditions at each POTW.
3. Once identified, the toxic contributors can be required through the industrial pretreatment program to reduce the discharge of toxicity. Practical control techniques are available to industries, including substitution of toxic chemicals, waste minimization, and pollution prevention.

Introduction

The City of Reidsville was required by the North Carolina Division of Environmental Management (NCDEM) to conduct a TRE based on evidence of chronic effluent toxicity at its POTW. Monthly NOECs for *C. dubia* have averaged about 35% effluent since 1992. These values show that chronic effluent toxicity has consistently exceeded the discharge permit NOEC limit of 90% effluent.

Background

In 1992, the City submitted a TRE plan and initiated TIE studies to determine the cause(s) of the effluent toxicity. Chronic TIE Phase I (Tier I) tests indicated that surfactants were the principal toxicant group. This evidence was based on toxicity reduction by filtration, aeration, and C18 SPE in the Phase I tests. TIE Phase II tests were performed to try to identify the toxic surfactant compounds; however, the results were inconclusive because of the difficulty in isolating the toxicants and the lack of conventional analytical techniques for surfactant compounds. The toxicants removed by the C18 SPE column were recovered by eluting the column with methanol, but toxic compounds could not be identified in the column extract (Burlington Research Inc., 1993).

In cases where the TIE is not successful in identifying the effluent toxicants, other TRE steps can be used to gather information on the nature and sources of effluent toxicity. USEPA and several municipalities have worked together in USEPA funded studies to develop the RTA method, which can be used to assess the potential toxicity contribution from indirect dischargers in sewerage collection systems (USEPA, 1989a; Botts et al., 1987; Morris et al., 1991; Fillmore et al., 1990; Collins et al., 1991). The RTA procedure involves treating industrial wastewater samples in a bench-scale, batch simulation of the POTW, and measuring the resulting toxicity. The toxicity remaining after batch treatment, referred to as refractory toxicity, represents the toxicity that passes through the POTW and is discharged in the effluent. Several municipalities have successfully used the RTA procedure to identify industrial sources of toxicity (Botts et al., 1992; Morris et al., 1991; and Engineering-Science, Inc., 1992).

Description of Treatment Plant

The major treatment processes at the Reidsville POTW are extended activated sludge treatment and filtration. Influent wastewater, which averages 2.8 mgd, is initially screened and then treated in two activated sludge aeration basins equipped with mechanical surface aerators. Both carbonaceous BOD and ammonia are removed in this single-stage aeration system. After 48 hours contact time, the basin effluent flows to the final clarifiers for solids clarification. The clarified effluent is then passed through sand filters to remove remaining suspended solids that may contribute to effluent BOD. The filter effluent is disinfected with chlorine gas and dechlorinated and aerated prior to discharge. Waste activated sludge is thickened and aerobically digested for land application.

Refractory Toxicity Assessment Procedure

Selection of Industries for Testing

Acute and chronic toxicity tests were performed on raw wastewater from the seven permitted significant industrial users in the Reidsville collection system. The industrial wastewater samples were tested at concentrations that reflected the average flow contribution of the industries to the POTW (dilutions were made with reconstituted lab water).

The results showed that five of the seven industries were contributing chronic toxicity to the POTW (Table C-1). It is possible that at least some of the raw wastewater toxicity would be removed by treatment at the POTW; therefore, the five toxic industrial users were selected for further evaluation by RTA testing. A description of the industries evaluated in the RTA is provided in Table C-2.

Test Procedure

A step-by-step description of the RTA procedure is given in Section 5 and Appendix J. The generic procedure must be adapted to simulate the treatment processes and operating conditions at each POTW. Several types of treatment processes can be simulated, including conventional activated sludge systems (Botts et al., 1987; Morris et al., 1991; and Fillmore et al., 1990), single and two-stage nitrification processes (Collins et al., 1991), and BNR systems (Botts et al., 1992).

Table C-1. Chronic Toxicity of Raw Industrial Wastewaters

	C. dubia **Chronic Pass/Fail Result***			
Industry	**May 1992**	**June 1992**	**July 1992**	**April 1993**
A	Fail	Fail	Fail	Fail
B	Fail	NT†	Fail	Fail
C	Fail	Fail	Fail	Fail
D	Fail	NT	Fail	Fail
E	Pass	Pass	Fail	Fail
F	Pass	Pass	Pass	NT
G	Pass	Pass	Pass	NT

* Tests were conducted using industrial wastewater diluted according to its percent contribution to the total POTW influent.

† NT = Not tested.

Table C-2. Description of Industries Evaluated in the RTA

Industry	**Type**	**Flow (mgd)**	**%Flow* to POTW**
A	Textile	1.072	65
B	Tobacco Products	0.308	28
C	Can Making	0.085	10
D	Food Processing	0.189	12
E	Metal Finishing	0.031	2
Domestic			38

* Based on maximum industrial flow and minimum POTW influent flow, except for domestic, which is based on average flow and minimum POTW influent flow.

The RTA simulated the two main treatment processes at the Reidsville POTW: the activated sludge and sand filtration processes. Wastewater samples were first treated in biological reactors and then the clarified effluents were passed through a bench-scale sand filter column.

Two types of simulations were tested as shown in Figure 5-2 (see Section 5). A control simulated the existing treatment conditions and treated only the POTW influent. The second simulation evaluated the addition of the industrial discharge to the POTW and treated the industrial wastewater spiked into the POTW influent.

The amount of industrial wastewater spike represented the conservative condition of maximum industrial flow and minimum total influent flow at the POTW. The operating conditions for the simulations are described in Table C-3.

Table C-3. Comparison of Operating Conditions for the City of Reidsville POTW Processes and RTA Simulation Tests

POTW Process Specifications	Treatment Plant	RTA Simulation
Activated Sludge Process		
Mixed liquor solids (mg/L)	2,200–2,500	2,240–2,740
DO (mg/L)	>2	2.4–9.2
Treatment period (hours)	48	48
Sand Filter Process		
Filtration rate (gpm/sf)	0.8	0.8
Total filter area (sf)	2,520	0.09
Sand particle size (mm)	0.45	0.45
Sand depth (inches)	10	10
Water depth on filter (ft)	0–7	0.1–2.5
Backwash rate (gpm/sf)	12	5 (estimated)

The results of the control and spiked simulations are compared to determine whether addition of the industrial wastewater increases effluent toxicity. An industry would be considered a source of toxicity if the effluent of the spiked simulation is more toxic than the control effluent.

Sampling

Three rounds of RTA tests were performed over a 4-month period. Twenty-four hour composite samples of the industrial wastewaters, POTW influent, domestic wastewater, and POTW effluent were collected for testing. In addition, a grab sample of the POTW RAS was collected on the day of testing. Domestic wastewater was tested because TRE studies at other municipalities have shown that domestic sources can contribute to effluent toxicity (Botts et al., 1990). The POTW effluent served as a baseline for comparison with the RTA control to determine if the treatment performance of the simulations and the POTW were similar.

Toxicity Monitoring

Following biological treatment, the clarified reactor effluents were passed through the sand filter column and the resulting filtrates were tested for chronic toxicity using *C. dubia*, the test species specified in the NPDES permit. Each RTA effluent sample was used for both test initiation and renewals on days 3 and 5 of the toxicity test (USEPA, 1989b).

Results

Source Characterization

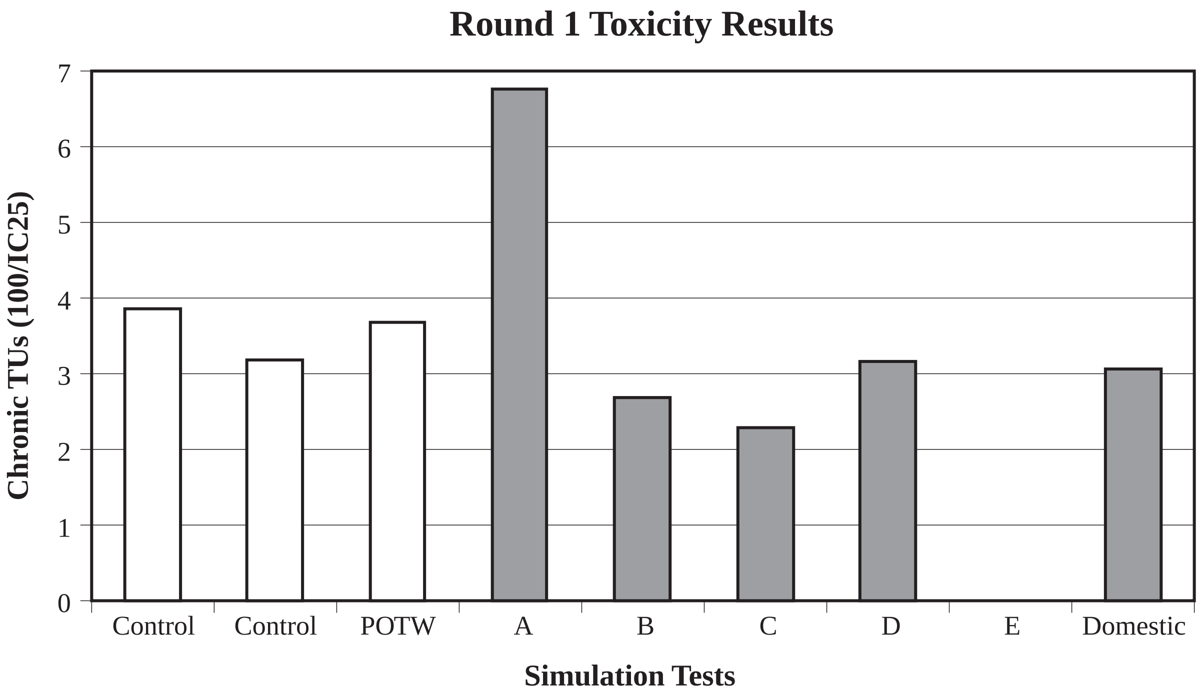
Two rounds of RTA tests were used to characterize the sources of toxicity. As shown in Figure C-1, the effluent TUc for the two control simulation tests in Round 1 were 3.8 and 3.1. These values compare well with the POTW effluent (TUc =3.6). The control simulation effluents in Round 2 also exhibited similar toxicity (TUc =3.0 and 2.9) as the POTW effluent (TUc =3.4). These results indicate that the RTA test accurately simulated the POTW with respect to toxicity treatment.

As shown in Figure C-1, the effluent from the simulation spiked with Industry A wastewater was about twice as toxic (TUc=6.7) as the control effluents in both rounds of tests. Effluent TUc values for the simulations spiked with other industrial wastewaters were similar to or less than the effluent TUc for the controls.

The results of both rounds of testing indicate a potential for Industry A to contribute toxicity to the POTW. The results for the simulations spiked with the other industrial wastewaters suggest that Industries B, C, D, and E do not contribute measurable toxicity to the POTW.

Toxicity Confirmation

A recent study for a New Jersey municipality found that an industry was contributing toxicity in amounts



Note: Industry E was not tested in round 1.

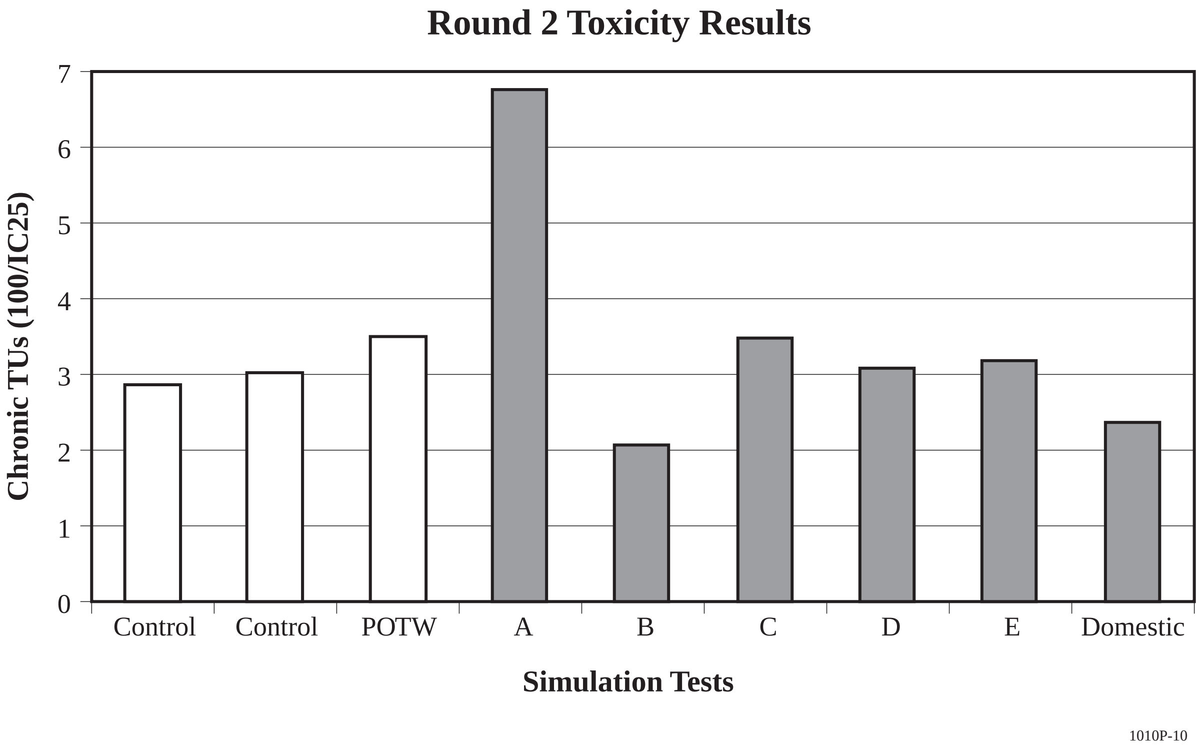


Figure C-1. Results of RTA (rounds 1 and 2).

high enough to mask other smaller sources of toxicity (Morris et al., 1991). It was necessary to remove the larger source of toxicity from the RTA test regime before other significant sources could be identified. The City of Reidsville decided to conduct a third round of tests to determine if a similar situation was occurring at their POTW.

Round 3 involved using a mock influent that did not contain Industry A wastewater. The mock influent was used in lieu of the POTW influent for the controls and the spiked simulations. The mock influent consisted of samples collected from each major sewer line with the exception of the sewer receiving Industry A wastewater.

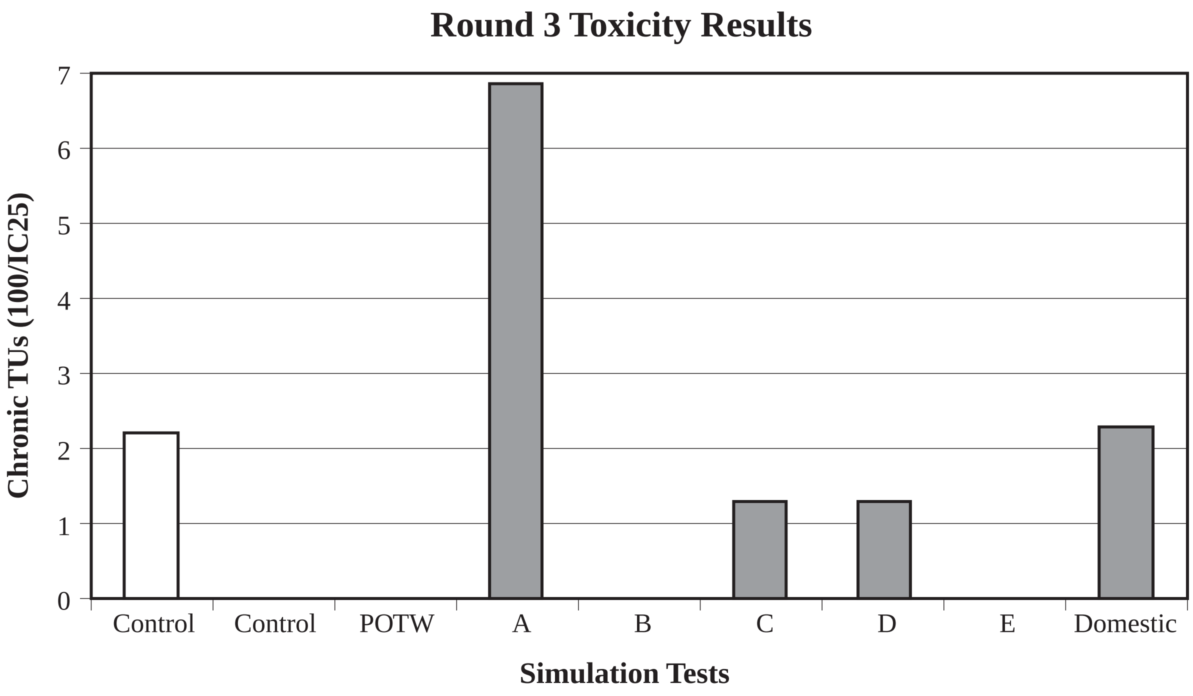
Toxicity results for the RTA simulation effluents are presented in Figure C-2. A comparison of results shows that the effluent of the Industry A spiked simulation was several times more toxic (TUc=6.8) than the control effluent (TUc =1.2). These results provide further evidence that Industry A is a source of toxicity. The simulations spiked with Industry C and D wastewater had similar effluent toxicity (TUc=1.3 for both) compared to the control. These data indicate that Industries C and D are not contributing significant toxicity to the POTW.

The simulation spiked with domestic wastewater had greater effluent toxicity (TUc=2.3) than the control (TUc=1.2). These results suggest that this waste stream may be a source of toxicity; however, results of Round 1 and 2 indicate that domestic wastewater collected from other areas of the collection system is not a problem. Further studies are planned to evaluate the potential toxicity contribution from domestic sources throughout the collection system.

Discussion

The results of this study indicate that Industry A is a major contributor to chronic effluent toxicity at the Reidsville POTW. None of the other industries (B, C, D, and E) were found to discharge measurable toxicity even after the potential toxicity interference from Industry A was removed.

In January 1994, the City of Reidsville implemented a program to minimize or eliminate the discharge of industrial chemicals that may contribute to the POTW effluent toxicity. Although the RTA results indicated



Note: A replicate control and POTW effluent were not tested in round 3. Industries B and E were not indicated to be sources of toxicity in rounds 1 and 2; therefore, these industries were not tested in round 3.

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Figure C-2. Results of RTA (round 3).

that Industry A is the major contributor of chronic toxicity, all of the City's eight permitted industrial users were requested to participate. The program involved:

- An evaluation of current chemical usage and the selection of alternative materials of low toxicity, low inhibition potential, and high biodegradability.
- An on-site evaluation of waste-minimization practices by the North Carolina Office of Waste Reduction.

Particular attention was given to surfactant products or chemicals with surfactant constituents because the TIE had indicated surfactants to be the principal toxicant in the POTW effluent. Industries were requested to maintain chronological records of changes in chemical usage, production, and housekeeping practices. These records were used to compare the timeline of industry modifications to results of chronic toxicity monitoring at the POTW.

Follow-up monitoring results showed a substantial reduction in effluent toxicity. Beginning in March 1994, the IC25 values (an endpoint that approximates the NOEC) for 7 of 10 monthly *C. dubia* toxicity tests were $\geq$90%. A review of the industries' chronological records established a correlation between toxicity reduction and chemical optimization practices, especially those implemented at Industry A.

However, in 1995 occasional chronic effluent toxicity was again observed. Since early 1997, the effluent has exhibited consistent chronic toxicity (NOEC=30–45%). Current studies are focusing on treatment with polymer, which has shown to reduce toxicity in bench-scale tests. The City is also working with the industries to implement additional chemical optimization and waste minimization practices. In addition, construction is underway to extend the outfall from a small creek to a river, which will afford greater dilution. In 1998, the City will need to meet a revised chronic toxicity limit of an NOEC of approximately 61%.

Summary

The RTA protocol was initially developed as part of TRE research studies funded by the USEPA Risk Reduction Engineering Laboratory in Cincinnati, Ohio. The procedure was intended to be used by municipalities as a tool for tracking sources of toxicity in sewer collection systems; however, the RTA approach has evolved to suit other purposes. In addition to toxicity tracking (Collins et al., 1991), the RTA protocol has been used to determine the compatibility of planned discharges to POTWs (Engineering-Science, Inc., 1992, 1993) and to establish compliance with toxicity-based pretreatment limits (Morris et al., 1991).

Acknowledgments

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The material presented in this appendix includes copyrighted data presented in a technical paper for the 67th Annual Water Environment Federation Conference (Botts et al., 1994). WEF has granted permission to include the data in this document.

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Appendix D

TRE Case Study:
City of Durham, North Carolina

Abstract

TRE Goal:	NOEC = 100%
Test Organism:	*C. dubia*
TRE Elements:	Toxicity treatability evaluation
Toxicant Identified:	TIE not performed
Toxicity Controls:	Proceeded with planned POTW upgrades

Summary

The City of Durham evaluated the expected toxicity reduction to be achieved by planned upgrades of their POTWs. Chronic toxicity reduction was evaluated through the use of bench-scale simulations of the upgraded POTWs. Results indicated that the new POTWs would reduce chronic toxicity to compliance levels. Based on this evidence, the TRE was waived until the new POTWs were online and effluent toxicity reduction could be confirmed. The upgraded POTWs became operational in late 1994 and effluent monitoring results have shown no chronic toxicity after consistent treatment performance was achieved.

Key Elements

The TRE study used a unique approach to evaluate chronic toxicity reduction. This approach may be useful to other municipalities that have TRE requirements, yet are planning upgrades of their POTWs. The key elements of interest in the City of Durham study include the following:

1. In cases where POTW staff are planning to upgrade their POTWs, it may be more practical to evaluate the toxicity reduction to be achieved by the upgrade than to conduct TIE tests on the existing POTW effluent. The treatability approach is recommended when the upgrade is expected to improve toxicity reduction, such as nitrification treatment for ammonia removal; however, additional evidence is needed to confirm the expectation.
2. A bench-scale simulation of the upgraded treatment system can be used to generate an effluent that is similar to the effluent expected for the new POTW. Calibration tests should be performed to ensure that the quality of the simulation effluent is similar to that of the planned POTW effluent.
3. The treatability approach should be thoroughly described in the TRE plan and the regulatory authority should accept the plan prior to testing.

Introduction

Permit Requirements

Since 1987, NCDEM has required the City of Durham to monitor the effluents of its four POTWs for chronic toxicity using the North Carolina pass/fail test. The pass/fail test consists of 10 replicates of the effluent at the critical instream waste concentration (IWC) and a control. The effluent test concentrations corresponding to the IWC were 63.8% for the Eno River POTW, 100% for Lick Creek POTW, 98.7% for Farrington Road POTW, and 100% for Northside POTW. The test results indicated unacceptable levels of chronic effluent toxicity for each of the four POTWs. In each case, a statistically lower number of *C. dubia* young were observed in the effluent concentration as compared to the control.

Based on the effluent toxicity monitoring results, NCDEM required the City of Durham to initiate a TRE in January 1990. The goal of the TRE was to identify methods for reducing chronic effluent toxicity to acceptable levels at each of the treatment plants by

January 1991. The City of Durham submitted a plan within 60 days that described a unique approach for implementing the TRE program.

Instead of the traditional TRE approach of testing the existing effluents, the City proposed to evaluate the expected chronic toxicity reduction to be achieved by planned upgrades to the POTWs. Toxicity reduction would be evaluated through the use of bench-scale simulations of the upgraded POTWs. This approach was favored over conventional TRE methods, such as TIE tests, because it was anticipated that the degree and nature of the effluent toxicity would change upon startup of the new treatment plants.

Description of the Treatment Plants

In 1990, the City of Durham, North Carolina, had four POTWs: Eno River (2.5 mgd), Farrington Road (13 mgd), Lick Creek (1.5 mgd), and Northside (10 mgd). In anticipation of the need for additional treatment capacity, the City decided to close the Eno River and Lick Creek treatment plants and divert the flow to an expanded Northside plant. At the same time, NCDEM established draft permit limits for several parameters, including phosphorus. The new permit limits would require advanced wastewater treatment; therefore, in addition to the Northside plant expansion, the City of Durham decided to upgrade the Northside and Farrington Road POTWs plants to include BNR treatment.

During the TRE, the Northside POTW comprised primary treatment followed by trickling filters, a single-stage nitrification process, secondary clarification, and chlorine disinfection. The Northside POTW upgrade involved building a new treatment system in parallel with the existing system, which would treat the flow diverted from the former Eno River and Lick Creek plants. The new treatment system was planned to consist of primary clarifiers and a five-stage BNR process designed to remove nitrogen and phosphorus. Effluents from the new and existing treatment systems will be combined, treated with aluminum sulfate (alum), passed through a filtration process, and disinfected by UV light prior to discharge to Ellerbe Creek.

The Farrington Road POTW was planned to be converted from a two-stage nitrification process to a five-stage BNR process similar in design to that planned for the Northside plant. Final effluent treatment, like the Northside plant, will involve alum treatment, filtration, and UV disinfection.

Wastewater Treatment Plant Simulations

The new treatment processes for the Northside and Farrington Road POTWs were planned to be similar; therefore, the simulation designs were nearly identical. A batch mode of operation instead of a continuous flow mode was selected to reduce study costs. Both simulations, as shown in Figure D-1, comprised a BNR process, followed by alum flocculation, settling, and effluent filtration. Phosphorus and nitrogen removal was achieved in the BNR process, which involved treating the influent wastewater with activated sludge in five consecutive stages (anaerobic, anoxic, aerobic, anoxic, and aerobic). The BNR process effluent was then treated with alum and passed through a dual media filter column to remove additional phosphorus. Chronic toxicity tests using *C. dubia* (USEPA, 1989) were performed on the final simulation effluents to evaluate the expected effluent quality of the full-scale treatment systems.

Simulation of the Northside POTW involved treating the combined influents of the three POTWs scheduled for consolidation: the Eno River, Lick Creek, and Northside plants. The influents were combined in proportion to their respective flow rates. The Farrington Road POTW influent was used directly in the simulation tests of the Farrington Road facility. Each simulation influent was settled for approximately 2 hours to simulate primary sedimentation.

The activated sludge used in the simulations was collected from a municipal treatment plant that had a BNR process similar to the system planned for the City of Durham POTWs. RAS was collected from the plant’s clarifier return line and mixed liquor solids were collected from the aeration basins. RAS was mixed with the simulated primary effluent in the first BNR simulation stage (anaerobic). Phosphorus removal was enhanced in the subsequent BNR stages by replacing a portion of the RAS with nitrate rich, aeration basin sludge. The nitrate was an essential source of oxygen for phosphorus removing bacteria in the BNR anoxic stage.

Following biological treatment, the activated sludge was settled and the clarified effluent was withdrawn and treated with alum. Alum treatment involved flash mixing and settling. The clarified supernatant was then

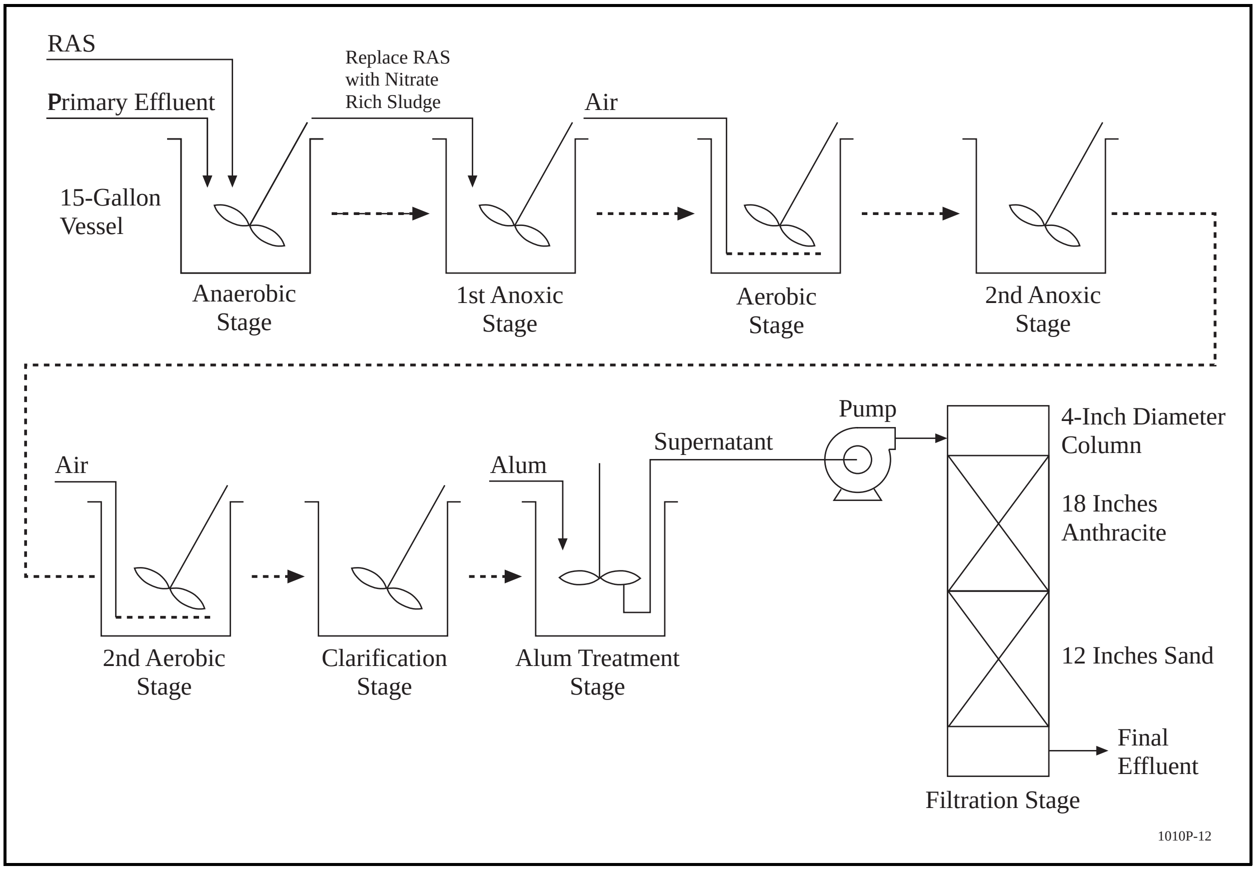

Figure D-1. Flow diagram for wastewater treatment simulations.

passed through an anthracite/sand filter column, which was operated in a constant headloss mode. Prior to testing, the anthracite and sand in the filter columns was distributed by backwashing the columns in the upflow direction using tap water. The filter columns were then rinsed with deionized water in the operational (downflow) mode.

The general operating conditions for the treatment simulations are shown in Table D-1. Some of the operating procedures for the simulations were modified during calibration testing to achieve the desired treatment performance.

Calibration of the Treatment Simulations

Prior to the toxicity evaluation, calibration tests were performed to match the simulation performance to expected performance for the upgraded POTWs. Also, several toxicity tests were performed during the calibration testing to verify that the simulation materials and additives (i.e., activated sludge, alum) would not introduce unexpected toxicity. The toxicity tests followed USEPA procedures (1989) for *C. dubia*, the test organism specified in the City's discharge permits.

The calibration testing involved varying the operation of the simulations and monitoring the resulting effluent quality. The objective was to achieve a reduction in influent concentrations of BOD_5, COD, TKN, NH_3-N, NO_3-N, TP, PO_4-P, and TSS to levels approximating those expected in the effluents of the planned treatment plants. Treatment performance was evaluated by varying the treatment times for each step.

The treatment times evaluated during the calibration testing were 90, 100, and 110% of the design HRT. A summary of the conventional pollutant results for the calibration study is shown in Tables D-2 and D-3. Also shown are the monthly average permit limitations and the design effluent characteristics for the planned facilities.

Biological Treatment

All BNR process simulations successfully achieved carbonaceous BOD_5 removal and nitrification. As shown in Table D-2, the batch biological process removed BOD_5, COD, and ammonia concentrations to well below design effluent levels. TKN concentrations in the simulation effluents also met the design levels.

Table D-1. Farrington Road and Northside Simulation Operating Conditions

Parameter	Farrington Road		Northside	
	POTW Design*	Simulation	POTW Design*	Simulation
Biological Treatment Step				
Primary effluent volume	24.5 mgd	100%		
Eno River			3.00 mgd	14.0%†
Lick Creek			6.94 mgd	32.3%†
Northside			11.53 mgd	53.7%†
Average MLSS	3,000 mg/L	3,508 mg/L	3,000 mg/L	3,481 mg/L
Minimum DO				
Anaerobic	0 mg/L	<0.2 mg/L	0 mg/L	<0.2 mg/L
1st Anoxic	0 mg/L	<0.2 mg/L	0 mg/L	<0.2 mg/L
1st Aerobic	2 mg/L	2 mg/L	2 mg/L	2 mg/L
2nd Anoxic	0 mg/L	<0.2 mg/L	0 mg/L	<0.2 mg/L
2nd Aerobic	4 mg/L	4 mg/L	4 mg/L	4 mg/L
Temperature (°C)	10–26	20–25	12–29	20–25
Alum/Filtration Treatment Steps				
Alum dose after biological treatment	10 mg/L	20 mg/L	5 mg/L	10 mg/L §
Depth of anthracite/sand in filter	8"/8"	8"/8"	18"/12"	18"/12"
Constant headloss level in filter	4 ft	4 ft	2–8 ft	4 ft
Average filtration rate	2.4 gpm/ft^2	2.4 gpm/ft^2 ‡	4 gpm/ft^2	4.1 gpm/ft^2 #

* Source: Hazen and Sawyer; R.L. Taylor, personal communication to J.A. Botts, *Design Information for the Treatment Plant Expansions*. December 10, 1990, Raleigh, North Carolina.
† Percent of total simulation influent volume.
‡ Filtration rate was 4.2 gpm/ft^2 for April 4–5 simulation.
§ Alum dosage increased to 20 mg/L for April 10–11 simulation.
\# Filtration rate was 7.1 gpm/ft^2 for April 4–5 simulation.

The BNR simulations did not consistently achieve the effluent permit levels for phosphorus (Table D-2). No phosphorus removal was observed in the April 4-5 test. For subsequent tests, the percentage of aeration basin sludge added to the anoxic stage was increased to stimulate phosphorus removal. This modification resulted in a decrease in phosphorus to near design levels in the April 10–11 test. As shown in Table D-3, phosphorus was initially released by the bacteria in the anaerobic stage, which is common in BNR systems. However, unlike the April 4–5 test, the phosphorus was re-assimilated in the anoxic and aerobic stages as would be expected. These results demonstrated that phosphorus removal can be achieved in the batch simulation tests. The lack of phosphorus removal in the April 18–19 test appeared to be related to the poor quality of the activated sludge on the day of testing.

The BNR simulations also did not achieve consistent denitrification (Table D-2). The Northside simulation test on April 10–11 reduced nitrate to a level (1.7 mg/L) close to the design effluent concentration (1.0 mg/L). All other simulation tests achieved only slight nitrate removal. The lack of nitrate removal in

Table D-2. Comparison of Calibration Test Results to Permit Limitations and Design Criteria (mg/L)

Parameter	Monthly Average* Effluent Permit Limits	Design† Effluent Characteristics	Calibration Results		
			Apr 4–5	Apr 10–11	Apr 18–19
Northside POTW					
BOD$_5$	24.0/12.0 ‡	5	1	1	1
COD	NA ‡	51	21	17	26
TSS	30	10	0	5	0
TKN	NA	1.5	1.5	1.5	0.9
NH$_3$-N	16.0/8.0 §	0.5	0.2	0.1	0.05
NO$_3$-N	NA	4.75	5.9	1.7	12.4
TP	2	0.5	6	0.8	6
Farrington Road POTW					
BOD$_5$	10.0/7.0 ‡	5	1	1	1
COD	NA	45	23	26	23
TSS	30	10	1	5	2
TKN	NA	1.5	1.9	1	0.8
NH$_3$-N	4.0/2.0 ‡	0.5	0.1	0.1	0.1
NO$_3$-N	NA	1	7.1	6.5	14.7
TP	2	0.5	7.4	0.6	7.1

* Values are interim limits for the period beginning January 1, 1991, and lasting until 3 months after construction completion.
† Source: Hazen and Sawyer, R.L. Taylor, personal communication, to J.A. Botts, *Design Information for the Treatment Plant Expansions* December 10, 1990, Raleigh, North Carolina.
‡ Winter and Summer limits, respectively.
§ No limit established in permit.

the Farrington Road simulation may have been due to the short anoxic treatment time (approximately 3 hours) as compared to the Northside simulation (more than 4 hours). The simulation procedure was modified to increase the anoxic treatment time for the Farrington Road simulation to attempt to achieve denitrification during the effluent toxicity evaluation.

The toxicity test results indicated that the RAS supernatant used in simulation testing was not acutely toxic (LC50 ≥ 100%). Therefore, the activated sludge was not expected to cause an acute toxicity interference in the simulation tests.

Alum Treatment

As shown in Table D-3, only a slight removal of phosphorus was observed in the alum treatment step. Solids flocculation did not occur at the designed alum dosages (10 mg/L for Farrington Road POTW and 5 mg/L for Northside POTW). Alum dosages were

Table D-3. Total Phosphorus Results (mg/L) for the Calibration Tests Conducted on April 10–11, 1990

Wastewater/Sludge	Farrington Road Simulation	Northside Simulation
Influent	5.49	3.95
RAS	13.5	13.5
Basin sludge	4.13	4.13
Biological treatment		
Anaerobic effluent	32.2	20.7
1st aerobic effluent	2.33	3.05
2nd aerobic effluent (Clarifier effluent)	1.48	1.78
Alum treatment supernatant	1.06	1.55

increased two-fold; however, no additional phosphorus removal was achieved.

The effect of alum on effluent toxicity was evaluated by comparing the toxicity of the wastewater before and after alum treatment. The results show that the alum did not add acute toxicity to the wastewater (i.e., LC50 >100% before and after alum addition).

Filtration Treatment

The filter columns were very efficient in removing suspended solids (Table D-2). As a result, nutrients and COD associated with the solids were further reduced. Total phosphorus concentrations decreased by nearly half after filtration (Table D-3).

The deionized water rinsates from the filter columns were analyzed for toxicity prior to testing. The results indicated that the filter media would not add acute toxicity to the simulation effluent (rinsate LC50 >100%).

Discussion of Calibration Results

The calibration results indicated that bench-scale tests could effectively simulate the effluent quality expected for the new POTWs. Pollutant removal was similar whether the simulations were tested at 90, 100, or 110% of the design HRT. BOD_5, COD, TKN, ammonia, and TSS were consistently reduced to levels expected to be achieved by the planned facilities. Although nitrate and phosphorus were not treated to design effluent levels, no adverse effects on toxicity treatment in the simulations were anticipated. The calibration results also indicated that the simulation materials would not contribute artifactual toxicity.

Toxicity Treatment Evaluation

Tests of the calibrated simulations were performed to determine if the new POTWs would eliminate chronic toxicity. The operating parameters for the simulations were based on the design HRT treatment condition (100%). An exception was the treatment time for the second anoxic treatment stage of the Farrington Road simulation, which was increased to stimulate denitrification. In addition, the alum dosages for both simulations were increased to enhance the flocculation necessary for phosphorus removal.

The treatment plant simulations were implemented on two occasions. Performance criteria were applied to ensure that the effluent quality was sufficient for toxicity evaluation. These criteria, shown in Table D-4, were based on the treatment performance that was consistently achieved in the calibration tests.

Treatment Performance Results

A summary of the conventional pollutant results for the simulation effluents is shown in Table D-4. The results show that the simulations consistently achieved the design effluent concentrations for BOD_5, COD, TSS, and ammonia. Effluent TKN concentrations were within the simulation performance criterion of 5 mg/L. The effluent concentrations of total phosphorus and nitrate also were within the simulation performance criteria levels. Overall, the simulation effluents were judged to be suitable for toxicity analysis based on the simulation performance criteria.

Toxicity Evaluation Results

Results of toxicity tests, presented in Table D-5, show that the simulation effluents were not acutely toxic to *C. dubia* (48-hour LC50$\geq$100% effluent). Chronic toxicity results show that the simulation effluents did not inhibit *C. dubia* reproduction (NOEC of 100% effluent). Only the effluent of the Farrington Road simulation on May 29–30, 1990, adversely affected *C. dubia* survival (NOEC = 75% effluent). The chronic toxicity of this effluent was due to significant mortality in the 100% effluent concentration.

Sulfide was detected in the May 29–30 Farrington Road simulation effluent at a concentration that may be chronically toxic to *C. dubia* (1.6 mg/L). The sulfide NOEC for *D. magna* at pH 7.6–7.8 is reported to be 1.0 mg/L (USEPA, 1990). Although the toxicity of sulfide to *C. dubia* is unknown, the sensitivities of *D. magna* and *C. dubia* to many classes of toxicants are similar (Mount and Norberg, 1984). The pH values of the Farrington Road simulation effluent and the value used for the reported test also were similar (i.e., 7.85 versus 7.6 to 7.8); therefore, the potential toxicity of sulfide in the simulation sample should be comparable to that of the reported test (Note: the concentration of hydrogen sulfide, the most toxic form of sulfide, increases when pH decreases). Based on this evidence, the chronic toxicity observed in the May 29–30 Farrington Road simulation effluent may be related to sulfide.

Discussion

The TRE study was completed within the 1-year time frame specified by NCDEM. The results of this study indicated that the addition of new BNR and filtration treatment processes at the City of Durham POTWs

Table D-4. Comparison of Simulation Test Results to Performance Criteria

Parameter	Simulation Performance Criteria (mg/L)*	Simulation Effluent Results (mg/L)	
		May 29–30	June 6–7
Northside POTW			
BOD_5	5	1	1
COD	51	22	21
TSS	10	3	2
TKN	5	2	NA †
NH_3-N	0.5	0.1	0.1
NO_3-N	15	5.5	11.3
TP	8	1.2	3.2
Farrington Road POTW			
BOD_5	5	1	1
COD	45	22	22
TSS	10	4	1
TKN	5	2.3	NA
NH_3-N	0.5	0.1	0.1
NO_3-N	15	5.2	9.3
TP	8	1.5	3.8

* Simulation performance criteria based on calibration results and design effluent levels (Hazen and Sawyer; R.L. Taylor, personal communication, to J.A. Botts, *Design Information for the Treatment Plant Expansions.* December 10, 1990, Raleigh, North Carolina).

† NA = not available.

Table D-5. Toxicity of Simulation Effluents to *C. dubia**

Date	Simulation	48-hour LC50 (%Effluent)	NOEC † (%Effluent)	LOEC ‡ (%Effluent)
May 29–30, 1990	Farrington Road Northside	100 >100	75 § 100	100 § >100
June 6–7, 1990	Farrington Road Northside	>100 >100	100 100	>100 >100

* 7-day chronic toxicity test (USEPA Method 1002.0) according to USEPA (1989).

† NOEC for Northside is based on survival and reproduction. Results for Farrington Road are based on survival.

‡ LOEC for Northside is based on survival and reproduction. Results for Farrington Road are based on survival.

§ Denotes statistically significant inhibition of survival.

would reduce chronic effluent toxicity to levels required under the North Carolina discharge permit. Sulfide, a potential effluent toxicant, was not expected to be a problem because the final effluents of the new treatment plants are aerated to meet instream DO standards. The sulfide should be volatilized or oxidized in this aeration step.

The POTW upgrades were implemented beginning in November 1994. Results of effluent monitoring through the second quarter of 1997 show that the POTWs are in compliance with the chronic toxicity limits. The limits were revised to NOECs $\geq$90% for both plants. One test failure was observed in January 1995; however, this result may have been related to the start-up of the new treatment processes. Since then, the City has passed all quarterly tests at both POTWs.

Bench-scale batch tests were successfully used to simulate the treatment processes planned for the new POTWs, including the BNR treatment process. In addition to carbon removal and nitrification, the simulations achieved phosphorus removal to near permit levels. Although nitrate was not reduced to permit levels, the observed concentrations did not cause chronic toxicity.

The study findings suggest an alternative TRE approach is appropriate in cases where POTW staff is planning upgrades or improvements to their WWTPs. Toxicity reduction can be evaluated by conducting bench-scale batch simulations of the planned upgrades. This testing can be used to determine the potential for compliance with discharge limits for toxicity. If non-compliance is anticipated, further testing can be performed to evaluate the additional improvements necessary for toxicity reduction. In cases where the conclusions of a bench-scale toxicity evaluation are uncertain, pilot-scale tests may be warranted.

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Appendix E

TRE Case Study:
Michigan City Sanitary District, Indiana

Abstract

TRE Goal:	LC50 ≥100%, NOEC ≥62%
Test Organisms:	*C. dubia* and *P. promelas*
TRE Elements:	TIE
Toxicant Identified:	Metals
Toxicity Controls:	Pretreatment requirements

Summary

Acute and chronic TIE studies indicated that metals were the primary cause of effluent toxicity. An industrial user was identified as a major source of metals loadings to the POTW. The POTW staff required the industrial user to discontinue a cadmium plating operation and, as a result, the POTW effluent has achieved compliance with the acute and chronic toxicity limits (MCSD, 1993).

Key Elements

1. Less expensive acute TIE procedures can be used in lieu of chronic TIE procedures to help characterize the causes of chronic effluent toxicity. However, chronic TIE testing is needed to confirm the acute TIE results.
2. C18 SPE can remove toxicity caused by compounds other than non-polar organic compounds. In this study, C18 SPE treatment removed toxicity caused by metals. These results demonstrate the importance of needing to recover toxicity from the C18 SPE column before concluding that non-polar organic compounds are causing effluent toxicity.
3. TIE Phase I data may provide sufficient information to proceed to the selection of pretreatment controls for toxicity reduction. Although specific toxic metals were not identified in this study, evidence of metals toxicity was successfully used to set pretreatment requirements.

Introduction

Permit Requirements

The NPDES permit for the Michigan City Wastewater Treatment Plant (MCWTP) requires acute and chronic toxicity monitoring using *C. dubia* and *P. promelas*. The permit specifies that the effluent must not demonstrate chronic effluent toxicity at effluent concentrations of 62% or less (≤1.6 TUc) and that the effluent must not be acutely toxic (e.g., LC50 ≥100%, ≤1.0 TUa). Based on evidence of unacceptable acute and chronic toxicity, Michigan City was required to perform a TRE. The Michigan City Sanitary District submitted a TRE plan and initiated TIE testing. The objective of the TIE was to characterize, identify, and confirm the causes of acute and chronic effluent toxicity so that an appropriate toxicity reduction strategy could be developed and implemented.

Description of Treatment Plant

The MCWTP comprises an activated sludge process with single-stage nitrification and advanced waste treatment of the secondary effluent. The facility is designed for an average wastewater flow of 12-million gallons per day (mgd) and 96.7% removal of BOD_5 and 96% removal of suspended solids. Monthly average effluent limits for ammonia are 2 mg/L in summer and 6 mg/L in the winter. Influent phosphorus is reduced with an iron salt added at the aeration tanks. Additional phosphorus and suspended solids removal is accomplished by sand filtration of the secondary effluent. Total phosphorus is reduced by 80%, which results in effluent concentrations of less than 1 mg/L. Post aeration equipment is provided to increase the effluent DO concentration prior to discharge to Trail Creek. During the months of June through September (which coincides with the seeding of Trail Creek with smolts and later fish migration up Trail Creek), a pure oxygen system supersaturates the plant effluent to a DO concentration in excess of 13.0 mg/L.

Toxicity Identification Evaluation

Initial Toxicity Characterization

When both acute and chronic toxicity requirements must be met, POTW staff must decide whether to use acute or chronic TIE procedures to determine the effluent toxicants. Acute TIE procedures can be used to provide information about the causes of chronic toxicity and may be preferred because they are simpler and less costly than chronic TIE tests. Follow-up confirmation tests can be performed using chronic TIE procedures to determine if additional toxicants are contributing to chronic toxicity. If an effluent exhibits marginal and intermittent acute toxicity, it may not be possible to identify the causes of effluent toxicity using acute TIE procedures. In this case, chronic TIE procedures should be used.

The initial TIE work at the MCWTP focused on characterizing the causes of acute effluent toxicity because previous testing indicated that the effluent exhibited consistent acute toxicity. *C. dubia* were used as the test organism based on previous tests showing it to be more sensitive to the MCWTP effluent than *P. promelas*.

The toxicity characterization tests conducted during the first quarter of the TIE program included the following effluent manipulations:

- Pressure filtration (1.0 µm filter).
- Submicron filtration (0.22 µm filter) following pressure filtration (performed on one sample)
- Aeration.
- C18 SPE following filtration.
- Cation resin treatment following filtration/C18 SPE treatment.
- Anion resin treatment following filtration/C18 SPE treatment.

As shown in Table E-1, the four effluent samples characterized from April through June 1991 were consistently toxic and the magnitude of toxicity was similar in each sample (1.5 to 2.5 TUa). Slight reductions in toxicity occurred following filtration and aeration and acute toxicity was completely removed by the C18 SPE column. Toxicity removal by the cation and anion resins could not be determined because the sample was first passed through the C18 SPE column, which removed all of the toxicity. In retrospect, it would have been preferable to treat the samples with the ion exchange resins following filtration rather than after C18 SPE treatment. Relatively nonpolar organic compounds are preferentially adsorbed onto the C18 SPE column; therefore, toxicity removal by the C18

Table E-1. Acute Toxicity Characterization Test Results from April 1991 Through June 1991

Characterization Test	***C. dubia* LC50 (TUa)***			
	4/18/91	**5/16/91**	**6/5/91**	**6/19/91**
Baseline (whole effluent)	42 (2.4)	40 (2.5)	46 (2.2)	67 (1.5)
Filtration	51 (2.0) †	79 (1.3) ‡	54 (1.9) †	§
Aeration ω	40 (2.5)	62 (1.6)	51 (2.0)	§
Post C18 SPE ι	>100 (0.0)	>100 (0.0)	>100 (0.0)	>100 (0.0) #
Cation exchange τ	>100 (0.0)	>100 (0.4)	>100 (0.0)	§
Anion exchange ∉	>100 (0.0)	>100 (0.0)	>100 (0.2)	§

* *C. dubia* 48-hour LC50 values expressed as percent effluent with acute TUs (100/LC50) in parentheses.
† Effluent first pressure filtered through a Gelman A/E glass fiber filter (1.0 µm).
‡ Effluent first pressure filtered through a Gelman A/E glass fiber filter (1.0 µm), followed by filtration through a Micro Separation, Inc., 0.22 µm nylon filter.
§ Characterization manipulation not conducted.
# Fine stream of air bubbles passed through an effluent sample placed in a graduated cylinder.
ω Effluent sequentially pressure filtered (1.0 µm) and passed over a C18 SPE column.
ι Effluent passed directly over a C18 SPE column.
τ Effluent passed over a Bio-Rex MSZ 50 cation resin after pressure filtration and C18 SPE treatment.
∉ Effluent passed over a Bio-Rex MSZ 1 anion resin after pressure filtration and C18 SPE treatment.

SPE treatment during the initial characterization tests suggested that non-polar or semi-polar organic compounds were causes of effluent toxicity.

Evaluation of Toxicity Removed by C18 SPE

The C18 SPE column can remove toxicants other than non-polar organic compounds, including organometallic complexes, certain metal ions, surfactants, and some high molecular weight organic compounds. Accordingly, additional tests were performed from July through October 1991 to obtain information about the types of compounds removed by the C18 SPE treatment. In an attempt to recover toxicity from the C18 SPE column, sequential elutions were performed with methanol, methylene chloride, 3N hydrochloric acid, and 9N sodium hydroxide. Metals were evaluated as possible causes of toxicity concurrently with the C18 SPE tests. Metals toxicity was investigated by adding EDTA to whole effluent samples and testing for acute toxicity. EDTA forms complexes with many toxic metals and, when added at appropriate concentrations, can render metals non-toxic.

Results of the C18 SPE column and EDTA tests are summarized in Table E-2. In contrast to previous tests, the acute toxicity of the whole effluent from August through October 1991 was variable and intermittent (Table E-2). Four of the seven effluent samples were not acutely toxic. The three acutely toxic samples were rendered non-toxic by the C18 SPE treatment; however, toxicity was not recovered by eluting the C18 SPE columns with methanol, methylene chloride, 3N hydrochloric acid, or 9N sodium hydroxide. Toxicity could not be successfully eluted from C18 SPE columns using conventional organic extraction techniques; therefore, it was concluded that the toxicity removed by the column was not caused by typical non-polar or semi-polar organic compounds.

Addition of EDTA to the three acutely toxic samples eliminated acute toxicity, suggesting that toxicity was caused by metals. The EDTA results provide evidence that the toxicity removed by the C18 SPE column was not caused by non-polar or semi-polar organic compounds. Instead, it indicated that metals or organometallic complexes were removed in the C18 SPE column tests. These results demonstrate the importance of needing to recover toxicity from the C18 SPE column before concluding that non-polar organic compounds are a cause of effluent toxicity.

Evaluation of Metal Toxicity

Additional testing was performed to evaluate metals as a cause of chronic effluent toxicity to *C. dubia*. Chronic tests were used to help avoid problems associated with the intermittent acute toxicity; however, acute toxicity endpoints (e.g., 48-hour LC50) were also obtained from the chronic tests. During October 1991 through January 1992, 7-day static renewal *C. dubia* survival and reproduction tests were performed on whole effluent samples and whole

Table E-2. Toxicity Characterization Test Results from July 1991 Through October 9, 1991

Sample Date	*C. dubia* LC50 (TUa) *		
	Baseline (Final Effluent)	Post C18 SPE †	EDTA ‡
7/10/91	>100 (0.2)	§	§
7/24/91	>100 (0.0)	>100 (0.2)	>100 (0.0)
8/07/91	61 (1.6)	>100 (0.0)	>100 (0.0)
8/22/91	52 (1.9)	>100 (0.4)	>100 (0.0)
9/11/91	>100 (0.4)	§	§
9/25/91	>100 (0.2)	>100 (0.0)	>100 (0.0)
10/09/91	<100 (>1) #	§	>100 (0.0) ω

* *C. dubia* 48-hour LC50 values expressed as percent with TUs (100/LC50) in parentheses.
† Effluent passed over a C18 SPE column.
‡ EDTA was added to the final effluent at a concentration of 186 mg/L.
§ Characterization manipulation was not conducted.
\# Test conducted only in 100% effluent; as a result, LC50 and TUa values could not be calculated.
ω EDTA concentration in the 10/09/91 sample was 18.6 mg/L.

effluent samples with EDTA added. As shown in Table E-3, three of the five samples exhibited acute toxicity and four of the five were chronically toxic. The 48-hour LC50 values for all of the EDTA treated samples were greater than 100% effluent. EDTA addition also eliminated chronic toxicity in two samples and reduced chronic toxicity in a third sample. These results provided additional evidence that metals cause acute effluent toxicity, and also suggested that metals were a primary cause of chronic effluent toxicity.

The correlation approach and spiking approach described by USEPA (1989a) were used to confirm that metals were causing effluent toxicity. The correlation approach is intended to evaluate the relationship between the concentration of suspected toxicants and effluent toxicity. Toxicity and metals data (aluminum, Cd, Cu, Ni, and Zn) for six effluent samples were compared by correlation analysis. All metals were measured as total metals.

Linear regression analysis indicated a good correlation (regression coefficient of 0.72) between effluent toxicity and effluent Cd concentrations. However, when data from May 1991 through December 1992 were pooled with the data set, the correlation between effluent toxicity and effluent Cd concentrations was not statistically significant. A comparison of the mean Cd concentrations from samples collected during a toxic period (May 1991 to December 1991), and those taken during a non-toxic period (May 1992 to December 1992) indicated a trend. The mean Cd concentration was 4.1 µg/L during the toxic period and 0.47 µg/L during the non-toxic period. These data provide evidence that Cd was contributing to effluent toxicity. No significant correlation was observed between effluent toxicity and the concentration of the other metals or the sum of all the metals.

The objective of the spiking approach was to determine whether an increase in the concentration of a suspected toxicant would cause a proportional increase in toxicity. Chronic *C. dubia* toxicity tests were performed on three chronically toxic effluent samples both with and without added Cd, Cu, Ni, and Zn. The metals were added in nominal concentrations approximating those typically found in the MCWTP effluent. The results indicated that effluent toxicity did not consistently increase when the metals were spiked individually or in combination. Therefore, the results of the spiking tests did not confirm that Cd or other metals were contributing to effluent toxicity.

Toxicity Control Evaluation and Implementation

Although the TIE did not conclusively identify the specific causes of effluent toxicity, the weight of evidence indicated that effluent toxicity was caused by metals. As a result, Michigan City investigated possible sources of metals in the collection system. Pretreatment program data indicated that a cadmium plating facility in the MCWTP service area was consistently out of compliance with pretreatment limitations for metals. Based on the persistent pretreatment permit violations, the cadmium plating

Table E-3. Acute and Chronic Toxicity of MCWTP's Effluent (with and without added EDTA) from October 1991 Through January 1992

	Final Effluent		Final Effluent with EDTA Added *	
Sample Date	Acute LC50 (TUa) †	Chronic NOEC ‡	Acute LC50 (TUa) †	Chronic NOEC ‡
10/30/91	73 (1.4)	50	>100 (0.0)	100
11/14/91	>100 (0.0)	62	>100 (0.0)	62
12/04/91	>100 (0.0)	100	>100 (0.0)	100
12/18/91	84 (1.2)	<50	>100 (0.0)	100
01/08/92	60 (1.7)	<50	>100 (0.0)	50

* EDTA concentration in the 10/30/91 and 11/14/91 tests was 5 mg/L. EDTA concentration in the 12/04/91, 12/18/91, and 01/08/92 tests was 10 mg/L.

† *C. dubia* 48-hour LC50 values expressed as percent effluent with acute TUas (100/LC50) in parentheses.

‡ Reproduction NOEC values expressed as percent effluent calculated from 7-day static-renewal chronic tests with *C. dubia*.

company was issued a consent decree to terminate their cadmium plating operation. The cadmium plating operation was shut down in April 1992.

The impact of the shutdown on effluent toxicity was evaluated by performing 4-day modified chronic *C. dubia* tests on whole effluent samples at approximately 2-week intervals from May through September 1992 (total of nine tests). The 4-day modified chronic tests consisted of four concentrations and a control, five replicate test chambers per concentration, and the tests were initiated with 3-day old *C. dubia*. This modified approach has been demonstrated to produce results that are comparable to the 7-day test (Masters et al., 1991). The results of these tests showed that acute and chronic effluent toxicity to *C. dubia* had been eliminated.

Discussion

Subsequent chronic testing with *C. dubia* and *P. promelas* using compliance monitoring procedures (USEPA, 1989b) confirmed the reduction in effluent toxicity following shutdown of the cadmium plating operation. The acute and chronic toxicity of the MCWTP effluent from inception of the TRE through December 1992 is summarized in Figure E-1. The correlation between the cadmium plating operation shutdown and improved effluent toxicity is clearly evident. Based on the improved effluent toxicity, the TRE was terminated and semiannual acute and chronic toxicity compliance monitoring was initiated.

However, starting in August 1996 significant reproductive effects were observed in 100% effluent as compared to the test control. Subsequent TIE testing was inconclusive because effluent samples were nontoxic. Michigan City has submitted a letter to the Indiana Department of Environmental Management (IDEM) requesting changes in the effluent monitoring program. The requested changes include the use of reconstituted laboratory water as dilution water in lieu of receiving water to minimize potential contamination and reducing the frequency of monitoring if no toxicity is observed in three consecutive tests. As of October 1997, a decision from IDEM was still pending.

Acknowledgments

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References

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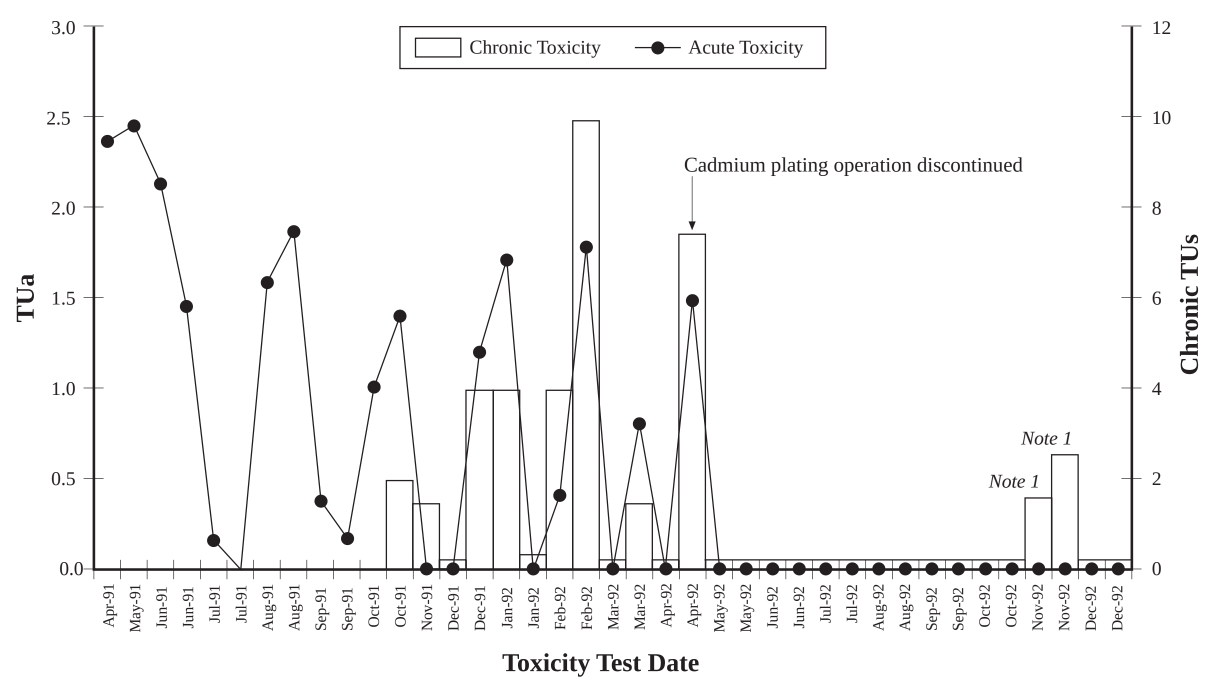

Note 1. Recorded toxicity is an artifact of the statistical analysis.

Note 2. TUa's and chronic TUs are based on 48-hour LC50 and NOEC values for *C.dubia*, respectively.

Figure E-1. Acute and chronic effluent toxicity: 1991 through 1992.

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Appendix F

TRE Case Study:
Central Contra Cost Sanitary District, Martinez, California, and Other San Francisco Bay Area POTWs

Abstract

TRE Goal:	No significant acute toxicity at 100% effluent
Test Organism:	*C. dubia*
TRE Elements:	TIE and source identification
Toxicants Identified:	Diazinon and chlorpyrifos
Toxicity Controls:	Multi-faceted public awareness program; ongoing program to identify and control sources; ongoing effort to identify POTW processes and operations that effectively remove organophosphate insecticides.

Summary

Acute toxicity to *C. dubia* was consistently detected in a POTW effluent. Application of Phase I, II, and III TIE procedures showed that the toxicity was caused by diazinon and one or more additional organophosphate insecticides. Follow-up studies, which required development of more sensitive analytical methods, showed that chlorpyrifos was present at levels that exceeded the NOEC in all effluent samples that were toxic to *C. dubia*. Influent and effluent monitoring studies of San Francisco Bay Area POTWs identified large differences in both influent loading and removal of the two insecticides between the POTWs. All the POTWs sampled achieved substantial removal of diazinon and chlorpyrifos from influent wastewater. Higher removal of both insecticides were generally associated with POTWs that had filtration treatment, extended mean cell residence times, chlorine contact times, and/or long retention in ponds. Source identification studies showed that the majority of the influent mass loading of the two insecticides was from residential sources. A multi-faceted outreach program was initiated within the POTW service area. Monitoring of effluent toxicity and insecticide concentrations to assess the effectiveness of the public outreach program is on-going.

Key Elements

1. The organophosphate insecticides, diazinon, and more recently, chlorpyrifos, have been implicated as causes of toxicity to *C. dubia* in POTW effluents.
2. Published TIE procedures are available to identify organophosphate insecticide toxicity (USEPA 1991, 1992, 1993a, 1993b, 1996). Application of new methods and procedures assisted in providing a more quantitative assessment of the role of diazinon and chlorpyrifos in effluent toxicity.
3. Source identification studies at the CCCSD demonstrated that the majority of the diazinon and chlorpyrifos influent loading was from residential sources.
4. Regional influent and effluent monitoring studies demonstrated patterns in influent diazinon and chlorpyrifos loadings at the CCCSD, which suggest there were demographic differences in use and disposal practices for organophosphate insecticides.
5. A multi-faceted public outreach program was implemented in the POTW service area. The effectiveness of the program is being assessed by frequent measurements of influent and effluent levels of diazinon and chlorpyrifos and effluent toxicity tests.

6. Monitoring studies showed that San Francisco Bay Area POTWs achieve substantial removal of both diazinon and chlorpyrifos. The highest levels of removal are associated with systems that have filtration systems, extended MCRTs, and/or longer chlorine contact times.

Introduction

Permit Requirements

During 1990–1991, the CCCSD conducted an effluent toxicity characterization program in which 18 acute toxicity tests were performed. The effluent produced detectable acute toxicity to *C. dubia* in 12 of the 18 test events. The CCCSD's NPDES permit requires no significant acute toxicity at 100% effluent; therefore, a TRE study was required by the California State Water Quality Control Board, San Francisco Bay Region, to determine the causes and sources of the acute toxicity.

This study was performed in addition to the TRE study that addressed effluent toxicity caused by Cu (see Appendix B). The CCCSD was required to meet permit limits based on toxicity testing using both *C. dubia* and echinoderms.

Description of the Treatment Plant

A description of the treatment plant is presented in Appendix B.

Facility Performance Evaluation

As part of the TRE study, the CCCSD conducted an internal facility performance evaluation to determine if the treatment system was operating at design performance specifications. A review of all relevant operating parameters indicated that there were no obvious performance deficiencies. During this period, monthly effluent tests showed intermittent acute toxicity to *C. dubia*, but no toxicity was detected to juvenile *P. promelas* (15- to 60-day-old).

Toxicity Identification Evaluation

USEPA TIE methods were used as guidance in conducting the Phase I (1988a), Phase II (1988b) and Phase III TIE studies (1988c).

Phase I TIE – Toxicity Characterization

A total of five Phase I TIE studies were conducted with the CCCSD final effluent to characterize the class of the toxicant(s) responsible for the acute toxicity to *C. dubia*. Tests were 48–72 hours in duration and TIE treatments were not renewed during the tests. TIE treatments were conducted on 100% effluent. The results, shown in Table F-1, indicated that the toxicity was consistently reduced by treatment with C18 SPE columns at pH*i* (initial pH of the sample) and PBO addition. Treatments that produced a partial decrease in toxicity in two or more samples included adjustment to pH 3 and aeration. Treatments that consistently did not decrease toxicity included pH adjustments, sodium thiosulfate, EDTA, or graduated pH treatment.

The results of the Phase I TIE studies showed that acute toxicity was consistently reduced by the C18 SPE column treatment, which removes non-polar organic chemicals. The methanol eluates from the C18 SPE column were toxic when added to dilution water at a concentration equivalent to 1.5 times (1.5X) the concentration in the effluent sample. It is important to note that the 1.5X calculation assumes that the toxicity was completely removed from the effluent sample by the C18 SPE column and further, that the toxicity was completely recovered from the column in the methanol eluate.

PBO was effective in preventing acute toxicity to *C. dubia* in all five samples. PBO blocks the metabolic activation and subsequent toxicity of organophosphate insecticides, which require metabolic activation to exhibit toxicity (Ankley et al., 1991). The ineffectiveness of sodium thiosulfate and EDTA suggest that oxidants and/or cationic metals were not implicated in the toxicity. The results of the graduated pH test also suggested that ammonia did not contribute to toxicity. Overall, the Phase I TIE results indicated that the effluent toxicity was due to non-polar organic toxicant(s), specifically one or more organophosphate insecticides, which require metabolic activation to produce toxicity. Diazinon, a metabolically activated organophosphate insecticide, has been reported to cause toxicity in municipal effluents (Norberg-King et al., 1989; Amato et al., 1992); therefore, subsequent Phase II studies focused on identifying organophosphate insecticides. Effluent and diazinon-spiked laboratory water were used to determine if the TIE treatments produced similar effects.

Phase II – Toxicity Identification

A total of four effluent samples were processed in Phase II. PBO completely prevented toxicity in all four effluent samples, suggesting that metabolically activated organophosphate insecticides were responsible for the acute toxicity. The Phase I TIE showed that the toxicity could be both removed by and

Table F-1. Matrix of Results of Phase I TIE Conducted on Five Effluent Samples with *C. dubia*

Treatment	Reduces Toxicity Due To	Samples with Substantially Reduced Toxicity				
		1	2	3	4	5
C18 SPE column (pH*i*)*	Non-polar organics, metals	■	■	■	■	■
C18 eluate toxic	Confirms non-polar organics	■	■	■	■	■
PBO addition	Organophosphate insecticides	■	■	■	■	■
Filtration	Filterable toxicants	■		■		
Aeration	Volatile/oxidizable toxicants		■		■	
Adjustment to pH 3	Acid hydrolyzable toxicants	■	■		■	
Adjustment to pH 11	Base hydrolyzable toxicants					
Thiosulfate addition	Oxidants, some metals					
EDTA addition	Cationic metals					
Graduated pH test	Ammonia, metals					

* pH*i* = initial pH.

recovered from C18 SPE columns; therefore, the Phase II TIE procedures focused on the use of the columns to fractionate the sample for further characterization. Aliquots of the samples were concentrated on C18 SPE columns and the columns were eluted with a series of methanol:water mixtures (USEPA, 1993a). Acute toxicity tests were then conducted on each fraction at 1.5X the original effluent concentration.

The 75% fraction from all the effluent samples was acutely toxic. In some samples, adjacent fractions (e.g., 70, 80, and 85%) also exhibited acute toxicity. The toxic fractions were combined, concentrated, and sequentially fractionated using HPLC. For comparison, an analytical standard of diazinon was run immediately prior to each effluent sample HPLC run. A total of 30 fractions were collected during the HPLC linear gradient (30–100% methanol:water for 25 minutes with 5 minutes at 100% methanol). Each fraction was assayed at 1.5X the original effluent concentration with *C. dubia*, and toxic fractions were treated with PBO to ascertain the presence of organophosphate insecticides. This procedure was similar to that described by USEPA (1993a). The results are summarized in Table F-2.

The diazinon standard consistently produced acute toxicity in one fraction (19), and in one HPLC run, toxicity also was observed in another fraction (18). All four effluent samples also produced acute toxicity in fraction 19 and occasionally in adjacent fractions (18 and 20).

As shown in Table F-2, in all cases, PBO provided protection against acute toxicity in the HPLC fractions in which toxicity occurred (18–20). However, PBO did not protect against the toxicity of fractions 12 and 13. The results of the PBO treatment of the toxic fractions suggested that one or more metabolically activated organophosphate insecticides, such as diazinon, had a role in the toxicity of all four effluent

Table F-2. Summary of TIE Phase II Results

Sample	Toxic Fractions
Diazinon (Runs 1–4)	18*$^{+}$, 19*$^{+}$
Effluent 1	18*$^{+}$, 19*$^{+}$
Effluent 2	12^{-}†, 19*$^{+}$
Effluent 3	18*$^{+}$, 19*$^{+}$, 20‡
Effluent 4	13†$^{-}$, 19*$^{+}$

* PBO provided full protection against toxicity.
† PBO provided no substantial protection against toxicity.
‡ PBO provided partial protection against toxicity.

samples. Diazinon consistently eluted in the same fractions that were identified in the effluent samples; therefore, further studies focused on confirming the presence of diazinon in the HPLC fractions and refining procedures for the accurate determination of diazinon in effluent samples. This latter aspect was challenging because diazinon is toxic to *C. dubia* at low concentrations (LC50=0.26–0.58 µg/L) (USEPA, 1991; Ankley et al., 1991; Bailey et al., 1997), and the C18 SPE column extracts of the effluent samples contained numerous interferences which made analysis by gas chromatography (GC) problematic. Diazinon analysis generally followed procedures described by USEPA (1993a). Diazinon was quantitated by GC/MS using selected ion monitoring. The detection limit for this procedure in the CCCSD effluent matrix was 0.010 µg/L of diazinon.

Phase III – Toxicity Confirmation

The role of diazinon in the CCCSD's effluent toxicity was assessed using the correlation approach (USEPA, 1988c). The purpose of the correlation approach is to determine whether there is a consistent relationship between the concentration of the suspected toxicant and the degree of effluent toxicity. If the correlation is not robust, the role of the suspect toxicant in the effluent toxicity should be re-examined.

A total of seven CCCSD effluent samples collected during July and August 1992 were evaluated by comparing the expected toxicity based on diazinon (48-hour LC50=0.38 µg/L) with the measured effluent toxicity. The 48-hour toxicity of the effluent samples ranged from 1.25–2.17 TUa. Diazinon concentrations in these samples ranged from 0.120–0.280 µg/L, which corresponds to 0.32–0.74 TUa based on the 48-hour LC50 for diazinon (i.e., 0.12 µg/L ÷ 0.38 µg/L and 0.28 µg/L ÷ 0.38 µg/L). The oxygen analog of diazinon (diazinon oxon) was not detected (<0.010 µg/L) in any of the effluent samples analyzed. Treatment of the toxic samples with PBO resulted in full reduction of toxicity in five samples, partial reduction in one sample, and no reduction in one sample. The effluent TUa and diazinon TUa values for the seven toxic samples are plotted in Figure F-1 along with the theoretical regression line, which depicts the case where all of the toxicity measured in the sample is due to diazinon (diazinon TUa = effluent TUa).

The linear regression of effluent TUa versus diazinon TUa had an R^2 value of 0.75 ($p \leq 0.01$), which indicates that diazinon concentrations can account for 75% of the variability in the toxicity of the effluent samples. However, the regression is above the theoretical regression line, which suggests that either the analytical procedure for diazinon was consistently detecting less than the actual effluent concentration, and/or there were one or more additional toxicants present in the effluent samples. Further studies were undertaken to assess both possibilities.

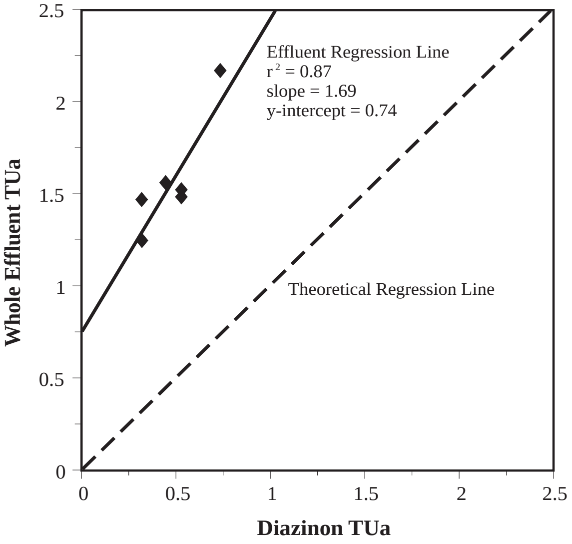

Figure F-1. Effluent TUs versus diazinon TUs in the CCCSD effluent samples.

Analytical procedures were reviewed by the CCCSD and were found to have acceptable levels of precision and accuracy. In an effort to identify the missing toxicant(s), more rigorous extraction procedures were applied to additional samples of effluent that were toxic to *C. dubia*. The effluent samples were exhaustively extracted with methylene chloride, evaporated to dryness, and resolubilized in hexane. Analysis of the extracts by GC/MS revealed the presence of chlorpyrifos, a metabolically activated organophosphate insecticide, in all the toxic effluent samples at concentrations greater than the NOEC of 0.030 µg/L (AQUA-Science, 1992; Bailey et al., 1997).

Follow-Up TIE Studies

Before further Phase III studies were initiated, a series of studies were conducted to validate the Phase I and II TIE findings for diazinon and to determine why the Phase II TIE process failed to identify chlorpyrifos as a toxicant in the CCCSD effluent. The results of these studies are summarized in Table F-3.

Table F-3. Summary of Follow-Up TIE Studies

TIE Treatment	Effect on Organophosphate Insecticides
pH adjustment	Diazinon is degraded rapidly at pH 3, but is relatively stable at pH 11
PBO addition	PBO at 100–700 µg/L effectively protects against three times LC50 concentration of diazinon and chlorpyrifos (1.6 and 0.24 µg/L, respectively). Effectiveness of PBO is affected by the matrix; therefore, use a range of PBO additions (USEPA, 1991a, 1993a).
C18 SPE	Diazinon is well recovered (80–100%) from C18 SPE columns Diazinon elutes sharply in specific methanol/water fractions: 75–80% methanol fractions for C18 SPE columns Chlorpyrifos is poorly recovered from C18 SPE columns (40–50% recovery) Chlorpyrifos tends to elute in broad bands: 80–95% methanol fractions for C18 SPE columns
HPLC fractionation	Diazinon is well recovered in specific fractions from C18 HPLC columns Recovery of chlorpyrifos from C18 HPLC columns is highly variable (20–60% recovery)
Sample stability studies	Significant amounts (20–40%) of diazonin and chlorpyrifos are lost from influent and effluent samples stored in either glass or plastic containers for 48 hours Effluent samples should be analyzed or extracted within hours of collection

The follow-up studies provided additional insight into the initial Phase I and II TIE results. The instability of diazinon at pH 3 is consistent with the reduction in effluent toxicity after pH 3 treatment. Diazinon is well recovered through the Phase II concentration and fractionation steps (Bailey et al., 1996); therefore, toxic fractions corresponding to those produced by diazinon standards should be present in all toxic effluent samples, as was demonstrated in the TIE.

On the other hand, the low overall recovery of chlorpyrifos from C18 SPE columns would explain the failure to detect chlorpyrifos toxicity in the effluent C18 SPE and HPLC fractions. For example, using the values in Table F-3, the recovery of chlorpyrifos in HPLC fractions could be as low as 8% (i.e., 40% recovery from 3 mL SPE column × 40% recovery from 1 mL SPE column × 50% recovery from HPLC column). This level of recovery would require an add-back of more than 12X to ensure that concentrations of chlorpyrifos in the HPLC fractions and the effluent samples were comparable. This study indicated that add-backs of fractions at levels substantially greater than 1.5X should be avoided because of the potential to amplify the toxicity due to toxicants that are below the toxic threshold in the effluent, but are well conserved through the TIE process. This could lead to erroneous identification of chemicals that do not have a causal role in the effluent toxicity.

A critical issue facing the investigator is how to identify toxicants that are not well recovered through the TIE process. Recently, procedures have been developed to selectively remove diazinon and chlorpyrifos from effluent samples using antibody-mediated processes (Miller et al., 1996; Miller et al., 1997). This process involves treating the effluent sample with the chemical-specific antibody preparation that selectively removes up to 95% of the target chemical (either diazinon or chlorpyrifos). By conducting effluent toxicity tests before and after the antibody treatment, the exact contribution of the target chemical to the overall toxicity can be determined. In addition, use of sequential antibody treatments to remove both diazinon and chlorpyrifos from the effluent matrix can indicate the extent to which toxicity is not due to either compound. The residual toxicity can be further characterized through the TIE.

Alternative Analytical Procedures

A major limitation of the TIE study was obtaining accurate and timely analytical information on levels of insecticides in effluent samples and TIE treatments. The GC/MS methods that were available involved tedious extractions, clean-up, and the use of expensive analytical equipment that was fully scheduled for compliance-related purposes. ELISA procedures were evaluated as an alternative analytical method for the analysis of diazinon and chlorpyrifos in subsequent Phase III TIE and source identification studies.

Commercially available ELISA kits (Beacon Analytical, Scaresborough, Connecticut) have some distinct advantages over GC or GC/MS methods, including cost ($40–70 versus $250–500 per sample), sample volumes (100 µL versus liters), sample turn-around (hours versus days or weeks), and equipment costs ($3,000 versus >$50,000). The detection limit for ELISA kits for diazinon and chlorpyrifos (0.030 µg/L) is also comparable to that for GC/MS. An interlaboratory study involving 6 laboratories and a total of 19 influent samples was conducted to compare the performance of ELISA, GC, and GC/MS procedures for diazinon and chlorpyrifos. The study showed that ELISA values for both insecticides were highly correlated ($R^2 > 0.95$) with GC and GC/MS results for those laboratories (Singhasemanon et al., 1997). The results were comparable over a wide range of concentrations (i.e., 0.030 to 31.5 µg/L for diazinon and 0.030 to 9.8 µg/L for chlorpyrifos).

Based on the excellent performance of the ELISA procedures in the interlaboratory study, ELISA procedures were used to monitor diazinon and chlorpyrifos concentrations in the CCCSD influent and effluent samples during follow-up studies, including source identification, POTW influent removal studies, and monitoring the effectiveness of public outreach programs.

Source Identification Studies

Source Study 1

A reconnaissance study was conducted in August 1995 to identify potential sources of diazinon and chlorpyrifos in wastewater from selected residential and commercial sources within the CCCSD collection system. A total of 36 24-hour composite samples of influent were analyzed for the two insecticides by ELISA. The samples included daily and/or hourly composite samples collected from a residential community, and from selected businesses within the CCCSD collection system, including self-service pet grooming facilities, operations centers for pest control operators, and kennels.

The measured levels of diazinon and chlorpyrifos were coupled with estimated flows from the various sources to provide estimates of overall contribution of the two insecticides to the CCCSD's influent. The results are shown in Table F-4.

Diazinon and chlorpyrifos concentrations in the wastewater from the residential sources were highly variable (0.050–0.720 µg/L and <0.050–0.520 µg/L, respectively). Peak concentrations of both insecticides in the residential samples were measured in the samples collected on Saturday afternoon. The cause of the spikes of the insecticides in the residential wastewater is under further study and may be related to home use and/or improper disposal of these chemicals during weekend activities (e.g., lawn care operations for diazinon and pet flea control for chlorpyrifos).

Diazinon and chlorpyrifos levels in wastewater samples collected from commercial sources also were highly variable (<0.030–16.0 µg/L and 0.040–5.4 µg/L, respectively). The highest concentrations of both insecticides were measured in wastewater samples from a commercial kennel.

Overall, the reconnaissance study showed that although high levels of diazinon and chlorpyrifos were detected in some of the wastewater samples from commercial sources, the vast majority of the loading of the insecticides into CCCSD influent during the sampling period was from residential sources. This finding agrees with an earlier study of sources of diazinon in Fayetteville, NC (Fillmore et al., 1990).

Table F-4. Diazinon and Chlorpyrifos Concentrations in Wastewater Samples from Selected Residential and Commercial Sources in the CCCSD

Source		Diazinon		Chlorpyrifos	
		µg/L	% of Total Influent Loading	µg/L	% of Total Influent Loading
Residential		0.050–0.720	101	<0.05–0.52	94
Commercial:	Pest control operators	<0.03–1.10	3	0.060–1.80	4
	Pet groomers	<0.03–0.10	<1	0.04–7.00	2
	Kennels	0.070–16.00	2	3.10–5.40	1

Source Study 2

Results of the reconnaissance study were used by the CCCSD and the California Department of Pesticide Registration (CADPR) to develop a plan for a more definitive study that was conducted from June to September 1996 (Singhasemanon et al., 1997). In this study, over 200 flow-proportional 24-hour composite samples were collected from each of 5 residential areas and 12 businesses (pet groomers, pest control operators, and kennels) within the CCCSD collection system. Flow measurements were made at selected sampling points in order to calculate mass loadings of diazinon and chlorpyrifos. The measured flows in residential areas were compared with modeled flow data obtained from a computer program [Sewer Network Analysis Program (SNAP) 1989, developed by the CCCSD]. The SNAP program applies modeled land use, groundwater infiltration, and CCCSD plant influent data to determine flow rates from the sampled areas. Concentrations of the insecticides were measured using ELISA, GC, and/or GC/MS procedures. The loading of diazinon and chlorpyrifos in the CCCSD influent from residential sources was estimated by multiplying the mean insecticide concentrations measured from the residential sites by the SNAP flow rates from the sampled sources. The commercial loading was estimated by multiplying the mean insecticide concentrations measured at each business by the measured flows and the number of similar businesses in the sewer service area. The data were analyzed using a computer program (SAS®, SAS Institute, Inc, 1994, Version 6.1, Cary, North Carolina), which calculated the Uniformly Minimum Variance Unbiased Estimator (UMVUE) for the mean influent loading concentrations for the insecticide (Singhasemanon et al., 1997). The mean UMVUE influent concentrations and associated loading for diazinon was 0.230 µg/L and 34.7 g/day, respectively. Corresponding values for chlorpyrifos were 0.145 µg/L and 15.0 g/day. The percentage of the total loading contributed by residential, commercial and unknown sources is shown in Figure F-2.

The CADPR study concluded that:

- Levels of diazinon and chlorpyrifos were highly variable in wastewater samples from both residential and commercial sources.
- Residential neighborhoods contributed the majority of diazinon and chlorpyrifos to the CCCSD's influent.
- Although relatively high concentrations of both insecticides were found at commercial sources, low flows from these sources resulted in relatively small mass loadings.
- A mass balance showed that a significant mass of chlorpyrifos and, particularly, diazinon was unaccounted for. Uninvestigated sources such as restaurants, nurseries, and industrial facilities should be sampled in future studies.
- Future source reduction strategies should focus on residential customers to identify and correct behaviors that contribute to disposal of organophosphate insecticides to the sewer system.

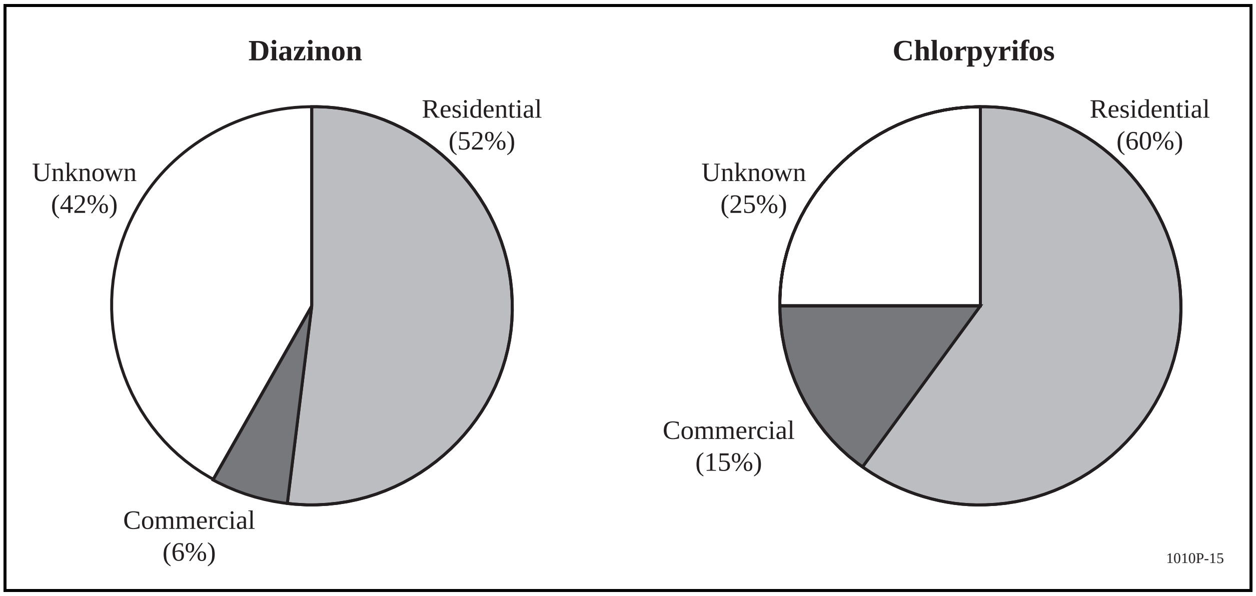

Figure F-2. Percent mass contribution of sources to the CCCSD influent.

As a result of the source identification studies, the CCCSD:

- Initiated a multi-faceted public outreach program targeting residential costumers to increase public awareness of the proper use of disposal of insecticides. The initial program included point of sale information sheets, newspaper articles, television ads, and billboards. A program to enhance public awareness of proper insecticide use by promoting integrated pest management practices is on-going.
- Shared study information with interested POTWs and State and Federal regulatory agencies.
- Initiated frequent effluent monitoring of diazinon and chlorpyrifos coupled with an effluent toxicity program to monitor the success of the public outreach program.
- Planned further studies to identify homeowner practices that contributed to the discharge of insecticides to the collection system.
- Reviewed disposal practices with pest control operators, pet care businesses, and kennels within the District.
- Conducted a study to identify the toxicity of alternative products for pet flea control.

Loading and Removal of Diazinon and Chlorpyrifos

Study 1

As an ancillary part of the CADPR source identification study, diazinon and chlorpyrifos were measured in seven consecutive daily samples of influent and effluent from CCCSD and two nearby POTWs [Union Sanitary District (USD), Fremont, California, and the Regional Water Quality Control Plant (RWQCP), Palo Alto, California]. The purpose of the study was to assess differences in loading and removal efficiencies for the POTWs. The three POTWs had similar influent flows (25–38 mgd), aeration detention times (3.8–5.6 hours), and clarifier detention times (2.0–4.2 hours). However, the CCCSD and the USD had shorter MCRTs (1.6–1.8 days versus 11.6 days) and shorter chlorine contact time (30–50 minutes versus 90 minutes) when compared to the RWQCP. In addition, the RWQCP treatment process incorporates two-stage aeration and dual media filtration to optimize particulate removal. The results of the study are shown in Figure F-3.

Daily concentrations of both diazinon and chlorpyrifos in the three POTWs varied widely during the sampling period. The CCCSD consistently had the highest influent and effluent concentrations of both insecticides, followed by the USD and the RWQCP. The CCCSD and the USD, which have similar treatment processes, had similar removal efficiencies for diazinon (32 and 24%, respectively), and chlorpyrifos (53 and 49%, respectively). The RWQCP, which has longer chlorine contact time, two-stage aeration, and dual media filtration had the highest removal efficiencies for diazinon (82%) and chlorpyrifos (71%). The effect of these parameters on the removal and/or degradation of diazinon and chlorpyrifos in municipal influent was further evaluated in a subsequent study.

Study 2

A larger scale study was conducted to confirm the findings of the CADPR study, which suggested that there may be demographic and/or microclimatic differences in influent loadings of diazinon and chlorpyrifos to POTWs within the same region and moreover, there may be differences in removal efficiencies of the two insecticides in POTWs using different treatment systems. Seven daily 24-hour composite samples of influent and effluent were collected from 9 Bay Area POTWs during August 1997. The POTWs included the CCCSD and the cities of Fairfield-Suisun, Hayward, Palo Alto, Petaluma, San Francisco, San Jose, Union City, and Vallejo. Samples were analyzed for diazinon and chlorpyrifos within 24 hours of collection using ELISA (AQUA-Science, 1997). The results for diazinon and chlorpyrifos are shown in Figure F-4. Information on the characteristics of each POTW treatment system is shown in Attachment 1.

The results of this study confirmed and extended the findings of the previous study. A summary is provided below.

- Mean influent concentrations for both diazinon and chlorpyrifos were highly variable and ranged from 0.278–1.211 µg/L and 0.030–0.176 µg/L, respectively. These results suggest that there are regional demographic, and possibly, climatic differences in use and disposal practices for the insecticides.
- All the POTWs achieved substantial removal of the two insecticides from influent (up to 98% for diazinon and up to 86% for chlorpyrifos). These removal rates are generally higher for both insecticides than were observed in the previous study. The highest levels of removal were

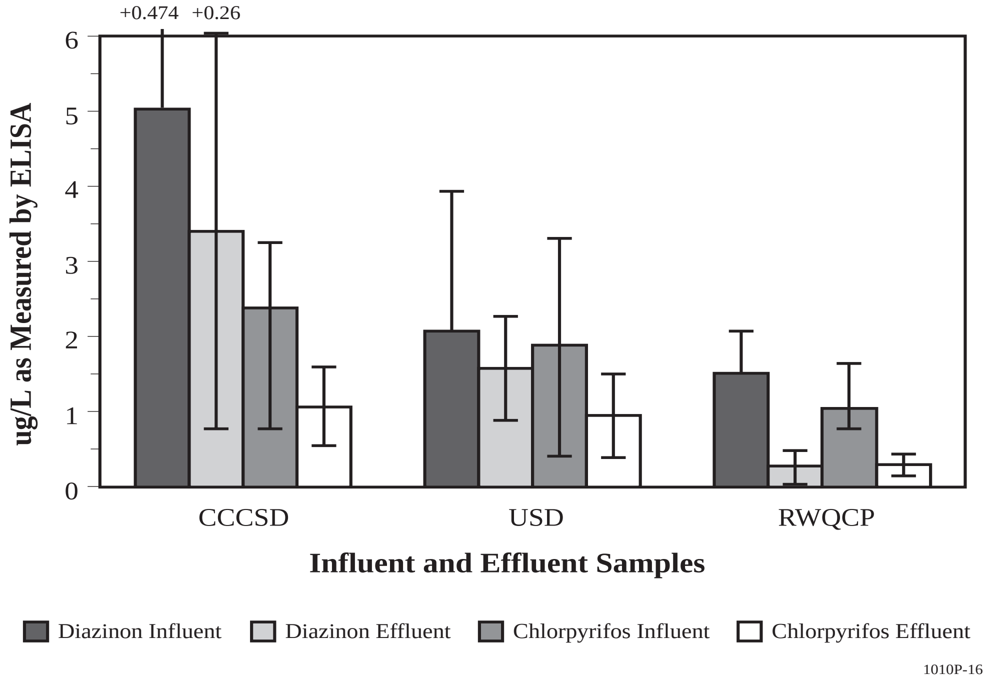

Figure F-3. Mean diazinon and chlorpyrifos concentrations (±std) in influent and effluent from three Bay Area POTWs.

associated with POTWs that had filtration, longer MCRTs and chlorine contact times, and long retention in ponds.

- Mean effluent concentrations for diazinon and chlorpyrifos ranged from <0.030–0.241 µg/L and <0.030–0.085 µg/L, respectively. The combined mean effluent concentrations for both insecticides exceeded 1.0 TUa in only three of the nine POTWs sampled (including the CCCSD).
- Overall, the results showed that all the POTWs sampled during this period had potentially toxic levels of diazinon and chlorpyrifos in their influents. However, all the POTWs achieved substantial removal of both insecticides.

Another round of sampling was scheduled for February 1998 to assess seasonal effects on influent levels and removal rates from the POTWs.

Alternative Pet Flea Control Products

Toxicity source investigations by the CCCSD suggested that pet flea control products were a major source of chlorpyrifos in the influent (AQUA-Science 1995a and 1995b). Before the CCCSD could recommend alternative products, it was necessary to conduct studies to determine the toxicity of several commonly used pet flea dips and shampoos. The acute toxicity of six flea shampoos and four dips was evaluated with *C. dubia* (AQUA-Science, 1995a; Miller et al., 1994). Although the products tested varied widely in toxicity, shampoos were generally less toxic than the dips. The most toxic products tested contained chlorpyrifos (IC25s of 0.800 to 2.30 µg/L as product), which were 2,500–7,000 times more toxic than the least toxic product tested, which contained D-limonene (IC25 of 5.687 µg/L). The products containing pyrethrins and permethrin had intermediate levels of toxicity (IC25s of 0.149–4.683 µg/L**)**. Calculations (with the associated assumptions on use rate, system losses, and dilution) indicated that only flea dip products containing chlorpyrifos were sufficiently toxic to produce measurable effluent toxicity to *C. dubia*.

Effects of Household Bleach on Aqueous Concentrations of Diazinon and Chlorpyrifos

A study was conducted to determine if household bleach could be recommended to residential customers

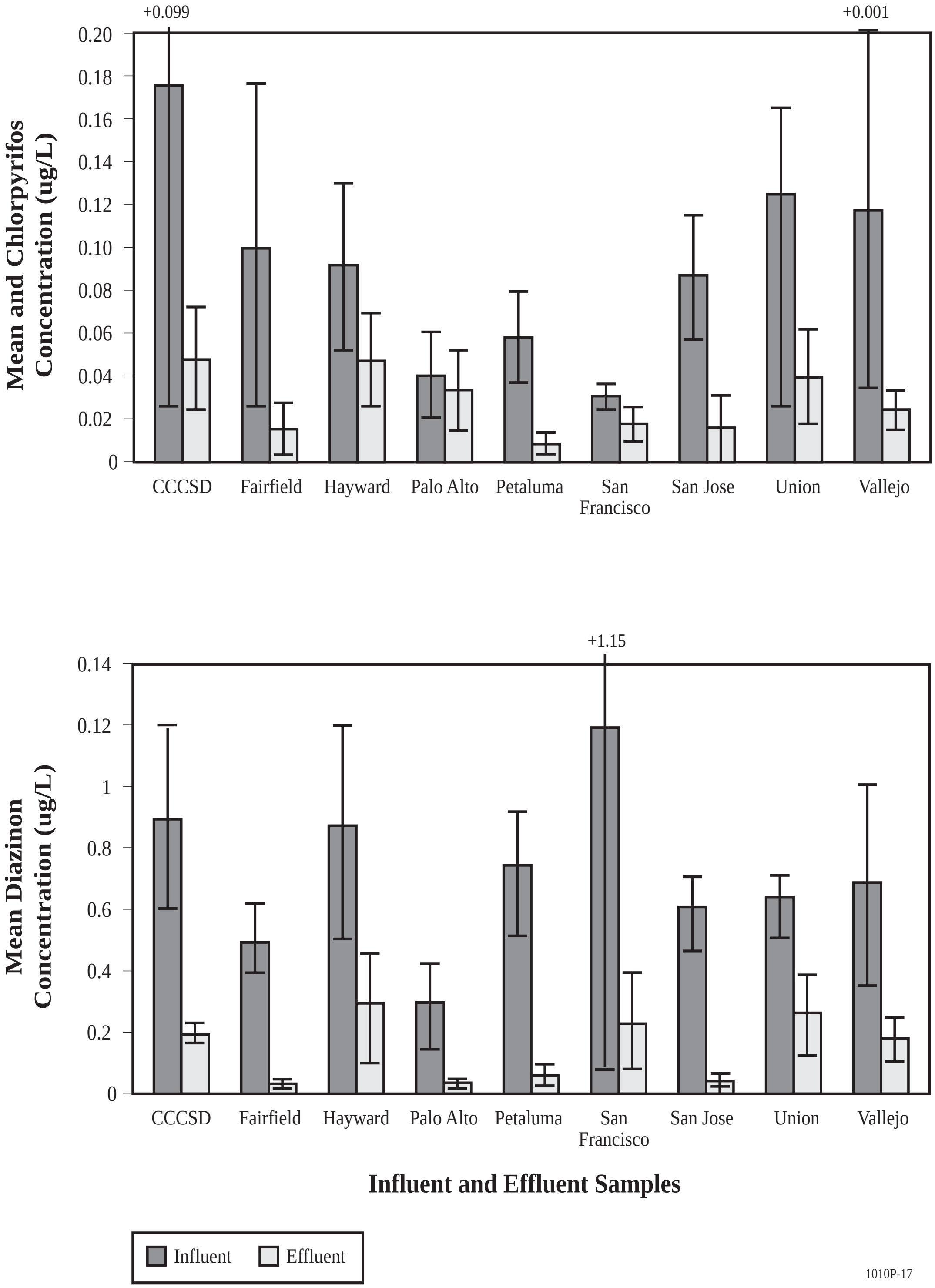

Figure F-4. Mean chlorpyrifos and diazinon concentrations (±std) in influent and effluent from nine Bay Area POTWs during August 1997.

as a measure to degrade diazinon in spray container rinsate and chlorpyrifos from pet flea washes prior to disposal into the sewer. Samples of tap water were spiked with high concentrations of diazinon (60.0 µg/L) and chlorpyrifos (10.0 µg/L) and treated with either 0.005 or 5% solutions of household bleach for 24 hours. After neutralization, concentrations of the insecticides were measured by ELISA (AQUA-Science, 1995a). Both bleach concentrations reduced concentrations of the insecticides by 86–92%. The study suggested that household bleach may be a effective pretreatment for waste solutions of diazinon and chlorpyrifos prior to disposal. Additional studies are planned to further define bleach exposure times and concentrations under actual use conditions, and to characterize the chemical oxidation products produced by the chlorine treatment.

Diazinon and Chlorpyrifos Concentrations in Water Samples from Restaurant Grease Traps

The CADPR source identification study recommended follow-up studies to determine concentrations of diazinon and chlorpyrifos in wastewater from restaurants. Water samples were collected from the grease traps of eight restaurants in the CCCSD service area (AQUA-Science, 1997). ELISA was used to measure concentrations of the two insecticides. Diazinon and chlorpyrifos concentrations ranged from 0.192–4.197 µg/L and 0.265–4.313 µg/L, respectively. The highest concentrations of both insecticides were found in wastewater from the same restaurant. The uses that contributed to these insecticide residues in the wastewater are currently being investigated by the CCCSD.

Regulatory Activities

Chlorpyrifos-Related

In January 1997, Dow-Elanco, as part of an agreement with USEPA, announced the following actions associated with the registered uses of chlorpyrifos (L. Goldman, USEPA Assistant Administrator for Prevention, Pesticides and Toxic Substances. Press Release on January 16, 1997):

- Withdrawal of chlorpyrifos from indoor broadcast and fogger flea control markets.
- Withdrawal of chlorpyrifos from direct application pet-care uses (shampoos, dips, and sprays).
- Increase marketing of ready-to-use products to replace concentrated formulas.
- Increase training and supervision of pest control operators.
- Revise chlorpyrifos labels to limit retreatment intervals.

If the chlorpyrifos in POTW influent loading is due to indoor and pet-care uses and/or misapplications by pest control operators, these actions should substantially reduce influent loadings of this chemical.

Diazinon-Related

In 1996, Novartis Crop Protection, Inc., the major U.S. registrant of diazinon, submitted voluntary label changes to USEPA to warn users not to dispose of this product into sanitary or storm drains. Novartis also developed educational materials with this message and provided the materials to selected cities in Texas and California. In 1997, Novartis completed a 4-year study with several POTWs in USEPA Region VI on diazinon occurrence and treatability (Novartis, 1997). A follow-up study is on-going with a California POTW to identify treatment processes that consistently optimize removal of diazinon (D. Tierney, personal communication, Novartis Crop Protection, 1997).

Discussion

In this case study, USEPA TIE procedures were used to identify organophosphate insecticide toxicity in a POTW effluent. Phase I and II TIE procedures identified diazinon as a candidate toxicant. Phase III TIE studies determined that effluent diazinon concentrations were significantly correlated with the extent of the effluent toxicity, but diazinon only accounted for approximately half of the effluent’s toxicity. The follow-up TIE studies identified chlorpyrifos at potentially toxic concentrations in the toxic effluent samples. ELISA procedures were shown to provide sensitive and accurate measurements of the two insecticides in samples of POTW influent and effluent, and these procedures were used extensively in follow-up TIEs and source identification studies. Additional TIE experiments found chlorpyrifos to be poorly recovered through the Phase I and II TIE processes, which may explain why it has not been identified as a toxicant in other effluent TIEs.

The source identification studies at the CCCSD and other Bay Area POTWs showed that the influents contained highly variable, and often potentially toxic, levels of diazinon and chlorpyrifos, which appear to be originating primarily from residential rather than commercial sources. However, only a relatively small number of commercial sources have been sampled to date. Thus, it is possible that certain business types (e.g., restaurants and nurseries) may be significant contributors of the two insecticides into wastewater. All of the POTWs that were sampled to date have demonstrated substantial removal of both insecticides from their influents. This was surprising because it was generally believed that these insecticides were poorly treated by POTWs (J.L. Miller, personal communication, Aqua-Science, Inc., Davis, California, April 1998). The available data suggest that there were substantial differences in influent loadings of diazinon and chlorpyrifos between POTWs within the San Francisco Bay region. Further studies are planned to explore the demographic basis for these differences to evaluate patterns of insecticide use. Seasonal trends in insecticide removal efficiencies are currently being monitored in nine Bay Area POTWs. Public outreach programs, supported, in part, by the manufacturers of diazinon and chlorpyrifos, have been implemented by the CCCSD and other POTWs across the country to increase awareness of the proper use and disposal of insecticides. Recent regulatory actions have resulted in the withdrawal of chlorpyrifos from the pet flea control market, and this action, coupled with the enhanced training of applicators and the increased use of prediluted insecticide products, may eventually reduce the influent loadings. Monitoring studies are in place at the CCCSD and elsewhere to determine if these programs will result in reduced influent loadings and decreased incidences of insecticide-related effluent toxicity.

Acknowledgments

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Attachment I
Summary of POTW Treatment System Characteristics

CCCSD
39 mgd
Primary sedimentation
Air-activated sludge (MART 1.6 days)
Secondary clarification
UV disinfection

Fairfield
13 mgd
Primary sedimentation
Oxidation towers with clarification
Air-activated sludge (MART 12–14 days)
Secondary clarification
Tertiary filtration with dual media
Chlorine disinfection (90–120 minutes)

Hayward
12 mgd
Valuators
Primary sedimentation
Fixed film reactors (sludge age n/a)
Anaerobic digester
Final clarifiers
Chlorine disinfection (~100 minutes)

Palo Alto
26 mgd
Primary sedimentation
Fixed film reactor to mixed aeration basins with activated sludge (MART 11.6 days)
Secondary clarifiers
Mixed media filtration
Chlorine disinfection (90 minutes)

Petaluma
6 mgd
Primary clarification
41% to activated sludge
32% to trickling filter
27% bypasses to ponds where retention time is about 100 days

San Francisco
17 mgd
Primary sedimentation
Air-activated sludge (MART ~ 0.86 days)
Secondary clarification
Sodium hypochlorite disinfection

San Jose
137 mgd
Primary sedimentation
Air-activated sludge (MART ~ 4 days)
Secondary clarification
Nitrification and clarification (MART ~ 11 days)
Tertiary filtration with backwash to clarification (for flow equilibrium)
Chlorine disinfection (40–60 minutes)

Union
31 mgd
Primary sedimentation
Air-activated sludge (MART ~ 1.75 days)
Secondary clarifiers
Chlorine disinfection (30 minutes)

Vallejo
12 mgd
Primary sedimentation
Biological filters
Aeration basins (MART ~ 3 days)
Clarification
UV disinfection and sodium hypochlorite contact (8 minutes)

Appendix G

TRE Case Study:
Linden Roselle Sewerage Authority, New Jersey

Abstract

TRE Goal:	96-hour LC50 ≥50% Interim goal of LC50 ≥30%
Test Organism:	*M. bahia*
TRE Elements:	Facility performance evaluation, TIE, toxicity source evaluation
Toxicants Identified:	Ammonia, non-polar organic compounds, surfactants
Toxicity Controls:	Pretreatment limits

Summary

Ammonia was confirmed as the primary cause of toxicity, and pretreatment limits were developed to reduce effluent ammonia concentrations. Secondary causes of toxicity were complex and highly variable. Toxicity-based procedures were used to identify industrial sources of toxicity and develop pretreatment limits to control secondary causes of toxicity.

In 1997, a major source of ammonia was eliminated. An acute toxicity test performed since then showed a reduction in effluent toxicity (LC50 = 72%) to compliance levels (i.e., LC50 >50%). Additional tests are planned to confirm this initial result.

Key Elements

1. TIE procedures may need to be modified to evaluate multiple causes of effluent toxicity. In this study, it was necessary to remove toxic effluent concentrations of ammonia in the TIE before other causes of toxicity could be identified and confirmed.
2. If TIE analyses are successful in confirming causes of effluent toxicity (e.g., ammonia), chemical-specific analyses can be used to identify sources and pretreatment limits can be developed for controllable toxicants.
3. If the TIE is inconclusive or the causes of toxicity are variable and complex, the RTA approach can be used to track the industrial sources of toxicity in the collection system. Once identified, the toxic dischargers can be required to meet pretreatment limits for toxicity.
4. If effluent toxicity is contributed by controllable industrial sources, pretreatment controls are more practical than in-plant controls.

Introduction

Permit Requirements

The LRSA New Jersey Pollutant Discharge Elimination System (NJPDES) permit contains an acute whole effluent toxicity limit of LC50 >50% effluent. A 96-hour static renewal *M. bahia* (mysid) test is used to monitor compliance with the limit. Based on observed toxicity to mysids, the NJPDES permit was amended to include a requirement to perform a TRE. In July 1992, the LRSA entered into an administrative consent order (ACO) with the New Jersey Department of Environmental Protection (NJDEP) to establish a compliance schedule for reducing acute effluent toxicity. The ACO established a compliance date of October 31, 1996, if pretreatment controls are implemented and a compliance date of December 31, 1997, if in-plant controls are implemented. The ACO also includes TRE milestones and an interim whole effluent toxicity limitation of an LC50 of 30%. The acute effluent toxicity limit of an LC50 of 50% becomes effective on May 1, 2000.

Description of the Treatment Plant

The LRSA POTW serves a 13-square-mile area in northeastern New Jersey. The POTW has a design flow of 17 mgd and is presently treating a wastewater flow of about 13 mgd. Approximately 20% of the influent flow is contributed by 40 industrial users. Primary treatment consists of screening and degritting followed by primary sedimentation. The primary effluent is then treated by roughing (trickling) filters and conventional activated sludge treatment. Following secondary clarification, the effluent is disinfected with chlorine and then discharged to the Arthur Kill estuary. The NJPDES permit specifies that samples for toxicity testing be collected prior to chlorination.

Plant Performance Evaluation

A limited POTW performance evaluation was conducted during a USEPA TRE research study to determine if POTW operations or performance was contributing to the observed acute toxicity. The POTW performance evaluation findings showed that industrial wastewater contributions have a significant effect on the variability and concentration of influent constituents. For example, in 1987, influent BOD_5 varied from 292 to 636 mg/L, oil and grease ranged from 11 to 132 mg/L, and ammonia-nitrogen varied from 17 to 119 mg/L (Morris et al., 1990). The influent variability requires the LRSA to make significant modifications to plant operations, such as operating one or two aeration basins, to maintain optimum treatment. Despite this variability, the LRSA has consistently met NJPDES permit effluent limits for conventional pollutants.

Overall, the POTW performance evaluation indicated that the operation and performance of the LRSA POTW was satisfactory and the treatment processes did not appear to be contributing to effluent toxicity (Morris et al., 1990). The POTW performance evaluation also indicated that the ammonia concentrations observed in the effluent warranted further evaluation as a cause of effluent toxicity.

Pretreatment Program Review

Monthly average influent ammonia concentrations at the LRSA have been as high as 150 mg/L. A review of the influent ammonia data indicated consistently lower ammonia levels in July of each year (LRSA, 1990a). The decreased ammonia concentrations were related to the temporary shutdown of a manufacturing process at a major industrial contributor.

Toxicity Identification Evaluation

An objective of the LRSA TRE was to identify the causes of effluent toxicity in order to select controls for reducing toxicity. Initial TIE Phase I and Phase II testing was performed in 1989 using *C. dubia* as a surrogate test species. *C. dubia* were used because little information was available at the time for using mysids as a TIE test organism. Subsequent TIE testing in 1991 was performed using mysids to confirm that the causes of toxicity identified using *C. dubia* were also causes of toxicity to mysids.

TIE Phase I

During the USEPA study, three effluent samples were tested using the TIE Phase I procedures (USEPA, 1988). The Phase I results and ammonia data indicated that ammonia was a primary cause of effluent toxicity. Toxicity reduction by C18 SPE suggested that non-polar organic compounds were also contributing to effluent toxicity (Morris et al., 1990).

TIE Phases II and III

TIE Phase II (USEPA, 1989b) and Phase III (USEPA, 1989c) analyses were performed using *C. dubia* and mysids to identify and confirm ammonia and non-polar organic toxicants as causes of effluent toxicity (LRSA 1990b, 1991; Morris et al., 1992). It was necessary to remove ammonia toxicity in the TIE before other toxicants could be evaluated. A serial treatment approach was used to evaluate the contribution of non-polar organic toxicants to acute effluent toxicity. Effluent samples were first treated with zeolite to remove ammonia and then non-polar organic toxicity was evaluated using C18 SPE column treatment and GC/MS analyses. A separate C18 SPE column test was performed using whole effluent to determine if zeolite treatment had removed non-polar organic toxicity.

Results of the non-polar organic toxicant confirmation tests, presented in Table G-1, show that filtration, C18 SPE column treatment, and zeolite treatment reduced toxicity to both mysids and *C. dubia*. The combined treatment steps removed all of the acute toxicity to both species. Following filtration, zeolite treatment removed 1.3 to 2.0 TUa, while the C18 SPE column removed 1.5 to 4.3 TUa. Acute toxicity to both species was recovered in the 80 to 100% methanol/water fractions from the C18 SPE column. Although only 0.3 TUa were recovered from the column, previous tests had shown greater recovery (>2 TUa). The lower recovery of non-polar organic toxicity in this sample may be due to the presence of toxicants that are difficult to elute from the C18 SPE column (e.g., surfactants were indicated as a possible toxicant based

Table G-1. TIE Phase III Results: Non-Polar Organic Compound Confirmation (LRSA POTW)

Sample Description*	TUa (100/LC50)	
	***C. dubia*†**	***M. bahia*‡**
Baseline toxic units	4.3	8.5
Post-filtration treatment	2.8	6.3
Aliquot No. 1		
Post-filtration and C18 SPE column treatment (original pH)	100 §	100 §
Combined toxic methanol/water C18 SPE column fractions#	0.3	0.3
Aliquot No. 2		
Post-zeolite treatment	1.5	4.3
Post-zeolite and C18 SPE column treatment	<1.0	<1.0
Combined toxic methanol/water fractions from zeolite/ C18 SPE column treatment#	0.3	0.3

* Effluents of serial treatment steps.
† 48-hour *C. dubia* acute toxicity test.
‡ 96-hour *M. bahia* acute toxicity test.
§ Percent mortality in 100% sample after 48 and 96 hours for *C. dubia* and *M. bahia*, respectively.
# Methanol/water fractions were evaluated at 5 times and 2.5 times whole effluent concentration for *C. dubia* and *M. bahia*, respectively.

on the toxicity removed by filtration). Overall, the results showed that mysids were sensitive to the same non-polar organic toxicity as *C. dubia*. These tests confirmed non-polar organic toxicants as a cause of effluent toxicity to mysids.

Difficulties were encountered in trying to identify and confirm the specific non-polar organic toxicants. TIE Phase II procedures (USEPA, 1989b), which included HPLC separation and GC/MS analyses, tentatively identified more than 20 non-polar organic compounds as potential causes of toxicity. In addition, many potentially toxic unknown compounds were detected. The results suggested that the majority of the compounds were related to industrial sources because the compounds are not typically found in domestic wastewater. Further work was not performed to identify the toxic non-polar organic compounds because:

- Little or no toxicity data were available for most of the non-polar organic compounds identified in the effluent (e.g., no LC50 values for the specific non-polar organic compounds); therefore, it was not possible to determine if the concentrations present in the effluent were acutely toxic.
- The non-polar organic toxicants varied from sample to sample, which made it difficult to determine consistent causes of non-polar organic toxicity.
- Many of the compounds detected were unknowns.

The TIE results indicated that, in addition to ammonia, non-polar organic toxicity may need to be controlled to achieve compliance with the acute toxicity limit. Due to the difficulty in determining the non-polar organic toxicants, the LRSA decided to use a toxicity-based approach to identify the sources of non-polar organic toxicity and other non-ammonia effluent toxicity.

Toxicity Source Evaluation

The available information indicated that both ammonia and non-ammonia (e.g., non-polar organic) toxicity was being contributed by controllable industrial sources. Therefore, pretreatment controls were deemed to be feasible and source evaluation studies were performed to identify the sources of ammonia and non-ammonia toxicity. Sources of ammonia were identified by a chemical-specific approach and sources of non-ammonia toxicity were identified by a toxicity-based approach. The resulting information was used to develop appropriate pretreatment limits.

Chemical-Specific Source Evaluation

The LRSA conducted studies to locate the major sources of ammonia in the collection system. Key manholes and industrial discharges were sampled and tested for total ammonia from 1990 through 1992. The results indicated one major industrial source of ammonia in the collection system. Based on the survey results, the LRSA developed and implemented pretreatment limits to reduce effluent ammonia concentrations (LRSA, 1993a).

Toxicity-Based Source Evaluations

The toxicity-based approach used RTA procedures that involved treating industrial wastewater samples in bench-scale, batch simulations of the POTW activated sludge process and measuring the resulting toxicity (USEPA, 1989a). The toxicity remaining after batch treatment, referred to as "refractory" toxicity, represented the toxicity that passes through the POTW and causes effluent toxicity. As shown in Figure 5-2 (Section 5), two types of batch reactors are tested. A control reactor simulated the treatment plant and treated only the POTW influent. The second reactor evaluated the addition of the industrial discharge to the POTW by treating industrial wastewater spiked into the POTW influent. An industrial discharge would be considered a source of toxicity if effluent from the spiked reactor was more toxic than the control reactor effluent.

Initial RTA tests conducted during the USEPA study indicated that refractory toxicity was limited to an industrialized area of the collection system. Following the USEPA study, ammonia was confirmed as the primary cause of effluent toxicity and the major source of ammonia was identified. Accordingly, subsequent RTA tests focused on identifying sources of non-ammonia toxicity. In 1992, RTA testing was performed to evaluate sources of non-polar organic toxicity because non-polar organic compounds had been identified as a major cause of non-ammonia toxicity.

The procedure for measuring non-polar organic toxicity involved passing the RTA batch effluent samples through a C18 SPE column, eluting the column with methanol, and performing a toxicity test on the methanol elution (LRSA, 1992a). This procedure provided a direct means of measuring non-polar organic toxicity and it eliminated interferences associated with toxic ammonia concentrations (i.e., ammonia was not captured by or eluted from the C18 SPE column).

The toxicity source evaluation identified two industrial dischargers of non-polar organic toxicity (LRSA, 1992b). Nonpolar organic toxicity tests performed on the effluent during this period suggested that non-polar organic toxicity was variable and that there may be other causes of non-ammonia toxicity. Therefore, further RTA testing was conducted in 1993 to identify sources of non-ammonia toxicity that may be caused by non-polar organic compounds and other unidentified compounds.

The ammonia pretreatment limits were not to become effective until after July 1995; therefore, the LRSA influent and effluent ammonia concentrations remained high during 1993. It was necessary to remove ammonia toxicity in RTA testing in order to identify sources of non-ammonia toxicity (LRSA, 1993b). Zeolite treatment of the batch effluent samples to remove ammonia was considered, but previous studies indicated that zeolite also may remove non-ammonia toxicity. Therefore, two alternative approaches were used to remove ammonia toxicity in the RTA. First, testing was conducted during periods of low influent ammonia concentrations, which occurred during the annual summer shutdown of the ammonia-contributing industrial process. During this period, ammonia concentrations were not acutely toxic; therefore, RTA testing would provide a direct measure of the non-ammonia toxicity contributed to the POTW. The second approach was used when the ammonia contributing process was fully operational and involved using a simulated plant influent (SPI). The SPI consisted of sewer wastewater collected from all major trunk lines except the sewer line serving the ammonia discharger. It was also necessary to wash the RAS used in the RTA to reduce the ammonia concentrations associated with the RAS (LRSA, 1993c).

The 1993 RTA testing was intended to identify those industries that would be required to meet pretreatment requirements to control non-ammonia toxicity. Thirty-two of the 40 industrial users were evaluated either directly or indirectly by testing sewer wastewater samples collected from key manholes. Previous RTA results and information obtained in an industrial user waste survey were used to select the industries to be tested.

The results of RTA tests performed in July and October 1993 are presented in Table G-2. If the effluent toxicity of the sewer wastewater spiked reactor was greater than that of the control reactor on two occasions, the discharge was considered a source of toxicity. Industries A, B, E, and F were indicated as sources of non-ammonia toxicity based on the results of direct testing of their industrial discharges. These results support the findings of the USEPA study, which identified industries A, B, and E as sources of toxicity, and the 1992 study, which identified industries B and E as sources of non-polar organic toxicity. Six other industries were identified as suspected sources based on the results obtained for key manholes 9 and 12. LRSA plans to test these suspected sources directly to determine which industries are contributing toxicity.

Table G-2. Results of Refractory Toxicity Assessment, July and October 1993*

RTA Reactor Effluent	**96-Hour Mysid TUa (100/LC50)**						**Source of Refractory Toxicity?**ω
	Jul 15	**Jul 16**	**Jul 22**	**Jul 23**	**Oct 19**	**Oct 20**	
Control Reactor	<1.0	<1.0	**1.63**	**1.05**	**2.0**	**1.75**	n/a
Spiked Reactors							
Industry A	<1.0	NT	**1.92**	NT	**3.39**	1.22	YES
Industry B	**1.45**	NT	**1.89**	NT	NT	NT	YES
Industry C	<1.0	NT	NT	NT	NT	NT	NO
Industry D	NT	<1.0	NT	NT	NT	NT	NO
Industry E	NT	<1.0	NT	**1.19**	NT	1.75	YES
Industry E 5× †	NT	NT	NT	**4.0**	NT	NT	YES
Industry F	NT	<1.0	NT	**2.18**	1.55	**1.86**	YES
Industry G	NT	NT	<1.0	NT	NT	NT	NO
Industry H	NT	NT	NT	NT	**2.28**	NT	NO
Industry I	NT	NT	NT	NT	NT	1.29	NO
Industry J	NT	NT	NT	NT	NT	**1.81**	NO
Key manhole 1	<1.0	NT	NT	NT	NT	NT	NO
Key manhole 3	NT	NT	NT	**1.12**	‡	NT	NO
Key manhole 4	NT	<1.0	NT	NT	NT	NT	NO
Key manhole 7A	<1.0	NT	NT	<1.0	NT	NT	NO
Key manhole 9 §	**1.1**	NT	NT	<1.0	**6.1**	NT	YES
Key manhole 10	NT	NT	1.33	NT	NT	NT	NO
Key manhole 12 #	**1.33**	NT	**1.81**	NT	1.71	1.63	YES
Key manhole 14	NT	NT	1.33	NT	NT	NT	NO
Key manhole 15	NT	<1.0	NT	NT	NT	NT	NO
Roselle flume	NT	<1.0	NT	NT	NT	NT	NO

* Spiked reactor results shown in **bold** indicate greater TUa than the control. Increased toxicity in the spiked reactor effluent compared to the control indicates a source of refractory toxicity.

† Tested at five times the normal flow contribution to evaluate anticipated increase in flow.

‡ Toxicity test was invalid based on unacceptable control survival.

§ Key manhole 9 receives wastewater from three industries.

\# Key manhole 12 receives wastewater from three industries.

ω If a spiked reactor result was greater than that of the control on two occasions then the discharge was considered a source of refractory toxicity.

NT Not tested.

Toxicity Control Evaluation

The LRSA evaluated control options for ammonia and non-ammonia toxicants. The objective was to identify and assess the available options and to determine the most cost effective and pragmatic approaches for reducing effluent toxicity to acceptable levels.

Ammonia Toxicity Control Evaluation

A modified acute toxicity test procedure was developed by the LRSA and approved by the NJDEP to control pH drift in the toxicity test. The pH in previous LRSA compliance tests typically drifted up to 8.0 to 8.5, which resulted in an overestimation of ammonia toxicity (i.e., unionized ammonia concentrations increase as pH increases). The modified test procedure maintains pH in the toxicity test at the receiving system pH of 7.4. This modification provides a more accurate measurement of instream ammonia toxicity.

Using ammonia toxicity values for mysids published by USEPA (1989d), a linear regression model was prepared to predict the concentration of ammonia in the effluent which, in the absence of other toxicants, should result in compliance with the acute toxicity limit. The ammonia value generated by the model accounts for toxicity test conditions that affect the concentration of unionized ammonia (e.g., pH, temperature, and salinity). The model determined that the acute toxicity limit could be met with an effluent ammonia concentration of 35 mg/L (LRSA, 1991).

Several options for in-plant treatment of ammonia were evaluated to achieve the ammonia target level. As shown in Table 6-1 (Section 6), none of the six options evaluated was practical based on technical and cost considerations. In addition, significant inhibition of nitrification was observed during treatability tests, indicating that inhibitory compounds would need to be controlled if nitrification was selected as a control option (LRSA, 1991). Based on these results and the results of the ammonia source evaluation, chemical-specific pretreatment limits were selected as the best approach for controlling toxicity caused by ammonia (LRSA, 1993a).

Non-Ammonia Toxicity Control Evaluation

The TIE indicated that the causes of non-ammonia toxicity were complex and highly variable and the specific compounds causing non-ammonia toxicity could not be identified and confirmed. Consequently, the necessary information was not available to develop chemical-specific pretreatment limits.

As an alternative to pretreatment limits, activated carbon treatment at the POTW was evaluated based on its effectiveness in reducing effluent toxicity caused by a variety of compounds including non-polar organic toxicants. Both PAC and GAC treatment were considered and found to be cost prohibitive (T.L. Morris, Technical Memorandum to LRSA, *Evaluation of Granular Activated Carbon at LRSA*, January 19, 1993). It also was determined that the use of PAC treatment would result in unacceptable sludge quality.

The LRSA elected to implement pretreatment controls because controllable industrial sources of non-ammonia toxicity had been identified and practical in-plant treatment options were not available. It was determined that the pretreatment limits must be toxicity-based because of the lack of specific information on the causes of non-ammonia toxicity. The proposed pretreatment approach involved RTA testing to determine which industries should be issued limits and which industries should be monitored to assess the need for future limits (LRSA, 1993c).

Implementation Of Toxicity Controls

Ammonia Pretreatment Limits

The approach used to develop pretreatment limits for ammonia was relatively straightforward. As required by the ACO, the LRSA submitted a work plan for developing ammonia pretreatment limits to the NJDEP in April 1992 and the plan was approved in May 1992 (LRSA, 1992c). Using the target ammonia level of 35 mg/L and the ammonia survey data, an allowable headworks loading approach (USEPA, 1987) was followed to develop draft pretreatment limits. The LRSA published the draft limits for public notice and comments were received and reviewed. In January 1993, the proposed ammonia pretreatment limits and the LRSA's response to public comments were submitted to the NJDEP. The limits were approved in March 1993 and industrial users were to comply with the limits by July 1995 (LRSA, 1993a).

Toxicity-Based Pretreatment Limits for Non-Ammonia Toxicity

The LRSA is one of the first municipalities to develop toxicity-based pretreatment limits to control non-ammonia toxicity. At the time of this study, toxicity-based pretreatment limits had not been applied elsewhere and there was no specific guidance on developing such limits. The selected approach was based on the available TRE information and involved several aspects of various pretreatment approaches recommended by USEPA (1987).

The LRSA submitted a work plan for development of the limits to the NJDEP in June 1993 (LRSA, 1993b). The proposed approach was designed to address both major and minor sources of non-ammonia toxicity (LRSA, 1993c) and to ensure compliance without unnecessary controls. The proposed limits will consist of the following components referred to collectively as a toxicity management program (TMP):

- Narrative local pretreatment limit of "no discharge of refractory toxicity."
- Pass/fail toxicity-based limit using the RTA procedure as a compliance test (i.e., the effluent LC50 of the industrial user spiked reactor may not be less than the LC50 of the control reactor effluent).
- Industrial user (if toxicity is found) may be required to implement a toxicity reduction program comprising requirements to identify causes and sources of toxicity, implement industrial user management practices, and evaluate and establish other controls to ensure compliance with the toxicity-based limits.
- RTA monitoring requirements and decision criteria for determining if an industrial user needs to continue with the TMP.
- Provisions to allow industries to be relieved from the TMP requirements if toxicity requirements are met.
- Compliance schedule including milestones and progress reports.
- Reopener clause stating that the pretreatment permit will be modified to include chemical-specific limits if the causes of toxicity are identified.

The proposed pretreatment limit approach falls under the case-by-case/best professional judgment approach described by USEPA (1987), but also includes toxicity-based requirements, industrial user management practice, and chemical-specific components. The TMP approach is consistent with USEPA recommendations for monitoring and controlling effluent toxicity through the NPDES.

The RTA procedures had not been used for compliance monitoring purposes in New Jersey. Therefore, a site-specific RTA protocol (LRSA, 1994) was submitted to the NJDEP for review and approval prior to development of the draft pretreatment limits. The RTA protocol was approved by the NJDEP in June 1996. Pretreatment program permits for several industries were modified to include the TMP provisions. These industries are currently required to conduct quarterly monitoring using the RTA protocol.

Discussion

Chemical-specific pretreatment limits are being implemented to control toxicity caused by ammonia and toxicity-based pretreatment limits are in place to control non-ammonia toxicity. The major source of ammonia ceased its discharge of the ammonia-laden waste stream in 1997. As a result, effluent ammonia concentrations at the LRSA treatment plant decreased to about 30 mg/L. A compliance test performed after the ammonia source was eliminated showed improved effluent quality (i.e., LC50 = 72%). Additional tests are planned to confirm this initial result.

It is possible that the ammonia pretreatment limits alone will achieve compliance with the acute effluent toxicity limit. However, due to the complex and variable nature of the non-ammonia toxicity, it is not possible to accurately predict if the ammonia reduction will achieve consistent compliance with the permit limit LC50 $\geq$50%). The LRSA has established pretreatment requirements for non-ammonia toxicity to ensure full and timely compliance with the toxicity limit. The need for industrial users to control non-ammonia toxicity is ultimately tied to compliance with the acute effluent toxicity limit. If necessary, industrial users may request relief from these requirements if the effluent consistently complies with the acute effluent toxicity limit.

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Appendix H

Toxicity Control Options for Organophosphate Insecticides

Organophosphate insecticides, including diazinon, chlorpyrifos, malathion, and chlorfenvinphos, have been found to cause effluent toxicity at POTWs throughout the United States (Norberg-King et al., 1989; Amato et al., 1992; USEPA, 1987; Botts et al., 1992; Fillmore et al., 1990). A case study of the occurrence of organophosphate insecticide toxicity at POTWs in the San Francisco Bay area is presented in Appendix F. Although procedures are available for identifying organophosphate toxicants, less is known about how to control organophosphate insecticides in POTW effluents. This section describes approaches for organophosphate toxicity control that have been successfully implemented at POTWs. Information is also presented on ongoing research into POTW operational improvements that may reduce effluent concentrations of organophosphate toxicants.

A review of the literature suggests that two approaches may be successful in reducing organophosphate compounds at POTWs:

- Public education to limit the discharge of organophosphate compounds to the POTW.
- POTW modifications, particularly involving enhancements to the biological treatment and chlorine disinfection processes.

The latter approach has been the subject of a research study being funded by the two principal manufacturers of organophosphate compounds in North America: Novartis Crop Protection, Inc., and Makhteshim-Agan of North America, Inc.

Public Education Approach

Organophosphate insecticides are used widely for pest control by homeowners, restaurants, veterinarians, and other commercial businesses. These sources are not readily controlled by pretreatment program regulations. Alternative efforts to minimize the use or disposal of organophosphate insecticides must have broad appeal to the public at large.

Organophosphate insecticide control measures that have been considered by POTW staff include public outreach and education programs and approaches to restrict the use of organophosphate compound applications. Efforts to ban or restrict the use of organophosphate insecticides have not been successful, largely because of concern about legal issues and the difficulty in controlling the sale of organophosphate compounds outside of the community.

Restrictions on Organophosphate Insecticide Use

In 1990, the City of Largo, Florida, evaluated the feasibility of banning the use of diazinon and other organophosphate insecticides (malathion and chlorfenvinphos) to control effluent toxicity (C. Kubula, personal communication, City of Largo, Florida, 1992). It was determined that a diazinon ban would likely increase the use of other, equally toxic, insecticides. For example, Dursban®, a likely alternative insecticide, contains chlorpyrifos, which has been found to be more toxic than diazinon. Also, restrictions on diazinon use would apply only to new supplies, not to insecticides already in stock at stores. The City of Largo estimated that the stockpiled diazinon would last for more than a year. An effective control program would also require the cooperation of neighboring communities in limiting the purchase of diazinon outside of the community. In addition, the local banning of federally approved insecticides would be controversial. It was anticipated that insecticide manufacturers and distributors would challenge the City's authority to implement such controls. Based on this analysis, the City of Largo determined that banning diazinon would not be a practical control option.

Public Education Campaigns

Based on the impracticality of insecticide bans, the City of Largo elected to pursue a public awareness approach to control diazinon toxicity. The City of Greenville, Texas, also implemented a public education program in 1990 (City of Greenville, 1991). The first year of the program focused on determining significant users of the insecticide and developing educational materials. The following years have involved distributing the materials and conducting other informational activities.

The City of Greenville initially identified nine groups of diazinon users: pest control businesses, lawn care businesses, veterinarians, animal shelters, janitorial services, apartment complexes, restaurants, hotels, and retail stores (City of Greenville, 1991). The residential population also was added as a target user group. The City service area was divided into sections, and a telephone survey was conducted. Information was gathered on diazinon use, including existing supplies and application and waste disposal practices, and business owners and homeowners were notified of the importance of controlling diazinon wastes. The program involved the following public education activities:

- Brochures and handouts
- Pest control fact sheets describing integrated pest management methods, which focused on minimizing insecticide usage
- Mass mailings
- Newspaper articles
- Public service announcements
- Occasional talk shows on local radio stations
- Biweekly presentations to schools and business groups
- A telephone information line.

The City of Greenville also enacted an ordinance to encourage environmentally sound use of insecticides. The ordinance requires retail vendors, pest control services, and apartment managers to distribute educational material to customers and to periodically report insecticide applications to the City.

The results of the Greenville education campaign are encouraging. Beginning in December 1993, the treatment plant effluent was not toxic to *C. dubia* for 3 consecutive months. The public awareness effort is continuing and the City will monitor its effect on toxicity reduction.

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Two types of POTW biological treatment processes were investigated in the Novartis study: fixed film (trickling filter and RBC) and activated sludge. Influent and effluent concentrations at several POTWs in the southwestern United States were compared to determine removals of diazinon and chlorpyrifos.

Overall, the data indicated that diazinon reduction could be achieved in conventional POTW treatment processes. A statistical analysis of the data showed that the fixed film process had a significantly lower percent removal (p=0.95) for diazinon than the activated sludge process or a combined fixed film/ activated sludge process. A similar trend was observed for chlorpyrifos, although no significant differences were found between the process types.

Bench-scale treatability testing was conducted to further evaluate the fate of diazinon in typical POTW processes. These tests considered the effect of design and operating conditions for biological treatment processes on diazinon removal and effluent toxicity. Additional tests were performed to investigate the effect of physical/chemical processes, including chemical precipitation, chlorination/dechlorination, and post aeration on diazinon concentrations and toxicity.

As shown in Figure H-1, a correlation was found to exist between diazinon removal and sludge retention time (SRT), HRT, and MLSS concentration in activated sludge treatment tests. The primary removal mechanism in the activated sludge tests was adsorption onto the biological solids. These results suggest that diazinon removal may be improved by increasing the SRT, HRT, and/or MLSS concentration of the treatment process.

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Additional tests evaluated the fate of diazinon in POTWs (Novartis, 1997). Anecdotal evidence from other studies (Fillmore et al., 1990) and the treatability studies suggested that adsorption onto solids was the dominant removal mechanism. Therefore, the tests focused on partitioning of diazinon and chlorpyrifos onto primary and mixed liquor solids. These tests showed that about 30% of the diazinon and 85 to 90% of the chlorpyrifos present in POTW primary influent samples is adsorbed onto primary influent solids. Mixed liquor adsorption results revealed that approximately 65 to 75% of the diazinon added to the mixed liquor adsorbed onto the biomass. Diazinon adsorption was greater for a 30-day SRT biomass than for a 15-day biomass. Chlorpyrifos strongly adsorbed to the biomass; 100% was removed.

Summary

Studies have shown that organophosphate compounds can be effectively controlled through public education (City of Greenville, 1991; Engineering Science, Inc., 1993) . This effort may vary from the distribution of educational materials to the enactment of ordinances that require strict accounting of insecticide use. The studies conducted to date indicate that characterization of the sources of organophosphate compounds is key to the development of a successful toxicity control program.

Recent information shows that relatively simple enhancements to POTWs may help to reduce organophosphate compounds. Factors affecting diazinon and chlorpyrifos removal include the SRT, HRT, and MLSS concentrations in activated sludge processes, chlorination/dechlorination, and post aeration. Further studies are in progress to better define the operating conditions that will promote organophosphate compound removal (D. Tierney, personal communication, Novartis Crop Protection, Inc., 1997).

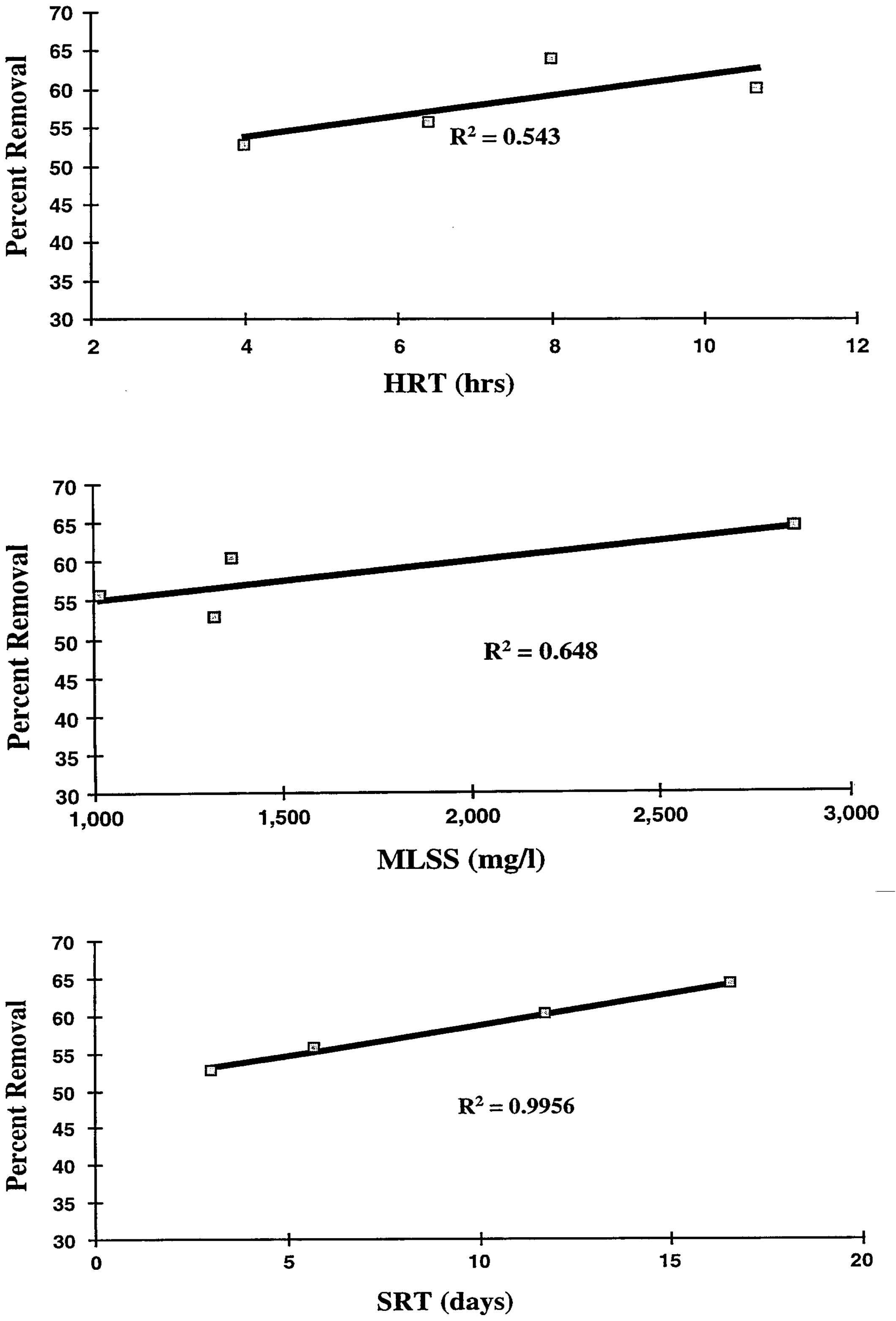

Figure H-1. Diazinon removal as a function of SRT, HRT, and MLSS concentration (reprinted with the permission of Novartis Crop Protection, Inc.) (Source: Novartis, 1997).

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Appendix H

Toxicity Control Options for Organophosphate Insecticides

Organophosphate insecticides, including diazinon, chlorpyrifos, malathion, and chlorfenvinphos, have been found to cause effluent toxicity at POTWs throughout the United States (Norberg-King et al., 1989; Amato et al., 1992; USEPA, 1987; Botts et al., 1992; Fillmore et al., 1990). A case study of the occurrence of organophosphate insecticide toxicity at POTWs in the San Francisco Bay area is presented in Appendix F. Although procedures are available for identifying organophosphate toxicants, less is known about how to control organophosphate insecticides in POTW effluents. This section describes approaches for organophosphate toxicity control that have been successfully implemented at POTWs. Information is also presented on ongoing research into POTW operational improvements that may reduce effluent concentrations of organophosphate toxicants.

A review of the literature suggests that two approaches may be successful in reducing organophosphate compounds at POTWs:

- Public education to limit the discharge of organophosphate compounds to the POTW.
- POTW modifications, particularly involving enhancements to the biological treatment and chlorine disinfection processes.

The latter approach has been the subject of a research study being funded by the two principal manufacturers of organophosphate compounds in North America: Novartis Crop Protection, Inc., and Makhteshim-Agan of North America, Inc.

Public Education Approach

Organophosphate insecticides are used widely for pest control by homeowners, restaurants, veterinarians, and other commercial businesses. These sources are not readily controlled by pretreatment program regulations. Alternative efforts to minimize the use or disposal of organophosphate insecticides must have broad appeal to the public at large.

Organophosphate insecticide control measures that have been considered by POTW staff include public outreach and education programs and approaches to restrict the use of organophosphate compound applications. Efforts to ban or restrict the use of organophosphate insecticides have not been successful, largely because of concern about legal issues and the difficulty in controlling the sale of organophosphate compounds outside of the community.

Restrictions on Organophosphate Insecticide Use

In 1990, the City of Largo, Florida, evaluated the feasibility of banning the use of diazinon and other organophosphate insecticides (malathion and chlorfenvinphos) to control effluent toxicity (C. Kubula, personal communication, City of Largo, Florida, 1992). It was determined that a diazinon ban would likely increase the use of other, equally toxic, insecticides. For example, Dursban®, a likely alternative insecticide, contains chlorpyrifos, which has been found to be more toxic than diazinon. Also, restrictions on diazinon use would apply only to new supplies, not to insecticides already in stock at stores. The City of Largo estimated that the stockpiled diazinon would last for more than a year. An effective control program would also require the cooperation of neighboring communities in limiting the purchase of diazinon outside of the community. In addition, the local banning of federally approved insecticides would be controversial. It was anticipated that insecticide manufacturers and distributors would challenge the City's authority to implement such controls. Based on this analysis, the City of Largo determined that banning diazinon would not be a practical control option.

Public Education Campaigns

Based on the impracticality of insecticide bans, the City of Largo elected to pursue a public awareness approach to control diazinon toxicity. The City of Greenville, Texas, also implemented a public education program in 1990 (City of Greenville, 1991). The first year of the program focused on determining significant users of the insecticide and developing educational materials. The following years have involved distributing the materials and conducting other informational activities.

The City of Greenville initially identified nine groups of diazinon users: pest control businesses, lawn care businesses, veterinarians, animal shelters, janitorial services, apartment complexes, restaurants, hotels, and retail stores (City of Greenville, 1991). The residential population also was added as a target user group. The City service area was divided into sections, and a telephone survey was conducted. Information was gathered on diazinon use, including existing supplies and application and waste disposal practices, and business owners and homeowners were notified of the importance of controlling diazinon wastes. The program involved the following public education activities:

- Brochures and handouts
- Pest control fact sheets describing integrated pest management methods, which focused on minimizing insecticide usage
- Mass mailings
- Newspaper articles
- Public service announcements
- Occasional talk shows on local radio stations
- Biweekly presentations to schools and business groups
- A telephone information line.

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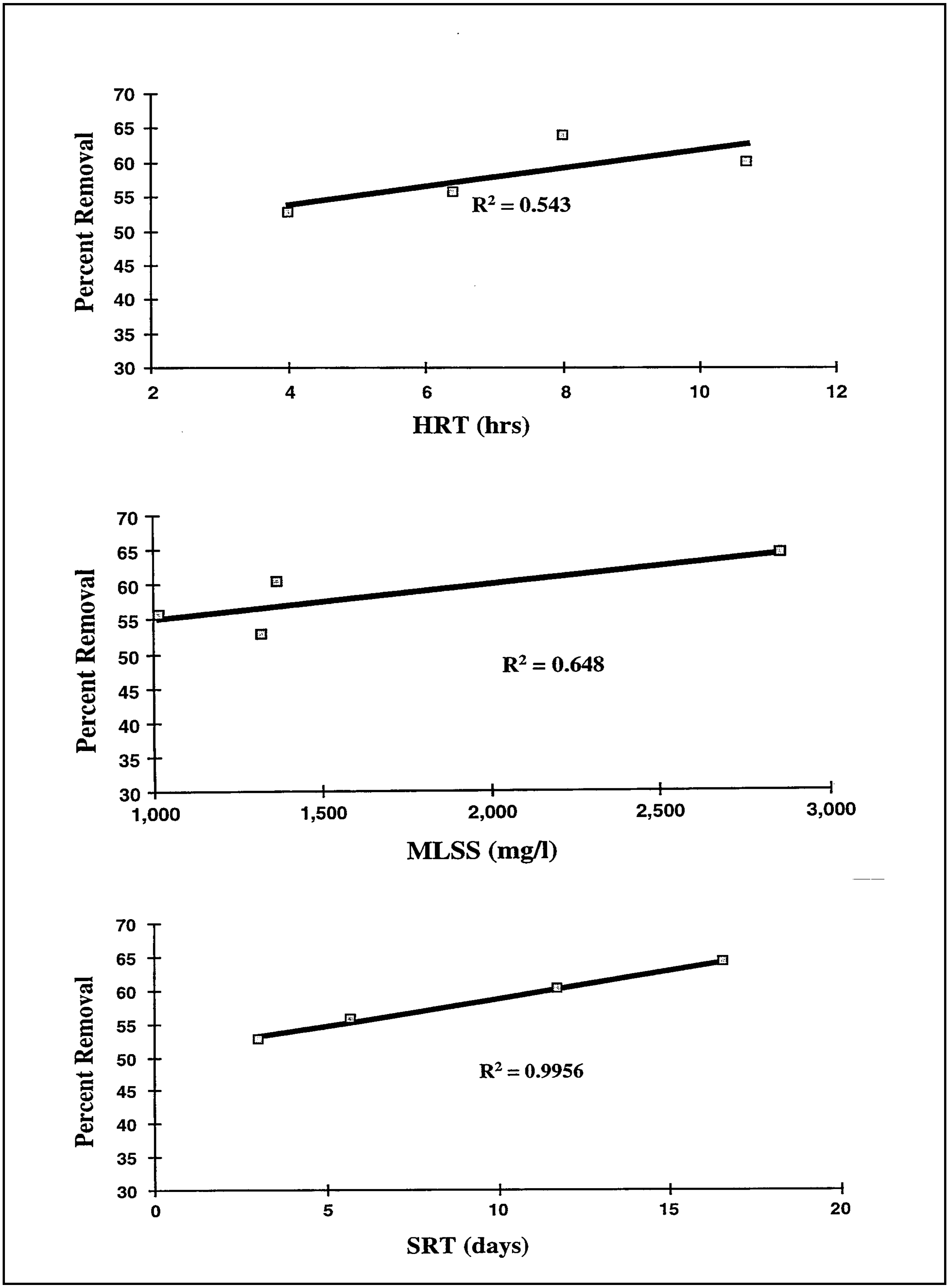

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Appendix I

Pretreatment Program Chemical Review

Introduction

It may be possible in limited cases to identify the toxic influent sources by comparing pretreatment program data on suspected sources to chemical-specific and toxicity data on the POTW effluent. The objective of the PPCR is to determine the sources of toxicity by comparing chemical data on industrial dischargers to toxicity data reported in the literature. The pretreatment program information should include flow and chemical monitoring data on the industrial users, descriptions and schedules of industrial production campaigns, and inventories of chemicals used in production. The final outcome of this review should be an improved understanding of the industries' processes and chemical usage, and the possible identification of sources of toxicity. Source identification through the PPCR approach has been successful in reducing effluent toxicity at POTWs with a limited number and type of industrial inputs (Diehl and Moore, 1987).

General Procedure

The main steps in a PPCR are to:

- Gather the pertinent pretreatment program data
- Compare the data to POTW effluent toxicity results and/or TIE data
- Identify potential influent source(s) of toxicity
- Evaluate and recommend a toxicity control option(s).

A brief description of each of these steps follows.

Collect Data on Industrial Users

Data on all categorical, significant non-categorical and other potential toxic dischargers (e.g., industrial users with local limits and RCRA and CERCLA inputs) should be collected. A list of pertinent information that should be considered in a PPCR is presented in Table 2-3. The data collection effort should include a survey of each industrial user, using the example checklist shown in Table I-1.

Information on chemicals that may be used in manufacturing processes can be obtained from the *Encyclopedia of Chemical Technology* (Kirk-Othmer, 1982). Although OSHA regulations require that information on hazardous chemicals is to be made available to the public on MSDSs, information on various "specialty" chemicals can be difficult to obtain. When data on a "specialty" chemical are not disclosed, a literature review can be performed to determine the chemical's acute toxicity and biodegradability. This information allows assumptions to be made concerning the biodegradability of the chemical at the POTW and the potential for the chemical to cause effluent toxicity. An initial indication of the possible toxic pollutants causing effluent toxicity can be made by comparing expected or actual effluent concentrations to toxicity values provided in the literature.

Compare PPCR Data to POTW Effluent Toxicity Results

Information on the magnitude, variability, and nature of the POTW effluent toxicity can be compared with the PPCR data to determine the sources(s) of possible problem chemicals. This comparison can be made using statistical analyses to determine if the variability in the source characteristics can be related to the variability in the POTW effluent toxicity. A description of data analysis techniques for comparing POTW and industry pretreatment data follows.

Two types of statistical analyses can be used to compare the pretreatment program chemical data and POTW effluent toxicity data: linear regression (Draper and Smith, 1966) and cluster analysis (Pielou, 1984; Romesburg, 1984).

Table I-1. PPCR Data Sheet

Industry Name
Notes:

Address
Notes:

Industrial Category (SIC Code)
Notes:

TRE Objectives
Notes:

Manufactured Products
Notes:

Chemicals Used
Notes:

Amounts (write on MSDS)
Notes:

MSDS _____ All Attached _____ Partial Available

Process in which chemical is used (write on each MSDS)
Notes:

Aquatic toxicity/biodegradability information on all chemicals used. Review MSDS, supplier information, and literature _____ None _____ Some
Notes:

Engineering drawings of facility
Notes:

Production flowchart and line schematic _____ Available _____ No
Notes:

All floor and process drains with schematic _____ Available _____ No
Notes:

Wastewater pretreatment system schematic _____ Available _____ No
Notes:

Facility records
Notes:

Water usage, water bills _____ Available _____ No
Notes:

DMRs for 24 months _____ Available _____ No
Notes:

Pretreatment system operations data _____ Available _____ No
Notes:

Pretreatment system operator interview _____ Available _____ No
Notes:

Spill prevention control plan _____ Available _____ No
Notes:

RCRA reports, hazardous waste manifests _____ Available _____ No
Notes:

Linear regression analysis is used to find correlations among the variables in the data base and to relate changes in POTW effluent toxicity to the variables. A cluster analysis using pattern recognition software can weigh and evaluate the significance of toxics/toxicity correlations. The determination of concentration /response relationships through statistical analysis should not be considered as a definitive answer to toxicity tracking because of the complexity of the factors contributing to toxicity in POTW effluents.

The following example illustrates how a stepwise linear regression technique can be used in a PPCR assessment. The technique is used to identify how changes in several variables can impact the presence and variability of effluent toxicity. Table I-2 presents an example data sheet for a POTW serving one manufacturing plant. In this example, only a few POTW effluent industry variables were used in the linear regression analysis; however, additional variables also could be added in the regression analysis.

The following variables are the "X" variables:

Industry variables:

- LBS is the manufactured product per month (millions of pounds).
- INFLOW is the discharge flow based on water usage (mgd).

POTW effluent variables:

- OFLOW is the recorded effluent flow (mgd).
- COD is the chemical oxygen demand concentration (mg/L).
- BOD_5 is the biochemical oxygen demand concentration (mg/L).
- Cu is the copper concentration (mg/L).
- Cr is the chromium concentration (mg/L).
- Zn is the zinc concentration (mg/L).

The following variable is the "Y" variable:

- LC50 is the acute LC50 as percent effluent.

By applying standard stepwise linear regression, the variables OFLOW, BOD_5, Cr, and Cu were eliminated because they were insignificant to toxicity. Stepwise linear regression showed that the remaining (X) variables were significant as regressed versus (Y) LC50. This analysis indicated that Zn, COD, LBS, and INFLOW were correlated with POTW effluent toxicity.

Identify Source(s) of Toxicity

Based on the data analysis, a list of the possible contributors to effluent toxicity at the POTW can be developed. Sources of suspected toxicants should be selected based on toxicant loading calculations. Industrial users who contribute potentially toxic

Table I-2. Data Sheet for Regression Analysis

Month	Parameter								
	LBS	**INFLOW**	**OFLOW**	**COD**	**BOD_5**	**Cu**	**Cr**	**Zn**	**LC50**
Jan	0.80	1.2	1.0	30	10	0.73	0.02	1.6	20
Feb	1.01	1.5	1.2	33	11	0.61	0.02	1.9	20
Mar	1.20	1.7	1.4	41	15	0.78	0.02	2.0	18
Apr	1.25	1.7	1.5	39	14	0.65	0.02	1.6	18
May	1.16	1.6	1.4	30	12	0.66	0.02	1.5	22
Jun	0.90	1.2	1.0	28	11	0.68	0.02	1.4	30
Jul	0.90	1.2	0.9	25	10	0.71	0.02	1.8	40
Aug	1.20	1.6	1.4	23	9	0.72	0.02	1.9	38
Sep	1.30	1.8	1.6	25	15	0.69	0.02	2.0	40
Oct	1.27	1.7	1.4	26	18	0.72	0.02	2.1	33
Nov	1.10	1.6	1.4	30	17	0.71	0.02	1.9	28
Dec	0.90	1.2	1.0	40	21	0.75	0.02	2.0	22

loadings of suspected toxicants would be candidates for a toxicity control evaluation.

Recommend Toxicity Control Option(s)

Of the potential toxicity control options, toxic chemical substitution or elimination is usually the most pragmatic approach. Thus, a follow-up interview with the toxic discharger(s) should be conducted to develop information concerning techniques for the preferred use of problem chemicals. A list of useful interview questions is shown in Table I-3. These questions may enable the industry to identify problem areas and possible corrective actions in the use of toxic chemicals in manufacturing. Source control may include substitution or elimination of problem chemicals, flow reduction, equalization, spill control, and manufacturing process changes.

Table I-3. Summary of the PPCR Chemical Optimization Procedure

1. Objectives
    a. Optimize chemical usage amounts in production and water treatment processes.

    b. Optimize chemical structures in process chemicals ensuring biodegradability or detoxification is possible.

    c. Establish process controls over incoming raw materials, measuring possible toxic components. Example: corrosion-resistant finish put on steel by manufacturer that must be removed prior to part fabrication.

2. Strategy
    a. Determine the role of each chemical in the process. This is done by supplier interviews and review of data gathered during the initial survey. Ask the questions:

        Can less of this chemical be used?

        Has the optimum amount been determined for each process?

        Do other suppliers offer compounds that will perform as well at lesser concentrations?

        Is the compound in reality a part of the manufacturer's water treatment system and independent of product production?

        OBJECTIVE: Use less chemicals per pound of product produced.

    b. Discover the biodegradability and toxicity of the process chemical. This is done by supplier interview, review of MSDS information, and literature search. Suppliers may not want to supply exact chemical formulations. In this case, ask industry to request supplier to perform tests to develop needed data. Questions to ask:

        What are the components in the product?

        What is its aquatic toxicity?

        Is the product biodegradable?

        What is the rate of biodegradation or half-life?

        Are there other component chemicals on the market that meet manufacturing requirements, but are low in toxicity and highly biodegradable?

        OBJECTIVE: Use chemicals that will not create or contribute to toxicity problems.

    c. Establish process controls over incoming raw materials. Many raw materials have chemicals used in their manufacturing that are removed in the production of the final product. Many raw materials may have trace contaminants that may cause toxic problems. Questions to ask:

        What chemicals are used in the manufacturing of the raw material?

        What are the residual amounts of these raw material contaminants or by-products?

        Are there quality-control procedures that measure the amounts of these chemicals?

        What are the statistical process measures used in the monitoring of these chemicals in the raw materials?

Table I-3. Summary of the PPCR Chemical Optimization Procedure (continued)

If these chemicals are required to be removed before the raw materials can be used in manufacturing the final product, what purpose do the chemicals serve in raw material manufacturing?
Can they be eliminated?

Can they be made less toxic or more biodegradable?

OBJECTIVE: Understand all raw materials being used and encourage development of QA procedures to monitor toxic chemicals removed during processing.

3. Outcome of Investigations
    - a. There will be a list of all chemicals used in processing and manufacturing of products. Included will be the amounts used, why the chemicals are used, and if optimization is being practiced.
    - b. MSDS sheets for all chemicals used will be on file.
    - c. A list of chemicals applied or used in the manufacturing of all raw materials will be on file under that raw material with the residual amounts noted if possible.
    - d. There will be a list of all chemicals and raw materials purchased on a monthly basis and the amount of product produced.

    OBJECTIVE: Hard information to be used in data analysis.

4. Use of opportunities available due to past experience
    - a. With experience in various industries, certain chemicals will become “known” as typically used in some process of manufacturing.
    - b. These known compounds can be categorized and toxicity determinations made. Once found toxic, the first information the industry must supply to the POTW staff conducting the TRE is whether these chemicals are used in its manufacturing process, in raw materials, or in water treatment processes.
    - c. Letters also are sent to raw material suppliers asking if these compounds are used in raw material production. If they are, the supplier is asked to submit prototype alternative raw materials that do not contain these compounds.
    - d. This can be done at the beginning of the TRE for known problem chemicals. Indeed, control regulations also usually involve establishing limits for selected known toxics in industrial operations.
    - e. What is accomplished by this process can be remarkable. First, the supplier is alerted that these compounds can cause his or her customers problems, resulting in a search for an alternative raw material source that is free of these objectionable chemicals. A successful market search reduces the market demand for contaminated or objectionable raw material.

5. Tests to help assess toxicity/biodegradability on speciality formulated chemicals and mixtures and to help evaluate competitive products
    - a. BOD_5, BOD_{20}.
    - b. BOD_5, BOD_{20} performed at LC50 concentration with toxicity test performed on settled effluent from test.
    - c. COD before and after BOD_5, BOD_{20} at LC50, EC50 concentrations.
    - d. Estimate biodegradability by using BOD_5 and COD tests and the calculation $(BOD_5 - COD)/COD \times 100$ of 10 or 20 mg/L solutions of chemical; this can be repeated at a 20-day BOD.
    - e. Biomass inhibition tests (see detailed procedures given in Section 5).
    - f. LC50 on products; screening dilutions 1–10,000 ppm.

    OBJECTIVE: Help industry determine relative biodegradability and toxicity of various raw materials, products, and by-products.

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Appendix J

Refractory Toxicity Assessment Protocol: Step-by-Step Procedures

The following protocol provides step-by-step procedures for designing and executing RTA studies to track sources of acute and/or chronic toxicity in POTW collection systems. This protocol describes the following steps:

- Using characterization data to evaluate waste streams of concern.
- Accounting for toxicity in the activated sludge biomass to be used in testing.
- Adapting and calibrating the protocol to site-specific conditions.
- Collecting and analyzing samples to be used in testing.
- Preparing RTA test mixtures.
- Performing RTA tests.
- Evaluating the inhibitory potential of waste streams.
- Performing TIE Phase I tests on RTA effluents (optional).

The RTA protocol was first developed in the USEPA TRE research study at the City of Baltimore's Patapsco POTW (Botts et al., 1987) to evaluate the potential for indirect dischargers to contribute refractory toxicity. Additional USEPA TRE research studies in Linden, New Jersey; High Point, North Carolina; Fayetteville, North Carolina; and Bergen County, New Jersey were conducted to improve the RTA approach (Morris et al., 1990; DiGiano, 1988; Fillmore et al., 1990; Collins et al., 1991). The RTA protocol described below is a refined version of the method given in the first edition of the Municipal TRE Protocol (USEPA, 1989).

The RTA procedure has been used to track sources of acute and chronic toxicity using both freshwater and estuarine/marine species (Morris et al., 1990; Botts et al., 1992, 1993, 1994). Examples of RTA studies are presented in Appendices C, D, and G. The RTA protocol has been designed to simulate conventional activated sludge processes, although it has also been adapted to other POTW treatment processes including single and two-stage nitrification systems (Collins, et al. 1991), BNR processes (Appendix D), and filtration treatment systems (Appendices C and D).

A. POTW Wastewater Profile

Characterization data are generated for each waste stream to be tested in the RTA.

1. Collect grab samples of RAS and 24-hour composite samples of POTW primary effluent and selected sewer wastewaters (i.e., sewer line wastewater or indirect discharges).

2. Analyze RAS samples (filtrate) for TSS, VSS, NH_3-N, and pH.

3. Analyze primary effluent and sewer wastewater samples for BOD_5, COD, TSS, TKN, TP, NH_3-N, and pH.

4. Determine the type of unit processes, type of discharge (e.g., continuous versus intermittent), operations schedule, and flow rate for the discharge points selected for evaluation (see Section 5).

5. Repeat above steps on several samples to characterize variability over time.

B. Biomass Toxicity Measurement

Biomass toxicity is measured to evaluate the potential for toxicity interferences in the RTA.

1. Collect 5 liters of fresh RAS and aerate vigorously for 15 minutes.
2. Prepare glass fiber filter [same type used for TSS analysis (APHA, 1995) by rinsing two 50 ml volumes of high purity water through the filter.
3. Filter sufficient volume of RAS for two acute or chronic toxicity tests.[1]
4. Centrifuge a portion of the RAS filtrate at 10,000 xg for 10 to 15 minutes. Alternatively, filter RAS filtrate through a 0.2 µm pore-size filter if blank tests show that the filter does not remove soluble toxicity or add artificial toxicity (see Section 5).[1]
5. Test RAS filtrate and RAS centrate/fine filtrate for acute toxicity using procedures described by USEPA (1991a, 1991b) or for chronic toxicity using limited-scale methods provided by USEPA (1992a, 1992b, 1992c, 1995, 1996).
6. Repeat above steps on several RAS samples to characterize variability over time.
7. If RAS filtrate is more toxic than the RAS centrate/fine filtrate, obtain non-toxic biomass (e.g., another POTW biomass or a freeze-dried preparation) (see Section 5).

C. RTA Reactor Calibration Testing[2]

Calibration tests are performed to select the RTA test operating conditions that most closely simulate the POTW operation and performance.

1. As described in Section 5, estimate MLVSS concentration for RTA batch tests using mathematical models (Grady and Lim, 1980; Kornegay, 1970). Alternatively, use the average MLVSS concentration for the POTW.
2. Select a series of MLVSS concentrations (e.g., four) that includes the model MLVSS concentration. Calculate the volumes of RAS (Vr) needed to yield the MVLSS concentrations in the batch reactors. If the RAS was found to be toxic (i.e., RAS filtrate is more toxic than RAS centrate in step B-5 above), also select appropriate volumes of non-toxic biomass (Vnb). An equation for calculating Vb and Vnb is:

$$\text{Vb or Vnb (L)} = \frac{\text{Target MLVSS (mg/L)}}{\text{RAS VSS (mg/L)}} \times \text{Vr (L)},$$

where: Vr is the reactor test volume.

3. Add each RAS volume (Vb and Vnb, if needed) to pre-cleaned glass or clear plastic containers. Add diffused air using air stones and gently aerate. Note that it may be necessary to filter the air supply to prevent contamination (e.g., compressor oil) of the reactor mixed liquors.
4. Add primary effluent (Vpe) to each reactor containing Vb and Vnb. Vpe can be calculated using the following equation:

$$\text{Vpe} = (\text{Vr} - \text{Vb}).$$

5. Adjust aeration rate to maintain DO at concentrations typically observed in POTW activated sludge process. Mechanical mixing using a magnetic stirrer and teflon-coated stir bars may be required to ensure complete mixing. Periodically check and adjust DO level.
6. Periodically check the batch reactor pH. Adjust pH to 6–9 range, if necessary.
7. Periodically collect 50–100 ml samples of batch reactor mixed liquor from each reactor (e.g., 1- to 2-hour intervals).
8. Allow mixed liquor samples to settle for 15 minutes. Rinse glass fiber filters as stated in

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[1] Positive pressure filtering is recommended. Chronic toxicity measurement will require larger filtrate volumes than acute tests.

[2] RTA calibration is recommended. If resources are limited, POTW staff may select test conditions that reflect POTW operating conditions. However, RTA reactor performance should be compared to POTW performance to ensure that the RTA procedure effectively simulates the POTW processes.

step B-2 above. Filter each mixed liquor supernatant using separate filters.

9. Stop aeration after the required reaction period, allow the Vb (and Vnb) to settle for 15 minutes, and filter the clarified batch effluents as described in step C-8.

10. Analyze filtered batch mixed liquor and effluent samples to determine COD removal over time.

11. Decant additional clarified batch effluent for toxicity analysis. Filter each batch effluent using rinsed filters.[1] Wash filter apparatus between each sample filtration using high-purity water.

12. Batch filtrates that were treated with toxic biomass (Vb) must be centrifuged at 10,000 xg for 10 to 15 minutes to remove colloidal size particles. Viscous mixtures may require faster or longer centrifugation (ASM, 1981). Alternatively, the batch filtrates may be filtered through a 0.2 µm pore-size filter if the filter does not remove soluble toxicity (see Section 5).[3] Filter blank analyses should be performed for each filter type using high-purity water.

13. Analyze the batch effluent filtrates, centrates, and filter blanks for acute or chronic toxicity using the procedures referenced in step B-5.

14. Calibration test results can be used to select a batch MLVSS concentration that achieves a level of COD and toxicity removal similar to that provided by the POTW activated sludge process (see Section 5).

D. Sample Collection

Representative samples are collected from each waste stream to be tested in the RTA.

1. Upon completion of the RTA calibration, tests can be conducted to evaluate the refractory toxicity of sewer wastewaters.

2. Obtain 24-hour, flow-proportioned composite samples of sewer wastewater (i.e., sewer line wastewater or indirect discharger effluent) and POTW primary effluent. If possible, lag collection of the primary effluent sample by the estimated travel time of the sewer wastewater to the POTW.

3. Collect 10 liters of RAS (and non-toxic biomass, if needed) on day of test.

E. Sample Characterization (performed on day of sample collection)

Sample characterization data are collected to set the operating conditions for the RTA.

1. Analyze sewer wastewater for BOD_5, COD, TSS, TKN, TP, NH_3-N, and pH.

2. Prepare glass fiber filter as stated in step B-2. Filter RAS and test filtrate for acute or chronic toxicity using the procedures referenced in step B-5.[4]

3. Determine percent volume of sewer wastewater in POTW influent based on flow data gathered in the wastewater profile (step A above).

F. Preparation of RTA Test Mixtures

Two types of batch reactors are prepared: one consisting of the POTW influent (primary effluent) and RAS, which serves as a control, and another consisting of the sewer wastewater spiked into the POTW influent and RAS.

1. Calculate the volume of sewer wastewater (Vw) based on the sewer wastewater flow and the desired flow concentration factor (Fw). Information on selecting an appropriate Fw is presented in Section 5. Vw can be calculated using the following equation:

$$\mathrm{Vw\,(L)} = \frac{\mathrm{Qw}}{\mathrm{Qi}} \times (\mathrm{Vr} - \mathrm{Vb}) \times \mathrm{Fw},$$

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[3] Positive pressure filtering is recommended. Also, chronic toxicity measurement will require larger filtrate volumes than acute toxicity tests.

[4] Positive pressure filtering is recommended. Also, chronic toxicity measurement will require larger filtrate volumes than toxicity tests.

where: Qw is the sewer wastewater flow rate (mgd).
Qi is the average POTW influent flow rate (mgd).
Fw is the sewer wastewater flow concentration factor (e.g., 1, 2, 10 times the sewer wastewater flow).

2. Calculate the volume of primary effluent (Vpe) using the following equation:

$$\mathrm{Vpe} = (\mathrm{Vr} - \mathrm{Vb} - \mathrm{Vw}).$$

3. Prepare spiked batch reactor influent by mixing Vw with Vpe and measure Vpe for control batch reactor influent.

4. If necessary, add nutrients to adjust the $\mathrm{BOD_5}$/TKN/TP ratio of the spiked batch influent to equal the average $\mathrm{BOD_5}$/TKN/TP ratio of the POTW influent (or 100:5:1). An equation for calculating $\mathrm{BOD_5}$, TKN, and TP concentrations in the spiked batch influent is:

$$\mathrm{BOD_5, TKN, or\ TP\ (C, mg/L)} = \frac{(\mathrm{Vpe} \times \mathrm{Cpe}) + (\mathrm{Vw} \times \mathrm{Cw})}{(\mathrm{Vpe} + \mathrm{Vw})},$$

where: Cpe is the $\mathrm{BOD_5}$ or nutrient concentration in primary effluent (mg/L).
Cw is the $\mathrm{BOD_5}$ or nutrient concentration in sewer wastewater (mg/L).

5. If necessary, adjust pH of batch influents to pH range for POTW influent.

6. Test sample toxicity (using methods referenced in step B-5) after nutrient addition and pH adjustment to determine if the batch influent toxicity is changed by these steps.

7. Select volume of RAS (Vb) to yield the MVLSS concentration determined in calibration testing (step C above). If RAS is toxic (i.e., RAS filtrate is more toxic than RAS centrate), also select appropriate volume of non-toxic biomass (Vnb). The equation for calculating Vb and Vnb is provided in step C-2.

8. Add each RAS volume (Vb and Vnb, if needed) to pre-cleaned glass or clear plastic containers.

9. Add spiked batch influent and control batch influent to reactors containing Vb (and reactors containing Vnb, if needed).

G. Performance of RTA Tests

The spiked batch reactor influent and control batch reactor influent are treated and the resulting effluents are tested for toxicity.

1. Add diffused air to reactors using air stones and gently aerate. Note that it may be necessary to filter the air supply to prevent contamination (e.g., compressor oil) of the reactor mixed liquors.

2. Adjust aeration rate to maintain DO at concentrations typically observed in the POTW activated sludge process. Mechanical mixing may be required to ensure complete mixing. Periodically check and adjust the DO level.

3. Periodically check the batch reactor pH and adjust to pH 6-9 range, if necessary.

4. The treatment period for the control reactor should be equal to the average HRT of the POTW aeration system. For the spiked reactor, calculate the required reaction period necessary to achieve a batch F/M ratio (F/Mb) equal to the nominal F/M ratio determined in calibration testing (step C above). F/Mb can be calculated using the following equation:

$$\mathrm{Test\ Period\ (days)} = \frac{\mathrm{Batch\ Influent\ COD\ (mg/L)}}{(\mathrm{MLVSS\ (mg/L)} \times \mathrm{F/Mb}},$$

where: F/Mb is equal to the calculated F/M of the control (primary effluent) reactor.
F/Mb = CODpe/(MLVSS × test period, days).

5. Stop aeration after the required reaction period and allow the Vb (and Vnb) to settle for 1 hour. Decant the clarified batch supernatant for toxicity analysis. Filter each batch supernatant using rinsed filters.[5] Wash filter apparatus between each sample filtration using high-purity water.

6. Batch filtrates that were treated with toxic biomass (Vb) must be centrifuged at 10,000 xg for 10 to 15 minutes to remove colloidal size particles (ASM, 1981). Alternatively, the batch filtrates may be filtered through a 0.2 µm pore size filter if the filter does not remove soluble toxicity (see Section 5).[1] Filter blank analyses should be performed for each filter type using high-purity water.

7. Analyze the batch filtrates, centrates, and filter blanks for acute or chronic toxicity using the procedures referenced in step B-5 above.

H. Synthetic Wastewater Testing (Optional)

Synthetic wastewater can be used in lieu of POTW influent (primary effluent) in the RTA to determine the toxicity of the sewer wastewater.

1. Select non-toxic synthetic wastewater. Confirm that the synthetic wastewater is non-toxic using toxicity test procedures referenced in step B-5 above.

2. Prepare synthetic wastewater solution with SCOD concentration equal to the average SCOD of the POTW primary effluent.

3. Prepare volume of synthetic wastewater (Vsw) equal to the volume of primary effluent (Vpe) used above for the sewer wastewater/primary effluent batch test.

4. Add Vw and Vsw to a reactor containing Vb (and a reactor containing Vnb, if needed).

5. After batch treatment, analyze batch effluent toxicity as described in step G above.

I. Inhibition Testing (Optional)

The RTA protocol can be used to evaluate the inhibitory potential of the sewer wastewater.

1. Add equal volumes of Vb to four reactors. Add diffused air and gently aerate.

2. Prepare a series of four sewer wastewater concentrations (e.g., 100, 50, 25 and 12.5% wastewater) by adding sewer wastewater to toxicity test dilution water (freshwater).

3. If necessary, add nutrients to adjust batch influent BOD_5/TKN/TP ratio.

4. Add sewer wastewater volumes (e.g., Vw100, Vw50, Vw25 and Vw12.5) to the reactors.

5. Adjust aeration rate to maintain DO at concentrations typically observed in the POTW activated sludge process. Mechanical mixing may be necessary to ensure complete mixing. Periodically check and adjust DO level.

6. Periodically check the batch reactor pH and adjust to pH 6–9 range, if necessary.

7. Subsample 300 ml from each reactor at 30 minutes and every 2 hours following test initiation. Immediately measure oxygen utilization using the BOD bottle method (APHA, 1995). Return the subsamples to the reactors immediately following oxygen utilization measurement. Alternatively, oxygen utilization can be measured using respirometric techniques.

8. Subsample 50 ml from each reactor at 5 minutes and every 2 hours following test initiation, and at completion of the test. Also, subsample 50 ml of the original undiluted RAS. Filter the subsamples through a 0.45 µm pore-size filter and measure the SCOD of the filtrates.

9. Calculate oxygen and COD utilization rates, as described in Section 5 of this manual, and plot rates versus sewer wastewater concentration. Lower oxygen and COD removal rates with increasing wastewater concentration may indicate inhibition.

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[5] Positive pressure filtering is recommended. Also, chronic toxicity measurement will require larger filtrate volumes than acute toxicity tests.

J. Phase I Toxicity Characterization (Optional)

1. TIE Phase I tests may be conducted on RTA test effluents using indirect discharger wastewater spiked into primary effluent. Additional volumes are required for TIE Phase I testing; therefore, the batch reactor volume will need to be increased accordingly (USEPA 1991a, 1992a, 1996).

2. TIE Phase I tests should be performed on effluent filtrates from RTA tests that use non-toxic POTW biomass.

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