UTILIZATION OF THE MICROTOX® TOXICITY ANALYZER IN DELINEATING SIMULATED GROUND-WATER CONTAMINANT PLUMES

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The National Environmental Policy Act of 1969 established a national policy to protect public health and the environment. The policy declared the use of all practicable means and measures to create and maintain conditions where man and nature can exist in harmony fulfilling the social, economic, and other requirements of present and future generations. The Solid Waste Disposal Act (SWDA) as amended by the Resource Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA, aka "Superfund") as amended by Superfund Amendments and Reauthorization Act (SARA) codified corrective action requirements for the removal of imminent threat and substantial endangerment to insure the protection of human health and the environment.

The passage of such federal laws to protect public health and the environment has generated the rapid growth of the field of quantitative risk assessment to provide a framework for developing the necessary information required for environmental decision-making. Risk assessment is an estimation of the severity and likelihood of harm to human health or the environment occurring from exposure to a risk agent. As set by the United States Environmental Protection Agency (EPA), the specific objectives of a risk assessment are to:

1. provide an analysis of baseline risks and help determine the need for action at sites;
2. provide a basis for determining levels of chemicals that can remain onsite and still be adequately protective of public health;
3. provide a basis for comparing potential health impacts of various remedial alternatives; and
4. provide a consistent process for evaluating and documenting public health threats at sites (USEPA, 1989a).

The initial step necessary for a risk-based evaluation is to develop a general understanding of the site. Site characterization involves collecting and analyzing field data to determine the nature and extent of potential threats to human health and the environment and to determine characteristics of the site. The endpoint of site characterization is identifying the chemicals at a particular site that, based on concentration and toxicity, are most likely to contribute significantly to risks calculated due to exposure.

The identification and delineation of contaminants at sites has traditionally been obtained through the use of chemical analysis of various exposure media to indicate contamination. The costs associated with chemical-based analysis are high and include a considerable time constraint to obtain results. Another factor to consider lies in determining the meaning of these results on a site specific basis due to the inability of this chemical analysis to define the environmental impact of contamination. Risk calculation requires the determination of toxicity values to assist in quantifying environmental impact by measuring the potential for contaminants to cause adverse effects.

A common result of environmental contamination is the threat to the nation's groundwater supply. Groundwater serves as a major source of water for domestic, industrial, and agricultural uses. Contamination of groundwater

has become a prevalent and potentially deleterious environmental impact from contaminant release or disposal at National Priorities List (NPL) sites, RCRA sites, and federal facilities around the country. The extent of the problem is indicated by nationwide estimates of contaminant release to groundwater at approximately 25% of the thousands of underground storage tanks (USEPA, 1988) and most of the 1200+ Superfund sites in the country.

The high cost and long turnaround time required for chemical analysis to accurately define the extent of contamination leads to alternative analytical methods. A need exists to provide more rapid, less expensive, environmentally meaningful methods to determine the presence of contamination and delineate the extent in support of evaluation and remediation of the environment.

Identifying the extent of a groundwater contaminant plume with respect to potential exposure points is the objective of initial site characterization. The study undertaken attempts to determine if a toxicity-based approach, using the Microtox® Toxicity Analyzer, can be used to delineate a simulated groundwater contaminant plume. The specific objectives of this study include:

1. Determine the sensitivity of the Microtox to selected industrial chemicals typical of groundwater releases;
2. Examine the sensitivity of the Microtox to identify toxicity in unknown samples containing a specific contaminant; and
3. Evaluate the efficacy of the Microtox to track a simulated groundwater contaminant plume.

Chapter II.

LITERATURE REVIEW

The potential application of this research project in providing quick, inexpensive evaluation of groundwater sources attempts to examine the use of toxicity testing as a means to receive near real-time data. The purpose of the literature review is to examine the existing body of work. Initially, toxicity and aquatic toxicity testing is examined. The Microtox® Toxicity Analyzer is then examined as a means to provide accurate and sensitive measurements of toxicity. The current applications of the Microtox are then summarized including comparison data to determine the efficacy of the method when compared to other toxicity tests.

Toxicity and Toxicity Testing

Toxicology is the basic science of poisons that attempts to establish the limits of safety for chemical agents. Rand and Petrocelli (1985) defined toxicity as the relative property of a chemical which determines its potential to have a harmful effect on a living organism. Any substance can have a toxic effect depending on the concentration of the chemical and the duration of exposure. A highly toxic chemical can be ingested by an organism without any harmful effects if the quantity of the substance is small enough. Likewise, a supposedly

innocuous substance can have harmful effects when taken up by an organism in a large enough dose.

A toxicity test is a means to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions which permit a comparison with other chemicals tested (Rand and Petrocelli, 1985). Toxicity tests can be separated into either short term (acute) or long term (chronic) and by the effect that is being measured. Lethal effects tests measure the mortality of an organism due to exposure, while sublethal effects tests examine adverse physiological and behavioral changes. A standard endpoint of these tests measure the concentration required to result in the death of 50 percent (LC50) of the test organisms. The standardization of aquatic toxicity tests have been established by the U. S. Environmental Protection Agency (EPA) (1991a and 1989b) and by the American Public Health Association (APHA), as described in Standard Methods for the Examination of Water and Wastewater (APHA, 1989).

Today several laws require ecotoxicological testing to predict the hazards that chemicals may pose to the aquatic environment. Most aquatic hazard evaluation currently available are based on data derived from acute toxicity tests with fish and daphnids (Sloof et al., 1986). Stephan (1982) acknowledged that the usefulness of the simple acute toxicity test is obvious to anyone who has tried to assess hazard to aquatic organisms or who has tried to derive water quality criteria for the protection of aquatic life. Buikema (1982) considered acute lethality tests to be ecologically significant, most scientifically and legally defensible, modest in predictive capability, simple and cost effective, and to have the greatest utility.

The most significant shortcoming of using the acute toxicity test with death of the test organism as the endpoint stems from differences in the

biological basis for lethal and sublethal responses. Therefore, Sloof et al. (1986) felt that acute lethality tests are often insufficient as a means of evaluating the potential hazard of chemicals. The development of another endpoint to be used in chemical hazard assessment was developed using chronic lethality testing. The no-observed-adverse effects concentration (NOAEC) is defined as the highest concentration of a toxicant that fails to demonstrate an acute or chronic effect compared to a control (Skalski, 1981). The no-effect level provided the means of estimating a "safe" level for a pollutant.

The standardization of toxicity tests has led to an increase in their usefulness. Stephan (1982) suggested that by standardization, the variability of the tests can be decreased. The EPA takes the position that toxicity test methods, if properly followed, exhibit an acceptable range of variability (USEPA, 1991b).

Buikema (1982) described how the information generated from various toxicity tests can be of use in the prediction of environmental effects of a waste, comparison of toxicants or animals or test conditions, or the regulation of discharges. A common problem lies in the determination of the appropriate species to be used in the toxicity test. A major requirement for optimal test-organism selection is the identification of the organism or organisms that both respond to a contaminant of interest and provide a basis for extrapolating effects to determine the risk of environmental damage (Herricks, 1992). The EPA (1979) used four criteria for the basis of selecting test organisms in their preproposal for guidance for premanufacture of new chemical compounds testing:

1. The organism is representative of an ecologically important group (in terms of taxonomy, trophic level or realized niche).

2. The organism occupies a position within a food chain leading to man or other important species.

3. The organism is widely available, is amenable to laboratory testing, easily maintained, and genetically stable so uniform populations can be tested.

4. There is adequate background data on the organism (i.e.., its physiology, genetics, taxonomy, and role in the natural environment are well understood).

These criteria are desirable but very few organisms will meet all of them. Herricks (1992) describes two general approaches to test organism selection. The first approach focuses on the characterization of the chemical and its environmental fate. The determination of concentration/effect relationships provides the basis for environmental management and control. This approach is similar to the chemical-specific approach to water quality-based toxics control (USEPA, 1991b). The second approach is based on a comprehensive analysis of the ecosystem. This approach is similar to the whole effluent approach for aquatic life protection coupled with comprehensive biosurveys (USEPA, 1991b). The test organisms used typically involve experimental manipulation using unaffected areas as references.

The toxicity of mixtures of chemicals is an important consideration since it is unrealistic to assume that natural aquatic communities are exposed to only single chemicals. Additive toxicity was defined by Rand and Petrocelli (1985) as the toxicity of a mixture which approximately equals the summation of the known toxicities of the individual chemicals in the mixture. Further, the effects on an organism resulting from exposure to a chemical mixture can exhibit a phenomenon where the effect can be either greater than (synergism) or less than (antagonism) additive toxicity. The realization that

understanding the mixtures toxicity was fundamental to the establishment of effective water quality standards and hazard assessment programs has led to the development of mathematical methods designed to assess and predict the toxicities of mixtures.

Marking (1977) attempted to quantify additive toxicity and to determine the significance and meaning of the obtained values. The method used, based on the work of several British researchers, measured the toxic effect of chemical mixtures in water by summing toxic units of individual chemicals (Lloyd, 1961, Herbert and Schurben, 1964, Herbert and Vandyke, 1964, Brown, 1968, Brown et al., 1968, Brown and Dalton, 1970). The procedure used the following summation:

$$(A_m/A_i) + (B_m/B_i) + \ldots = S$$

where

A and B = chemicals

i and *m* = toxicities (LC50s) of the individual chemicals and mixtures, respectively, and

S = sum of biological activity.

When the sum of the biological activity of chemicals A and B is 1.0, the toxicity is simply additive. Sums less than 1.0 indicate greater than additive toxicity (synergism), and sums greater than 1.0 indicate less than additive toxicity (antagonism). Marking (1977) then devised a system of data interpretation where zero indicates simple additive toxicity, negative values indicate less than additive toxicity, and positive values indicate greater than additive toxicity. The point of the data interpretation system was to enable the expression of toxicity and synergism to both be increasing values. Thus,

greater than additive toxicity was represented by values greater than zero which eases data interpretation.

The Microtox®Test

Background

The extensive cost, time, and expertise needed to perform acute toxicity tests has prompted the search for alternatives. Microbial and biochemical toxicity screening procedures are attractive for reasons of rapidity and sensitivity (Elnabarawy, 1986). Further, these short-term toxicity methods have been used for screening single compounds as well as complex mixtures such as industrial effluents and extracts of soils and wastes. Elnabarawy (9186) also found that microbial and biochemical toxicity methods show potential in the environmental hazard evaluation process, especially in the screening and predictive process. One such example of a short-term microbial toxicity method is the Microtox system.

The Microtox toxicity analyzer tests the toxicity of aqueous samples. The system uses a lyophilized marine bacteria (*Photobacterium phosphoreum*) which after reconstitution, emits light. The Microtox Test System measures the light output of the luminescent bacteria after they have been challenged by a sample of unknown toxicity, and compares it to the light output of a control (reagent blank) that contains no sample (Atkinson et al., 1985). McDowell and Boardman (1986) found the Microtox bioassay had the positive points of being: 1) rapid (5 to 60 minutes), 2) simple (mathematical evaluation of results are straightforward), 3) inexpensive (5 to 20 dollars per test), 4) sensitive, 5) reproducible, and 6) easy to maintain.

Currently, the Microtox system is under consideration by the United States Environmental Protection Agency for becoming an approved method of measuring the toxicity of effluents and receiving waters (USEPA, 1991a). The agency has proposed the inclusion of the Microtox system among toxicity methods for 1) predicting land treatability of organic wastes, and 2) performing bioassessments of waste disposal sites (Elnabarawy, 1986).

Applications of the Microtox®

Bulich (1988) describes several validated environmental applications of the Microtox acute toxicity test. The Microtox has been used to quantitate the relative toxicity of industrial and municipal waste water and small municipal and industrial dischargers. The Microtox has proven to be an effective and cost saving analytical tool which allowed the high volume of toxicity testing required for these applications. The Microtox has been used to assess the relative toxicity of sediment samples. The toxicity data derived from these studies were compared with analytical data to verify the utility of the Microtox. Bulich (1988) states that the data showed a very high correlation between toxicity level and concentration of chemicals present in the sample extracts. In terms of hazardous waste remediation, the Microtox has been used during site evaluation and cleanup processes.

McDowell and Boardman (1986) found that the Microtox could provide a means to screen large numbers of samples for contamination more rapidly, more economically, and provided a clearer idea of groundwater quality than any screening test available. The usefulness of the Microtox bioassay as a screening test lies in reducing the number of extensive and costly chemical analyses required by identifying the samples for which further analysis was

necessary. Likewise, Sanchez et al. (1988) found that the quick response of the Microtox made it a potentially useful screening tool for future investigations of effluent quality.

Ribo et al. (1985) used the Microtox to provide a general overview of contamination levels in a survey area and allowed a quick recognition of local "hotspots". They felt that since the Microtox measures the overall toxicity of an aquatic sample, it integrates the toxicities of single compounds and possible synergistic effects of their mixtures. The screening of a large number of water samples allowed the determination of areas where more sampling and other analyses are required.

Kaiser et al. (1988) found that plumes of toxic effluents and zones of toxic effects could be detected in receiving waters with the Microtox bioassay. The applicability and usefulness of the Microtox in aquatic freshwater of generally high water quality required operation of the instrument close to its detection limit. They found a survey involving hundreds of samples, analyzed under identical conditions, provides the necessary statistical basis for reliable interpretation of the results.

Lankford et al. (1988) also found that for the initial screening stages the Microtox would work well as a shortened, simplified bioassay technique due to the quicker data turnaround and lower cost. However, they felt that determination of a correlation between the surrogate test and the required test was necessary.

Comparison Data

The Microtox system has been compared to fish and invertebrate bioassays for the evaluation of the toxic effects of a wide variety of substances

by numerous investigators and found to have comparable precision and accuracy. Williams et al. (1986) compared the Microtox to amphipod and oyster embryo bioassays to evaluate marine sediment toxicity. Results indicated that the three bioassays were in overall agreement (Kendall's coefficient of concordance = 0.64, $P < 0.001$). However, they felt that the magnitude of individual correlations suggested considerable heterogeneity among the bioassays. This degree of variation was attributed to interspecies differences in sensitivity to the kinds of contaminants in the various sediment samples, heterogeneity among sampling sites in quantities and kinds of contaminants, and differences in exposure routes inherent in the experimental design of each bioassay. The proportion of toxic sediments, as determined by the Microtox bioassay (63%), was significantly greater ($P<0.05$) than that determined by either of the other assays. It was concluded that these studies indicated the Microtox bioassay was a reliable screening technique for a variety of pollutants, may be useful for effluent monitoring and toxicity testing, and may be used in a battery of tests to characterize contaminated waters (Williams et al., 1986).

The Microtox test was compared with other toxicity assays including the oxygen uptake procedure, daphnid acute toxicity test, algal bioassay, root-elongation test, and earthworm acute toxicity test for their ability to assess the toxicity of chemicals and to define the areal extent of contamination for a specific site (Walker 1987). Walker found that the algal bioassay was generally the most sensitive test. They also found that toxicity values (LC50s) for fathead minnows and invertebrates (daphnids) used to evaluate specific classes of organics ($r^2 \geq 0.88$)and metals ($r^2 \geq 0.77$) used in the study were similar to the Microtox values. The authors concluded that toxicity tests may be helpful in

identifying classes of toxic chemicals in contaminated soil and that selected bioassays can be used to map chemical contamination at a site.

The objectives of Thomas et al. (1986) were to 1) assess the comparative sensitivity of test organisms to known chemicals, 2) determine if the chemical components in field soil and water samples of unknown composition could be inferred from laboratory studies using pure chemicals, and 3) investigate kriging (a statistical mapping technique) of bioassay results as a method to define the areal extent of contamination. All pure chemical, waste site soil, surface water and groundwater samples were assayed using algae, daphnia, earthworm, root elongation, modified Neubauer seed germination phytoassay, Microtox, and dissolved oxygen depletion rate tests. The algal bioassay were generally most sensitive, but their results indicated it may not be possible to estimate the toxic constituents for all samples in the absence of other bioassays. The results indicate that the bioassay of soil samples from a statistically designed field study area, accompanied by kriging, can aid in defining the extent of contamination and in site cleanup decisions.

Qureshi et al. (1982) compared the Microtox with three conventional bioassays for determining the toxicity of eleven chemical compounds and industrial effluents, including those from an oil refinery, a chemical plant, and a pulp and paper mill. The specific compounds tested included copper, zinc, mercury, arsenate, ammonia, cyanide, phenol, chloroform, 1,2-dichloroethane, and styrene. The results indicated that the rainbow trout bioassay was the most sensitive for several single toxicants. Furthermore, the daphnid assay was more sensitive than Microtox when the trout bioassay was not the most sensitive. The Microtox generally demonstrated the greatest sensitivity to industrial effluents. In the majority of cases, the Microtox test sensitivity was determined to be comparable to the rainbow trout and daphnid

static bioassays. The authors felt that the Microtox has potential as a reliable test for the detection of an effluent or chemical toxicity which is relatively rapid, inexpensive, and simple to perform. They recommended that the Microtox be used in a battery of screening tests or to supplement data obtained from other bioassays.

Plotkin and Ram (1984) attempted to assess the potentially adverse environmental impact of a sanitary landfill leachate by conducting toxicity tests with organisms from different trophic levels. The test organisms included fathead minnows (*Pimephales promelas*), zooplankton (*Daphnia magna*), green algae (*Selenstraum capricornutum*), and bacteria (*Photobacterium phosphoreum*, the Microtox organism). The leachate was highly toxic to the algae and bacteria, moderately toxic to daphnids, and only slightly toxic to fathead minnows. The considerable variation between toxicity test results demonstrated the importance of conducting several toxicity tests using organisms from several trophic levels.

Lebsack et al. (1981) compared the Microtox with fish bioassays to study the effects of fossil-fuel process water and their constituents. The comparative data shows that in most cases the toxicity values from both tests were similar (correlation coefficient = 0.82, $P < 0.05$). A comparison of the bacterial and fish results for phenolic compounds indicates somewhat more variability (correlation coefficient = 0.67), but the general trends remain similar. The authors concluded that this type of toxicity testing system has potential value for semicontinuous monitoring of industrial and municipal effluents. They recommended the Microtox testing method as a supplement or, in some cases, replacement for routine chemical water quality analyses.

Dutka and Kwan (1988) studied the application of a battery of microbiological and toxicant screening tests to sediments. All of the sediment

water extracts, when tested for toxicant or stressing activity by the Microtox test, Algal-ATP test, ATP-TOX system, *S. volutans* test, and SOS chromotest, were essentially negative. However, when *D. magna* tests were performed significant toxicity values were determined that would have been reported as not toxic. Bulich and Kwan felt these findings supported their practice of using a battery of tests to evaluate samples for the presence of toxicants. However, the authors stated that a problem lies in attempting to establish a minimum battery of tests that would provide maximum information with the fewest number of tests.

The object of Calleja et al. (1986) was to examine the toxicity, using the Microtox and *D. magna*, of leachates from a waste generated by the pesticide manufacturing industry and sludge from an electroplating process wastewater. They found a good agreement in toxicity assessment between the two test, but the *D. magna* test appears to be more sensitive, with a greater difference found in the analysis of pesticides than metals. They also supported the use of several acute toxicity tests to assess the potential contamination impact of a waste.

Couture et al. (1989) used results from the Microtox, algae, and fish bioassays to assess the toxicity of zirconium. The algal microtest was found to be the most sensitive species to zirconium toxicity. They found that the Microtox inhibition showed comparable results, slightly less sensitive, to the other test while the fish bioassay proved to be significantly less sensitive than the Microtox.

Curtis et al. (1982) found that the Microtox could be used to screen compounds for potential fish toxicity. The correlation between fish and bacterial toxicity for a composite of industrial chemicals and pesticides had a correlation coefficient value of 0.65. However, the relationship between the

two tests for a somewhat similar series of industrial chemicals appeared to be firm, with the correlation coefficient value being 0.96 for common alcohols. The authors felt that this correlation demonstrates that the Microtox could be used in a regulatory-oriented tier testing scheme as an acute toxicity trigger.

Summary

The Microtox Toxicity Analyzer appears to be a sensitive bioassay. The results of the comparison studies appeared to show that the Microtox is at least as sensitive to chemicals when compared to the other EPA approved bioassays. Further, several studies show the Microtox is significantly more sensitive than the other test organisms.

One issue of concern about the use of the Microtox centers with the nature of translating the method to compare freshwater to marine environments. The Microtox bacteria is a marine bacteria which requires osmotic adjustment to all samples before testing. The physical, chemical, and biological differences between freshwater and marine environments could effect the results obtained using the Microtox. Williams et al. (1986) found that bacterial luminescence deviated with a change in salinity but quickly re-equilibrates. Studies concerning the differences due to the use marine nature of the Microtox are lacking in the literature.

The current study involves the use of simulated data based on a contaminated groundwater site located at the Building 3001 complex on Tinker Air Force Base, Midwest City, Oklahoma. Releases of industrial chemicals from previous activities have contaminated the area. The site has been placed on the Federal Facility National Priority List for cleanup and has undergone remedial investigation (U. S. Army Corps of Engineers, 1988). The primary contaminants

at the site are trichloroethene (TCE) and chromium. The study also included benzene, lead, and tetrachloroethene (PCE) as contaminants found at the site.

The endpoint used in the analysis were the NOAEC values determined for each compound. The use of the NOAEC restricted comparisons with existing data due to the limited use of the NOAEC in the literature. However, examination of current LC50 values for the specific contaminants used in the analysis where there was not NOAEC data allows for indirect comparison of the Microtox to acute *Daphnia magna* toxicity tests.

Amodor (1993) determined NOAEC values for lead and chromium using both *Daphnia magna* and the Microtox. The Microtox showed greater sensitivity to both the lead and chromium. The NOAEC values for lead were determined to be 0.10 mg/L for the Microtox and 6.0 mg/L for the *Daphnia magna*. Likewise, the Microtox (0.17 mg/L) was slightly more sensitive to chromium than Daphnia (0.69 mg/L).

Comparison of the organics used in the analysis requires the use of LC50 values due to lack of existing data. For benzene, the EPA (1980a) reports Daphnia values ranging from 203 to 620 mg/L while the toxicity data index compiled by Kaiser and Palabrica (1991) found Microtox data ranging from 2 to 394 mg/L. The EPA (1980b) reports a Daphnia LC50 range for PCE between 17.7 mg/L and 30.8 mg/L while Kaiser and Palabrica (1991) found Microtox PCE values ranging from 19.5 to 117 mg/L. Likewise, Kaiser and Palabrica report TCE values for the Microtox from 117 to 610 mg/L while the EPA (1980c) reported *Daphnia magna* values ranging from 41 to 100 mg/L.

The toxicity data found in the literature for these specific chemicals shows comparable sensitivity of the Microtox bacteria to the *Daphnia magna*. The Microtox bacteria appears to be slightly more sensitive than the Daphnia to both benzene and TCE. However, the PCE concentration ranges found for

both organisms are very similar. Overall, the Microtox appears to be at least as sensitive to these compounds as *Daphnia magna*.

CHAPTER III

MATERIAL AND METHODS

Study Design

A comprehensive study was undertaken to determine the applicability of the Microtox analyzer to delineate groundwater contaminant plumes of industrial chemicals. The proposed study is an investigation to be developed from laboratory evaluation of sensitivity and prediction to field confirmation of laboratory results. The focus for the initial phase of this study was to determine the sensitivity of the Microtox to typical groundwater chemical concentrations and to determine the applicability of the Microtox to delineate a contaminant plume using sample concentrations based on field data from the proposed site.

The proposed site of field study was Building 3001, located in the northeast portion of Tinker Air Force Base, Midwest City, Oklahoma. Past industrial practices within and in the vicinity of Building 3001 have resulted in groundwater contamination of an underlying perched aquifer and upper zones of the Garber-Wellington Aquifer. The EPA has placed the site on the Federal Facilities National Priorities List of hazardous waste sites. The extent and magnitude of contamination has been defined through remedial investigations conducted by the United States Army Corps of Engineers (COE), Tulsa District. An initial study by the COE (1988) established that the primary

contaminants were trichloroethene (TCE) and chromium (Cr) although other organic compounds and trace metals were found.

The initial phases of the study restricted the findings of the site remedial investigation to limit the number of specific contaminants for study and establish concentration ranges from monitoring well data. Simulated contaminant plumes were developed using the monitoring well data to develop sample concentrations of the representative compounds.

The chemical compounds selected for use in the study are typical industrial compounds found in groundwater releases including benzene, trichloroethene (TCE), tetrachloroethylene (PCE), lead, and chromium. Stock solutions of all inorganic and organic compounds were freshly prepared from analytical-grade material from Fisher Scientific. The organic solvents were dissolved using methanol as a carrier solvent. Rand and Petrocelli (1986) limit the use of carrier solvents to less than 2% by volume. In the analysis, methanol concentrations in all organic samples never exceeded 1% by volume. The methanol was tested for toxicity to the Microtox bacteria with the lowest toxic response found at 5 percent. Nominal concentrations for each chemical compound were used in evaluating toxicity to the luminescent bacteria *Photobacterium phosphoreum* and 24-hour acute toxicity to *Ceriodaphnia magna* to establish comparison values. The nominal concentrations of the compounds were prepared by gravimetric analysis. The daphnid tests and NOAEC determinations for the two metals were performed by another analyst (Amodor, 1993).

A very hard (280 mg/L as $CaCO_3$) reconstituted water was used in the analysis as dilution water (Amodor, 1993). The choice of dilution water was determined to be representative of the uncontaminated groundwater at the proposed site of study.

Microtox® Standard Assay

Simulated groundwater samples were tested for acute toxicity, using the Microtox analyzer, to determine the sensitivity of the bioassay to nominal concentrations of specific chemical compounds. The bioassay was performed using the Microbics model 2055 Microtox Toxicity Analyzer system, a temperature-regulated photometer equipped with photo-multiplier. Freeze-dried bacteria, reconstitution solution, diluent, and other necessary materials were purchased or obtained from Microbics Corporation. The Microtox photometer measures the light output of the luminescent bacteria (*Photobacterium phosphoreum*) after they have been challenged by a sample of unknown toxicity, and compares it to the light output of a control (reagent blank) that contains no sample.

All Microtox bioassays were performed a minimum of four repetitions per sample following the manufacturers operating instructions and procedures for their basic assay (Microbics, 1992) to establish the NOAEC concentrations. A screening protocol was also developed for use in testing the potential of using the Microtox in defining the areal extent of contamination in a model matrix (Refer to Appendix B).

The protocol for the assay conducted can be summarized as follows. A vial of freeze-dried bacteria was rehydrated with 1.0 mL of reconstitution solution (toxic material free distilled water) and kept in a 4.0° C, temperature-regulated well in the Microtox analyzer. All assays were conducted within 3 hours of rehydration. For all tests, 2.5 mL of sample (from stock solutions) and 0.25 mL of 20 percent sodium chloride were mixed to yield an initial concentration of 91 percent of the sample's concentration. Serial dilutions

were prepared from this solution, using the Microtox diluent (toxicity free 2% NaCl solution), and stored in 15° C, temperature-regulated wells. The dilution's consisted of 45, 22, and 11 percent of the original stock solution concentration. Resulting chemical concentrations are expressed in milligrams per liter (mg/L).

Aliquots (10 uL) of the reconstituted Microtox reagent (lyophilized bacteria) at 4° C were transferred to cuvettes containing 0.5 mL diluent equilibrated at 15° C. The reconstituted reagent was allowed to equilibrate for 15 minutes to insure maximum light production. Initial light measurements were made using the Microtox analyzer. The Microbics procedure ordinarily uses Microtox diluent (0.5 mL) as a control. The control used in our analysis was a 0.5 mL sample of very hard (280 mg/L as CaCO3) reconstituted dilution water osmotically adjusted, using the Microtox Osmotic Adjusting Solution (MOAS), to distinguish natural toxicity. Osmotic adjustments to the controls were prepared by mixing ten parts sample to one part MOAS. The control and the sample dilutions, both equilibrated to 15° C in the incubator wells, were added (0.5 mL) from the corresponding cuvettes to luminescent bacterial suspensions (0.5 mL). Light measurements were then made cycling cuvettes through the turret, at 5 and 15 minutes, to obtain the final light output readings for each cuvette. The NOAEC (No-observed-adverse-effects concentration) values for each sample were calculated using the EPA's (1991a) analysis scheme for statistical determinations. The NOAEC values were expressed in milligrams per liter (mg/L).

Daphnid Bioassays

Daphnia magna 24-hour static bioassays were performed on several of the chemical compounds to determine NOAEC values to be used in comparison with the Microtox results. The *D. magna* tests were conducted according to the latest EPA methods (USEPA, 1991a). The choice of *D. magna* was due to a higher survival rate in comparison to *D. pulex* in an initial test performed using the very hard dilution water. The daphnids used for all tests were subcultured from a stock reared using consistent methods. The brood was maintained in dilution water at approximately 20° C under a 16-h light and 8-h dark regimen. The daphnids were fed, immediately before exposure to toxicants, a diet of *Selenastrum capricornutum* algae and trout chow-yeast. Only young daphnids, 24 h or less, were used in all tests.

All the tests were unaerated due to the daphnids' characteristic low requirement for dissolved oxygen. Test concentrations were based on a series of screening tests to determine the appropriate range. All of the tests were performed a total of four replicates per concentration. Controls of reconstituted dilution water, without added toxicant, were also included for comparison.

All bioassays were conducted at approximately 20° C with a 16-h light and 8-h dark cycle. All daphnids remained unfed for the duration of each test. The organisms were considered to be dead if no movement was observed. The number of dead organisms were always recorded after 24-h, but periodic observations were also made. The relative toxicity endpoint, calculated as the NOAEC, was based on the nominal concentration of toxicants in terms of mg/L.

Statistical Analysis

Calculation of the NOAEC

The statistical endpoint for the purposes of this research was the highest concentration at which survival was not significantly different from the control (NOAEC). The NOAEC was determined by hypothesis testing. Dunnett's procedure is currently used in the EPA's data analysis methods to determine the NOAEC (USEPA 1989b). However, Rand and Petrocelli (1985) found that Dunnett's procedure only considers each comparison of a concentration with the control separately, without regard to the concentration-response curve. The authors feel the failure to consider the logical ordering of the responses in the alternative hypothesis testing leads to loss of power of the test to detect such alternatives.

Like the Dunnett's test, the Williams' test also compares each of the group means to the control. However, the Williams' test is designed to be sensitive to a response due to increasing concentration of toxicants. Rand and Petrocelli (1985) state that Dunnett's procedure is not the most powerful test available; Williams' test is preferable.

The determination of the NOAEC values for each of the chemical compounds used in the analysis followed the EPA's (1989b) statistical analysis method (Appendix A). The only exception is the use of the Williams' test for the organics instead of the Dunnett's procedure. Amodor (1993) determined the NOAEC endpoint for chromium and lead using Dunnett's procedure. All data analyses were determined using TOXSTAT software.

Toxicity Determination

The statistical endpoint from the screening procedure (Appendix B) was simply to determine whether a sample was toxic or not. A comparison was made between the sample mean and the control mean to determine if the two were significantly different. The procedure used was based on comparing the 95% confidence limits about the median light output value for both sample and control. A sample was determined to be toxic if there were a clear break between the two confidence ranges.

CHAPTER IV

RESULTS

Overview of Study

The purpose of the study was to attempt to determine whether or not a toxicity based approach could be used to define the extent of contamination at a site. The specific objectives to be studied were established to determine the ability of the Microtox Toxicity Analyzer to accurately distinguish toxicity in simulated groundwater samples. Initially, the sensitivity of the Microtox to specific contaminants was to be defined. Next, a series of blind samples, using TCE, were tested for toxicity to confirm the accuracy of the Microtox bacteria to differentiate samples with concentrations above or below the NOAEC endpoint. Finally, a simulated groundwater plume was analyzed to verify if the method could adequately define the edges of a contaminated plume.

Sensitivity of the Microtox®

Determination of NOAEC Values

Five chemical compounds were analyzed using the Microtox Toxicity Analyzer to determine the NOAEC values. The NOAEC values and 95 percent confidence values about the means are summarized in Table 1. The values show

Table 1
15 min Microtox Test
NOAEC values - 95% confidence limits

Compound	NOAEC (mg/L)	lower limit (mg/L)	upper limit (mg/L)
Lead	0.10	0.06	0.15
Chromium	0.17	0.10	0.23
PCE	1.91	1.17	2.65
Benzene	3.58	1.77	5.39
TCE	8.13	5.87	10.38

quite a bit of a difference between the response of the *Photobacterium phosphoreum* to the various compounds used in the analysis.

A comparison of toxicity results between the organic compounds tested showed some variability between the individual 95% confidence intervals in the sensitivity of the bacteria to TCE, PCE, and benzene. The chlorinated compounds show a definite increase of toxicity as the number of chlorines per molecule increases. The bacteria appeared to be more sensitive to PCE when compared to TCE, with NOAEC values of 1.91 mg/L and 8.13 mg/L, respectively. Likewise, the NOAEC for benzene had a 95% confidence interval about the mean from 1.77 mg/L to 5.39 mg/L. A comparison of 95% confidence ranges for all three organic compounds is depicted in Figure 1.

The sensitivity of the Microtox to chromium and lead is significantly greater than the organic compounds tested. NOAEC values were established by Amodor (1993) for lead (0.104 mg/L) and chromium (0.165 mg/L) which are very similar, especially when the confidence intervals are examined. Figure 2 plots the relationship of the 95% confidence intervals between the two metals while Figure 3 compares the sensitivity of the Microtox to all five compounds used in the analysis. The NOAEC values for lead, chromium, and PCE correlated to the ranges found at the proposed site of study (Building 3001, Tinker Air Force Base). However, the lower limits established using the NOAEC 95% confidence limits for PCE and benzene were above the concentration ranges found at the site. The relatively high sensitivity of the Microtox to several of the compounds studied allowed actual well data (COE, 1988) to be used in later phases of the study.

Figure 1 - NOAEC 95% Confidence Intervals - Organics

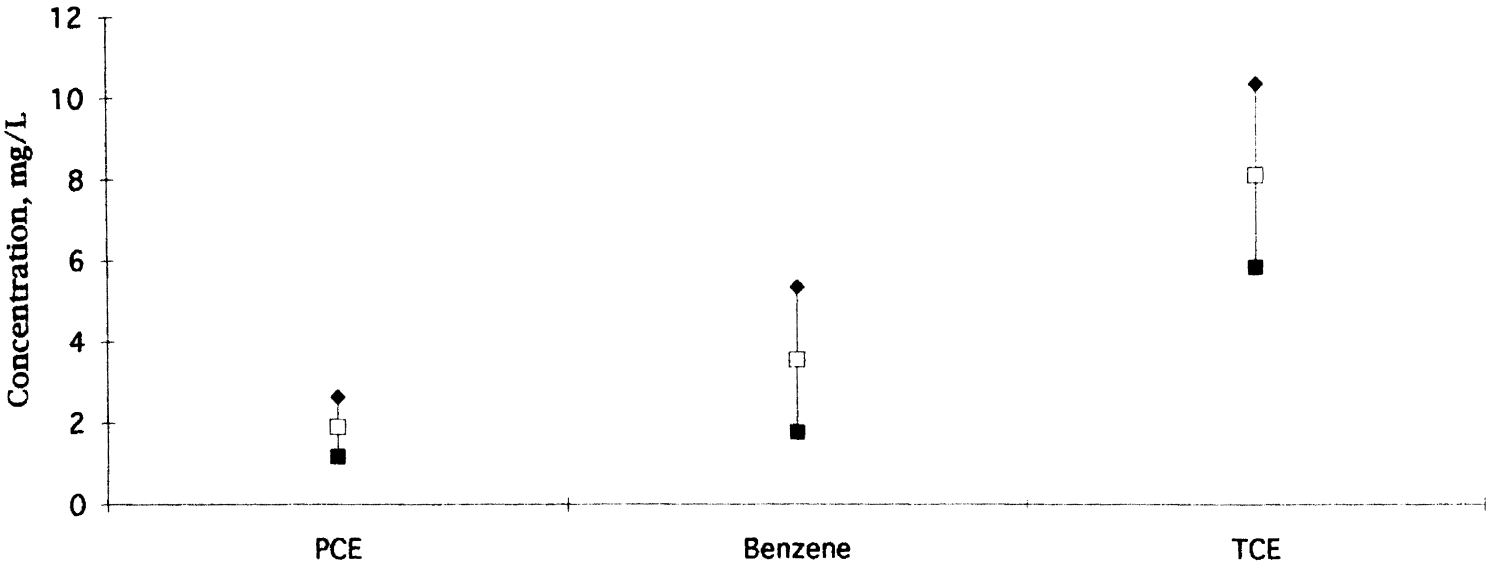


Figure 2 - NOAEC 95% Confidence Intervals - Metals

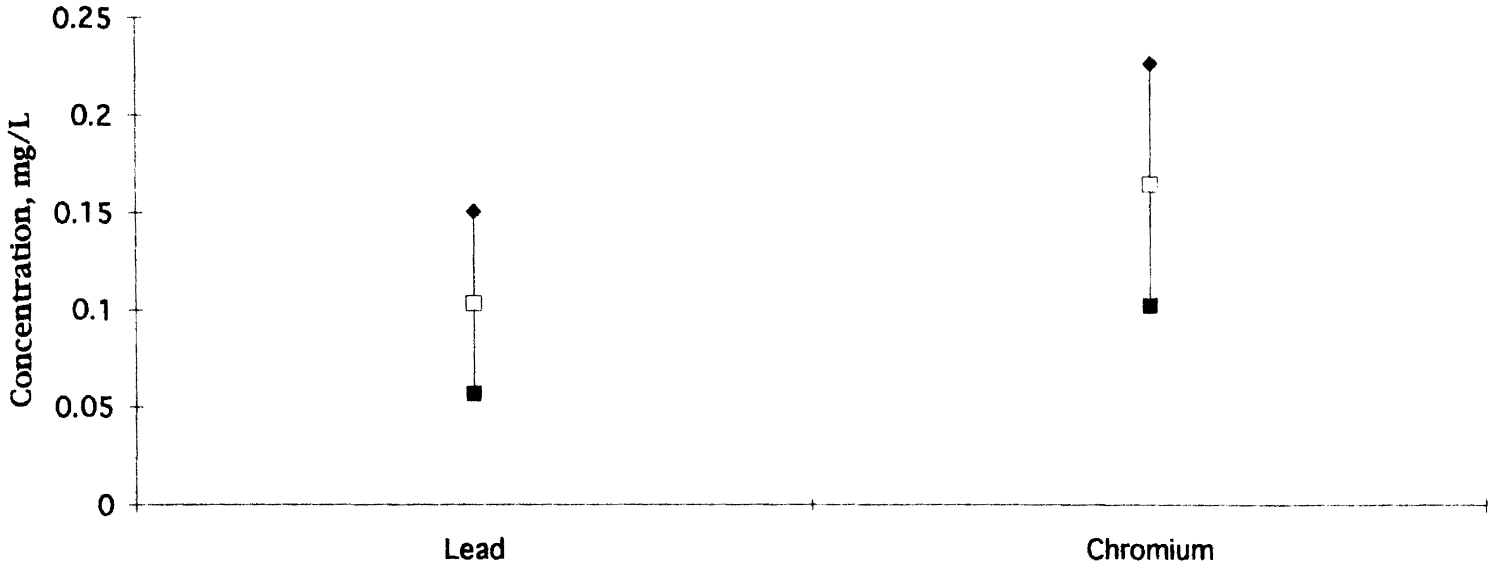
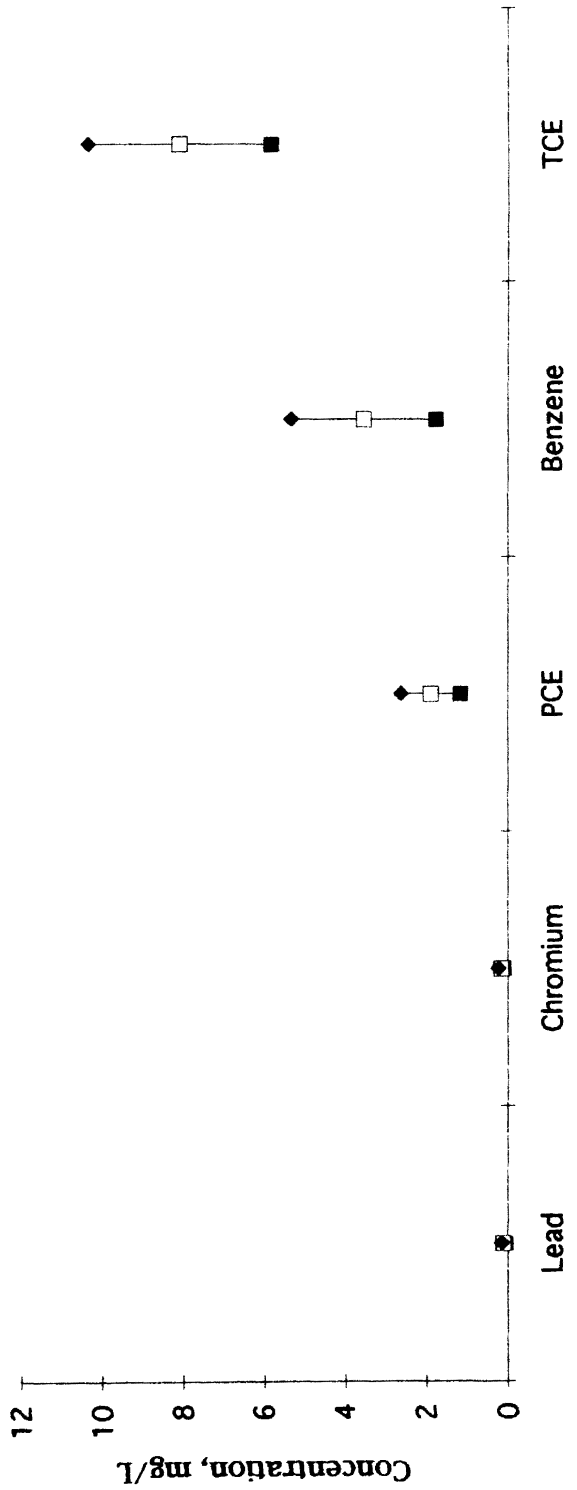


Figure 3 - NOAEC 95% Confidence Intervals



Comparison of Microtox and Daphnia Values

The Microtox data was reinforced by comparing the results to NOAEC values determined from 24 h acute toxicity tests using *Daphnia magna* (Amodor, 1993). Overall, the Microtox showed greater sensitivity to the compounds tested when compared to the daphnids toxic response to the compounds. The NOAEC values and the confidence intervals about the mean determined by the Daphnia tests are summarized in Table 2.

The sensitivity of the Microtox to lead is substantially higher than the daphnids with NOAEC values of 0.104 mg/L and 6.0 mg/L, respectively. The variations between the confidence ranges for both tests are plotted in Figure 4. The response of both test organisms to chromium was very similar. However, the Microtox values showed the bacteria appeared to be slightly more sensitive than the Daphnia (Figure 5).

Contaminant Identification

The ability of the Microtox to differentiate toxicity between TCE samples with concentrations greater than or less than the NOAEC confidence interval was then tested. The 95% confidence interval for TCE was previously determined to be from 5.87 mg/L to 10.38 mg/L with a mean value of 8.13 mg/L. Each test was designed using a total of 8 samples with varying TCE concentrations per test. The TCE samples were prepared from stock solutions and stored in 50 mL glass sample containers with Teflon® sealed lids. Each sample was randomly labeled before being given to the analyst performing the Microtox screens. All samples were analyzed using the screening protocol described in Appendix B.

Table 2
24 h Acute Toxicity Test (Daphnia magna)
NOAEC values - 95% confidence limits

Compound	NOAEC (mg/L)	lower limit (mg/L)	upper limit (mg/L)
Lead	6.00	4.87	7.13
Chromium	0.69	0.25	1.23

Figure 4 - Comparison Data - Lead

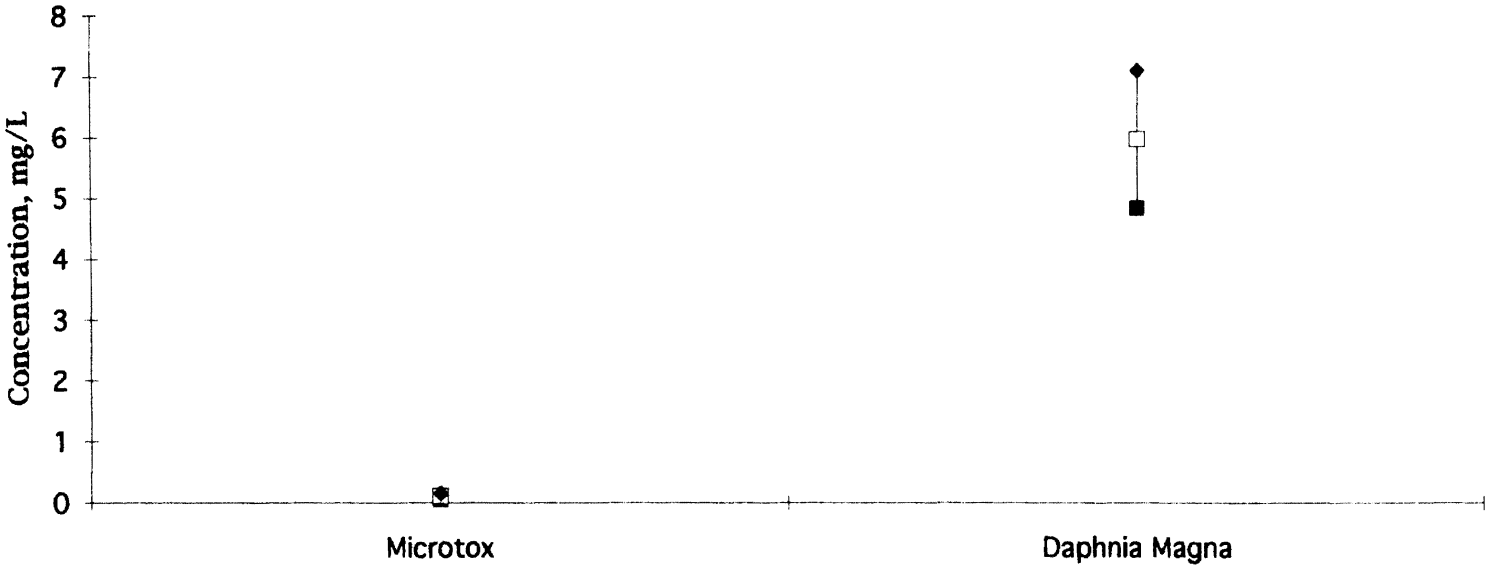
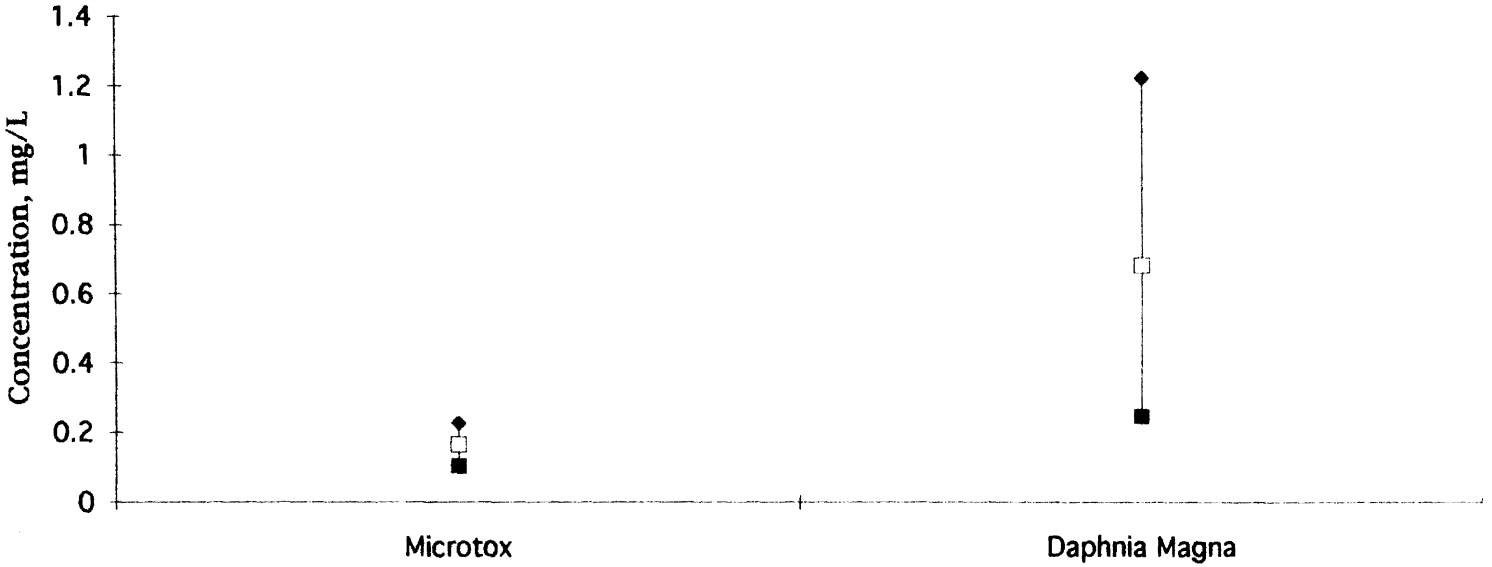


Figure 5 - Comparison Data - Chromium



Two experiments were performed to quantify the accuracy percentage for the screening method to distinguish toxicity in unknown samples. The calculation of the percentage was determined using the total number of correct responses divided into the total number of samples. A correct response was based on the ability of the bacteria to accurately distinguish toxicity in samples with concentrations either within the 95% confidence interval previously established or greater than the 95% confidence interval. Further, all samples with concentrations below the 95% confidence interval were expected not to elicit a toxic response from the Microtox. However, another possible approach would only consider toxic responses correct to concentrations greater than the 95% confidence limit established for TCE. The former method was considered to insure the most conservative approach in attempting to quantify the ability of the Microtox to accurately discriminate toxicity in unknown samples.

Determination of toxicity for each sample was based on comparing 95% confidence intervals calculated from replicate Microtox luminescence readings for both samples and controls. A sample was considered toxic when there was clear separation between the confidence ranges determined for the sample and the control. Samples were determined nontoxic when there was not a distinct difference between samples and controls.

Experiment 1

The results from the first experiment are summarized in Table 3. The TCE concentrations used in the first experiment were all either above or within the established NOAEC 95% confidence interval. Correct responses to the specific TCE concentrations in individual samples for both runs were denoted

Table 3
15 min Microtox Test
Experiment 1 - Summary Table
Compound: Trichloroethene
Date Run: 8/26/93

Sample	Concentration (mg/L)	Toxic? (y or n)	Correct? (+ or -)
A	6	n	-
B	18	y	+
C	6	y	+
D	15	y	+
E	15	n	-
F	6	n	-
G	12	y	+
H	12	y	+

percent correct: 62.5

Sample Calculation of Percent Correct:

percent correct = (correct sample response / total samples) * 100

= 5 correct responses / 8 samples

= 62.5

by an addition sign (+) while incorrect responses were represented using a negative sign (-). Samples A, C, and F were all near the low end of the sensitivity range of the bacteria to TCE. Both A and F were determined not to be toxic while C resulted in a toxic response. The rest of the sample concentrations were above the upper limit of the NOAEC range and were found to be toxic with one exception. Sample E (15 mg/L) did not elicit a toxic response from the bacteria. The accuracy of the Microtox in this test was calculated as 62.5 percent. However, when only the samples outside the NOAEC range were considered the accuracy of the model increased to 80 percent.

Experiment 2

The second experiments results showed greater accuracy in comparison to the first model. Table 4 summarizes the toxicity screen results for the second model. The TCE concentrations used in this model completely bracketed the NOAEC interval established previously. Samples E and F with TCE concentrations of 6 mg/L and 10 mg/L, respectively, were the only samples that showed incorrect bacterial responses. These samples were considered to be incorrect simply because they were not determined toxic. The results of the second experiment reinforced the ability of the Microtox to distinguish between toxic and nontoxic TCE sample concentrations. Overall, the bacteria exhibited a 75 percent accuracy in the second run. Again, samples with TCE concentrations outside the confidence interval resulted in accurate responses (100%) from the bacteria.

The ability of the Microtox to discriminate toxicity in samples below the NOAEC range appeared to be substantiated. Only one sample (Sample E in experiment 1) outside the confidence interval was incorrectly designated. The

Table 4
15 min Microtox Test
Experiment 2 - Summary Table
Compound: Trichloroethene
Date Run: 9/28/93

Sample	Concentration (mg/L)	Toxic? (y or n)	Correct? (+ or -)
A	3	n	+
B	10	y	+
C	15	y	+
D	12	y	+
E	6	n	-
F	10	n	-
G	15	y	+
H	6	y	+
percent correct:	75		

variances for both models were exhibited in samples that had concentrations within the 95% confidence interval developed for the contaminant. The level of uncertainty for correct responses to samples within the confidence range reinforced the use of the established interval as an endpoint.

Plume Tracking

The ability of the Microtox to track a contaminant plume was then tested. Theoretical model plumes were laid out on a 20 sample matrix using nominal concentrations of lead, chromium, PCE, TCE and benzene. The contaminant concentrations used in the analysis were established from concentration ranges found by the U. S. Army Corps of Engineers (1988) at the proposed site of study. Samples were prepared from stock solutions and placed in 50 mL glas containers with Teflon® sealed lids. Seven samples per model had concentrations above the NOAEC value for at least one compound while the other sample concentrations were all below the NOAEC range for each of the individual chemicals. The specific compound above the NOAEC interval for the known toxic samples was randomly varied. The experiment was performed using three different sets of matrices and samples. After initial designation, the samples were randomly assigned a designation before being given to the analyst performing the Microtox evaluations.

To test the effect of typical field conditions one model was repeated twice (Models 3 and 5). The individual samples for both models were prepared from stock solutions of the five chemical compounds and then divided into two sample containers. Toxicity screens were performed immediately for one set while the second set of samples were tested after a 24 h storage period at 8° C. The samples were stored using Teflon® sealed air-tight lids and head space free

to minimize volatility. Prior to toxicity screening the stored samples were allowed to equilibrate to room temperature.

Model 3

The sample concentrations used in model 3 are summarized in Table 5. Figure 6 shows the physical layout of the sample matrix. The known toxic samples are highlighted by the solid contour line to show the expected extent of contamination (toxicity). Also, the random designations for each sample given to the analyst performing Microtox screens are included in parentheses as part of Figure 6.

Table 6 summarizes the results from the toxicity screens for model 3. Samples a1 through a7 had concentrations in excess of at least one of the contaminants NOAEC values. A toxic response was considered correct for these samples. All other samples were not expected to elicit toxic responses from the bacteria. As previously mentioned, a correct response was based on accurately determining toxicity in samples where toxicity was expected. A correct response was denoted with a positive sign (+) while incorrect responses received a negative sign (-). The Microtox response to the expected toxic samples was correct with the single exception of sample a4 (#15).

However, four samples that were designated incorrect elicited toxic responses from the bacteria. The samples that were determined toxic were samples a9, a11, a13, and a14. Each of these samples were not expected to be found toxic since the individual contaminant concentrations in each of these samples were below the NOAEC for any of the individual compounds. The bacterial responses to the samples indicated the mixture of contaminants caused the toxic response.

Table 5
15 min Microtox Test
Model 3 - Sample Concentrations
Date Run: 10/16/93

Sample (number)	Concentrations lead (ug/L)	chromium (ug/L)	tce (ug/L)	pce (ug/L)	benzene (ug/L)
a1	83	35	47,000		
a2	345	330	27		
a3	410	370	97		
a4	73	15	19,000		
a5	120	240			
a6	400	930			
a7	58	100	18,000	170	
a8	63	45	35		
a9	63	58			
a10	60	45	27		
a11	38	83	2,400		
a12	43	55	130		
a13	50	90	96		
a14	70	43			
a15	50		95		
a16	63	13			
a17	30		490		
a18	40	38	100		
a19	35	68			
a20	25	10	360	50	50

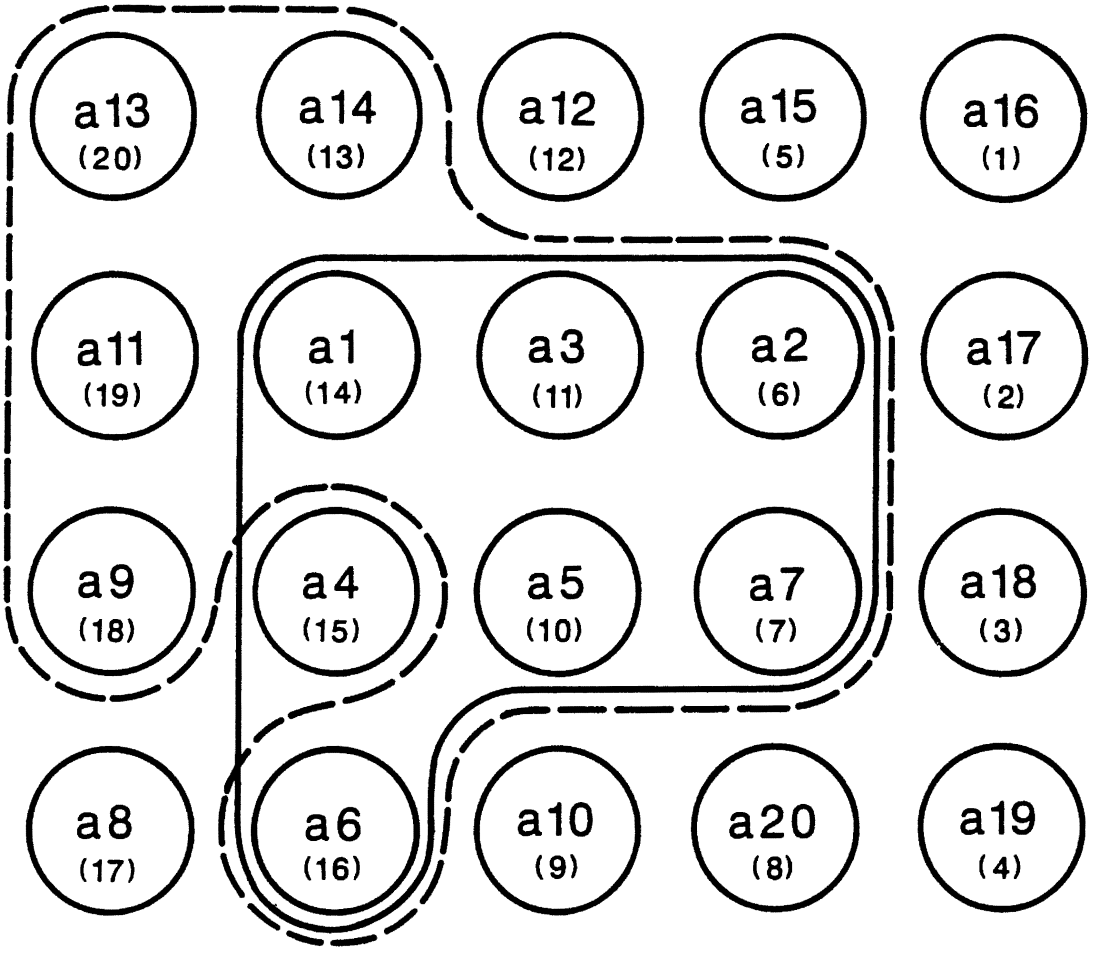


Figure 6. Model 3 Matrix

Table 6
15 min Microtox Test
Model 3 - Summary Table
Date Run: 10/16/93

Sample	Random Designation	Toxic? (y or n)	Correct? (+ or -)
a1	14	y	+
a2	6	y	+
a3	11	y	+
a4	15	n	-
a5	10	y	+
a6	16	y	+
a7	7	y	+
a8	17	n	+
a9	18	y	-
a10	9	n	+
a11	19	y	-
a12	12	n	+
a13	20	y	-
a14	13	y	-
a15	5	n	+
a16	1	n	+
a17	2	n	+
a18	3	n	+
a19	4	n	+
a20	8	n	+

The results of model 3, with the exception of sample a4, accurately delineated the expected area of contamination. A larger extent of contamination was determined due to the sensitivity of the bacteria to register toxicity in complex samples with contaminant mixtures. As previously mentioned, Figure 6 plots the expected area of contamination. Figure 6 also illustrates the larger area, inside the dashed lines, determined to be contaminated after screening the samples for toxicity.

Model 4

Sample concentrations used in this model are summarized in Table 7. Figure 7 develops the plot of the samples with the area of expected contamination highlighted by a solid contour line. The designations for each sample given to the analyst performing the toxicity tests were also included as part of Figure 7.

Table 8 shows the results from the tests performed for each sample. The samples with concentrations in excess of NOAEC values were b1 through b7. The bacterial response to these samples correctly indicated toxicity.

The only other sample to be determined toxic in model 4 was b10. Again, the sample was designated incorrect solely on the basis of not expecting to find toxicity. Figure 7 plots the area determined to be toxic with a dashed contour line. The Microtox was again able to distinguish toxicity over a larger area when compared to the expected contamination. Mixture toxicity allows the detection of toxicity in more samples which increases the ability to define the edges of a contaminated plume. The results of the model again reconfirms the ability of the Microtox to distinguish toxicity above NOAEC levels.

Table 7
15 min Microtox Test
Model 4 - Sample Concentrations
Date Run: 10/16/93

Sample (number)	Concentrations lead (ug/L)	chromium (ug/L)	tce (ug/L)	pce (ug/L)	benzene (ug/L)
b1	80	390	6,900	33	
b2	80	300	14,000	1,200	
b3	570	1,100			260
b4	220	340	35		
b5	580	950	36		
b6	110	210	370		
b7	290	870	1,200		
b8	48	35	490		
b9	45	50		5	
b10	38	83	2,400		
b11	43	55	130		
b12	60	45	27		
b13	63	45	35		
b14	63	58			
b15	35		510		
b16	30	12	300	25	25
b17	50		90		
b18	38	64			
b19	65	10			
b20	45	33	95		

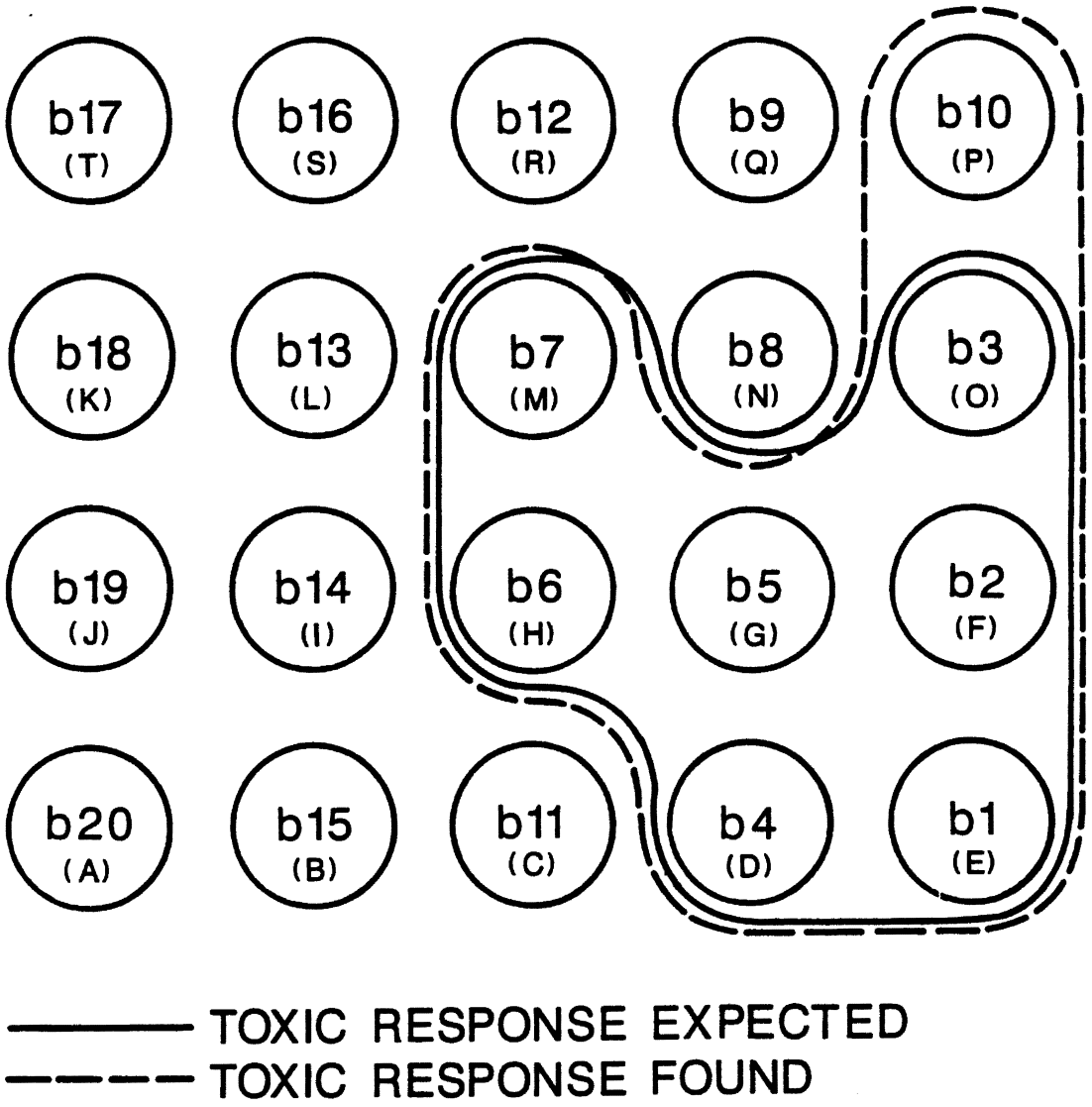


Figure 7. Model 4 Matrix

Table 8
15 min Microtox Test
Model 4 - Summary Table
Date Run: 10/16/93

Sample	Random Designation	Toxic? (y or n)	Correct? (+ or -)
b1	E	y	+
b2	F	y	+
b3	O	y	+
b4	D	y	+
b5	G	y	+
b6	H	y	+
b7	M	y	+
b8	N	n	+
b9	Q	n	+
b10	P	y	-
b11	C	n	+
b12	R	n	+
b13	L	n	+
b14	I	n	+
b15	B	n	+
b16	S	n	+
b17	T	n	+
b18	K	n	+
b19	J	n	+
b20	A	n	+

Model 5

As previously mentioned, model 5 repeated the specific parameters used in model 3. The only variation was a 24 h storage period for the samples. Table 9 and Figure 8 summarize the sample concentrations used and the layout of the matrix. Samples a1 through a7 were the samples where a toxic response was expected.

Table 10 summarizes the results from the screening procedure for model 5. The bacterial response to the toxic samples was correct with the exception of sample a4. Sample a4 produced negative responses for both models 3 and 5. The inability of the bacteria to determine toxicity in this sample for both models casts doubt on the integrity of sample preparation. Considering the accuracy of the Microtox to indicate toxicity for the other known toxic samples, the high concentration of TCE in sample a4 should have caused an inhibition of light production in the bacteria. Unfortunately, the use of nominal concentrations in the analysis did not allow confirmation of this assumption.

Six other samples were determined to be toxic by the Microtox. Each of these samples were not expected to be toxic due to all the contaminants concentrations were below NOAEC levels. The toxic samples were a9, a11, a12, a13, a14 and a17. Figure 8 shows the area determined to be toxic with a dashed contour line. Two of the samples, a12 and a17, showed variance to the results determined in model 3. The differences in responses between the two models reinforced the use of multiple tests and samples to insure an accurate delineation of site contamination.

Short term storage appeared to have little if any effect on the Microtox to differentiate toxicity for these compounds. There were not any samples

Table 9
15 min Microtox Test
Model 5 - Sample Concentrations
Date Run: 10/17/93

Sample (number)	Concentrations lead (ug/L)	chromium (ug/L)	tce (ug/L)	pce (ug/L)	benzene (ug/L)
a1	83	35	47,000		
a2	345	330	27		
a3	410	370	97		
a4	73	15	19,000		
a5	120	240			
a6	400	930			
a7	58	100	18,000	170	
a8	63	45	35		
a9	63	58			
a10	60	45	27		
a11	38	83	2,400		
a12	43	55	130		
a13	50	90	96		
a14	70	43			
a15	50		95		
a16	63	13			
a17	30		490		
a18	40	38	100		
a19	35	68			
a20	25	10	360	50	50

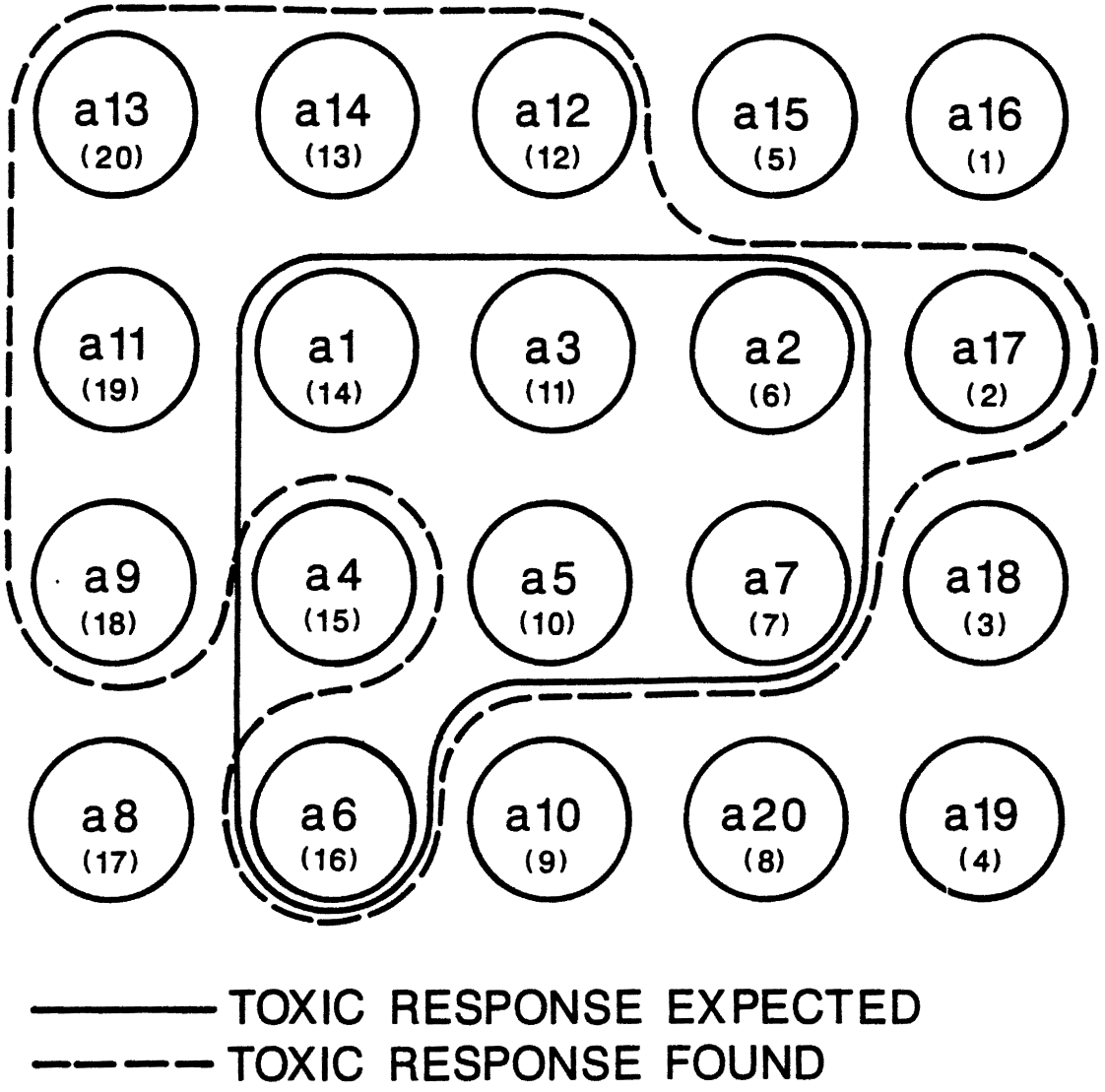


Figure 8. Model 5 Matrix

Table 10
15 min Microtox Test
Model 5 - Summary Table
Date Run: 10/17/93

Sample	Random Designation	Toxic? (y or n)	Correct? (+ or -)
a1	14	y	+
a2	6	y	+
a3	11	y	+
a4	15	n	-
a5	10	y	+
a6	16	y	+
a7	7	y	+
a8	17	n	+
a9	18	y	-
a10	9	n	+
a11	19	y	-
a12	12	y	-
a13	20	y	-
a14	13	y	-
a15	5	n	+
a16	1	n	+
a17	2	y	-
a18	3	n	+
a19	4	n	+
a20	8	n	+

found toxic in model 3 that were not also toxic after the 24 hour storage in model 5. The reproducibility of the results between the two models indicated volatility of the organics had a minimum effect on test efficacy. As previously mentioned, without any supporting data the apparently minimal effect of volatility can only be inferred due to the high level of precision between the results for both models.

CHAPTER V

SUMMARY

The primary objective of the experiment was to determine if the Microtox toxicity analyzer would prove to be a reproducible method to define areas of contamination based on toxicity. The study was based on finding a more cost effective means to perform an initial site characterization for risk assessment. In order to determine the suitability of the Microtox to define areal contamination three specific objectives were defined: to quantify the sensitivity of the Microtox to specific chemicals, to determine the ability to distinguish whether an unknown sample is toxic or not, and to evaluate the ability of the Microtox to track a simulated groundwater contaminant plume.

Based on the results of the study, it would appear that the Microtox is quite sensitive to the specific compounds used in the analysis. The actual NOAEC range determined for lead, chromium, and TCE were similar to the concentrations found at the proposed site of study. However, the benzene and PCE NOAEC confidence ranges were above the site's specific concentration ranges. Comparison to the acute toxicity values determined by the 24-hour acute test using *Daphnia magna* reinforced the relative sensitivity of the *Phosphobacterium phosphoreum*. For the metals tested the bacteria proved to be more sensitive than the daphnids. Overall, the results, including data from later phases of the study, support the reproducibility of the bacterial response to the five compounds used in the study.

The study found the identification of toxic concentrations of various chemicals and in mixtures in unknown samples could be distinguished with reasonable accuracy. Accuracy percentages were determined to be reasonable (62.5 and 75 percent) for single chemical samples given the fact that the calculations were based on the assumption that samples with concentrations within or above the defined NOAEC range should have been found toxic. The accuracy of the bacterial response increased significantly (greater than 80%) when only samples with contaminant concentrations outside the NOAEC 95% confidence interval were considered.

The Microtox proved to be an effective means to track a simulated groundwater contaminant plume. The bacteria were able to accurately determine contamination in samples exceeding the NOAEC values for any specific contaminant. The results of experiments with the plume models showed the Microtox was able to distinguish a larger area of contamination due to the sensitivity of the bacteria to complex mixtures of contaminants.

The ability of the Microtox to detect contamination substantiates the use of the test as an initial method to characterize a site. Due to the relatively lower cost of the Microtox when compared to specific chemical analysis, the test appears to be able to provide quick information concerning areas requiring further testing.

CHAPTER VI

RESEARCH RECOMMENDATIONS

The findings of this study led to the conclusion that the Microtox would be suitable to define the extent of contamination at a site. However, the specific parameters used in the study limit the inferences that can be made concerning the general application of the test. Logical subsequent studies would examine the further usefulness of the application.

Questions concerning the suitability of the use of unconfirmed, gravimetrically prepared (nominal) concentrations of volatile concentrations need to be addressed. Although the use of nominal concentrations in toxicity tests appears to be a common practice, the actual concentration that caused the toxic responses from the bacteria was not quantified. Future studies could attempt to determine the actual concentration that causes a toxic response to the *Phosphobacterium phosphoreum*. The short time frame of the Microtox system definitely allows less variability than the traditional toxicity tests which have duration's in days not hours. To increase the general application of the methods used in the study to spatially define a contaminant plume further analysis needs to examine the effect of other source background waters and increase the number of chemical compounds used in the analysis.

SELECTED BIBLIOGRAPHY

American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1989). Standard Methods: For the Examination of Water and Wastewater, 17th edition. Franson, M. A. H., Ed., APHA.

Amodor, L. (1993). A Comparison Study of Toxicity Based Approach to Evaluate Groundwater Contamination Using Microtox and *Daphnia Magna*. Master's Thesis, Oklahoma State University, December, 1993. pp.

Atkinson, D. S., Ram, N. M., and Switzerbaum, M. S. (1985). Evaluation of the Microtox Analyzer for Assessment of Sediment Toxicity. Dept. of Civil Engrg. Env. Eng. Report No. 86-85-3, University of Massachusetts, Amherst, Massachusetts.

Baker, B. L. (1985). Surface Water Contamination by Landfill Leachates. Prepared for Alberta Environment, Research Management Division by Kananaskis Centre for Environmental Research, University of Calgary. RMD Report L-90. 27 pp.

Brown, V. M. (1968). The Calculation of the Acute Toxicity of Mixtures of Poisons to Rainbow Trout. Water Res 2:723-733.

Brown, V. M., Milrovic, V. V., and Stark, G. T. C. (1968). Effects of Chronic Exposure to Zinc on Toxicity of a Mixture of a Detergent and Zinc. Water Res 2:255-263.

Brown, V. M. and Dalton, R. A. (1970). The Acute Lethal Toxicity to Rainbow Trout of Mixtures of Copper, Phenol, Zinc, and Nickel. J Fish Biol 2:211-216.

Brungs, W. A. and Mount, D. I. (1978). Introduction to a Discussion of the Use of Aquatic Toxicity Tests for Evaluation of the Effects of Toxic Substances. Estimating the Hazard of Chemical Substances to Aquatic Life, ASTM STP 657, Cairns, J. , Dickson, K. L., and Maki, A. W., Eds., American Society for Testing and Materials. pp. 15-26.

Buikema, A. L., Neiderlehner, B. R., and Cairns, J. (1982). Biological Monitoring Part IV - Toxicity Testing. Water Res. 16:239-262.

Bulich, A. A., Greene, M. W., and Isenberg, D. L. (1981). Reliability of the Bacterial Luminescence Assay for Determination of the Toxicity of Pure Compounds and Complex Effluents. Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 338-347.

Bulich, A. A. (1988). Analytical Applications of the Microtox System. Analytical Techniques and Residuals Management in Water Pollution Control Specialty Conference, April 19-20, Atlanta, Georgia.

Burkhard, L. P. and Ankley, G. T. (1989). Identifying Toxicants: NETAC's toxicity-based approach. Environ. Sci. Technol. 23:1438-1443.

Cairns, J. (1981). Biological Monitoring Part IV - Future Needs. Water Res. 15:941-952.

Calleja, A., Baldasano, J. M., and Mulet, A. (1986). Toxicity Analysis of Leachates from Hazardous Wastes via Microtox and Daphnia Magna. Toxicity Assess. 1:73-83.

Couture, P., Blaise, C., Cluis, D., and Bastien, C. (1989). Zirconium Toxicity Assessment Using Bacteria, Algae and Fish Assays. Water, Air, and Soil Pollution. 47:87-100.

Curtis, C., Lima, A., Lozano, S. J., and Veith, G. D. (1982). Evaluation of a Bacterial Bioluminescence Bioassay as a Method for Predicting Acute Toxicity of Organic Chemicals to Fish. Aquatic Toxicology and Hazard Assessment: Fifth Conference. ASTM STP 766. Pearson, R. B., Foster, R. B., Bishop, W. E., Eds., American Society for Testing and Materials. pp. 170-178.

DeZwart, L. and Slooff, W. (1983). The Microtox as an Alternative Assay in the Acute Toxicity Assessment of Water Pollutants. Aquatic Toxicology. 4:129-138.

Durkin, P. R. (1981). Approach to the Analysis of Toxicant Interactions in the Aquatic Environment. Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 388-401.

Dutka, B. J. and Kwan, K. K. (1988). Battery of Screening Tests Approach Applied to Sediment Extracts. Toxicity Assessment. 3:303-314.

Elnabarawy, M. T. (1986). Short-Term Microbial and Biochemical Assays for Assessing Chemical Toxicity. Hazardous Substances, November, 1986.

Grange, D. and Pescheux, F. (1985). Utilisation de bacteries luminescentes pour evaluer la toxicite d'une eau Test de l'appareil Microtox. Bull. liaison Labo. P. et ch. 136:37-48.

Herbert, D. W. M. and Shurben, J. M. (1964). The Toxicity to Fish of Mixtures of poisons. I. Salts of Ammonia and Zinc. Ann Appl Biol 53:33-41.

Herbert, D. W. M. and Vandyke, J. M. (1964). The Toxicity to Fish of Mixtures of Poisons. II. Copper-ammonia and Zinc-phenol Mixtures. Ann Appl Biol 53:415-421.

Herricks, E. E. (1992). Optimizing Bioassay Use in Environmental Management Through Test System Selection. Water Sci. Tech. 25(11):479-489.

Kaiser, K. L. E., Lum, K. R. and Palabrica, V. S. (1988). Review of Field Applications of the Microtox Test in Great Lakes and St. Lawrence River Waters. Water Poll. Res. J. Canada. 23(2):270-278.

Kaiser, K. L. E., and Palabrica, V. S. (1991). Photobacterium phosphoreum Toxicity Data Index. Water Poll. Res. J. Canada. 26(3):361-431.

Koblenz, F. K. (1988). The pT-Value as a Classification Index in Aquatic Toxicology. GIT-Fachzietschrift fur das Laboratorium. 32:1-12.

Kwan, K. K., Dutka, B. J., Rao, S. S., and Liu, D. (1990). Mutatox Test: A New Test for Monitoring Environmental Genatoxic Agents. Environmental Pollution. 65:323-332.

Lankford, P. W., Eckenfelder, W. W., and Torrens, K. D. (1988). Reducing Wastewater Toxicity. Chem. Eng., Nov. 7, 1988, pp. 72-82.

Lebsack, M. E., Anderson, A. D., DeGraeve, G. M., and Bergman, H. L. (1981). Comparison of Bacterial Luminescence and Fish Bioassay Results for Fossil-Fuel Process Waters and Phenolic Constituents. Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 348-356.

Lewis, M. A. and Perry, R. L. (1981). Acute Toxicities of Equimolar and Equitoxic Surfactant Mixtures to Daphnia magna and Lepomis macrochirus. Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM

STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 402-418.

Lloyd, R. (1961) The Toxicity of Mixtures of Zinc and Copper Sulfates to Rainbow Trout. Ann Appl Biol 49: 535-538.

Marking, L. L. (1977). Method for Assessing Additive Toxicity of Chemical Mixtures. Aquatic Toxicology and Hazard Assessment ASTM STP 634. Mayer, F. L. and Hamelink, J. L., Eds., American Society for Testing and Materials. pp. 99-108.

McDowell, A. S. and Boardman, G. D. (1986). Three Screening Tests for the Evaluation of Ground Water Quality. Toxis and Hazardous Wastes'. G. D. Boardman, Ed. , Technomic Publishing Company, Inc., Lancaster, Pennsylvania.

Microbics Corporation (1992). Microtox® Manual, volumes 1 through 5.

Parkhurst, M. A., Onishi, Y., and Olsen, A. R. (1981). A Risk Assessment of Toxicants to Aquatic Life Using Environmental Exposure Estimates and Laboratory Toxicity Data. Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 59-71.

Plotkin, S. and Ram, N. M. (1984). Multiple Bioassays to Assess the Toxicity of a Sanitary Landfill Leachate. Arch. Environ. Contam. Toxicol. 13:197-206.

Qureshi, A. A., Flood, K. W., Thompson, S. R., Janhurst, S. M., Inniss, C. S., and Rokosh, D. A. (1982). Comparison of a Luminescent Bacterial Test with Other Bioassays for Determining Toxicity of Pure Compounds and Complex Effluents. Aquatic Toxicology and Hazard Assessment: Fifth Conference. ASTM STP 766. Pearson, J. G., Foster, R. B., and Bishop, W. E., Eds., American Society for Testing and Materials. pp. 179-195.

Rand, G. M. and Petrocelli, S. R (1985). Fundamentals of Aquatic Toxicology. Hemisphere, New York.

Reynolds, L., Blok, J., de Morsier, A., Gerike, P., Wellens, H., and Bontinck W. J. (1987). Evaluation of the Toxicity of Substances to be Assessed for Biodegradability. Chemosphere. 16:2259-2277.

Ribo, J. M., Zaruk, B. M., Hunter, H., and Kaiser, K. L. E. (1985). Microtox Toxicity Test Results for Water Samples from the Detroit River. J. Great Lakes Res. 11(3):297-304.

Roop, R. D. and Hunsaker, C. T. (1985). Biomonitoring for Toxics Control in NPDES Permitting. Journal WPCF. 57(4):271-277.

Sanchez, P. S., Sato, M. I., Paschoal, C. M. R. B., Alves, M. N., and Furlan, E. V. (1988). Toxicity Assessment of Industrial Effluents from San Paulo State, Brazil, Using Short-Term Microbial Assays. Toxicity Assessment. 3:55-80.

Skalski, J. R. (1981). Statistical Inconsistencies in the Use of No-Observed-Effect Levels in Toxicity Testing. Aquatic Toxicology and Hazard Assessment:

Fourth Conference. ASTM STP 737. Branson, D. R. and Dickson, K. L., Eds., American Society for Testing and Materials. pp. 377-387.

Slooff, W., van Oers, J. A. M., and de Zwart, D. (1986). Margins of Uncertainty in Ecotoxicological Hazard Assessment. Environ. Toxicol. Chem. 5(9):841-852.

Stephan, C. E. (1977). Methods for Calculating an LC50. Aquatic Toxicology and Hazard Assessment. ASTM STP 634. Mayer, F. L. and Hamelink, J. L., Eds., American Society for Testing and Materials. pp. 65-84.

Stephan, C. E. (1982). Increasing the Usefulness of Acute Toxicity Tests. Aquatic Toxicology and Hazard Assessment: Fifth Conference. ASTM STP 766. Pearson, J. G., Foster, R. B., and Bishop, W. E., Eds., American Society for Testing and Materials. pp. 69-81.

Thomas, J. M., Skalski, J. R., Cline, J. F., McShane, M. C., Simpson, J. C., Miller, W. E., Peterson, S. A., Callahan, C. A., and Greene, J. C. (1986). Characteristics of Chemical Waste Site Contamination and Determination of Its Extent Using Bioassays. Environmental Toxicology and Chemistry. 5:487-501.

U. S. Army Corps of Engineers, Tulsa District (1988). Tinker Air Force Base Installation Restoration Program, Building 3001: Remedial Investigations, vol. 1.

U. S. Council on Environmental Quality, Executive Office of the President (1989). Risk Analysis: A Guide to Principles and Methods for Analyzing Health and Environmental Risks. Cohrssen, J. J. and Covello, V. T., Eds.

U. S. Environmental Protection Agency (1979). Guidance for Premanufacture Testing: Discussion of policy issues, Alternative Approaches and Test Methods. Fed. Reg. 44, 16240.

U. S. Environmental Protection Agency (1980a). Ambient Water Quality Criteria for Benzene. EPA 440/5-80-018.

U. S. Environmental Protection Agency (1980b). Ambient Water Quality Criteria for Tetrachloroethylene. EPA 440/5-80-073.

U. S. Environmental Protection Agency (1980c). Ambient Water Quality Criteria for Trichloroethylene. EPA 440/5-80-077.

U. S. Environmental Protection Agency (1988). Underground Storage Facility (UST) Guidance, Seminar Document. Office of Solid Waste and Emergency Response.

U. S. Environmental Protection Agency (1989a). Risk Assessment Guidance for Superfund: Volume 1 - Human Health Evaluation Manual (Part A). EPA 540/1-89/002.

U. S. Environmental Protection Agency (1989b). Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA 600/4-89/001.

U. S. Environmental Protection Agency (1991a). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA 600 4-90/027.

U. S. Environmental Protection Agency (1991b). Technical Support Document for Water Quality-based Toxics Control. EPA 505/2-90-001. PB91-127415, March 1991.

Vasseur, P. J., Ferrard, J. F., Rast, C., and Larbaight, G. (1984). Luminescent Marine Bacteria in Exotoxicity Screening Tests of Complex Effluents. Toxicity Screening Procedures Using Bacterial Systems. Liu, D. and Dutka, B. J., Eds., Marcel Dekker, New York. pp. 23-35.

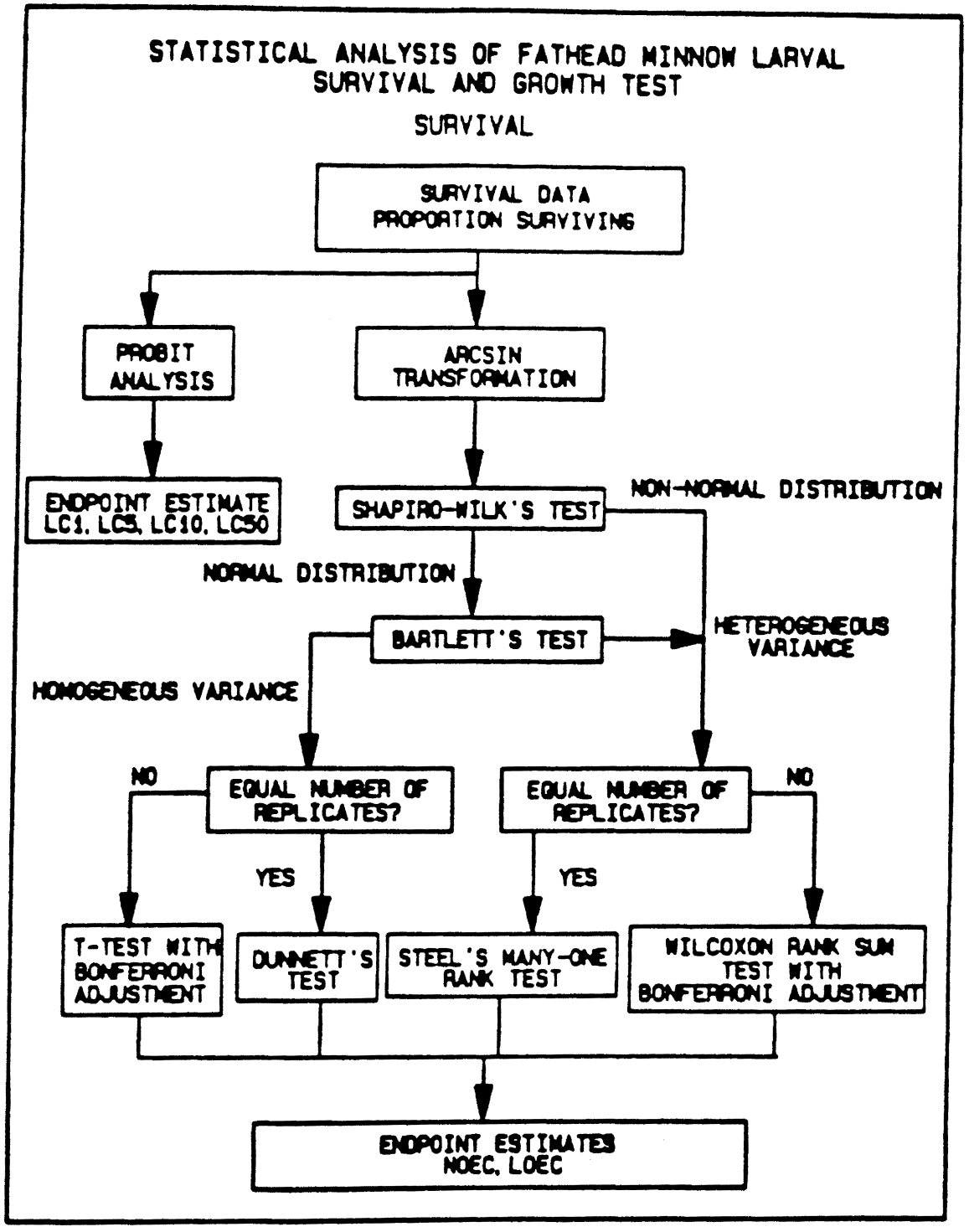
Walker, J. D. (1987). Effects of Chemicals on Microorganisms. Journal WCF. 59(6):614-626.

Williams, L. G., Chapman, P. M., and Ginn, T. C. (1986). A Comparative Evaluation of Marine Sediment Toxicity Using Bacterial Luminescence, Oyster Embryo and Amphipod Sediment Bioassays. Marine Environmental Research 19:225-249.

Winkless, N. (1988). Will it Hurt? Chemtech. 18:658-660.

APPENDIX A

USEPA 's Statistical Analysis Flowchart


STATISTICAL ANALYSIS OF FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST
SURVIVAL
SURVIVAL DATA PROPORTION SURVIVING
PROBIT ANALYSIS
ARCSIN TRANSFORMATION
ENDPOINT ESTIMATE LC1, LC5, LC10, LC50
SHAPIRO-WILK'S TEST
NON-NORMAL DISTRIBUTION
NORMAL DISTRIBUTION
BARTLETT'S TEST
HETEROGENEOUS VARIANCE
HOMOGENEOUS VARIANCE
NO
EQUAL NUMBER OF REPLICATES?
EQUAL NUMBER OF REPLICATES?
NO
YES
YES
T-TEST WITH BONFERRONI ADJUSTMENT
DUNNETT'S TEST
STEEL'S MANY-ONE RANK TEST
WILCOXON RANK SUM TEST WITH BONFERRONI ADJUSTMENT
ENDPOINT ESTIMATES NOEC, LOEC

APPENDIX B

Toxicity Screening Procedure

Toxicity Screening Procedure

1. Fill all wells except turret well with clean, empty cuvettes.
2. Perform Pre-test instrument check according to manufacturer's instructions. (Instrument check only necessary for Model 2055).
3. Add 1000 uL Reconstitution Solution to cuvette in precooling well.
4. Add 500 uL Diluent to A1 through C4.
5. Add 2500 uL of very hard reconstituted water (280 mg/L as CaCO3) and 250 uL Microtox Osmotic Adjusting Solution (MOAS) to A5. Mix 20 times with the 500 uL pipette.
6. Likewise, add 2500 uL of sample and 250 uL MOAS to B5 and C5.
7. Wait 5 minutes for temperature equilibrium.
8. Reconstitute Microtox reagent according to manufacturer's procedures.
9. Transfer 10 uL reconstituted reagent to A1 to C4.
10. Mix each cuvette 5 times with the 250 uL pipette.
11. Wait 15 minutes to allow maximum light output.
12. Place cuvette A1 into turret well and close. Adjust reading to approximately 90 with SPAN (100% ADJ) dial. Cycle remaining cuvettes to obtain I_0 light levels.

    Determine which nine cuvettes are the most closely grouped and place them in A2 through C4. Reset SPAN dial using highest cuvette as 100. Once again, cycle through cuvettes to establish initial (I_0) light levels.
13. Immediately transfer 500 uL from A5 to A2, A3, and A4. Likewise, transfer 500 uL from both B5 and C5 to the corresponding cuvettes. Mix each cuvette (A2 through C4) 5 times with the 500 uL pipette. Start timer.

14. After 5 minutes get I_5 light level readings from each of cuvettes A2 through C4. Start timer for 10 minutes.
15. Take I_{15} light level readings for each cuvette.
16. Repeat until all samples have been analyzed.

APPENDIX C

NOAEC Calculations

Lab Data
Determination of NOEC
(Organic compounds)

NOEC data

Benzene (mg/L)		PCE (mg/L)		TCE (mg/L)	
5.688		2.275		11.375	
2.275		0.546		11.375	
1.808		2.730		5.688	
4.550		2.275		5.688	
3.580	average	1.706		5.688	
1.846	std. dev.	1.906	average	11.375	
1.809	95% conf. limit	0.843	std. dev.	5.688	
5.389	upper limit	0.739	95% conf. limit	8.125	average
1.771	lower limit	2.645	upper limit	3.040	std. dev.
		1.168	lower limit	2.252	95% conf. limit
				10.377	upper limit
				5.873	lower limit

APPENDIX D

Raw Data

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Benzene
Test No.: 1
Date Run: 8/16/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	92	98	94	93
5.7	86	94	87	93
11.4	81	92	82	88
22.8	69	83	64	77
45.5	45	59	42	58

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Benzene
Test No.: 2
Date Run: 8/16/93
Initial Conc.: 20.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	98	102	92	102
2.3	91	94	93	95
4.6	85	95	87	92
9.1	82	93	78	85
18.2	72	83	71	82

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Benzene
Test No.: 3
Date Run: 8/16/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	105	119	100	114
5.7	98	92	95	93
11.4	87	92	89	93
22.8	80	83	78	79
45.5	67	68	64	65

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Benzene
Test No.: 4
Date Run: 5/24/93
Initial Conc.: 7.9
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	83	82	78	85
0.9	79	82	81	77
1.8	75	80	80	80
3.6	80	79	75	76
7.2	77	70	69	72

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Benzene
Test No.: 5
Date Run: 8/16/93
Initial Conc.: 20.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	100	98	93	91
2.3	89	94	86	93
4.6	90	94	83	92
9.1	76	92	74	92
18.2	68	78	68	77

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Chromium
Test No.: 1
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	78	76	78	77
0.91	44	41	30	33
0.45	57	59	51	51
0.22	73	72	68	68
0.11	79	82	71	80

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Chromium
Test No.: 2
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	76	71	71	71
0.91	65	64	56	58
0.45	66	66	70	63
0.22	72	70	69	68
0.11	73	73	71	73

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Chromium
Test No.: 3
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	68	71	64	68
0.91	30	28	28	26
0.45	47	43	50	44
0.22	59	62	50	61
0.11	64	69	64	57

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Chromium
Test No.: 4
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	73	78	74	71
0.91	25	28	27	25
0.45	41	39	42	38
0.22	50	57	56	52
0.11	71	70	67	73

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Lead
Test No.: 1
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	85	78	77	77
0.91	14	14	15	14
0.45	38	34	37	35
0.22	60	6	56	54
0.11	74	76	76	75

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Lead
Test No.: 2
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	81	83	81	81
0.91	27	30	31	31
0.45	48	42	42	55
0.22	59	63	64	61
0.11	80	79	77	80

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Lead
Test No.: 3
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	76	68	76	74
0.91	7	7	7	7
0.45	14	13	14	14
0.22	43	48	44	42
0.11	66	65	70	72

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: Lead
Test No.: 4
Date Run:
Initial Conc.: 1.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	63	69	68	67
0.91	33	35	37	32
0.45	50	53	47	45
0.22	62	57	61	58
0.11	71	65	69	63

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 1
Date Run: 6/14/93
Initial Conc.: 5.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	82	85	86	87
0.6	84	89	75	85
1.1	83	87	69	82
2.3	83	81	72	82
4.6	74	78	64	76

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 2
Date Run: 6/3/93
Initial Conc.: 1.2
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	85	85	91	88
0.1	80	84	84	93
0.3	80	88	81	89
0.5	80	90	81	84
1.1	83	86	74	80

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 3
Date Run: 6/18/93
Initial Conc.: 12.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	95	93	88	88
1.4	88	97	82	91
2.7	81	95	79	91
5.5	71	85	69	85
10.9	57	66	58	66

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 4
Date Run: 6/3/93
Initial Conc.: 10.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	90	91	90	90
1.1	90	85	90	83
2.3	86	92	87	85
4.6	85	90	82	84
9.1	79	81	77	77

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 5
Date Run: 6/18/93
Initial Conc.: 15.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	80	79	82	86
1.7	78	59	79	86
3.4	53	54	74	83
6.8	51	61	67	83
13.7	54	51	56	62

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 6
Date Run: 6/14/93
Initial Conc.: 1.2
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	83	89	85	90
0.1	84	88	89	88
0.3	82	84	85	91
0.5	80	82	79	91
1.1	80	87	89	84

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: PCE
Test No.: 7
Date Run: 6/28/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	102	106	102	98
5.7	87	85	82	80
11.4	77	83	72	75
22.8	60	64	57	64
45.5	36	39	37	40

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 1
Date Run: 8/16/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	92	85	90	99
5.7	86	88	88	88
11.4	88	88	86	93
22.8	79	81	71	72
45.5	66	68	63	64

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 2
Date Run: 8/4/93
Initial Conc.: 25.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	63	67	61	63
2.8	69	57	60	61
5.7	59	64	63	67
11.4	60	57	62	66
22.8	58	58	59	60

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 3
Date Run: 8/4/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	70	68	74	72
5.7	68	65	69	72
11.4	65	63	65	68
22.8	57	54	60	56
45.5	48	47	50	51

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 4
Date Run: 8/4/93
Initial Conc.: 12.5
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	55	54	51	53
1.4	49	55	54	49
2.8	48	51	48	56
5.7	53	52	56	51
11.4	49	48	42	50

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 5
Date Run: 8/4/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	71	73	72	70
5.7	70	69	71	68
11.4	63	70	62	71
22.8	52	64	55	66
45.5	37	37	37	38

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 6
Date Run: 6/21/93
Initial Conc.: 90.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	88	93	93	97
10.2	93	88	94	93
20.5	85	87	88	93
41.0	82	84	78	80
81.9	65	68	63	65

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 7
Date Run: 8/4/93
Initial Conc.: 12.5
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	53	50	55	49
1.4	45	49	45	36
2.9	44	47	45	33
5.7	44	47	51	47
11.4	44	45	39	48

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 8
Date Run: 8/4/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	70	70	69	70
5.7	67	70	67	65
11.4	60	67	61	70
22.8	51	64	52	65
45.5	34	35	37	33

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 9
Date Run: 6/21/93
Initial Conc.: 90.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	80	87	84	95
10.2	88	83	83	91
20.5	78	73	80	96
41.0	72	71	74	82
81.9	57	57	62	66

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 10
Date Run: 8/4/93
Initial Conc.: 25.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	71	76	69	70
2.8	63	62	64	66
5.7	63	71	69	73
11.4	63	62	67	72
22.8	63	63	62	63

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 11
Date Run: 8/4/93
Initial Conc.: 50.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	73	71	81	76
5.7	71	68	73	76
11.4	69	67	69	72
22.8	60	58	64	61
45.5	50	49	52	53

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 12
Date Run: 6/2/93
Initial Conc.: 100.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	82	87	87	88
11.4	75	75	81	87
22.8	76	70	78	85
45.5	62	72	71	73
91.0	57	57	52	69

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 13
Date Run: 6/21/93
Initial Conc.: 45.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	88	87	93	94
5.1	94	86	89	82
10.2	85	79	86	81
20.5	78	74	81	77
41.0	70	63	66	61

Raw Data / Luminescence Readings
Compound: TCE
Test No.: 14
Date Run: 6/21/93
Initial Conc.: 90.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	80	87	84	95
5.1	88	83	83	91
10.2	78	73	80	96
20.5	72	71	74	82
41.0	57	57	62	66

15 minute Microtox Test

Raw Data / Luminescence Readings
Compound: TCE
Test No.: 15
Date Run: 6/2/93
Initial Conc.: 10.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	89	93	85	85
1.1	88	93	90	89
2.3	89	88	92	86
4.6	84	83	95	91
9.1	88	87	91	87

15 minute Microtox Test
Raw Data / Luminescence Readings
Compound: TCE
Test No.: 16
Date Run: 6/28/93
Initial Conc.: 100.0
(mg/L)

concentration (mg/L)	replicate 1	replicate 2	replicate 3	replicate 4
control	95	98	91	96
11.4	86	93	88	98
22.8	83	93	84	92
45.5	74	86	74	89
91.0	58	73	63	76

24 hour D. magna Test
Raw Data
Compound: Chromium
Test No.: 1
Date Run:
Initial Conc.: 3.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
0.25	0/20	0/20	0/20	0/20	0
0.5	0/20	0/20	0/20	2/20	10
1.0	0/20	0/20	0/20	3/20	15
1.5	0/17	0/17	0/17	7/17	59
3.0	0/20	0/20	0/20	20/20	100

24 hour D. magna Test
Raw Data
Compound: Chromium
Test No.: 2
Date Run:
Initial Conc.: 3.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
0.25	0/20	0/20	0/20	0/20	0
0.5	0/20	0/20	0/20	1/20	5
1.0	0/19	0/19	0/19	6/19	31
1.5	0/19	0/19	0/19	16/19	84
3.0	0/19	0/19	0/19	19/19	100

24 hour D. magna Test
Raw Data
Compound: Chromium
Test No.: 3
Date Run:
Initial Conc.: 3.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
0.25	0/20	0/20	0/20	0/20	0
0.5	0/20	0/20	0/20	2/20	10
1.0	0/20	0/20	0/20	6/20	30
1.5	0/20	0/20	0/20	16/20	80
3.0	0/20	0/20	0/20	20/20	100

24 hour D. magna Test
Raw Data
Compound: Chromium
Test No.: 4
Date Run:
Initial Conc.: 3.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
0.25	0/20	0/20	0/20	0/20	0
0.5	0/20	0/20	0/20	1/20	5
1.0	0/20	0/20	0/20	4/20	20
1.5	0/20	0/20	0/20	15/20	75
3.0	0/20	0/20	0/20	20/20	100

24 hour D. magna Test
Raw Data
Compound: Lead
Test No.: 1
Date Run:
Initial Conc.: 12.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
5.0	0/20	0/20	0/20	0/20	0
7.0	0/20	0/20	0/20	9/20	45
9.0	0/20	0/20	0/20	11/20	55
12.0	0/20	0/20	0/20	20/20	100

24 hour D. magna Test
Raw Data
Compound: Lead
Test No.: 2
Date Run:
Initial Conc.: 12.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
5.0	0/20	0/20	0/20	0/20	0
7.0	0/20	0/20	0/20	1/20	10
9.0	0/20	0/20	0/20	11/20	55
12.0	0/20	0/20	0/20	19/20	95

24 hour D. magna Test
Raw Data
Compound: Lead
Test No.: 3
Date Run:
Initial Conc.: 12.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
5.0	0/20	0/20	0/20	0/20	0
7.0	0/20	0/20	0/20	2/20	10
9.0	0/20	0/20	0/20	14/20	70
12.0	0/20	0/20	0/20	20/20	100

24 hour D. magna Test
Raw Data
Compound: Lead
Test No.: 2
Date Run:
Initial Conc.: 12.0
(mg/L)

concentration (mg/L)	2 hour	4 hour	8 hour	24 hour	% mortality
control	0/20	0/20	0/20	0/20	0
5.0	0/20	0/20	0/20	2/20	10
7.0	0/20	0/20	0/20	7/20	35
9.0	0/20	0/20	0/20	14/20	70
12.0	0/20	0/20	0/20	20/20	100

APPENDIX E

Model Calculations

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: A
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	95	94
	96	89
	88	91
sum	279	274
average	93	91
std. dev.	4.359	2.517

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	93	4.933	88.067	97.933
sample	91	2.848	88.486	94.181

toxic? no

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: B
Concentration 18 (mg/L)

	control (I15)	sample (I15)
	95	85
	96	82
	88	83
sum	279	250
average	93	83
std. dev.	4.359	1.528

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	93	4.933	88.067	97.933
sample	83	1.729	81.605	85.062

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: C
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	90	76
	88	75
	87	74
sum	265	225
average	88	75
std. dev.	1.528	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	75	1.132	73.868	76.132

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: D
Concentration 15 (mg/L)

	control (I15)	sample (I15)
	90	73
	88	72
	87	72
sum	265	217
average	88	72
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	72	0.653	71.680	72.987

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: C
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	90	76
	88	75
	87	74
sum	265	225
average	88	75
std. dev.	1.528	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	75	1.132	73.868	76.132

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: D
Concentration 15 (mg/L)

	control (I15)	sample (I15)
	90	73
	88	72
	87	72
sum	265	217
average	88	72
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	72	0.653	71.680	72.987

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: E
Concentration 15 (mg/L)

	control (I15)	sample (I15)
	80	82
	77	76
	80	79
sum	237	237
average	79	79
std. dev.	1.732	3.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.960	77.040	80.960
sample	79	3.395	75.605	82.395

toxic? no

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: F
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	93	85
	80	85
	80	86
sum	253	256
average	84	85
std. dev.	7.506	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	84	8.493	75.840	92.827
sample	85	0.653	84.680	85.987

toxic? no

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: G
Concentration 12 (mg/L)

	control (I15)	sample (I15)
	85	75
	88	75
	84	76
sum	257	226
average	86	75
std. dev.	2.082	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	86	2.356	83.311	88.022
sample	75	0.653	74.680	75.987

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 8/26/93
Sample: H
Concentration 12 (mg/L)

	control (I15)	sample (I15)
	85	78
	88	79
	84	81
sum	257	238
average	86	79
std. dev.	2.082	1.528

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	86	2.356	83.311	88.022
sample	79	1.729	77.605	81.062

toxic? yes

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: A
Concentration 3 (mg/L)

	control (I15)	sample (I15)
	93	94
	94	92
	95	90
sum	282	276
average	94	92
std. dev.	1.000	2.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	94	1.132	92.868	95.132
sample	92	2.263	89.737	94.263

toxic? no

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: B
Concentration 10 (mg/L)

	control (I15)	sample (I15)
	93	91
	94	92
	95	91
sum	282	274
average	94	91
std. dev.	1.000	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	94	1.132	92.868	95.132
sample	91	0.653	90.680	91.987

toxic? yes

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: C
Concentration 15 (mg/L)

	control (I15)	sample (I15)
	90	81
	87	83
	88	83
sum	265	247
average	88	82
std. dev.	1.528	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	82	1.307	81.027	83.640

toxic? yes

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: D
Concentration 12 (mg/L)

	control (I15)	sample (I15)
	90	85
	87	85
	88	86
sum	265	256
average	88	85
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	1.729	86.605	90.062
sample	85	0.653	84.680	85.987

toxic? yes

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: E
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	91	90
	88	89
	86	88
sum	265	267
average	88	89
std. dev.	2.517	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	2.848	85.486	91.181
sample	89	1.132	87.868	90.132

toxic? no

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: F
Concentration 10 (mg/L)

	control (I15)	sample (I15)
	91	89
	88	89
	86	88
sum	265	266
average	88	89
std. dev.	2.517	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	2.848	85.486	91.181
sample	89	0.653	88.013	89.320

toxic? no

15 min Microtox Test
Model 2 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: G
Concentration 15 (mg/L)

	control (I15)	sample (I15)
	85	82
	84	75
	83	76
sum	252	233
average	84	78
std. dev.	1.000	3.786

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	84	1.132	82.868	85.132
sample	78	4.284	73.382	81.951

toxic? yes

15 min Microtox Test
Model 1 - Hide and Seek
Compound: Trichloroethene
Date Run: 9/28/93
Sample: H
Concentration 6 (mg/L)

	control (I15)	sample (I15)
	85	80
	84	81
	83	82
sum	252	243
average	84	81
std. dev.	1.000	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	84	1.132	82.868	85.132
sample	81	1.132	79.868	82.132

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 1

	control (I15)	sample (I15)
	80	78
	79	80
	78	79
sum	237	237
average	79	79
std. dev.	1.000	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.132	77.868	80.132
sample	79	1.132	77.868	80.132

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 2

	control (I15)	sample (I15)
	82	79
	79	82
	82	82
sum	243	243
average	81	81
std. dev.	1.732	1.732

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	81	1.960	79.040	82.960
sample	81	1.960	79.040	82.960

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 3

	control (I15)	sample (I15)
	76	77
	77	73
	74	76
sum	227	226
average	76	75
std. dev.	1.528	2.082

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	76	1.729	73.938	77.395
sample	75	2.356	72.978	77.689

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 4

	control (I15)	sample (I15)
	83	75
	80	80
	82	73
sum	245	228
average	82	76
std. dev.	1.528	3.606

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	76	4.080	71.920	80.080

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 5

	control (I15)	sample (I15)
	83	80
	80	81
	82	80
sum	245	241
average	82	80
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	80	0.653	79.680	80.987

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 6

	control (I15)	sample (I15)
	80	46
	78	47
	77	47
sum	235	140
average	78	47
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	78	1.729	76.605	80.062
sample	47	0.653	46.013	47.320

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 7

	control (I15)	sample (I15)
	82	71
	79	70
	82	72
sum	243	213
average	81	71
std. dev.	1.732	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	81	1.960	79.040	82.960
sample	71	1.132	69.868	72.132

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 8

	control (I15)	sample (I15)
	77	75
	75	76
	75	77
sum	227	228
average	76	76
std. dev.	1.155	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	76	1.307	74.360	76.973
sample	76	1.132	74.868	77.132

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 9

	control (I15)	sample (I15)
	76	68
	72	67
	71	77
sum	219	212
average	73	71
std. dev.	2.646	5.508

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	73	2.994	70.006	75.994
sample	71	6.232	64.434	76.899

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 10

	control (I15)	sample (I15)
	77	69
	75	68
	75	71
sum	227	208
average	76	69
std. dev.	1.155	1.528

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	76	1.307	74.360	76.973
sample	69	1.729	67.605	71.062

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 11

	control (I15)	sample (I15)
	76	44
	77	45
	74	44
sum	227	133
average	76	44
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	76	1.729	73.938	77.395
sample	44	0.653	43.680	44.987

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 12

	control (I15)	sample (I15)
	80	78
	79	78
	78	79
sum	237	235
average	79	78
std. dev.	1.000	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.132	77.868	80.132
sample	78	0.653	77.680	78.987

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 13

	control (I15)	sample (I15)
	102	84
	98	83
	96	88
sum	296	255
average	99	85
std. dev.	3.055	2.646

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	99	3.457	95.210	102.124
sample	85	2.994	82.006	87.994

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 14

	control (I15)	sample (I15)
	119	72
	98	77
	96	57
sum	313	206
average	104	69
std. dev.	12.741	10.408

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	104	14.418	89.916	118.751
sample	69	11.778	56.889	80.445

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 15

	control (I15)	sample (I15)
	91	92
	90	93
	90	81
sum	271	266
average	90	89
std. dev.	0.577	6.658

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	90	0.653	89.680	90.987
sample	89	7.535	81.132	96.201

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 16

	control (I15)	sample (I15)
	106	98
	102	97
	101	99
sum	309	294
average	103	98
std. dev.	2.646	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	103	2.994	100.006	105.994
sample	98	1.132	96.868	99.132

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 17

	control (I15)	sample (I15)
	106	96
	102	92
	96	89
sum	304	277
average	101	92
std. dev.	5.033	3.512

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	101	5.696	95.638	107.029
sample	92	3.974	88.359	96.307

toxic? no

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 18

	control (I15)	sample (I15)
	99	95
	98	89
	96	93
sum	293	277
average	98	92
std. dev.	1.528	3.055

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	98	1.729	95.938	99.395
sample	92	3.457	88.876	95.790

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 19

	control (I15)	sample (I15)
	92	91
	98	83
	95	85
sum	285	259
average	95	86
std. dev.	3.000	4.163

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	95	3.395	91.605	98.395
sample	86	4.711	81.622	91.045

toxic? yes

15 min Microtox Test
Model 3 - Plume Tracking
Date Run: 10/16/93
Sample: 20

	control (I15)	sample (I15)
	93	84
	90	85
	90	85
sum	273	254
average	91	85
std. dev.	1.732	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	91	1.960	89.040	92.960
sample	85	0.653	84.013	85.320

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: A

	control (I15)	sample (I15)
	109	103
	107	105
	102	103
sum	318	311
average	106	104
std. dev.	3.606	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	106	4.080	101.920	110.080
sample	104	1.307	102.360	104.973

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: B

	control (I15)	sample (I15)
	109	109
	107	107
	102	104
sum	318	320
average	106	107
std. dev.	3.606	2.517

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	106	4.080	101.920	110.080
sample	107	2.848	103.819	109.514

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: C

	control (I15)	sample (I15)
	109	105
	107	108
	102	104
sum	318	317
average	106	106
std. dev.	3.606	2.082

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	106	4.080	101.920	110.080
sample	106	2.356	103.311	108.022

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: D

	control (I15)	sample (I15)
	93	82
	90	83
	94	83
sum	277	248
average	92	83
std. dev.	2.082	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	92	2.356	89.978	94.689
sample	83	0.653	82.013	83.320

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: E

	control (I15)	sample (I15)
	84	51
	84	52
	85	51
sum	253	154
average	84	51
std. dev.	0.577	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	84	0.653	83.680	84.987
sample	51	0.653	50.680	51.987

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: F

	control (I15)	sample (I15)
	79	76
	86	71
	84	69
sum	249	216
average	83	72
std. dev.	3.606	3.606

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	83	4.080	78.920	87.080
sample	72	4.080	67.920	76.080

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: G

	control (I15)	sample (I15)
	82	71
	77	72
	79	73
sum	238	216
average	79	72
std. dev.	2.517	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	2.848	76.486	82.181
sample	72	1.132	70.868	73.132

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: H

	control (I15)	sample (I15)
	92	68
	85	62
	86	72
sum	263	202
average	88	67
std. dev.	3.786	5.033

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	4.284	83.382	91.951
sample	67	5.696	61.638	73.029

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: I

	control (I15)	sample (I15)
	80	82
	78	78
	78	77
sum	236	237
average	79	79
std. dev.	1.155	2.646

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.307	77.360	79.973
sample	79	2.994	76.006	81.994

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: J

	control (I15)	sample (I15)
	83	84
	81	81
	81	86
sum	245	251
average	82	84
std. dev.	1.155	2.517

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.307	80.360	82.973
sample	84	2.848	80.819	86.514

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: K

	control (I15)	sample (I15)
	83	83
	81	83
	81	83
sum	245	249
average	82	83
std. dev.	1.155	0.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.307	80.360	82.973
sample	83	0.000	83.000	83.000

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: L

	control (I15)	sample (I15)
	82	78
	77	78
	79	80
sum	238	236
average	79	79
std. dev.	2.517	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	2.848	76.486	82.181
sample	79	1.307	77.360	79.973

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: M

	control (I15)	sample (I15)
	86	53
	86	54
	84	53
sum	256	160
average	85	53
std. dev.	1.155	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	85	1.307	84.027	86.640
sample	53	0.653	52.680	53.987

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: N

	control (I15)	sample (I15)
	93	95
	90	96
	94	95
sum	277	286
average	92	95
std. dev.	2.082	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	92	2.356	89.978	94.689
sample	95	0.653	94.680	95.987

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: O

	control (I15)	sample (I15)
	84	47
	84	48
	85	48
sum	253	143
average	84	48
std. dev.	0.577	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	84	0.653	83.680	84.987
sample	48	0.653	47.013	48.320

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: P

	control (I15)	sample (I15)
	86	67
	86	66
	84	66
sum	256	199
average	85	66
std. dev.	1.155	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	85	1.307	84.027	86.640
sample	66	0.653	65.680	66.987

toxic? yes

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: Q

	control (I15)	sample (I15)
	79	76
	76	73
	74	72
sum	229	221
average	76	74
std. dev.	2.517	2.082

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	76	2.848	73.486	79.181
sample	74	2.356	71.311	76.022

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: R

	control (I15)	sample (I15)
	80	79
	78	80
	78	81
sum	236	240
average	79	80
std. dev.	1.155	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.307	77.360	79.973
sample	80	1.132	78.868	81.132

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: S

	control (I15)	sample (I15)
	92	83
	85	81
	86	83
sum	263	247
average	88	82
std. dev.	3.786	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	88	4.284	83.382	91.951
sample	82	1.307	81.027	83.640

toxic? no

15 min Microtox Test
Model 4 - Plume Tracking
Date Run: 10/16/93
Sample: T

	control (I15)	sample (I15)
	80	77
	78	78
	77	78
sum	235	233
average	78	78
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	78	1.729	76.605	80.062
sample	78	0.653	77.013	78.320

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 1

	control (I15)	sample (I15)
	78	75
	76	77
	78	78
sum	232	230
average	77	77
std. dev.	1.155	1.528

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	77	1.307	76.027	78.640
sample	77	1.729	74.938	78.395

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 2

	control (I15)	sample (I15)
	83	76
	80	77
	82	79
sum	245	232
average	82	77
std. dev.	1.528	1.528

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	77	1.729	75.605	79.062

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 3

	control (I15)	sample (I15)
	78	71
	76	72
	78	78
sum	232	221
average	77	74
std. dev.	1.155	3.786

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	77	1.307	76.027	78.640
sample	74	4.284	69.382	77.951

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 4

	control (I15)	sample (I15)
	82	91
	80	90
	80	91
sum	242	272
average	81	91
std. dev.	1.155	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	81	1.307	79.360	81.973
sample	91	0.653	90.013	91.320

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 5

	control (I15)	sample (I15)
	74	74
	72	73
	72	75
sum	218	222
average	73	74
std. dev.	1.155	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	73	1.307	71.360	73.973
sample	74	1.132	72.868	75.132

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 6

	control (I15)	sample (I15)
	80	47
	76	48
	78	47
sum	234	142
average	78	47
std. dev.	2.000	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	78	2.263	75.737	80.263
sample	47	0.653	46.680	47.987

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 7

	control (I15)	sample (I15)
	83	76
	80	77
	82	77
sum	245	230
average	82	77
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	77	0.653	76.013	77.320

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 8

	control (I15)	sample (I15)
	88	86
	82	84
	89	88
sum	259	258
average	86	86
std. dev.	3.786	2.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	86	4.284	82.049	90.618
sample	86	2.263	83.737	88.263

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 9

	control (I15)	sample (I15)
	88	86
	82	84
	89	88
sum	259	258
average	86	86
std. dev.	3.786	2.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	86	4.284	82.049	90.618
sample	86	2.263	83.737	88.263

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 10

	control (I15)	sample (I15)
	74	65
	72	64
	72	66
sum	218	195
average	73	65
std. dev.	1.155	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	73	1.307	71.360	73.973
sample	65	1.132	63.868	66.132

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 11

	control (I15)	sample (I15)
	82	50
	80	50
	80	52
sum	242	152
average	81	51
std. dev.	1.155	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	81	1.307	79.360	81.973
sample	51	1.307	49.360	51.973

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 12

	control (I15)	sample (I15)
	82	77
	83	79
	80	79
sum	245	235
average	82	78
std. dev.	1.528	1.155

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	78	1.307	77.027	79.640

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 13

	control (I15)	sample (I15)
	80	75
	78	74
	78	75
sum	236	224
average	79	75
std. dev.	1.155	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.307	77.360	79.973
sample	75	0.653	74.013	75.320

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 14

	control (I15)	sample (I15)
	80	63
	78	62
	78	61
sum	236	186
average	79	62
std. dev.	1.155	1.000

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	1.307	77.360	79.973
sample	62	1.132	60.868	63.132

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 15

	control (I15)	sample (I15)
	81	81
	80	82
	80	81
sum	241	244
average	80	81
std. dev.	0.577	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	80	0.653	79.680	80.987
sample	81	0.653	80.680	81.987

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 16

	control (I15)	sample (I15)
	81	75
	78	75
	77	76
sum	236	226
average	79	75
std. dev.	2.082	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	2.356	76.311	81.022
sample	75	0.653	74.680	75.987

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 17

	control (I15)	sample (I15)
	81	81
	80	82
	80	82
sum	241	245
average	80	82
std. dev.	0.577	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	80	0.653	79.680	80.987
sample	82	0.653	81.013	82.320

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 18

	control (I15)	sample (I15)
	81	75
	78	75
	77	76
sum	236	226
average	79	75
std. dev.	2.082	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	79	2.356	76.311	81.022
sample	75	0.653	74.680	75.987

toxic? yes

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 19

	control (I15)	sample (I15)
	82	82
	83	81
	80	82
sum	245	245
average	82	82
std. dev.	1.528	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	82	1.729	79.938	83.395
sample	82	0.653	81.013	82.320

toxic? no

15 min Microtox Test
Model 5 - Plume Tracking
Date Run: 10/17/93
Sample: 20

	control (I15)	sample (I15)
	80	73
	86	72
	78	72
sum	244	217
average	81	72
std. dev.	4.163	0.577

95% Confidence Limit Intervals

		interval	lower limit	upper limit
control	81	4.711	76.622	86.045
sample	72	0.653	71.680	72.987

toxic? yes

VITA

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