Technical Report

An Assessment Of The Microtox™ Toxicity Analyzer As A Screening Test For Acitvated Sludge Wastewater Treatment Plant Influents

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ABSTRACT

Many municipal activated sludge wastewater treatment systems must treat industrial discharges as well as domestic sewage and therefore have the potential problem of dealing with toxic pollutants which can inhibit and upset biological treatment systems. Monitoring influent waste streams becomes an important aspect of treatment plant operations where toxicity is a problem, but specific chemical analyses are a costly process. A more cost effective alternative to chemical species identification is acute toxicity testing.

This report discusses an examination of some toxicity tests proposed to screen activated sludge wastewater treatment plant influents. The Microtox™, BOD inhibition, and short term oxygen uptake tests were conducted on synthetic wastewaters containing a toxicant. Four categories of toxicants were tested: heavy metals, solvents, pesticides, and refractory organics. The Microtox™ test was the most sensitive. It was followed in sensitivity by the BOD inhibition test. The sensitivity of the Microtox™ test to toxicants was compared to the sensitivity of acitvated sludge to toxicants by comparing reported literature values. Microtox™ was equally or more sensitive to 83% of the chemical compounds. Wastewater treatment plant operators owning Microtox™ were surveyed. They described the Microtox™ test as rapid, easy to conduct, easy to maintain, and inexpensive. It was used at all the surveyed sites as a screening test, usually in a battery approach with other toxixity tests.

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CHAPTER 1

INTRODUCTION

Over 5,500 activated sludge wastewater treatment plants exist in the United States (USEPA 1982). One reason for the popularity of this treatment process is its relatively low land requirement. It is an economically attractive alternative in urban areas, where land availability is limited. Urbanly located activated sludge treatment plants must often treat industrial discharges as well as domestic sewage (Kurz et al. 1984, Slattery et al. 1985). As would be expected, many priority pollutants are found in publicly-owned wastewater treatment works (POTW's) (Burns and Roe Industrial Services Corp. 1982). Toxic pollutants can inhibit and upset biological treatment of wastewater. Upsets of wastewater treatment plants are a source of concern both in the U.S. and abroad (Russell 1983, Murakami 1980, Green 1975).

Pretreatment regulations are a means of preventing the introduction of pollutants in toxic concentrations. Federal pretreatment regulations as stated in 40 CFR 403 are enforced by monitoring specified quantities or concentrations of pollutants or pollutant properties according to industrial subcategory. Specific chemical analysis of wastewater is a costly process (Chapman et al. 1981, Alleman 1986). A more cost effective alternative to chemical species identification is acute toxicity testing (Szal 1985). Used as screening tests, acute toxicity

tests can eliminate non-inhibitory wastewater sources from further testing.

One analysis proposed as an influent toxicity screening test is the Microtox™ Toxicity Analyzer made by Microbics Corporation of Carlsbad, California. Microtox™ measures toxicity by reduction in light output of bioluminescent bacteria exposed to potential toxicants.

The objective of this research was to evaluate the appropriateness of the Microtox™ Toxicity Analyzer as an influent toxicity screening test for activated sludge wastewater treatment. This project assessed the use of Microtox™ as an influent toxicity screening test using published works, laboratory experiments, and wastewater treatment plant questionnaires. Work progressed in the following stages:

1) Discuss and compare Microtox™ and other proposed influent toxicity screening tests using the aforementioned criteria and publications

2) Compare the sensitivity of Microtox™ and activated sludge to toxicants using reported toxic concentrations of chemical compounds.

3) Conduct laboratory experiments to compare the toxic sensitivity of Microtox™ with two other toxicity tests (BOD inhibition and sludge respiration inhibition).

4) Investigate field use of Microtox™ through a questionnaire sent to wastewater treatment plant operators using Microtox™.

CHAPTER 2

BACKGROUND

Toxicity Testing

Specific chemical analysis, in combination with models can provide more complete insight into such things as chemical reactivity, biological availability, physiological and toxicological effects (Stumm and Morgan 1981), but has the following disadvantages (Szal 1985):

1) There is a lack of criteria for all but the most common toxicants.

2) Synergistic and/or antagonistic interactions of compounds in a complex waste cannot be accounted for.

3) Site specifics of receiving waters (pH hardness) cannot be incorporated into most chemical evaluations.

4) Chemical approach relies on the previous identification of toxic components of a waste.

Toxicity tests are the measurement of a test substance which produces a quantifiable poisonous effect on a test organism. Though toxicity tests do not provide information about the mechanisms associated with the fate of pollutants; toxicity tests do provide a prediction of the effects of toxicants on organisms. There are a large number of toxicity tests which use different combinations of effects on organisms. The tests can be classified into two groups: acute tests

which measure short term effects like death; and chronic tests, which measure long term effects such as cancerous growths.

Aquatic toxicity testing is traditionally done by freshwater organism bioassay. Test organisms such as Pimephales promelas (fathead minnows) or Daphnia (water fleas) are exposed to the toxicants for a 24 to 96 hour test period. The number of dead organisms are counted and the results are presented as an LC50, the concentration which is lethal to 50% of the test organisms. Fish bioassays cost $100-500+ dollars to run (Alleman 1986, Branson et al. 1981) and require trained personnel. Bioassays are sensitive and often specified as toxicity tests in discharge permits (Peltier and Weber 1980).

The high cost of fish bioassays is one reason why tier testing of toxicity has been proposed (Builema et al. 1982, Brandes et al. 1984). The first round of tests would include inexpensive toxicity tests and simple chemical analysis. The next round of tests, if required, would be more complicated and costly. With rapid turn around time and better allocation of laboratory resources (Kurz 1984) the tier approach offers less expense and more compliance (Branson et al. 1981). In pretreatment monitoring of industrial discharges to wastewater treatment plants, the first round toxicity test (the screening test) is an inexpensive, relatively crude test used to pretest discharge samples to determine whether more costly fish bioassays are required. An appropriate screening test for wastewater influent of industrial discharges would

have the following characteristics:

1. Inexpensive,
2. Rapid,
3. Operationally simple,
4. Reproducible,
5. Reliable (few false alarms), and
6. Sensitive

These characteristics will be used later in this project as the basis of comparison of various toxicity screening tests. Tests should cost less than $50 per test and should have a turn around time of less than 3 hours. The tests should be operationally simple enough that they do not require specially trained personnel. The tests must be reproducible for results to be meaningful. Correlation to the treatment works is provided by tests which are both reliable and sensitive. Reliable tests do not show toxicity for noninhibitory wastes and sensitive tests do show toxicity for inhibitory wastes.

Numerous tests have been proposed as toxicity screening tests for wastewater influent. Many are adapted either from aquatic toxicology or sanitary engineering process control. Some proposed tests include: bioassays on Lumbricillus rivalis (worms), Tetrahymena pyriformis (protozoa), and Zoogloea ramigera or Nitrobacter (bacteria); assessment of Spirillum volutans bacteria movement or Photobacterium phosphoreum light output; and measurement of dehydrogenase, ATP, respiration, and glucose activities of activated sludge (Green et al. 1974, Slabbert and Morgan 1982, Norberg and Molin 1983, Williamson and Johnson 1981, McElroy 1983, Bulich 1982, Lenhard 1964, Patterson et al. 1969, Arthur

1984, Olah and Princz 1986). The toxicity tests discussed in this study are: dehydrogenase activity, ATP activity, Microtox, and respirometry.

Dehydrogenase Activity

Dehydrogenase enzymes are intracellar enzymes which mediate reactions between electron donors and acceptors during the oxidation of substrate. Measurement of the enzyme activity is measurement of the metabolic activity of the cell itself. In the presence of readily degradable substrate, cell activity is high; in the presence of an inhibiting toxicant, cell activity is low.

Reduced nicotinamide adenine dinucleotide (NADH) is oxidized by a dehydrogenase, forming reduced flavin adenine dinucleotide ($FADH_2$) from FAD at the start of the electron transport chain which is shown in Figure 1 (Lehninger 1982).

Note that the $FADH_2$ can be linked with an electron acceptor instead of ubiquinone (coenzyme Q). The dehydrogenase activity test uses a reducible dye as an electron acceptor. Reduced dye concentration is a measure of the dehydrogenase activity. Some proposed dyes include 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT), 2,3,5 triphenyltetrazolium chloride (TTC), and methylene blue (MBRR). The advantages of the various dyes are described elswhere (Patterson et al. 1969, Jorgensen 1984, Lopez et al. 1986). A spectrophotometer is used to measure the amount of reduced dye present. Some substances, such as metal ions, sulfides, humic substances, hydrogen peroxide can

oxidize the reduced dye, interfering with the test (Lopez et al. 1986). Temperature, pH and oxygen concentration may affect the test results (Lenhard et al. 1964, Lopez et al. 1986).

Dehydrogenase activity measurement has been advocated for use as an influent screening test (Lenhard et al. 1964, Ryssov-Nielsen 1975). Using a spectrophotometer, it produces rapid, measurable responses and easily interpretable results. The equipment and materials are fairly inexpensive and easy to maintain.

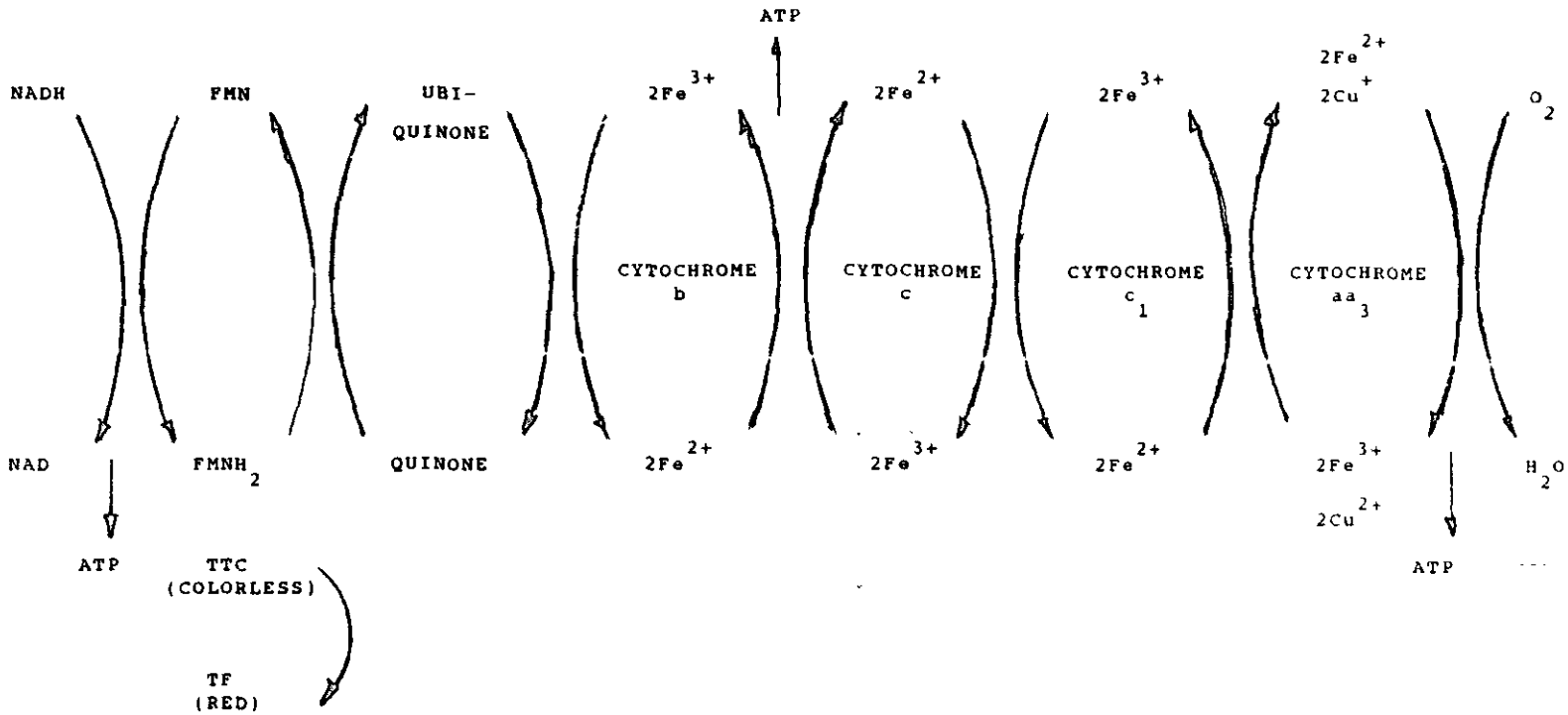
ATP Activity

Adenosine triphosphate (ATP) is an important compound in all cells. It transfers energy needed for cell synthesis and maintenance from substrate. ATP is synthesized by substrate level phosphorylation and oxidative phosphorylation during electron tranfer in the respiration chain as shown earlier (See Figure 1). Measurement of the ATP pool is a measurement of the number of viable cells and the activity of the cells (Patterson et al. 1970).

The test is conducted by first extracting ATP from activated sludge exposed to test material. Next, luciferin and luciferase enzyme are added to the ATP extraction. Finally, the resulting light output is measured after a specified time in a photometer.

In the presence of substrate, the ATP pool increases rapidly (Brezonik and Patterson 1971). In the presence of a toxicant, the ATP

FIG. 1: THE RESPIRATORY CHAIN



WHERE: NAD IS NICOTINAMIDE ADENINE DINUCLEOTIDE
FMN IS FLAVIN MONONUCLEOTIDE
TCC IS TRIPHENYLTETRAZOLIUM CHLORIDE DYE
TF IS TRIPHENYL FORMAZAN

pool decreases. This is caused by either efforts to maintain homeostasis, or reduction in viable cell mass (Patterson et al. 1969).

Because ATP concentration increases after substrate addition, it is difficult to measure ATP pool decreases marking the presence of inhibitory substances in the test material. It is necessary to measure response to toxicants during endogenous phase of cells and without substrate addition (Parker 1982), which is difficult in the case of wastewater because of its variability. Schneider (1987) observed this in an activated sludge pilot plant.

The ATP test is inexpensive after the initial investment and it is rapid. Automation may make it easy to perform (Picciolo et al. 1981). Results are reproducible and appropriate for screening wastewater toxicity (Brezonik and Patterson 1971).

Microtox™

The Microtox™ test consists of measuring the change in light output of the luminescent bacteria Photobacterium phosphoreum when exposed to a toxicant. The test uses constant temperature cooling wells, lyophized test organisms, and prepared osmotic adjustment, dilutent and reconstituion solutions in order to insure reproducible results (Beckman 1982). Results are generally presented as the 5EC50 concentration (i.e., concentration which caused 50% light diminution after exposure of 5 minutes).

Microtox™ has been proposed for a variety of uses including landfill leachate toxicity, tracking and pinpointing sources of industrial toxic sources, toxic levels of oil and gas drilling fluids, and quality control for package foods and medicines (Bulich and Isenberg 1981). Many researchers have proposed it as an influent screening test for wastewater treatment plants (Bulich and Isenberg 1981, Casseri et al. 1983, Dutka and Kwan 1983, Slattery 1983, Kurz et al. 1984).

The test is easy to run. It costs approximately $10-$50 per test (Alleman 1986, Sheehan et al. 1983, Kurz et al. 1984). It produces rapid results and is reproducible (Bulich et al. 1981, Atkinson 1987).

Respirometry

Respirometers make use of the stoichiometry of aerobic conversion of organic matter (Metcalf & Eddy 1979):

$$\underset{\text{matter}}{\text{Organic}} + O_2 + \text{bacteria} \rightarrow CO_2 + NH_3 + \underset{\text{cells}}{\text{new}} + H_2O + \text{energy} \qquad (1)$$

Oxygen is the terminal electron acceptor in the electron transport chain. A measurement of the oxygen used is a measurement of the amount of substrate consumed. Thus, oxygen consumption measures the degradability of the substrate or the health of the microorganisms. Respirometry test results are presented as either the total amount of oxygen consumed, the rate of oxygen consumed (oxygen uptake rate), or

the rate of oxygen consumed per mass of organisms present (specific oxygen uptake rate).

The change in oxygen can be measured directly by either a dissolved oxygen probe or the Winkler method (APHA 1975) titration. Alternatively, oxygen consuption may be measured by recording headspace gas pressure or volume changes in a sealed microbial reactor.

The three types of respiration tests discussed in this section are biochemical oxygen demand (BOD), respirometers, and sludge respiration.

BOD - The BOD_5 test is a sanitary engineering process control test adapted to toxicology. In the presence of a toxicant, the test organism, or seed is inhibited, consuming less oxygen and producing a lower BOD (Ingols 1954). Several experimental variations have been used (Busch 1982). The most common means is comparing calculated BOD_5 for a baseline control and different dilutions of test solutions.

Obtaining results usually takes five days, though some researchers produce 5 day BOD's by correlation with 3 hour data (Schowanek et al. 1987, Arthur 1984). The precision of results is poor (APHA 1975). The BOD inhibition test is, however, inexpensive and easy to perform.

One possible cause for inconsistent results in the BOD_5 test is that the number, type, and mass of microorganisms in the test change over the long test period (Blok 1974). Respirometers minimize the changes in microorganisms by using a larger, already established seed over a shorter test period.

Respirometers - Many different varieties of respirometers exist. Some use organisms in suspended growth (Arthur 1984) or fixed film (Shieh and Yee 1985). Some measure headspace pressure or volume change, while others measure dissolved oxygen change (Montgomery 1967). Respirometers run as batch reactors (Spanjers and Klapwijk 1987), plug flow reactors (Reeves 1976), or continously stirred tank reactors with recycle (Pagga and Gunthner 1981). Some respirometers reported in literature include: Simcar Respirometer (Abson et al. 1967), Robertshaw Respirometer (Reeves 1976), Sapormat (Blok 1976), Biomonitor (Clark et al. 1977), Toximeter (Pagga and Gunthner 1981), Arthur Techline (Arthur 1984), Toxigard (Roesler 1986), and WAZU Respiration Meter (Spanjers and Klapwijk 1987).

Inhibition is calculated by comparing the oxygen uptake rate of a biomass at endogenous respiration rate as baseline to the oxygen uptake rate of a biomass feeding on the test substance. Some researchers use a biomass in high growth phase as the baseline in order to better differentiate between a nonbiodegradable substance and an inhibitory substance (Arthur 1984, Slattery 1985).

Respirometers require trained technicians because of difficulty in operating and obtaining results (Williamson and Johnson 1981, Patterson et al. 1969). The tests are rapid. They can be insensitive if seed is acclimitized or previously upset (Pagga and Gunthner 1981). Respirometers are relatively inexpensive to run (Parker 1982).

Sludge Respiration - The standard sludge respiration test method attempts to eliminate some of the variabitity of respiration tests

(Painter 1986). It is a batch test in which a standard amount of standard synthetic sewage is spiked with different concentrations of test substance and added to aerated standard prepared sludge. Oxygen uptake rate is measured after 30 minutes and after 3 hours. The EC50 is the concentration which causes 50% inhibition of respiration activity.

The standard sludge respiration test is considered reproducible (King and Painter 1986), sensitive (Dutka et al. 1983), inexpensive, simple and rapid.

CHAPTER 3

EXPERIMENTAL METHODS

Literature Review

The objectives of the literature review were twofold. First, the literature review provided information necessary to compare proposed toxicity screening tests using the previosly established criteria. Second, the literature review provided data necessary to compare relative toxicities of chemical compounds to activated sludge and Microtox™.

Comparison of Toxicity Screening Tests - This project first compared the toxicity tests outlined in the previous chapter using the screening test criteria. Published articles provided opinions on the characteristics of the different tests. The results, recognized as secondary sources, yielded the "yes" or "no" answers to the questions posed by the criteria. The results of this work are presented in Table 3.

Comparison of Toxic Sensitivity of Activated Sludge and Microtox™ Published reports also gave information used to better assess whether Microtox™ is sensitive enough to measure inhibitory influents. The project compared reported concentrations of chemical compounds which were toxic to both the activated sludge process and Microtox™. The

comparison used the activated sludge threshold of inhibition concentration and the Microtox™ 5EC50 concentration.

Researchers report a wide range of activated sludge inhibition threshold concentrations. One reason is that several factors cause activated sludge toxic inhibition to vary from plant to plant and within plant including: temperature, pH, substrate concentration, and presence of complexing agents in the plant influent; age, concentration, population distribution, and previous exposure history of the plant biomass (Schneider 1987). Another reason for the wide range is that different researchers were looking at the toxicity to different aspects of the activated sludge process, such as carbonaceous material removal, nitrification, and subsequent anaerobic digestion of waste activated sludge (Russell et al. 1982).

Researchers also report a wide range of Microtox™ 5EC50's. Though reproducibility within laboratories has been good, reproducibility between laboratories has not been good (Dutka et al. 1983). Ansar Qureshi et al. (1987) is currently investigating this.

This project presented two comparisons of the toxic sensitivity of activated sludge and Microtox™. First, chemical compounds were listed according to the categories: 1)chemicals more toxic to activated sludge than Microtox™; 2)chemicals more toxic to Microtox™ than activated sludge; and 3)chemicals equally toxic to both activated sludge and Microtox™. Second, the activated sludge inhibiting concentrations were graphed against the lowest reported Microtox™ 5EC50 concentrations.

Laboratory Comparison of Microtox™, BOD_5, and Sludge Respiration Tests

Microtox™, BOD_5 inhibition, and sludge respiration experiments were conducted on chemical compounds of pollutant classifications found in municipal wastewater. Table 1 lists the chemical compounds. Each experiment was run using the Beckman (1983) reported 5EC50 concentrations in each test. The results provided a comparison of the the relative sensitivity of the three tests.

Microtox™ - The Microtox™ experiments were run according to the standard procedure outlined in the Beckman Microtox™ System Operating Manual (1982). Test samples were prepared with distilled water and osmotically adjusted using the Microtox™ Osmotic Adjusting Solution (MOAS). Sample dilutions were prepared by the 2:1 serial dilution method. Freeze-dried bacteria were reconstituted and equilibrated for 15 minutes before addition to the test cuvettes for another 20 minutes of equilibration. Initial light output was recorded on the strip chart before transfering test samples to test cuvettes. Light output was measured again 5 and 15 minutes after the introduction of the toxicant. If the initial light output readings of any test cuvettes was not between 80 and 100, or if the two blank ratios differed by more than 0.02, then that test was aborted.

Results were presented in a graphical form by plotting log

TABLE 1: LIST OF TOXICANTS

TOXICANT	SALT/SOLUTION	SUPPLIER
Heavy Metals:		
Hg(II)	$HgCl_2$	Fischer
Cu(II)	$CuCl_2 \cdot 2H_2O$	Mallinckrodt
Zn(II)	$ZnSO_4 \cdot 7H_2O$	Mallinckrodt
Cr(IV)	$K_2Cr_2O_7$	Mallinckrodt
Organic Solvent:		
Chloroform	Acetone	Fischer
Pesticides:		
Endrin	Acetone	USEPA
Malathion	Acetone	Aldrich
Priority Pollutants:		
Nitrobenzene	Water	Aldrich
Trichloroethylene	Acetone	Fischer

concentration vs. log Gamma. Gamma is the light loss divided by the light remaining.

$$\Gamma = \frac{R(t)I(o) - I(t)}{I(t)} \qquad (2)$$

Where: R(t) is mean blank ratio at time t
I(o) is initial light reading
I(t) is final light reading

The percent light loss, %Δ is calculated with Gamma:

$$\%\Delta = \frac{\Gamma}{1 + \Gamma} \qquad (3)$$

The concentration where Gamma is 1 and the percent light loss is 50% is the 50EC.

Fill and Draw Reactor - A 4 liter constantly aerated fill-and-draw reactor provided constant seed organisms for the BOD_5 inhibition test and the modified respiration test. Each day, 800 ml of mixed liquor was wasted and 800 ml of synthetic sewage was fed resulting in a 5 day solids retention time. The synthetic sewage feed solution was a mixture of glucose, phosphate and carbonate buffers, ammonia, nutrients and unchlorinated tap water. Daily, the effluent clarity was visually inspected. Table 2 shows the chemical constituents of the feed solution. While conducting the experiments, the fill and draw reactor's mixed liquor volatile suspended solids (MLVSS) and chemical oxygen

TABLE 2: FEED SOLUTION FOR FILL-AND-DRAW REACTOR

FEED COMPONENT	CONCENTRATION (MG/L)
Glucose	500
Phosphate Buffer	
A. KH_2PO_4	527
B. K_2HPO_4	1070
$NaHCO_3$	375
NH_4SO_4	125
$MgSO_4 \cdot 7H_2O$	50
$CaCl_2$	5
$FeCl_3$	5
Yeast	10

demand (COD) were monitored. Those tests were conducted according to standard methods (APHA 1975, APHA 1985).

BOD_5 Inhibition - The test procedure was run according to standard methods (APHA 1975). Seed, test solution, and dilution water were incubated in 300 ml bottles at 20°C for 5 days. The fill and draw reactor provided the seed. Dissolved oxygen concentrations were measured using the azide modification of the Winkler method. The BOD was calculated according to the formula (Sawyer and McCarty 1978):

$$BOD = \frac{(DO_b - DO_i)\text{Vol. of bottle}}{\text{Vol. of sample}} - (DO_b - DO_s) \qquad (4)$$

Where: DO_b is the dissolved oxygen in the blank
DO_i is the dissolved oxgen in the sample
DO_s is the dissolved oxygen initially present

Test samples were the synthetic feed solution spiked with a concentration of toxicants to provide the Microtox™ EC50 concentration. In each test, three series were run concurrently: two were the test samples; one was the blank of unspiked synthetic sewage. For each series, the bottles containing dilution water control (DWC), blank at time zero, blank at time 5 days, and dilutions of 1 ml, 2 ml, 5 ml, 10 ml were prepared. The test sample was inhibitory if either: 1) the residual dissolved oxygen of greater dilution volumes (e.g., 10 ml) were

greater than the residual dissolved oxygen of lesser dilution volumes e.g., 5 ml), or 2) if the calculated BOD_5 of greater dilution volumes (e.g., 10 ml) were less than the BOD_5 of lesser dilution volumes (e.g.,5 ml).

Tests were invalid if the dissolved oxygen concentration of the DWC was more than 1 mg/l less than the initial blank (Sawyer and McCarty 1978). Samples which did not show an oxygen depletion of more than 2 mg/l were not used for results.

Inhibition occurred if the dissolved oxygen concentration of the greater dilutions exceeded those of the lesser dilutions. A second means was to examine the BOD_5 of the different dilutions. If greater dilution volumes showed lower BOD_5's than the lesser dilution volumes than there was inhibition. Percent inhibition, %Δ, was calculated according to the formula:

$$\%\Delta = \frac{BOD_{2ml} - BOD_{5ml}}{BOD_{2ml}} \qquad (5)$$

Where: BOD_{2ml} is the BOD calculated for 2 ml dilution bottle.
BOD_{5ml} is the BOD calculated for 2 ml dilution bottle.

Sludge Respiration Test - This test differed from the BOD test in that it had a constant amount of biomass and no lag phase. It was run similarly to the standard sludge respiration test (Painter 1986) except

a smaller, less concentrated biomass was used. This test simulated the reactor shortly after feeding.

The test series was synthetic sewage spiked to provide the toxic concentration used in the Microtox™ test. The control, or baseline, was unspiked synthetic sewage.

The test consisted of adding 72 ml of spiked or unspiked synthetic sewage to 288 ml mixed liquor from the fill and draw reactor and placing the mixture immediately in 60 ml BOD bottles. Dissolved oxygen was measured by the Winkler method at time zero and each 30 minutes thereafter for two hours. The method required sacrificing a BOD bottle at every reading.

The spiked sewage series' 30 minute oxygen uptake rate was then compared to the baseline series' 30 minute oxygen uptake rate. The test samples which had a lower oxygen uptake rate than the baseline exhibited inhibition. Results were presented as percent inhibition, (%Δ) as follows:

$$\%\Delta = \frac{OUR_b - OUR_t}{OUR_b} \qquad (6)$$

Where: OUR_b is Oxygen Uptake Rate of Baseline
OUR_t is Oxygen Uptake Rate of Test Substance

Survey of Wastewater Treatment Plants Using Microtox™ In The United States

A survey of publicly owned treatment plants in the United States using Microtox™ was conducted in order to find out whether Microtox™ was used as toxicity screening test and why. A copy of the survey is included in Appendix B.

Microbics Corporation graciously provided a list of the wastewater treatment plants owning a Microtox™ Toxicity Analyzer. A questionnaire was mailed to treatment plant operators. This was followed up with phone calls to obtain more information, if necessary.

CHAPTER 4

RESULTS AND DISCUSSION

Literature Review

The literature review accomplished two tasks: comparing several proposed toxicity screening tests and comparing the toxic sensitivity of Microtox™ with activated sludge.

Comparison of Toxicity Screening Tests - The comparison of proposed toxicity screening tests showed that no one test was completely satisfactory in all categories (Table 3).

The dehydrogenase activity test was unreliable when the dye was toxic itself or when the dye was reactive with a reducing agent (Patterson et al. 1969). Though some researchers stated that dehydrogenase activity measurement was a sensitive toxicity test (Ryssov-Nielsen 1975, Patterson 1969, Lopez et al. 1985), one researcher said that it was not (Klapwijk et al. 1974).

ATP pool measurent was considered an expensive test by one researcher (Parker 1982). The test was unreliable because it produced false toxicity reading immediately after sludge was fed highly degradable substrate (Schneider 1987). Schneider (1987) also noted that ATP activity test was not sensitive to nickel toxicity.

TABLE 3: LITERATURE COMPARISON OF PROPOSED TOXICITY SCREENING TESTS

TEST	INEXPENSIVE	RAPID	OPERATIONALLY SIMPLE	REPRODUCIBLE	RELIABLE	SENSITIVE	SOURCES
Dehydrogenase activity	+	+	+	+	-	+/-	1-6
ATP activity	-	+	+	+	-	+/-	7-9,14
Microtox™	+	+	+	+/-	+/-	+	9-13
Respirometer	+/-	+	+/-	+	+/-	+	14-20
BOD	+	-	+	+/-		+/-	14,16,17, 21,22
Sludge Respiration	+	+	+	+	-	+	23,24

SOURCES

1. Lenhard 1964
2. Ford et al. 1966
3. Ryssov-Nielsen 1975
4. Patterson et al. 1969
5. Lopez et al. 1985
6. Klapwijk et al. 1974
7. Brezonik and Patterson 1971
8. Picciolo et al. 1981
9. Schneider 1987
10. Sheehan et al. 1984
11. Bulich 1982
12. Dutka and Kwan 1981
13. Kurz et al. 1984
14. Parker 1982
15. Summers and Sion 1981
16. Montgomery 1967
17. Williamson and Johnson 1980
18. Blok 1974
19. Pagga and Gunthner 1981
20. Arthur 1984
21. Mowat 1976
22. Busch 1982
23. Painter 1986
24. King and Painter 1986

Dutka and Kwan (1981) questioned the Microtox's reproducibility between different laboratories because of the wide range of reported EC50 concentrations One researcher thought the Microtox™ may be too sensitive, producing toxic reponses to noninhibitory substances (Schneider 1987). Bulich (1982) noted that the sensitivity of the test was variable depending on the data reduction method.

Reseachers had differing opinions on respirometry. This may be due to the differnent devices and operating procedures.

Researchers also had differing opinions on the BOD toxicity test. Again this may be due to the different test procedures. The BOD test is usually run for 5 days making it a slow test. One researcher even suggested incubating the bottles for 2 weeks (Mowat 1976). No reports were found stating that the BOD inhibition test can give false toxicity results, though one researcher described chromium interfering with the dissolved oxygen measurement and giving a false reading (Stones 1962).

The standard sludge respiration test was a recent test procedure which had not been investigated thoroughly. One researcher noted that the test procedure occasionally gave false toxicity results (King 1986). In a comparison with Microtox™, sludge inhibition was not as sensitive to toxicity as Microtox™ (Dutka et al. 1983).

Each toxicity test had its limitations. For that reason, some researchers suggested a battery approach to toxicity testing (Dutka and Kwan 1983, Schneider 1987). Others suggested using a sensitive test as the first step in a tiered toxicity protocol (Roesler 1986, Branson 1981).

Comparisons of Toxic Sensitivity of Activated Sludge and Microtox™

Comparison of reported chemical toxicities to activated sludge and

Microtox™ showed that most chemicals were approximately of equal toxicity to both. Table 4 lists chemicals in three categories according to relative toxicity. (Appendix A lists the reported concentrations of activated sludge threshold of inhibition and Microtox™ 5EC50 used in the comparisons. Appendix A also supplies the references for the concentration.) The largest category was the one of chemical compounds equally toxic to both activated sludge and Microtox™. Microtox™ would be an appropriately sensitive toxicity test for influents containing chemicals in this category. Many compounds were in this category because of the wide range of reported threshold of inhibition to activated sludge and the wide range of Microtox™ EC50's.

The literature survey placed 21 percent of the chemicals in the category of chemicals more toxic to Microtox™ than activated sludge. Chemicals in this category, present in wastewater influents, would cause false toxicity readings during screening tests. This is not desirable as possibly noninhibitory influents would require further testing.

Even less desirable is potentially inhibitory influents passing through screening tests without toxic readings. Chemicals in the category of more toxic to activated sludge than Microtox™ may possibly enter treatment plants at inhibitory concentrations without detection by Microtox™. Sixteen percent of the chemicals in the survey were in this category.

Several modifications of the Microtox™ test procedure may improve sensitivity. Changing the test exposure time from 5 minutes to 15 minutes increases the sensitivity of Microtox™. Calculating the

TABLE 4: LISTING OF CHEMICAL COMPOUNDS BY REPORTED TOXICITY TO ACTIVATED SLUDGE AND MICROTOX™

Chemical Compounds More Toxic To Activated Sludge	Chemical Compounds More Toxic To Microtox™	Chemical Compounds Equally Toxic To Both
Chloroform	Naphthalene	Benzene
Dimethylformamide	Carbon Tetrachloride	2,4,6-Trichlorophenol
2,4-Dinitrotoluene	Ammonia (Free)	2-Chlorophenol
Methylene Chloride	Acrolein	1,2-Dichlorobenzene
2,4-Dinitrophenol	1,1,1-Trichloroethane	1,3-Dichlorobenzene
Arsenic	Hexachloroethane	1,4-Dichlorobenzene
Nickel	1,1,2,2-Tetrachloroethane	2,4-Dichlorophenol
	para-Chloro-meta-Cresol	2,4-Dimethylphenol
	Arochlor-1242	Nitrobenzene
		Pentachlorophenol
		Phenol
		Toluene
		3,5-Dichlorophenol
		Trinitrotoluene
		Malathion
		Cadmium
		Chromium(VI)
		Chromate(III)
		Copper
		Cyanide
		Lead
		Mercury
		Silver
		Zinc
		Aluminum
		Ammonia (Free)

concentration which causes 25% inhibition (5EC25) instead of the 5EC50 also makes the Microtox™ a more sensitive test.

Laboratory Comparison of Toxicity Screening Tests

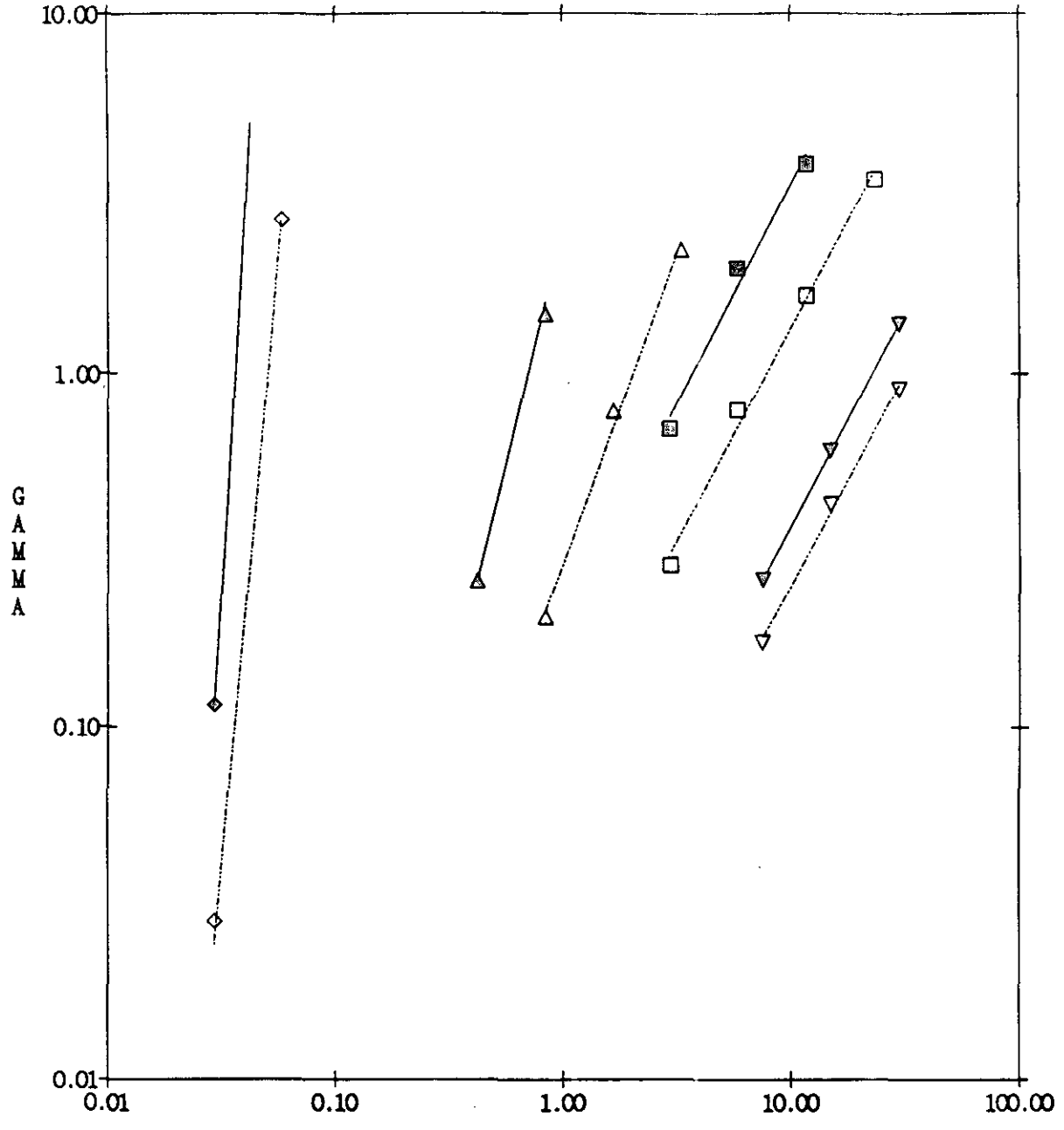
Microtox™ - In general, Microtox™ produced results comparable to the expected Beckman (1983) 5EC50 concentrations. Figures 2 - 5 show the results for the four classes of potential pollutants in wastewater. Table 5 summarizes the results. Both 5 minute and 15 minute EC50 concentrations were presented as suggested by Beckman (1981) and Sellers (1985).

Comparing the laboratory Microtox™ test results with literature reported activated sludge inhibitory thresholds, it appeared Microtox™ was not sensitive enough to measure potential toxicants at inhibitory concentrations for the chemicals tested. Table 6 presents reported activated sludge thresholds of inhibition concentrations and Microtox™ 5EC50 concentrations. Changing the test exposure time from 5 minutes to 15 minutes improved toxic sensitivity of Microtox™. Changing the percent inhibition from 50% to 25% also improved the toxic sensitivity of Microtox™. Using the 5 or 15EC25 concentration, Microtox™ was a sensitive enough toxicity test.

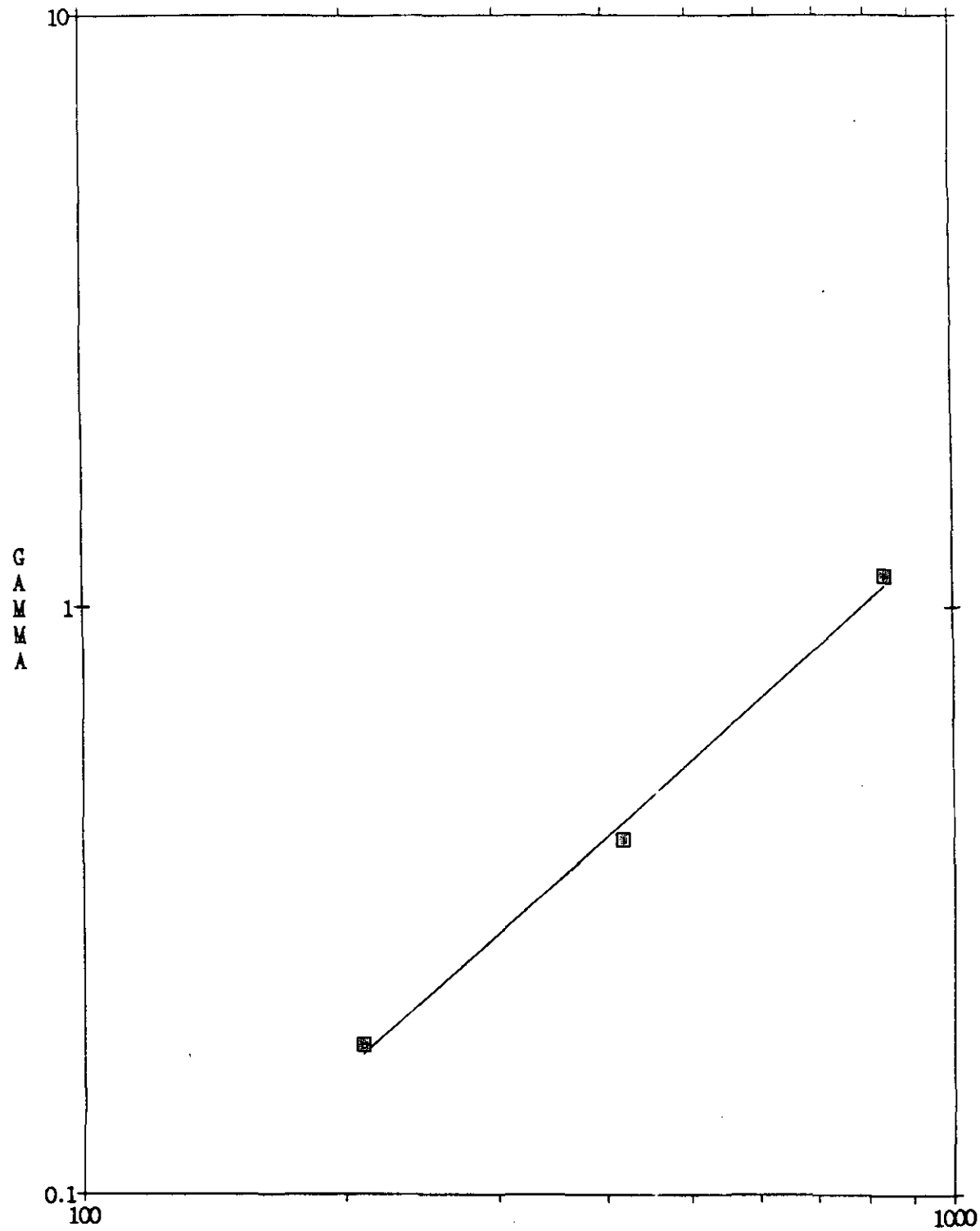
BOD_5 Inhibition - As mentioned earlier, the BOD inhibition test data were examined in two manners. First, the residual dissolved oxygen of each dilution volume was compared, inhibition being exhibited when there was a higher residual dissolved oxygen in a bottle containing a higher sample volume after the 5 day incubation. For instance, a test

TM

FIG 2: MICROTOX RESULTS FOR HEAVY METALS



TM
FIG. 3: MICROTOX RESULTS FOR ORGANIC SOLVENTS



TM
FIG. 4: MICROTOX RESULTS FOR PESTICIDES

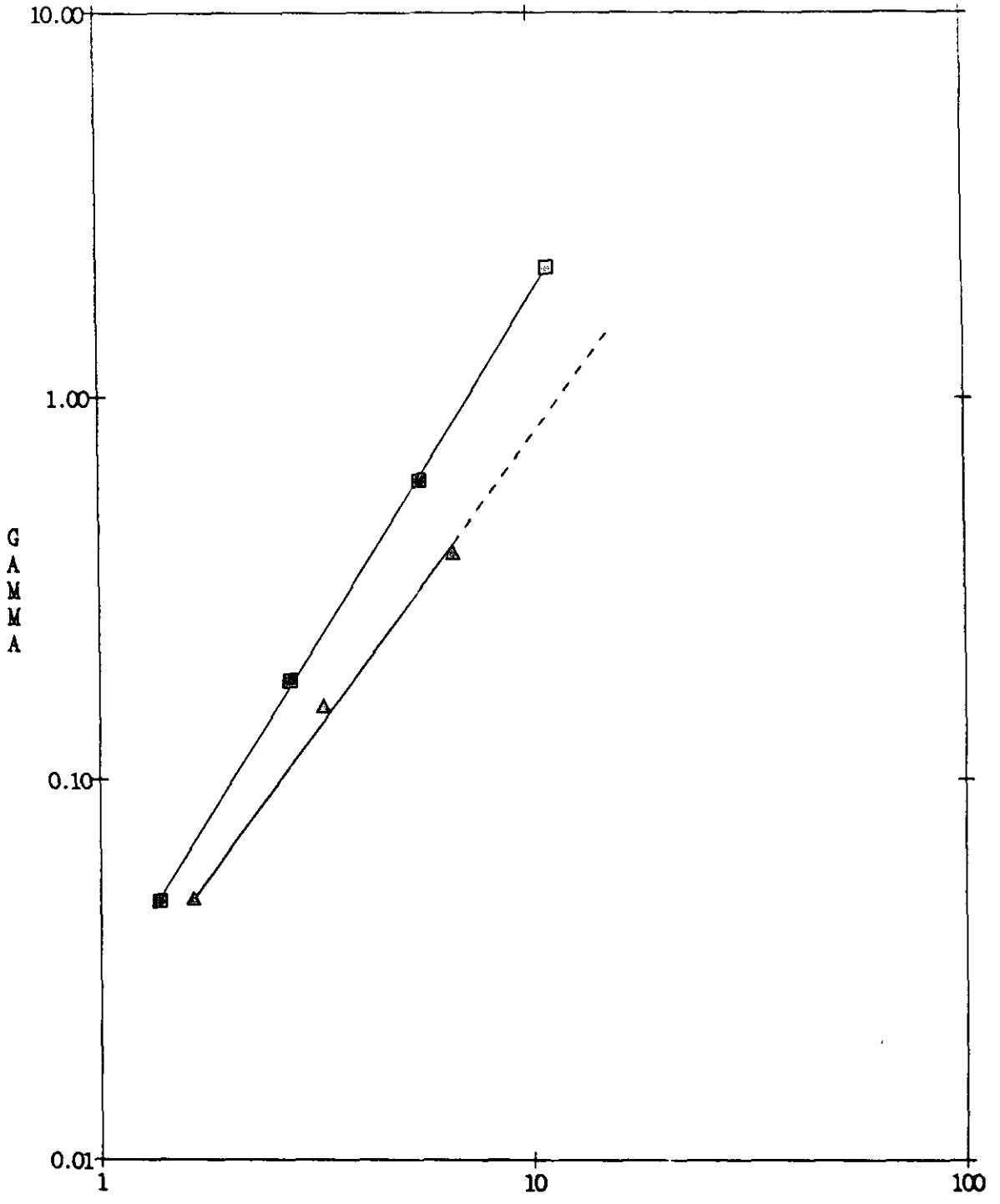


FIG. 5: MICROTOX™ RESULTS FOR PRIORITY POLLUTANTS

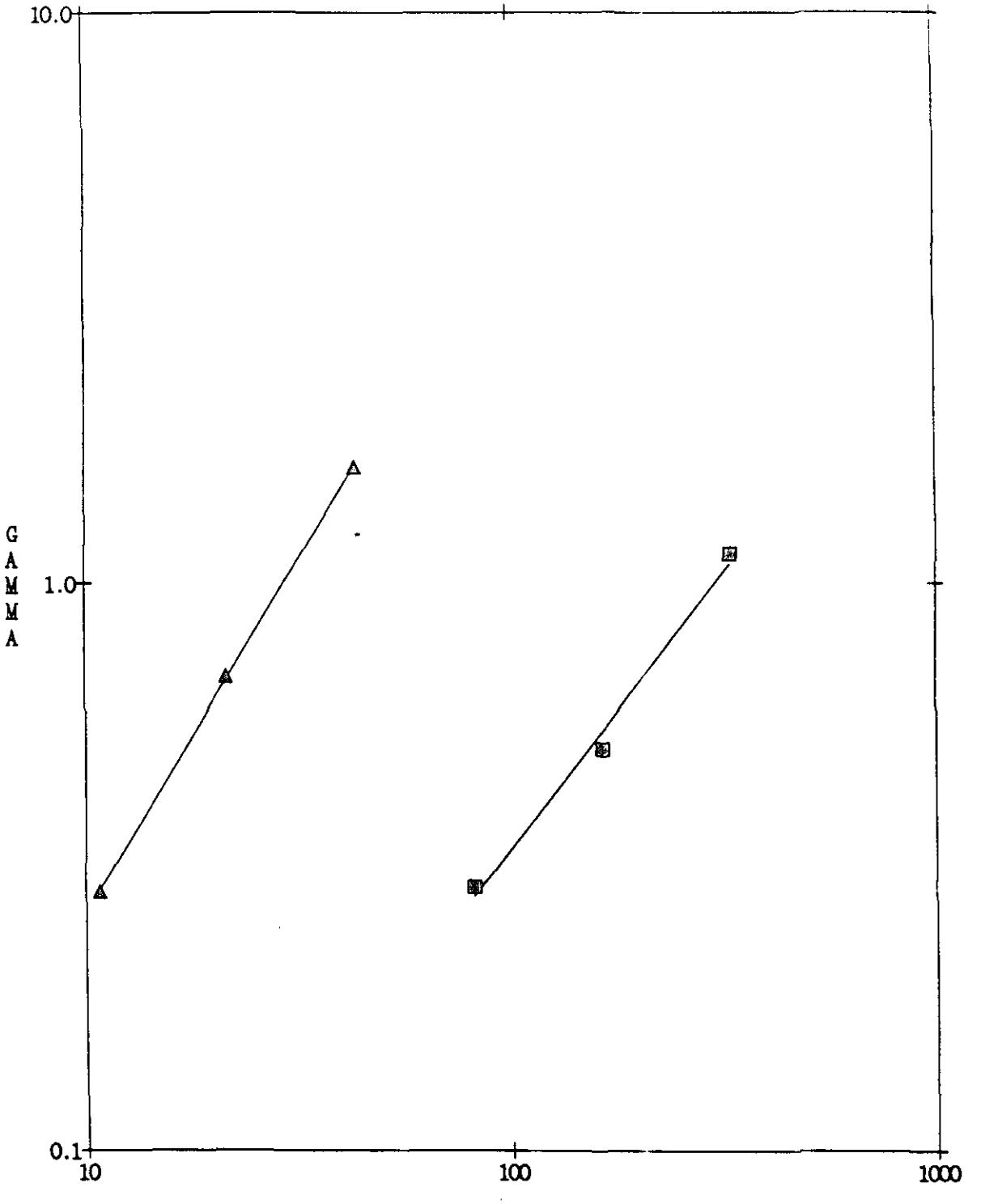


TABLE 5: MICROTOX™ LABORATORY RESULTS

TOXICANT	5EC50 (MG/L)	15EC50 (MG/L)
COPPER(II)	2.0	0.70
MERCURY(II)	0.051	0.036
ZINC(II)	7.8	3.7
CHROMIUM(VI)	32.	23.
CHLOROFORM	790[a]	-
ENDRIN	12.[a]	-
MALATHION	7.2	-
TRICHLOROETHYLENE	300	-
NITROBENZENE	29.	-

a. limit of solubility

TABLE 6: COMPARISON OF LITERATURE ACTIVATED SLUDGE INHIBITORY CONCENTRATIONS WITH LABORATORY MICROTOX™ TOXICITY CONCENTRATIONS USING DIFFERENT TEST MODIFICATIONS

TOXICANT	ACTIVATED[d] SLUDGE (MG/L)	5EC50 (MG/L)	15EC50 (MG/L)	5 or 15EC25[c] (MG/L)
COPPER(II)	0.05-2.	2.0	0.70	0.46
MERCURY(II)	0.1-5.	0.051	0.036	0.033
ZINC(II)	0.08-0.5	7.8	3.7	1.5
CHROMIUM(VI)	0.25-10.	32.	23.	9.1
CHLOROFORM	10.	790[a]		61.[a]
ENDRIN	---	12.[a]		5.7[a]
MALATHION	100.	7.2		3.9
TRICHLOROETHYLENE	>10.[b]	303		97.
NITROBENZENE	30.-500	29.		12.

a. Results calculated from data at limit of solubility
b. No inhibition at reported concentration
c. 15 minute data for heavy metals; 5 minute data for others
d. Literature values. References are in Appendix A

substance would be inhibitory if the 10 ml dilution volume bottle had a residual dissolved oxygen of 7.0 mg/l while the 5 ml dilution volume bottle had a residual dissolved oxygen of 6.8 mg/l. No toxicants exhibited this behavior. Results are not presented.

Second, the BOD_5 was calculated for each test dilution. Inhibition occurred where greater test volumes (e.g., 10 ml) showed lower BOD than smaller test volumes (e.g., 5 ml). In this test procedure, inhibition was exhibited. Table 7 list the results. The percent inhibition of malathion was equivocal because the control series also exhibited some inhibition, possibly the result of the acetone solvent. The chromium results may reflect some reaction with the Winkler titrant (Stones 1962).

Generally, the BOD inhibition laboratory results were less than literature reported BOD inhibition test results (Mowat 1976, Stones 1962). This was attributed to test procedure. The other two researchers used low substrate concentration which remained constant while the toxicant spike concentration varied. Mowats' test procedure was more sensitive, but required chemical analysis of the wastewater influent in conjucton with the BOD inhibition test. It was therefore not appropriate as a screening test.

Sludge Respiration - Only the toxicant trichloroethylene caused inhibition in this test at the concentration tested. Table 7 lists all the results. Over half the compounds caused stimulation rather than inhibition. Several researchers have observed stimulation at low toxicant doses (Brouzes et al. 1978, Poon and Bhayani 1971, Randall and Lauderdale 1967, Hastings et al. 1985). One possible explanation is that some toxicants can block a competing metabolic pathway before

TABLE 7: COMPARISON OF LABORATORY RESULTS FOR THREE TOXICITY TESTS

TOXICANT	CONCENTRATION (MG/L)	MICROTOX TEST (%Δ)	BOD TEST (%Δ)	RESPIRATION TEST (%Δ)
COPPER(II)	3.5	72	3	-33[a]
MERCURY(II)	0.065	84	17	13
ZINC(II)	26.	81	12	-5
CHROMIUM(VI)	22.	86	31	-23
CHLOROFORM	930.[b]	56	11	-37
ENDRIN	7.3[b]	33	-2	10
MALATHION	12.2	72	42[c]	-3
TRICHLOROETHYLENE	365.	54	-3	27
NITROBENZENE	47.8	65	1	-81

a. Negative values denote stimulation
b. Limit of solubility
c. Blank exhibited 34% inhibition

affecting the measured pathway. Without the competition, the measured pathway's activity would increase (Johnson et al. 1974).

The test modification used in this research project was not as sensitive as the standard sludge respiration test (Dutka and Kwan 1983). The modified test differed from the standard test by having a lower solids concentration and a more dilute test sample. Low solids concentration did not compensate enough for test sample dilution.

The results show this sludge respiration inhibition test is not as sensitive as the other two toxicity tests. One other researcher found that the standard sludge respiration test is not as sensitive as Microtox™ to toxicity (Dutka and Kwan 1983).

Summary Discussion - The Microtox™ test was the most sensitive toxicity test. The Microtox test procedure could be modified to change its sensitivity. The BOD inhibition test followed Microtox™ in sensitivity. In this study, the BOD inhibition toxicity test, run as a screening test, was not as sensitive as other researcher's BOD inhibition toxicity tests. The sludge inhibition test was not as sensitive as the other two toxicity tests.

Survey of Wastewater Treatment Plants using Microtox™

The purpose of this part of the research project was to examine field use of Microtox™. Results of the survey are in Appendix B. The project found 18 wastewater treatment plants possessing a Microtox™ Analyzer. Only one plant was located in New England. The treatment plants used different process types, treated various flows, and treated

different shares of industrial and domestic wastewater. The treatment plants treated wastewater from many of the industrial categories subject to national pretreatment regulations. Wastewater influents contained pollutants from the four classes used in this study in the following percentage:

Heavy Metals	87%
Organic Solvents	80%
Pesticides	53%
Refractory Organics	47%

The percentages of plant operators conducting Microtox™ tests for various uses were:

Toxicity Screening	82%
Effluent Monitoring	76%
Toxicity Reduction Evaluations	53%
Establishing the Treatibility of New Sources	50%
Pollutant Source Identification	24%
Establishing Operating Parameters	12%
Billing	6%

Most treatment plants owning Microtox™ use it for toxicity screening. The percentages of the treatment plants using other toxicity tests (who also use Microtox™) by type were:

Fish Bioassay	40%
Daphnia Bioassay	40%
Respirometry	20%
Selenastrum Algal Assay	13%
Ames Mutagenicity	7%

Other toxicity tests were conducted at treatment plants using a battery approach to toxicity testing. The battery approach recognizes that there is usually more than one type of organism influenced by a

potential toxicants, so more than one test organism may better predict toxicants' effects. The other toxicity tests's uses by percentage of all treatment works were:

Effluent Monitoring	70%
Establishing Treatibility of New Sources	70%
Toxicity Screening	50%
Toxicity Reduction Evaluations	50%
Pollutant Sources Identification	20%

Microtox™ was used more often in toxicity screening tests. Microtox™ users identified the following merits and faults of the test by percentage of respondents:

Merits:	
Quickness	100%
Ease of Operation/Maintenance	86%
Inexpensive	50%
Reproducible Results	33%
Sensitivity	29%
Faults:	
Lack of Government Regulations	80%
Expensive	27%
Time Required to Analyze Results	7%
Sample Preparation	7%
Not Reproducible	7%
No Chronic Test	7%
Difficult ot Interpret Complex Effluents	7%

The Microtox™ test was credited with being quick and easy to use. Most users considered it inexpensive. It was considered reproducible and sensitive by some, but not all users. Its most cited drawback was the lack of government regulation. This is in regards to effluent

monitoring where Microtox™ is rarely allowed as the toxicity test on N.P.D.E.S. discharge permits.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Using the criteria for a good influent wastewater toxicity screening test, the project compared some proposed toxicity tests by reviewing available literature. The dehydrogenase activity test was inexpensive, rapid, easily conducted, reliable, but not reproducible or sensitive. The ATP activity test was inexpensive, rapid, easily conducted, sensitive, but not reproducible or reliable. The Microtox™ was inexpensive, rapid, easily operated, reproducible, reliable, and sensitive. The BOD_5 inhibition test was inexpensive, easily conducted and sensitive. It was not, however, rapid, reproducible or reliable. Respirometers were found to be inexpensive, rapid and sensitive; they were not found to be easily operated, reproducible, or reliable. The sludge respiration test was inexpensive, rapid, easily conducted, reproducible, reliable, and sensitive.

The project also compared reported toxicities of chemical compounds to activated sludge and Microtox™. Activated sludge inhibitory concentrations and Microtox™ toxic concentrations were of the same order of magnitude according to a comparison of published literature

chemical concentrations of activated sludge threshold of inhibition and Microtox™ 5EC50's. Most reported inorganic compounds inhibited activated sludge at lower concentrations than the Microtox™ 5EC50.

Laboratory experiments compared three microbial toxicity tests. Based on the modifications used in these experiments, Microtox™ was the most sensitive of the three tests. The modified BOD_5 inhibition test was generally the next most sensitive test. The modified sludge respiration inhibition test exhibited stimulation for most of the chemicals tested.

The survey of wastewater treatment plant operators using Microtox™ showed that Microtox™ was considered rapid and easy to use. It was considered inexpensive and sensitive by the majority of treatment plant operators.

Recommendations

Recommended future work would be pilot studies using a bench scale activated sludge treatment plant. Microtox™ would be compared to treatment inhibition using different toxicant concentrations, chemical matrices, and operating parameters.

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APPENDIX A

INHIBITION THRESHOLD CONCENTRATIONS TO ACTIVATED SLUDGE BY PROCESS

TOXICANT	ACT. SLUDGE (MG/L)	NITRIFYING BACTERIA (MG/L)	ANAEROBIC DIGESTION (MG/L)	SOURCE
ORGANICS:				
BENZENE	125-880			2,3,10
BENZIDINE	5-500			2,3,4
HEXACHLOROBENZENE	5			3
2,4,6-TRICHLOROPHENOL	50			3
CHLOROFORM		10		3
2-CHLOROPHENOL	20-200			2
1,2-DICHLOROBENZENE	5			3
1,3-DICHLOROBENZENE	5			3
1,4-DICHLOROBENZENE	5			3
2,4-DICHLOROPHENOL	64-75	64		2,3
DICHLOROPHEN			1	1
DIMETHYLFORMAMIDE			400	1
2,4-DIMETHYLPHENOL	40-200			2
2,4-DINITROTOLUENE	5			3
2,6-DINITROTOLUENE	5			3
1,2-DIPHENYLHYDRAZINE	5			3
ETHYLBENZENE	200			2
METHYLENE CHLORIDE			1	1
TRICHLOROFLOUROMETHANE			0.7	1
NAPHTHALENE	500			2,3
NITROBENZENE	30-500			2,3
2,4-DINITROPHENOL	1	150		3,5
PENTACHLOROPHENOL	0.95-50		0.4	1,2,3
PHENOL	50-200	4-10		2,3,4,5
ANTHRACENE	500			3
PHENANTHRENE	500			3
TOLUENE	200			2
3,5 DICHLOROPHENOL	25			7
EDTA	25			4
TRINITROTOLUENE	20-25		60	4,10
NACCONOL	200			4
CEEPRYN	100	25		4,6
CRESOL		4-16		5
MALATHION	100			8
CARBON TETRACHLORIDE	800			10

TOXICANT	ACT. SLUDGE (MG/L)	NITRIFYING BACTERIA (MG/L)	ANAEROBIC DIGESTION (MG/L)	SOURCE
INORGANICS:				
ARSENIC	0.1	1.5		2,3,4,5
CADMIUM	0.5-100	0.02-5	1	1,2,3,4,5
CHROMIUM(VI)	1-10	0.25-10	5-10	1,2,3,4,5,10
CHROMATE(III)	10-50	50		2,3,4,5
COPPER	1	0.005-2	0.7-5	1,2,3,4,5,10
CYANIDE	0.1-5	0.34-4	2	2,3,4,5,10
IRON	5-1000		5	1,4,5,10
LEAD	0.1-10	0.5-10	50-70	1,2,3,4,5,10
MERCURY	0.1-5	1365		2,3,4,5
NICKEL	1-10	0.25-10	40	1,2,3,4,5,10
SILVER	0.25-5			2,3,4,5
ZINC	0.3-55	0.08-0.5	10-20	1,2,3,4,5,10
ALUMINUM	15-26			5
AMMONIA	480			4,5
BORATE	0.05-100			4,5,10
CALCIUM	2500			4
MAGNESIUM		50		5
MANGANESE	10			4,5
SULFATE		500		5
TIN			9	1

NO INHIBITION EXHIBITED TO ACTIVATED SLUDGE BY PROCESS

TOXICANT	ACT. SLUDGE (MG/L)	NITRIFYING BACTERIA (MG/L)	ANAEROBIC DIGESTION (MG/L)	SOURCE
ACENAPHTHENE	10			3
ACROLEIN	62			3
ACRYLONITRILE	152			3
CARBON TETRACHLORIDE	10			3
CHLOROBENZENE	1			3
1,2,4-TRICHLOROBENZENE	6			3
1,2-DICHLOROETHANE	258			3
1,1,1-TRICHLOROETHANE	10			3
HEXACHLOROETHANE	10			3
1,1-DICHLOROETHANE	10			3
1,1,2-TRICHLOROETHANE	5			3
1,1,2,2-TETRACHLOROETHANE	201			3
bis-(2-CHLOROETHYL)ETHER	10			3
2-CHLOROETHYL VINYL ETHER	10			3
2-CHLORONAPHTHALENE	10			3
para-CHLORO-meta-CRESOL	10			3
1,1-DICHLOROETHYLENE	10			3
1,2-trans-DICHLOROETHYLENE	10			3
1,2-DICHLOROPROPANE	182			3
1,3-DICHLOROPROPYLENE	10			3
FLOUROANTHENE	5			3
bis-(2-CHLOROISOPROPYL)ETH	10			3
CHLOROMETHANE	180			3
BROMOFORM	10			3
DICHLOROBROMOMETHANE	10			3
CHLORODIBROMOMETHANE	10			3
HEXACHLOROBUTADIENE	10			3
HEXACHLOROCYCLOPENTADIENE	10			3
ISOPHORONE	15.4			3
2-NITROPHENOL	10			3
4-NITROPHENOL	10			3
N-NITROSODIPHENYLAMINE	10			3
N-NITROSO-DI-N-PROPYLAMINE	10			3
bis-(2-ETHYL HEXYL)PHTHALA	10			3
BUTYL BENSYL PHTHALATE	10			3
DI-n-BUTYL PHTHALATE	10			3
DI-N-OCTYL-PHTHALATE	16.3			3
DIETHYL PHTHALATE	10			3
DIMETHYL PHTHALATE	10			3
CHRYSENE	5			3
ACENAPHTHYLENE	10			3
FLOURENE	10			3
PYRENE	5			3
TETRACHLOROETHYLENE	10			3
TRICHLOROETHYLENE	10			3
AROCLOR-1242	1			3
AROCLOR-1254	1			3
AROCLOR-1221	1			3
AROCLOR-1232	10			3
AROCLOR-1016	1			3

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3. RUSSELL 1983
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5. WPCF 1977
6. BOETHLING 1984
7. BROECKER AND ZAHN 1977
8. RANDALL AND LAUDERDALE 1967
9. WEBER AND SHERRARD 1980
10. RUDOLFS 1950

REPORTED MICROTOX™ 5EC50 CONCENTRATIONS

TOXICANT	5EC50 CONCENTRATION (MG/L)	SOURCE
ORGANICS:		
BENZENE	2-200	1,2,3,4,12,14
BENZIDINE		
HEXACHLOROBENZENE		
2,4,6-TRICHLOROPHENOL	7.1-7.2	1,7
CHLOROFORM	435-520	1,9,13
2-CHLOROPHENOL	22.1	1
1,2-DICHLOROBENZENE	10.25	14
1,3-DICHLOROBENZENE	1.35	12
1,4-DICHLOROBENZENE	9.7	1
2,4-DICHLOROPHENOL	3.6-15.8	1,7,8
DICHLOROPHEN		
DIMETHYLFORMAMIDE	18685	1
2,4-DIMETHYLPHENOL	4.4	1
2,4-DINITROTOLUENE	70	8
2,6-DINITROTOLUENE		
1,2-DIPHENYLHYDRAZINE		
ETHYLBENZENE		
METHYLENE CHLORIDE	1000	1,6
TRICHLOROFLOUROMETHANE		
NAPHTHALENE	2	3
NITROBENZENE	46.2	1
2,4-DINITROPHENOL	15.8-15.9	1,7
PENTACHLOROPHENOL	0.08-1.3	1,7,13
PHENOL	20-42	1,2,3,4,5,7,9,10,13,14,15
ANTHRACENE		
PHENANTHRENE		
TOLUENE	17-33833	1,3,4,12,13,14
3,5 DICHLOROPHENOL	3.2-4.4	5,13
EDTA		
TRINITROTOLUENE	20	2
NACCONOL		
CEEPRYN		
CRESOL	1.5-31.5	1,2,4
MALATHION	3-59.7	1,2,4,14
CARBON TETRACHLORIDE	5.6	1

TOXICANT	5EC50 CONCENTRATION (MG/L)	SOURCE
INORGANICS:		
ARSENIC	35-40	1,9
CADMIUM	44-154	1,10,11,13
CHROMIUM(VI)	22-124	1,2,10,13,14
CHROMATE(III)	10.7	10
COPPER	1.21-69	1,2,5,6,9,10,11,13,14
CYANIDE	2.8-13.3	1,2,3,6,8,9,11,13
IRON		
LEAD	1.4-31.5	1,5,6,10
MERCURY	0.044-0.08	1,2,5,6,9,11,13,15
NICKEL	155-1056	1,5,6,10
SILVER	9.5-13.1	1,14
ZINC	2.5-55.5	1,2,5,6,9,10,11,13
ALUMINUM	1.62	10
AMMONIA	1.2-2	1,2,9
BORATE		
CALCIUM		
MAGNESIUM		
MANGANESE		
SULFATE		
TIN		

REPORTED MICROTOX™ 5EC50 CONCENTRATIONS FOR SELECTED TOXICANTS

TOXICANT	5EC50 (MG/L)	SOURCE
ACENAPHTHENE		
ACROLEIN	0.67	1
ACRYLONITRILE	3910	1
CARBON TETRACHLORIDE	5.6	1
CHLOROBENZENE	0.24	12
1,2,4-TRICHLOROBENZENE		
1,2-DICHLOROETHANE	158	1,9
1,1,1-TRICHLOROETHANE	18.2-105	1,7
HEXACHLOROETHANE	0.14	7
1,1-DICHLOROETHANE		
1,1,2-TRICHLOROETHANE	324	1
1,1,2,2-TETRACHLOROETHANE	8.4	1,7
bis-(2-CHLOROETHYL)ETHER		
2-CHLOROETHYL VINYL ETHER		
2-CHLORONAPHTHALENE		
para-CHLORO-meta-CRESOL	0.58	1
1,1-DICHLOROETHYLENE		

1,2-trans- DICHLOROETHYLENE	1220	15
1,2-DICHLOROPROPANE		
1,3-DICHLOROPROPYLENE		
FLOUROANTHENE		
bis-(2-CHLOROISOPROPYL)ETHER		
CHLOROMETHANE		
BROMOFORM		
DICHLOROBROMOMETHANE		
CHLORODIBROMOMETHANE		
HEXACHLOROBUTADIENE		
HEXACHLOROCYCLOPENTADIENE		
ISOPHORONE		
2-NITROPHENOL	13	7
4-NITROPHENOL	13.5	8
N-NITROSODIPHENYLAMINE		
N-NITROSO-DI-N-PROPYLAMINE		
bis-(2-ETHYL HEXYL)PHTHALATE		
BUTYL BENSYL PHTHALATE		
DI-n-BUTYL PHTHALATE		
DI-N-OCTYL-PHTHALATE		
DIETHYL PHTHALATE		
DIMETHYL PHTHALATE	33.5	1
CHRYSENE		
ACENAPHTHYLENE		
FLOURENE		
PYRENE		
TETRACHLOROETHYLENE		
TRICHLOROETHYLENE	324	1
AROCLOR-1242	0.7	2
AROCLOR-1254		
AROCLOR-1221		
AROCLOR-1232		
AROCLOR-1016	2.05	14

SOURCES

1. Beckman 1983
2. Bulich and Isenberg 1981
3. Samak and Noiseux 1980
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5. Dutka and Kwan 1982
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7. Curtis et al. 1981
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11. Green et al. 1985
12. Kamlet and Doherty 1986
13. Einabarawy et al. 1987
14. McFeters et al. 1983
15. Atkinson 1987

APPENDIX B

SURVEY OF MICROTOX™ USE IN PUBLICLY OWNED WASTEWATER TREATMENT PLANTS

TREATMENT PLANT CHARACTERISTICS:

What is Plant Classification?

__ Conv. Activated Sludge
__ Pure Oxygen Act. Sludge
__ Contact Stabilization
__ Trickling Filter
__ Other. Please Specify:
__ Plug-Flow Act. Sludge
__ Extended Aeration Act. Sludge
__ Oxidation Ditch
__ Lagoon

What is Average Daily Dry Weather Flow? Please check one.

__ < 1 MGD
__ 1-10 MGD
__ 10-50 MGD
__ 50-100 MGD
__ > 100 MGD

What percentage of total influent flow is from industrial sources?

__ 0-10%
__ 10-20%
__ 20-30%
__ 30-40%
__ 40-50%
__ >50%

What industries contribute to influent flow? Please check.

__ Adhesives
__ Leather tanning and finishing
__ Soaps and detergents
__ Aluminum forming
__ Battery manufactoring
__ Coil coating
__ Copper forming
__ Electroplating
__ Foundries
__ Iron and steel
__ Nonferrous metals
__ Photographic supplies
__ Plastics processing
__ Porcelain enamel
__ Gum and wood chemicals
__ Paint and ink
__ Printing and publishing
__ Pulp and paper
__ Textile mills
__ Inorganic chemicals
__ Timber
__ Coal mining
__ Ore mining
__ Petroleum refining
__ Steam electric
__ Organic chemicals
__ Pesticides
__ Pharmaceuticals
__ Plastic and synthetic material
__ Rubber
__ Auto and other laundries
__ Mechanical products
__ Electric and electronic components
__ Explosives manufacturing

What are some identified pollutants in the influent? (i.e. Cyanide, Mercury)

__ Heavy metals
__ Pesticides
__ Other. Please specify:
__ Refractory organics
__ Organic solvents

MICROTOX™ USE

Please check uses:

__ Toxicity Screening
__ Toxicity Reduction Evaluation
__ Pollutant Source Identification
__ Establishment of Operating Parameters
__ Effluent Monitoring
__ Establishing Treatability of New Industry Effluents
__ Billing of Specific Industries
__ Other, Please Explain:

How often do you test toxicity?

OTHER TOXICITY MEASURING MEANS:

Are other toxicity measuring devices used at your plant? What are they?

__ Fish assays
__ ATP assays
__ Other. Please specify:
__ Daphnia assays
__ Respirometry

How are they used?

__ Toxicity Screening
__ Toxicity Reduction Evaluation
__ Pollutant Source Identification
__ Establishment of Operating Parameters
__ Effluent Monitoring
__ Establishing Treatability of New Industry Effluents
__ Billing of Specific Industries
__ Other, Please Explain:

PERMIT REQUIREMENTS:

What effluent discharge requirements must be met?

Is Microtox™ used to monitor any requirement in your permit?

ADDITIONAL COMMENTS:

What are the reasons that you use the Microtox™?

__ Ease
__ Sensitivity
__ Reproducibility
__ Quickness
__ Cost
__ Other. Please Specify:

What are the disadvantages of Microtox™?

__ Cost
__ Speed
__ Sample preparation
__ Lack of government standards
__ Results
__ Other. Please Specify:

Please add your own comments.

Please fill in name, address, and telephone number of a person we may contact at your facility:

Name: _____
Address: _____
_____
_____
_____
Telephone number: _____

Please return to:

Mike McGrath
Civil Engineering Department
University of Massachusetts
Amherst, MA 01003

RESULTS OF SURVEY OF WASTEWATER TREATMENT PLANTS USING MICROTOX™

POTW'S Using Microtox™:	18
Respondants:	17
Treatment Process Types:	
Primary Only	
Conventional Activated Sludge	
Pure Oxygen Activated Sludge	
Contact Stabilization	
Extended Aeration	
Oxidation Ditch	
Trickling Filter	
RBC	
Plant Size:	
1-10 MGD	32%
10-50 MGD	42%
50-100 MGD	1 POTW
>100 MGD	21%
Industrial Contribution to Plant Influent:	
0-10%	40%
10-20%	13%
20-30%	20%
30-40%	20%
40-50%	1 POTW
Toxicant Classes Identified in Plant Influent:	
Heavy Metals	87%
Organic Solvents	80%
Refractory Organics	53%
Pesticides	47%

Industrial Categories Contributing to Plant Influent:

- Aluminum Forming
- Battery Manufacturing
- Coil Coating
- Copper Forming
- Electrical and Electronic Components
- Electroplating
- Paint and Ink Formulating
- Inorganic Chemicals
- Iron and Steel Manufacturing
- Leather Tanning and Finishing
- Metal Molding and Casting
- Nonferrous Metals Forming and Manufactoring
- Pesticides
- Petroleum Refining
- Pharmaceuticals
- Porcelain Enameling
- Pulp and Pater
- Rubber Processing
- Soaps and Detergents Manufactoring
- Timber Products Manufactoring
- Plastics Molding and Forming
- Textile Mills

Microtox Uses™:

Toxicity Screening	82%
Pollutant Sources Identification	24%
Establishing Treatibility of New Sources	50%
Toxicity Reduction Evaluations	53%
Effluent Monitoring	76%
Establishing Operating Parameters	12%
Billing	6%

Testing Frequency:

Daily	38%
Weekly	31%
Monthly	1 POTW
Quarterly	15%
Irregularly	1 POTW

Other Toxicity Tests:

Fish Bioassay	40%
Daphnia Bioassay	40%
Respirometry	20%
Selenastrum Algal Assay	13%
Ames Mutagenicity	1 POTW

Other Toxicity Test Uses:

Toxicity Screening	50%
Pollutant Sources Identification	20%
Establishing Treatibility of New Sources	70%
Toxicity Reduction Evaluations	50%
Effluent Monitoring	70%
Establishing Operating Parameters	0
Billing	0

Discharge Permit Requirements:

Toxicity Testing Required in NPDES Permit	33%
Microtox™ Used to Meet Toxicity Testing Requirement	1 POTW

Merits of Microtox™ Test:

Quickness	100%
Ease of Operation/Maintenance	86%
Inexpensive	50%
Reproducible Results	33%
Sensitivity	29%

Faults of the Microtox™ Test:

Lack of Government Regulations	80%
Expensive	27%
Time Required to Analyze Results	1 POTW
Sample Preparation	1 POTW
Not Reproducible	1 POTW
No Chronic Test	1 POTW
Difficult to Interpret Complex Effluents	1 POTW