Three Screening Tests Used to Evaluate Groundwater Quality

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Sciences

in

Environmental Science and Engineering

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January, 1986

Blacksburg, Virginia

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(ABSTRACT)

The recent emergence of the critical problem of groundwater contamination due to materials leaching from toxic waste disposal sites and other sources has had a sobering impact on society. The rash of unexpected contamination has led to a plethora of legislation designed to address this problem. Along with this legislation has come the need to be able to accurately, effectively, and economically detect the contamination of groundwater.

The primary objectives of the research reported here were to investigate the use of the parameters total organic carbon (TOC), total organic halide (TOX), and the photoluminescent bacterial bioassay, Microtox (Beckman Instruments, Carlsbad, CA.) as screening parameters for the evaluation of groundwater quality. This objective was accomplished through an analysis of background information from the literature including a Virginia State Water Control Board study of groundwater quality in Southwestern Virginia, a laboratory study, a United States Department of Agriculture groundwater project, and analysis of five field samples collected from uncontaminated and contaminated sources. The TOC and TOX tests were shown to provide important information. Microtox bioassays

were performed on all samples and the results were correlated with the chemical analysis.

It was concluded that the Microtox bioassay was a useful screening test for groundwater quality and that this assay in combination with the TOC and TOX parameters provided a reasonable indication of groundwater quality.

ACKNOWLEDGEMENTS

This research project was a combination of a great deal of information and experience gained while the author worked for the Virginia State Water Control Board, United States Department of Agriculture, and the classrooms and in the laboratories of Virginia Tech.

I would like to thank Julie Petruska and Maryland Grender for their expertise and excellent management of the environmental laboratories at Virginia Tech.

The experience of working for the Virginia Water board was invaluable and an excellent first step. I would like to thank Bruce Davidson, Tim Liptak, and Holly Carroll especially for being well informed colleagues and friends.

This project would not have been possible without the USDA groundwater project that was primarily supported by Dr. Michael Rose. I would like to thank Dr. Rose particularly for being steadfast and knowledgeable. I would also like to mention Doug Campbell, Martin Patch, and Chuck Wright, without whom I would have missed much learning and conversation.

I am proud to have worked at Virginia Tech with its excellent faculty and intense learning atmosphere. I thank Dr. Greg Boardman for providing much guidance and for being someone I could communicate with. I also would like to thank Dr. Novak and Dr. Hoehn for their excellent suggestions and reasonable comments.

Lastly, I would like to dedicate this thesis to my parents, neither of whom could be here to see me receive my Masters degree.

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I. INTRODUCTION

Groundwater Contamination in the United States

The withdrawal of groundwater has tripled since 1950 and now accounts for one quarter of all fresh water used (15). In many rural areas, well water provides the majority of fresh water. The purity of this water source is then crucial to the economic and physical health of society.

Approximately six billion tons of toxic wastes have been disposed of in the U.S. since 1950 (51). The Environmental Protection Agency has listed about 25,000 toxic waste landfills nationwide and may eventually list 2,500 of these on the national priority list. In addition there are an estimated 133,000 waste lagoons (44). A major portion of toxic waste is pumped into injection wells (37). New Jersey alone, one of the smallest states in the union, produces more toxic waste than any other state (14) and was cited for 15 of 64 of the highest concentrations of toxic organic chemicals found in groundwater nationwide (44).

The disposal of toxic wastes causes serious problems that stem from the contamination of water used for drinking, irrigation, and industry by leaching of organic chemicals and metals into groundwater sources. This contamination can lead to chronic illnesses in individuals through a variety of vectors, and to economic stress to agriculture and industry (21). The names of Love Canal, Times Beach, and Valley of the Drums evoke emotions of fear and represent a critical problem. "The Committee on Environmental Quality, 1981, found that hundreds of drinking-water wells,

which had provided domestic water for millions of persons, had been closed over a period of three years due to contamination by synthetic chemicals and that the concentrations of these chemicals were often orders of magnitude higher than those found in surface water" (44). In a prophetic report to Congress in 1973 the EPA reported that "Current hazardous waste management practices are generally unacceptable, and that public health and welfare are unnecessarily threatened by the uncontrollable discharge of such waste materials into the environment" (20). The recognition of these problems has led to a series of legislation designed to control waste disposal.

The Solid Waste Disposal Act of 1970 (PL 89-272) was the first to delineate a plan for disposal of hazardous wastes including radioactive, toxic chemical, and biological solid wastes. Later in 1970, the Environmental Protection Agency was formed. The Federal Toxic Substances Control Act of 1976 (PL 94-469) delineated regulation of the chemical industry. The Resource Conservation and Recovery Act of 1976 (PL 94-580) capped off a series of laws attempting to correct past negligence of waste disposal problems by creating a well of funds called 'superfund' to clean up improper and/ or illegal dumps. It is evident, as toxic chemical problems continue, that until the economics of waste disposal are radically altered, the problem will persist.

Along with the passage of legislation regulating hazardous waste disposal and addressing groundwater contamination came the need to be able to accurately and effectively analyze groundwater samples for contaminants. Analytical methods for heavy metals are very well established, whereas analysis for organic chemicals is less well established and more

difficult in terms of time, expertise, and cost. The basic problem in detecting organic chemicals in the environment stems from the infinite variety of compounds that need to be identified. Environmental scientists have turned to the use of general parameters that indicate the presence of certain types of compounds or toxicity such as Total Organic Carbon (TOC), Total Organic Halide (TOX), and Chemical Oxygen Demand (COD). Bioassays using organisms such as rainbow trout are also being researched (8).

The author of a recently published text on Environmental Toxicology states, "There is a real need for the inexpensive test systems to evaluate both the acute and chronic toxicity of a large number of industrial chemicals, as evidenced by the recent passage of the Toxic Substances Act." (23). The concept of a bioassay has recently been investigated in depth (1, 31, 43, 54 - 56). Bioassay procedures utilize the natural reaction of organisms ranging from vertebrates to bacteria to detect toxicity. The Ames test is an example of a bacterial bioassay that has been utilized to determine whether or not certain compounds have carcinogenic properties but has not been applied to groundwater evaluation (1). A photoluminescent bacterial bioassay termed Microtox (Beckman Instruments, Carlsbad, CA.) has recently been investigated as a possible screening test to indicate groundwater quality (7).

The author was employed by the Virginia State Water Control Board for which he was responsible for extensive statistical analysis of groundwater data. This study elucidated the need for groundwater screening techniques. The author was also employed through a cooperative agreement with Virginia Polytechnic Institute with the United States De-

partment of Agriculture. The USDA project involved a groundwater sampling project for which the author was technical project leader. The objective of the groundwater sampling project was to determine whether or not groundwater sources that were utilized in food processing (i.e.-washing of meat and poultry and mixing into products such as sausage) were affected by anthropogenic contamination. It became evident that the amount of funding available for this project was much less than the amount of funds that were needed to adequately analyze the hundreds of food processsing plants that merited sampling. Therefore, the author designed the research reported herein to investigate the possible use of the parameters TOC and TOX and the bacterial bioassay Microtox as indicators of groundwater contamination.

The objectives of this work were to:

1) Determine the effectiveness of Total Organic Halogen (TOX), Total Organic Carbon (TOC), and Microtox bioassay to serve as screening procedures for groundwater quality.

2) Determine the influence of groundwater hardness, temperature of assay, and pH on the response of the Microtox bioassay.

3) Characterize the response of the Microtox test to parachlorophenol, phenol, and benzene, alone and in combination, in order to gain an estimate of the lowest concentrations that these compounds could reasonably detected by the Microtox bioassay.

II. LITERATURE REVIEW

Total Organic Halide (TOX)

The TOX analysis is a chemical method first introduced in German drinking water research that is used for detecting halogenated compounds in liquid matrices. It has recently received attention as an "indicator" of water quality (48). The discovery of halogenated compounds in chlorinated drinking water in the 1970's led to the use of measurement of trihalogenated methanes (THM's) as an indicator of the concentration of chlorinated compounds in drinking water. The legally defined upper limit of THM's is 0.10 parts per million (17). The TOX measurement reportedly will detect a much greater portion of the total concentration of halogenated compounds than the legal THM parameter (28). The THM measurment represents only approximately 5 per cent of the total ortganic halide concentration in chlorinated water sources (13). Gas chromatography identifies a specific group of compounds for each column and detector. The TOX test will detect virtually any chlorinated compound. The ability to detect chlorinated compounds, many of which are common contaminants and are accepted as indicators of pollution, provides the possibility of screening water samples for organic contamination. The theoretical detection limit is 10 micrograms per liter (ug/L) (13).

The versatility and sensitivity of the TOX procedure make it the most useful chemical method available for screening water samples for chemical contamination by chlorinated organics. It is readily applicable

to surface, ground, and tap water studies. Results proved conclusively the presence or absence and/or increase or decrease of organic halides in a sample (13). The cost, time and expertise involved in performing a TOX analysis is much lower than that for gas chromatographic methods. For these reasons, the TOX test is becoming a popular method of screening ground and surface waters and wastewaters for contamination (4). The TOX test is particularly suited to groundwater screening because of the generally low levels of solids and low concentration of organic halides. The TOX test works optimally under these conditions. The inclusion of the TOX parameter in the aforementioned State Water Control Board study might have provided some invaluable information. TOX might also be useful in the future for drinking water evaluations and National Pollution Discharge Elimination System permits.

Total Organic Carbon and COD

TOC and COD have been used as general indicators of groundwater quality (33). The parameter COD is generally used for evaluation of waste streams particularly domestic sewage but can be used for groundwater. The COD parameter is a measurement of the amount of compounds that are oxidizable by a strong chemical oxidant (54). The TOC and COD tests are subject to variability. The variability of the COD test was reported to be plus or minus 14 milligrams per liter (mg/L) for a 200 mg/L standard (54). It is apparent that the COD test is not reliable for detecting levels of contamination below 10 mg/L. The precision of the TOC test is greater than for COD because it is a direct chemical method and ranges

from 5 to 10 per cent for samples with a great deal of particulate matter to one to two per cent for clear samples (54). Total Organic Carbon will detect compounds that COD will not. For example, the TOC will detect benzene, but the COD will not. The TOC method consists of breaking down organic carbon into carbon dioxide by introducing heat, oxygen, and hydrochloric acid. The carbon dioxide is then measured directly by a nondispersive infrared analyzer. The ultra-low level TOC analyzer utilized in this work provided accurate determinations of TOC concentration below 1.0 mg/L.

State Water Control Board Study and Variability of Groundwater

The author's search for a screening test for toxic organic chemicals in groundwater began with a survey of well water performed by the Virginia State Water Control Board (33). This study consisted of a wide variety of parameters taken quarterly on many groundwater samples over the period from 1977 to 1984. These parameters included TOC, COD, conductivity, pH, trace elements, hardness, and others. The data collected for the disposal site wells and monitoring wells were compiled in tables for each well. Means and standard deviations were computed. A 'T' test was performed for the disposal site data that had eight or more samples taken. These data clearly showed those wells that violated the standard for metals such as iron, manganese, and zinc. The standards for iron and manganese are based on aesthetics whereas the standard for zinc is based on health. It was found that 14 of 16 wells that had some type of violation were near industrial sites. One of these industries was Vulcan Materials, a well

water sample source that was analyzed in this thesis. It was found that the pH of groundwater near four of seven landills decreased with time. It should be noted that municipal landfills, while not designated for toxic waste disposal, may contain organic compounds emanating from discarded pesticide containers and a variety of sources including material which is illegally dumped.

The results of the State Water Control Board study indicated that the variability of conductivity was greatest, followed by the variation of total dissolved solids, hardness, and alkalinity. The variability of groundwater quality must be taken into account when evaluating groundwater monitoring data. Concentrations of organic contaminants in groundwater have been known to vary by as much as 16 parts per billion (ppb) to 10,940 ppb in successive samples (5). Conductivity has been found to vary 50 per cent from day to day (5). Variations in groundwater data as extreme as those above may be explained as laboratory error, especially in cases where there are legal repercussions of finding high concentrations of contaminants. This variability further points out the need for relatively rapid, inexpensive screening tests that can be implemented on a continuous basis rather than at a single time.

It was very difficult to draw any concrete conclusions from the State Water Control Board well water survey. The pH of several of the samples was found to decrease over time, indicating the breakdown of soil alkalinity. Certain numbers such as 49 mg/L COD for one well indicated a pulse of contamination, but this could not be verified. It would seem that this value must be an error due to the fact that the TOC for the same

sample was only 1.0 mg/L. It was evident that more information was needed to make the groundwater monitoring study of use to the investigators.

Bioassays

The first and most obvious use of biological organisms as indicators of anthropogenic pollution is the observation of sick and/or dying organisms in their natural habitat. The dawn of environmental science was the observation of ecosystems that were ill. The signs of this illness range from mass scale death to more subtle clues such as decreased productivity. The destruction of the base of an ecosystem such as algae or phytoplankton is the key that threatens the upper levels of the food chain. The amount of pollutants entering an ecosystem is reflected in the overall ecological health of that ecosystem (8). Simple mathematical indices such as the Sequential Comaparison Index (SCI) provide the best method to evaluate an ecosystem's overall health (8). The SCI index is based on the concept that a healthy ecosystem supports a diverse biota. However, the presence or absence of any one species is not a reliable indicator of pollution (53).

The basic concept of observing the effects of unnatural pollution on living organisms has been developed into the bioassay. Any bioassay is a test that measures toxicity through the evaluation of the effects of a contaminated water on an organism. This effect can be measured by death, sickness, unnatural behavior, or as specific as changes in enzyme and protein content. Bioassays are perormed with fish (43, 55), algae (56), bacteria (1, 31, 54), as well as a variety of other organisms in-

cluding aquatic insects, copepods, crustaceans, and Daphnia (54). The refinement and interpretation of these bioassays has become a scientific discipline in itself.

Fish were first used because of their sensitivity (26) and obvious interaction with water. Fish have been known to be used to track down contamination of a river at a concentration as low as one part per billion (23). Although, fish are gennerally not that sensitive (27). Elaborate fish bioassays have been developed that determine lethal concentrations (LC's) of specific chemicals to specific species. These generally consist of tanks of dosed water in which the fish must survive. This type of test seems at first rather simple. The problems with this bioassay are related to species and environmental specificity. The main drawbacks of fish assays are: 1) small size of gene pool or in other words the possibility of gross statistical variation due to hypersensitive or overtolerant individuals, 2) long amount of time needed for chronic effects or difficulty in interpretation of acute effects to chronic effects, 3) large amount of equipment needed in bulk and price (cost varies with the complexity of apparatus), 4) variability of results due to the wide variety of possible variables including operator differences and inconsistency of testing conditions. A constant, uniform supply of organisms is also needed. Bioassays utilizing other macroorganisms have drawbacks equivalent to those of the fish bioassays.

Bioassays using bacteria have an advantage over bioassays using macroorganisms due to the large number of individuals that can be dosed at one time. In addition, they can be grown or obtained in large quan-

tities relatively rapidly with a genetically uniform population. Results from bacterial tests are obtained relatively rapidly.

Bacterial tests are not generally directly applicable to chronic studies. However, the Ames (1) test for carcinogenicity measures the amount of mutations of specific bacteria grown on a specific media upon dosing with a suspected mutagen. The method has been widely accepted as an indicator of carcinogenicity. A compound that is considered carcinogenic is considered to have chronic effects.

A test for the bacteriological quality of distilled water is included in Standard Methods that utilizes Enterobacter aerogenes (54). The basis for this test and many others is that a toxic compound will decrease the bacterial population to a measureable degree, in this case by 20 per cent, when compared to a control sample. This type of test could be modified for use on groundwater samples. A major drawback to this type of test is that it is difficult to produce a constant control population.

A bacterial bioassay that measures differences in phosphorescent light output in comparison to a control has been recently developed. This procedure, called the Microtox analysis, has received a great deal of attention due to its flexibility (i.e. - ability to be used for many different environmental purposes), and low cost, and ease of analysis.

Microtox

The Microtox test developed by Beckman Instruments, Inc., Carlsbad, California represents an important portion of the experimental analysis of this thesis. The Microtox bioassay is based on the relative

luminescence of the marine bacterium Photobacterium phosphoreum (3). These bacteria are specially grown and harvested by Beckman to insure genetic stability and to minimize differences in bacterial batches. The bacteria are lyophilized for long-term storage. The instrument used to measure luminescence is a spectrophotometer that measures the differences in light output of samples as compared by percentage to the light output of diluent blanks which represents a 100 per cent response. The result can be positive or negative. Tests can be run at temperatures ranging from 10 to 25 degrees Celcius (10 to 25 C). The temperature is precisely controlled by the instrument and can be exactly monitored to the tenth of a degree. The results for toxic solutions are expressed as effective concentrations causing 50 per cent light reduction (EC50s). The EC50 is analogous to the Lethal Concentration causing 50 per cent mortality (LC50) for fish bioassays.

The Microtox bioassay was originially developed as an alternative to fish bioassays. The Microtox bioassay has the positive points of: 1) rapidity of testing (5 to 60 minutes), 2) simplicity (the mathematical evaluation of results is straightforward), 3) low cost (5 to 20 dollars per test), 4) sensitivity, 5) reproduceability, 6) low maintenance requirements, 7) low genetic variability of the organisms, and 8) ready availability of bacterial reagent.

The toxic response of Photobacterium phosphoreum is directly related to the interaction of toxics with the cell membrane and internal metabolic processes. These internal metabolic processes include phosphorescence and diminuition of light output is an indicator that the normal processes of the bacteria are being inhibited. The effect is

connected to the osmolality of the surrounding solution (3). The light output mechanism is controlled by an independent system of enzymes that control physiological factors (3). Inhibition of any step in this complex enzyme mechanism may cause a toxic response. Enzymes have long been used in assays of chemicals (42). The multiplicity of possible mechanisms for the depression of light output is a positive point in that the Microtox test will respond to a wide variety of toxicants and a negative point in that the response is nonspecific (57). The mechanism of luminescence is only well understood in the case of the firefly (25). It is reasonable to assume that the "cold light" mechanism of Photobacterium phosphoreum is similar (26). The important points of consideration are: 1) molecular oxygen is needed, 2) adenosine triphosphate (ATP) is needed, and 3) the reaction is catalyzed by luciferase.

The Effect of pH and Temperature on Light Intensity

The acute effect of pH on the light output of P. phosphoreum demonstrates the direct connection between the osmolality of the surrounding solution and the Microtox response. Figure 1 shows luminescence intensity from P. phosphoreum versus pH (29). The figure shows that having the solution pH between 6.0 and 7.0 will insure that the light output will be relatively close to the maximum luminescence and that below these values the light output will fall off markedly. The pH effect is termed reversible because the light output can be altered up and down with pH adjustment.

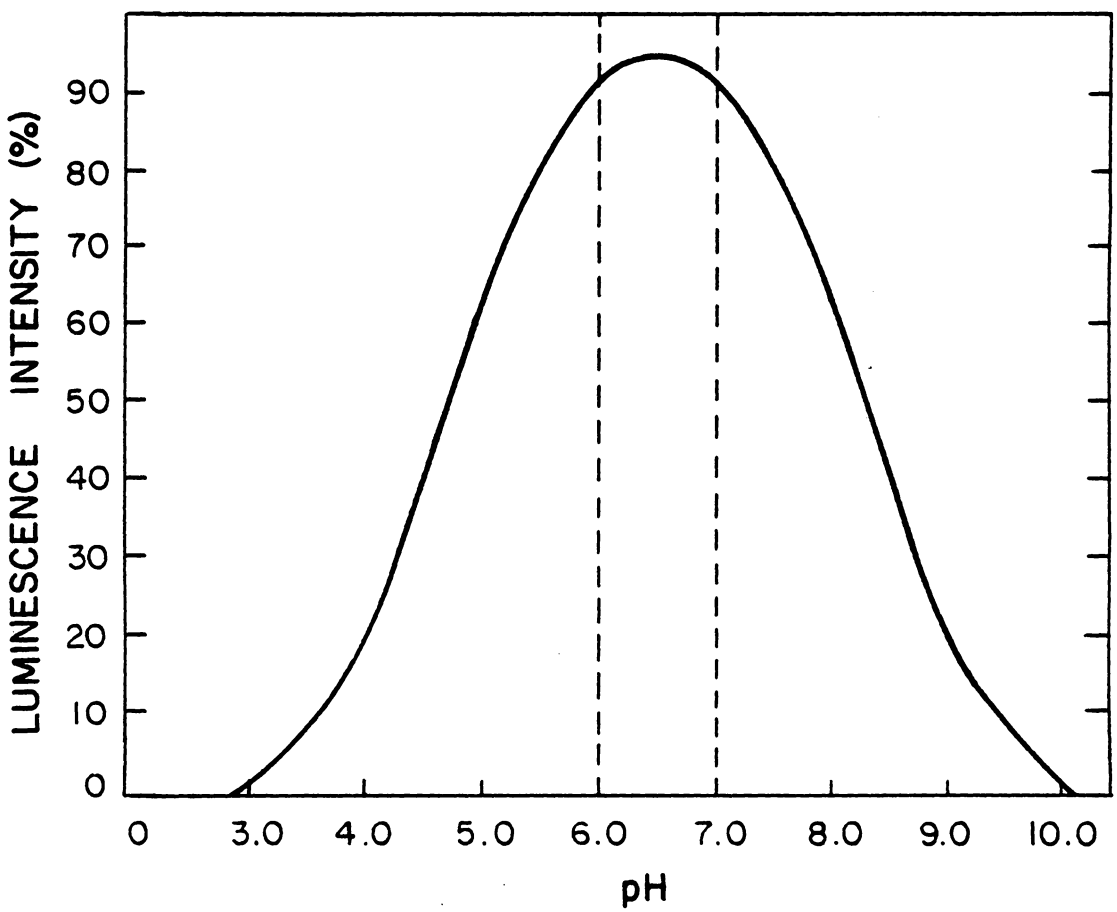

FIGURE 1. The Effect of Solution pH on Light Intensity of Photobacterium phosphoreum

The effect of temperature on the light intensity of P. phosphoreum is well documented (2). It has been reported that Microtox testing of organics is more sensitive at lower temperatures (2). However, testing of mycotoxins showed that the effective concentrations causing 50 per cent light reduction (EC50s) were lowest at 30 C (57). Apparently, the effect of temperature is related to the compound being studied.

The Microtox Test in Water Quality Monitoring

The coefficient of variation of the Microtox bioassay has been reported to be 18 per cent for 5 minute tests (8). This number represents the variability from solution to solution, instument to instrument, and operator to operator. A coefficient of variability of 10 per cent was reported for the same solutions tested simultaneously with the same instrument and operator (3, 4). The results can be affected, however, by the characteristics of the chemical being tested. For example, EC50's reported for benzene vary from 214 mg/L (11) to 2.0 mg/L (27). It is likely that this type of variance is due to the characteristics of the chemical. Other more stable chemicals such as chlorophenols yield very reproducible results (47). Therefore, it is important to use pure chemicals and not to compare toxicities of solutions that have been exposed to air and/or light for significant periods of time. It has been found that in the toxicity of water samples to the Microtox bioassay in general declines significantly during storage (46). Conditions of temperature, pH, and minor contaminants in distilled water can affect the results (57). Variability of bioassays using Daphnia, rainbow trout, and Spirillium volutans was reported to range from 13 to 26 per cent (6).

Several organic compounds have been tested using the Microtox bioassay. One of the most toxic compounds tested was 2,3,4,5-quadrachlorophenol with a 5 minute effective concentration for 50 per cent light diminuition (EC50) of 0.30 mg/L (47). Pentachlorophenate was reported to have an EC50 of 0.94 mg/L (11). Trichloroethene had a 5 minute EC50 of 156 mg/L. Acetone proved to have the highest EC50 of 22.3 grams per liter. Phenol and benzene were reported to have 15 minute EC50's of 39.5 and 4.1 mg/L, respectively (31). The 5 minute EC50 of 2,4,6-trichlorophenol was reported to be 6.0 mg/L (47). The 5 minute EC50 of parachlorphenol was reported to be 8.6 mg/L (47).

The toxicity of metals to the Microtox test has been well documented (2). Mercuric chloride is reported to be one of the most toxic substances with a 5 minute EC50 of 0.065 mg/L or 65 ppb (2). Copper, cadmium, and zinc chlorides were reported to have 5 minute EC50's of 3.5, 100, and 26 mg/L, respectively (2). The 15 and 30 minute EC50's for these metals were always lower than the 5 minute EC50s (2, 49). Mixtures of metals have been studied with a less than additive response being observed and no clear trend could be established concerning toxicant mixtures (49).

The Microtox bioassay has been utilized to evaluate the toxicity of complex wastewaters under a variety of circumstances. The ability of the Microtox bioassay to detect the toxicity of samples that are exceedingly difficult to analyze is one of the assay's strengths (2). It has been reported that the Microtox bioassay may be a poor indicator of toxicity for complex effluents containing certain compounds such as ammonia and cyanide (7). A paper by Bulich (7) relates some of the factors

that must be taken into account when using the Microtox to evaluate complex samples. The paper included a study on groundwater from 31 wells tested using the Microtox bioassay that were "strategically located near a large hazardous waste dumpsite" . These water samples were analyzed for six metals and six organics and a table of Microtox toxicity, pH, and metals and organics contamination was presented. Because the pH of these samples was not adjusted, the degree of toxicity did not correlate well with contamination. Well 18 had a pH of 11.0 that contributed to its toxicity according to the author. The author states that correlation was good. But examination of the table shows that five of the nine samples that exhibited high toxicity had low amounts of contamination. The pH of all five samples was greater than 10.0. Of four samples that showed low toxicity, three had a pH between 7.0 and 8.0 with the remaining sample having a pH of 10.0. The author ends the section by stating that, "Additional studies are in progress to determine the utility of the Microtox system as a functional test for screening groundwater samples."

The Microtox bioassay can be used to monitor landfill leachates (36, 41) or to evaluate the effectiveness of municipal (38) and chemical (9, 52) treatment. A study by Slatery (52) is notable because it does not include any analysis of organic pollutants by gas chromatography. The author relies on the ability of the Microtox bioassay to identify degrees of toxicity associated with a highly complex waste stream. This study was carried out due to the unusually toxic content of the waste treated. This treatment plant received effluents from several chemical manufacturing plants in the area and was termed, " the worst polluter of toxic materials to the Chesapeake Bay in Maryland". The plant management used

the Microtox bioassay as a warning of high toxicity of the waste stream and as an indicator of plant performance. The Microtox bioassay was used in another case to evaluate the effectiveness of several different methods of waste treatment, including aerobic treatment, oxidation, irradiation with ultraviolet light in the presence of hydrogen peroxide, and adsorption on carbon.

The Microtox bioassay has also been used to evaluate the toxicity of influent and effluent streams from two conventional activated sludge pilot wastewater treatment systems (38). One of the pilot systems was spiked with 16 priority pollutants (50 micrograms per liter of each). The authors concluded that the added priority pollutants "did not affect the acute toxicity of the samples". This finding seems to contradict the findings of Nadeau (36) who reported that low concentrations of mixtures could cause as significant a response as a single toxicant at a higher concentration. The samples involed in the Neihesel study were from complex waste streams. The Nadeau study was performed with landfill leachate samples.

The Microtox bioassay has also been used to evaluate the toxicity of contaminated marine sediments (50). The authors correlated acute toxicity with the sums of concentrations of hydrocarbons and napthalenes, using a linear regression analysis. They found the correlation coefficient to be 0.828 for the hydrocarbons and 0.796 for napthalenes. A similar study was accomplished on water tainted with oil (40). Microtox responses were correlated with analysis of organic compounds. Linear regression analysis was not done. No single class of compounds was found to be consistently responsible for toxic responses. Ribo et al. (46)

correlated Microtox bioassay results with phenols, volatiles, organic carbon, polychlorinated biphenyls (PCB's) and polyaromatic hydrocarbons found in Detroit River samples. The authors reported "only some general correlations" between observed Microtox responses and concentrations of organics. Wastewater treatment effectiveness was evaluated using the Microtox bioassay (9). The results showed consistent correlation of Microtox response with the presence of toxic organic compounds including phenol, benzoic acid, and ortho, meta, and parachlorobenzoic acid. Also included were TOC, TOX, and COD parameters. Again, the correlation reported was determined by inspection, not mathematical manipulation. A common premise among these studies was that the bacterial bioassay was utilized to evaluate the toxicity of a complex water sample for which even costly and extensive organic analysis might not adequately indicate the possible deleterious effects of the samples in the environment.

An important question to address is at what point of light diminuition does the investigator consider the sample "toxic"? A cutoff point of 20 per cent is the accepted limit (40, 36). It should be kept in mind that this limit is never absolute. In studies on complex samples, it is not possible to determine which toxicants are the major causes of toxic responses or what interactions are occurring. Ribo and Kaiser (47) reported that Microtox bioassay results generally correlate with Quantitative Structure Activity Relationships (QSAR's). This further demonstrates the overall correlation of Microtox responses to toxicity.

The Microtox Bioassay was originally developed as an alternative to bioassays such as those using fish or Daphnia. Therefore, the comparison of EC50's and variability of these bioassays is of concern.

Indorato et al. (27) reported a good correlation of chemical toxicity to fish bioassays, but there were some aberrations. Linear regresssion analysis revealed that the coefficient of correlation between Microtox and fathead minnow bioassays was 0.77. For the golden orfe the coefficient of correlation was 0.50. For bluegill sunfish the coefficient was 0.68. Ribo and Kaiser (47) reported good correlation of the Microtox bioassay with rainbow trout. A correlation coefficient of 0.92 was noted for para- substituted phenols. Other correlations with chlorophenols included 0.93 for bacteria, 0.73 for spore, 0.77 for bluegill, 0.92 for brown trout, 0.89 for guppy, 0.87 for daphnia, and 0.68 for shrimp. A number of other authors reported similar correlations (6, 9, 38, 41). Mcfeters et al. ,however, disagree. They reported that, "The Microtox test was somewhat more sensitive than the Tchan bioassay (uses bacteria also) in detecting most of the test chemicals and the fish bioassays were generally more sensitive than either of the microbial tests. Further, these test comparisons provided evidence that microbiological test systems, including the Microtox, are subject to some variation from laboratory to laboratory and so are not unlike fish bioassays in that regard". The argument here may be a product of differences in the definition of a good correlation. For example, Mcfeters et al. reported a Microtox bioassay EC50 for phenol of 40 mg/L as compared to a fish bioassay EC50 of 50 to 100 mg/L (34). Ribo and Kaiser (47) correlated Microtox EC50s of chlorobenzenes with the response of rainbow trout, bluegill, sheepshead minnow, guppy, and daphnia. The authors reported a correlation coefficient of 0.71 for rainbow trout even though the Microtox EC50 for monochlorobenzene was 11.4 mg/L, as compared to the trout EC50 of 1.1

mg/L. The authors stated that the correlations of chlorobenzenes were lower than the correlations with chlorophenols due to the "generally low solubilities of the chlorobenzenes with the resulting difficulties in obtaining stable solutions of the chlorobenzenes for the bioassay tests". This demonstrates the specificity of the Microtox and bioassays, in general, and that "good" correlations must be adequately defined. Indorato et al. (27) suggested that mathematical models with confidence limits be used to provide "general but not perfect" correlations.

It is imperative to establish the non-toxicity of blanks used in Microtox testing in order to use the method as a screening tool. Obviously, if the blank water used has a chemical composition identical to the Microtox diluent, then the light output should be the same. Qureshi (45) reported that "deionized water at pH 7.0 and 2 per cent osmolality effected negligible light diminuition". It is important to remember that distilled water can not be assumed to be exactly the same from laboratory to laboratory or even from day to day in the same laboratory.

One aspect of the Microtox bioassay that can not be ignored is the variation of the bacterial reagent over long time periods. The age of the reagent can grossly affect the light output. Quereshi (45) found that significantly lower EC50's were recorded using reagent that was four hours old. The author concluded that the aged reagent became "too sensitive" and could therefore could produce "false positive and/or erroneous results". They recommended that the reagent be used within an hour. This time would limit the testing to one per reagent batch. Due to the cost of the reagent (about $ 600.00 for a box of 40 vials), this time limit would increase the overall cost greatly. Another important consideration

is that the dry reagent loses its ability to phosphoresce over a period of months. The reagent is advertised as being good for up to one year.

III. METHODS AND MATERIALS

Experimental Design

The experimental design of the research resulted in the collection of two types of data: data gathered on 25 field samples and laboratory data gathered at Virginia Tech. Microtox bioassay responses were recorded for all field samples. Twenty of the field samples were analyzed for organics, trace elements, anions, TOC, and TOX by Webb Technology Labs, Inc. (Raleigh, N.C.) under contract with the USDA Groundwater Project. The author acted as technical project leader for the USDA Groundwater Project and was responsible for choice of sample sites and actual sampling. Five of the USDA samples were chlorinated prior to sampling and were therefore evaluated as a separate set of data. Figure 2 depicts the locations of sample sites and toxic waste sites (14). Conclusions from the field data were based on a comparison of Microtox bioassay responses to TOC, TOX, organic, trace elements, and anion concentrations (detailed in Appendix D). Five of the field samples were collected and analyzed for trace elements, anions, TOC, and TOX by the author at Virginia Tech. These five samples were not a part of the USDA contract and were treated as a separate group. The research was designed to obtain information from the field samples that was supplemented by data generated under carefully controlled conditions in the laboratory. The laboratory procedures are detailed below.

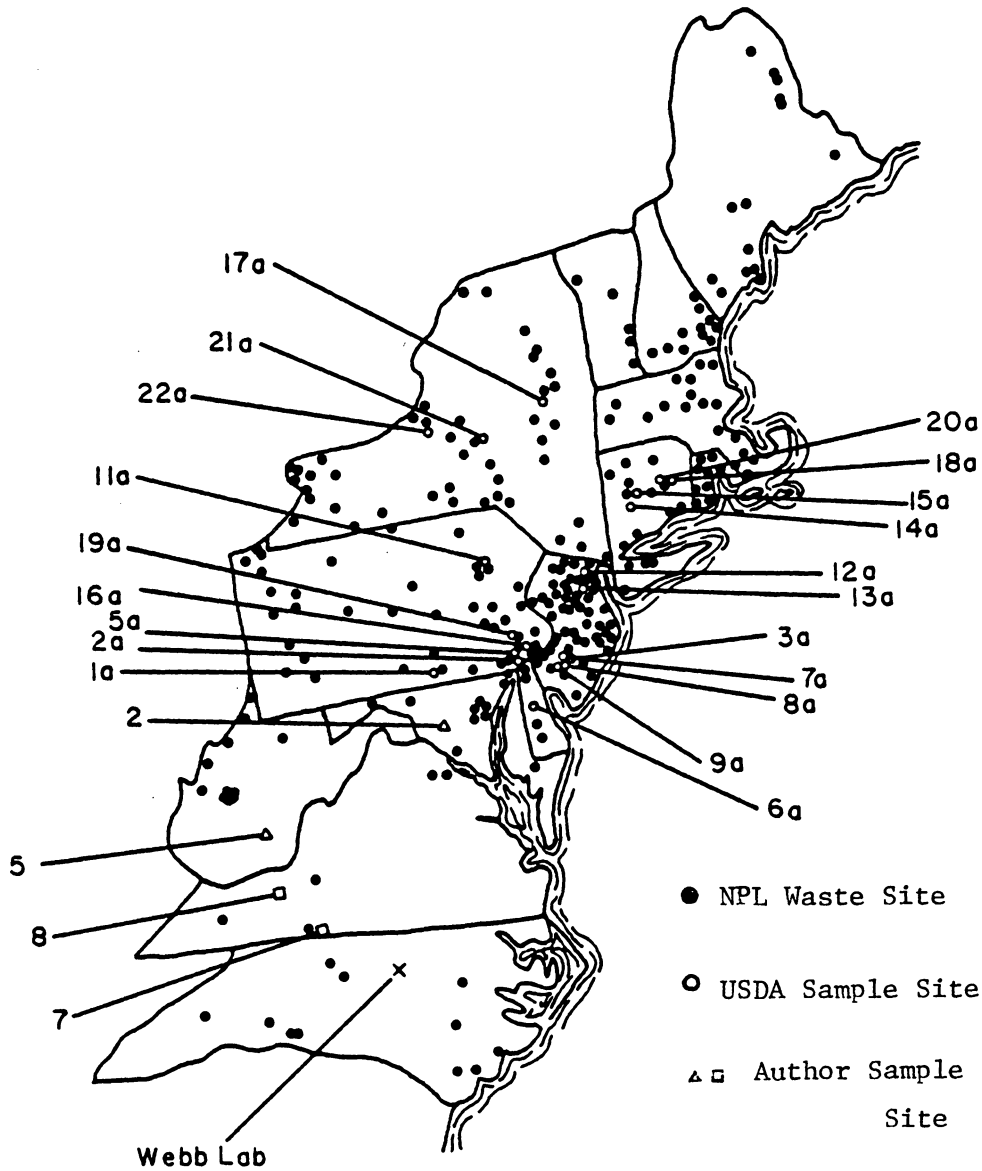

FIGURE 2 . MAP OF SAMPLE SITES AND WASTE SITES

Glassware

All glassware used in laboratory procedures was acid washed (25 per cent HCl) and then rinsed four times with tap water followed by four rinses with distilled water. Borosilicate glass bottles equipped with Teflon lined closures were used for all field sample MilliQ water (distilled water run through activated carbon columns) was used to prepare all blanks and stock solutions.

Laboratory Microtox Study

A laboratory study was performed using the Microtox bioassay to determine the sensitivity of the Microtox to parachlorophenol, phenol, benzene, trichloroethane, and 2,4,6-trichlorophenol. Solutions of parachlorophenol, phenol, and benzene were assayed alone and in combination at equal concentrations in order to characterize the Microtox bioassay response for each compound and mixture of compounds. The organic compounds were chosen because they represent common groundwater contaminants (14, 44), and are representative of aromatic compounds, phenols, chlorinated phenols, and alkanes. The organic compounds tested proved to be soluble in water at a level of at least 100 mg/L. The effect of groundwater hardness on the bacterial response was investigated by assaying each chemical in solution with 150 mg/L of hardness as calcium carbonate (CaCO3). The hardness solution consisted of 80 per cent calcium carbonate and 20 per cent magnesium carbonate (on basis of equivalents). The response of the Microtox bioassay to the hardness solution alone was

also assayed. The carbonates were dissolved by adjusting the pH up to approximately 8.0 with sodium hydroxide. All organics were assayed at concentrations of 5.0, 2.5, 1.25 and 0.63 mg/L. The EC50s were expressed as a concentration equal to the concentration for each compound in solution. For example, a solution of parachlorophenol, phenol, and benzene contained 5.0 mg/L of each compound, and the EC50 would be expressed as if the mixture were a single compound at a concentration of 5.0 mg/L. The effect of temperature on assays was investigated by testing each solution at temperatures of 10, 15, and 20 C. Comments with regard to the effect of pH were made possible by recording the pH for each sample.

Microtox testing was carried out using a model 2055 Beckman Microtox instrument (Beckman Instruments, Inc., Carlsbad, CA.) following the procedure given in the Microtox Manual (2). The Microtox instrument consists of 15 'wells' that hold cuvettes containing the solution to be tested, a spectrophotometer that measures the amount of light output from the luminescent bacteria (in solution in the cuvettes), and a strip chart recorder that records the relative light output of samples. The temperature for assays was accurately controlled by heating elements to a tenth of a degree Celcius.

The Microtox bacterial bioassay procedure consisted of dosing ten sterile cuvettes containing 0.5 mL of Microtox diluent with 10 microliters (uLs) of Microtox bacterial reagent. The reagent bacteria (Photobacterium phosphoreum), sterile cuvettes, diluent solution, and reconstitution solution were obtained from Beckman Instuments, Inc. (Carlsbad, CA.). The reagent, diluent, and reconstitution solution were kept at 4 C. Sodium chloride (ACS grade) was used to adjust all solutions

to the required two per cent salinity. MilliQ water was used to prepare all stock solutions. A 25 uL syringe was used to add the reagent to each cuvette. The lyophilyzed bacterial reagent was reconstituted with a specially prepared solution obtained from Beckman Instruments. The reconstituted reagent was allowed to stabilize for 15 minutes. The dosed cuvettes were then spectrophotometrically analyzed and a 'blank' light intensity was recorded on a data sheet and a strip chart recorder. Blank refers to diluent injected with reagent to which no toxicant has been added. A blank therefore yields the maximum light output. All responses were recorded as per cent light outputs relative to the light intensity of the blanks. The cuvettes were then dosed with 0.5 mL of the toxic agent being tested. The final volume was 1.0 mL and the initial dilution was 50 per cent. The dilution procedure produces a range of concentrations equal to 50, 25, 12.5, and 6.25 per cent of the original solution's strength. The reagent concentration was equal in each cuvette.

The cuvettes were then spectrophotometrically measured again at 5, 20, and 30 minutes after dosing with the toxic agent. A crucial point of the Microtox bioassay procedure is that light intensities are compared to the original light output prior to dosing with the toxic agent. The diluent blanks are used to determine the amount of light diminuition that is attributed to natural die off. The resulting amount is subtracted from any light diminuition caused by the toxic agent. The result is some level of response for each cuvette. This procedure accounts for the biological variation from reagent dose to reagent dose. Duplicate cuvette wells are available for each trial thus effectively producing a duplicate test for each run.

Results were expressed using the Gamma equation, as given in the Microtox manual, to determine effective concentrations causing fifty and twenty per cent light diminuition (EC50s and EC20s). Gamma is determined by the following equation:

$$\left[\Gamma = \frac{\%\Delta}{100-\%\Delta} \right]$$

WHERE Δ = THE CHANGE IN LIGHT LEVEL

where Δ = the difference in light emmission of test samples to blanks The result is plotted on log scales.

The effective concentrations are determined from regression analysis of the responses over a range of concentrations or from graphing the linear response.

Figure 3 shows an example of a strip-chart recording obtained in the toxicity testing. Figure 4 shows an example of the Gamma plot used to determine effective concentrations. Replicate tests were performed when it was felt that verification was needed or that the results were questionable. Due to the immense volume of data gathered, all graphs are not presented herein. Rather, examples of graphs are depicted, and effective concentrations are noted.

Initial experiments showed that pH greatly affected the light output. Therefore, both laboratory and field samples were adjusted to between pH 6.0 to 7.0 with sodium hydroxide and/or hydrochloric acid.

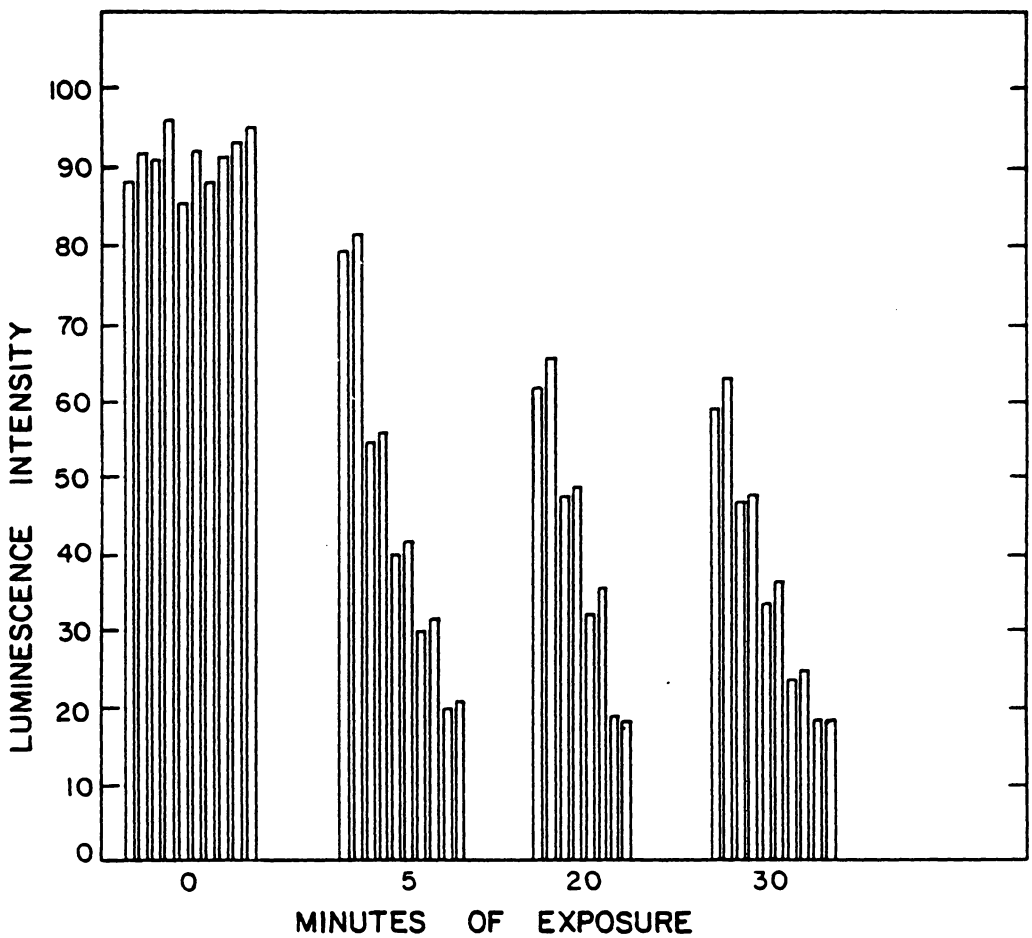

FIGURE 3. Example of Microtox Bioassay Strip Chart Recording of Effective Concentration Causing 50 and 20 Per Cent Light Reduction for Parachlorophenol
(Zero time bars represent diluent blank responses for each well and dosed bars represent increasing concentration from left to right.)

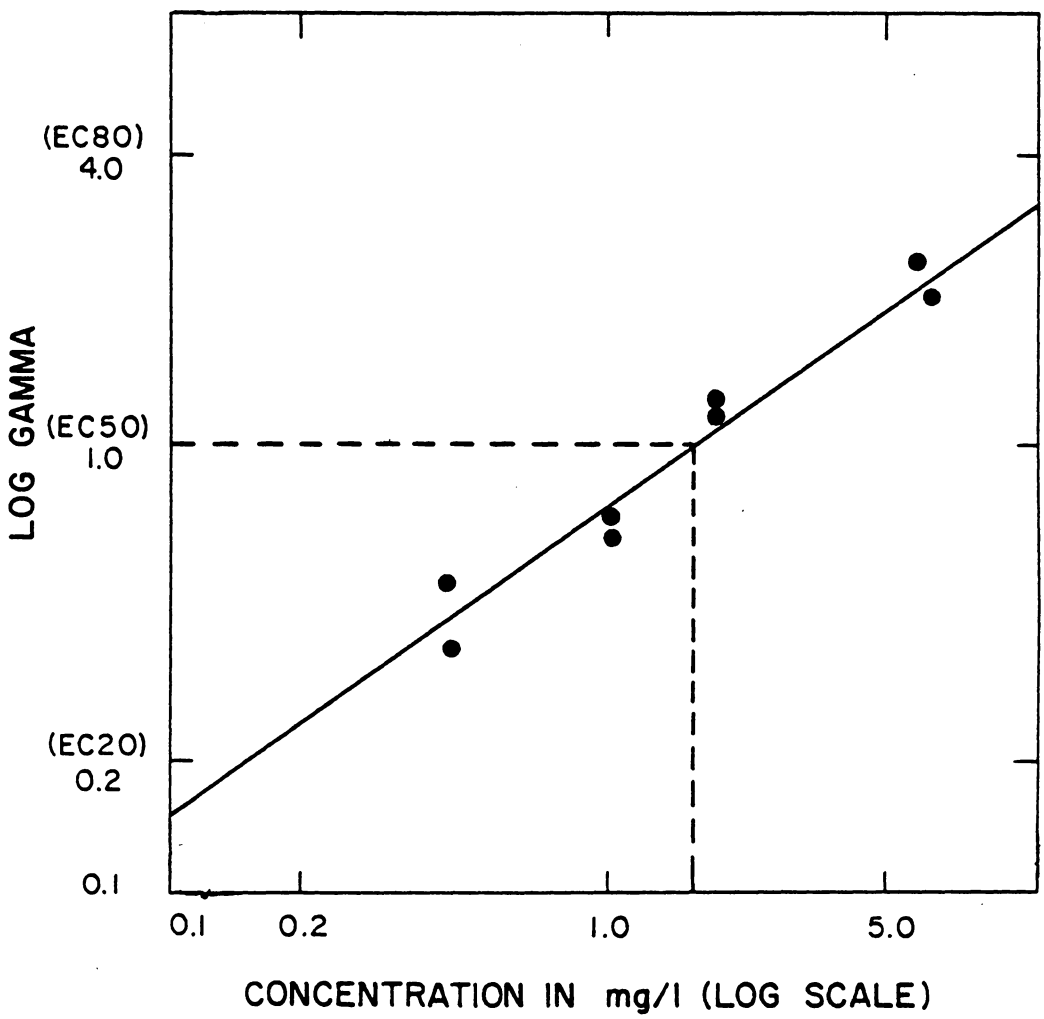

FIGURE 4. Example of Gamma Plot to Determine Effective Concentration Causing 50 Per Cent Light Reduction (EC50) for Parachlorophenol

Parafilm was used to cover cuvettes during testing to minimize volatilization and atmospheric contamination. All chemical solutions were stored in borosilicate glass bottles equipped with Teflon-lined closures.

Field Sampling

Field samples were collected following procedures outlined in the Federal Register (18). Figure 2 depicts the locations of sample sites and toxic waste sites identified from information obtained from the USEPA. The sites were chosen on the basis of proximity to waste sites (by township) and to a lesser extent, the degree of industrialization. The numbers given in Figure 2 represent each sample site. Numbers with an 'a' indicate USDA samples. Samples were taken as close as possible to the well heads and water sources were flushed for approximately five minutes prior to sampling. Hydrochloric acid was used to preserve aromatic hyrocarbons samples; nitric acid was used to preserve trace elements samples; and, sodium thiosulate was used to preserve chlorinated samples, as specified in the Federal Register (18). All preservatives were ACS grade or better. The samples were kept on ice or in a refrigerator prior to analysis. The samples were taken without headspace. United States Department of Agriculture groundwater project samples were shipped on ice to Webb Food Technology Labs, Inc. in Raleigh, North Carolina, and extracted and analyzed within time periods specified in the Federal Register. The five samples that were analyzed outside of the USDA project were

transported by the author on ice to Virginia Tech and analyzed within one week.

Laboratory Procedures

Total Organic Carbon (TOC) concentrations were obtained using a Dohrmann Envirotech (Santa Clara, CA.), Ultra Low Level Total Organic Carbon analyzer model DX20 following EPA method 415.1 (15).

Trace element values were obtained using a Perkin/Elmer model 703 Atomic Absorption Spectrophotometer with flame detection following EPA method 200.7 (15). Trace element analysis on Sample 8 (Dixie Caverns Landfill) was performed, after it was filtered and digested with nitric acid. These steps were neccessary because the sample contained a high level of suspended solids. Trace element values were obtained for cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, and zinc. The USDA sample analysis also included aluminum, barium, berylium, and mercury.

All USDA samples were analyzed by gas chromatography following procedures outlined in the Federal Register (18) for eight purgeable halocarbons, seven polychlorinated biphenyls, benzene, toluene, phenol, and pentachlorophenol (see Appendix D for details of lab contract). The other five field samples were not analyzed for organics.

Anion values were obtained using a Dionex (Sunnyvale, CA.) Ion Chromatograph (model 2010I) equipped with an anion separator column and fiber supressor (15). Values were obtained for chloride, fluoride,

nitrate and sulfate. USDA samples were analyzed for carbonate and bicarbonate.

Free chlorine residual values were obtained using a Fisher/Porter Amperometric Titrator following method 409C in Standard Methods (54).

Total Organic Halide (TOX) values were obtained using a Dohrmann Envirotech (Santa Clara, CA.) TOX analyzer (model DX20) following procedures given in the Dohrmann Envirotech TOX manual (12).

Details of Total Organic Halide Procedure

The TOX system is a device that can quantify chlorinated organics in water samples at levels as low as 10 ug/L. The procedure consisted of adsorbing chlorinated organics onto carbon columns containing 40 mg of powdered activated carbon, after the samples were preserved with nitric acid and sodium sulfite was added to complex any free chlorine present. Nitrogen gas pressure (30 pounds per square inch) was used to force the water sample through the carbon column. The optimum level of detection was 10 to 25 ug of TOX as chlorine. The carbon columns were then treated with potassium nitrate to desorb inorganic halides from the nitrate channel. The carbon was then pyrolyzed at 790 to 820 C in a carbon dioxide rich atmosphere in the microcoulometric analyzer module. The resulting chlorine ions were titrated with silver ions in the coulometric cell.

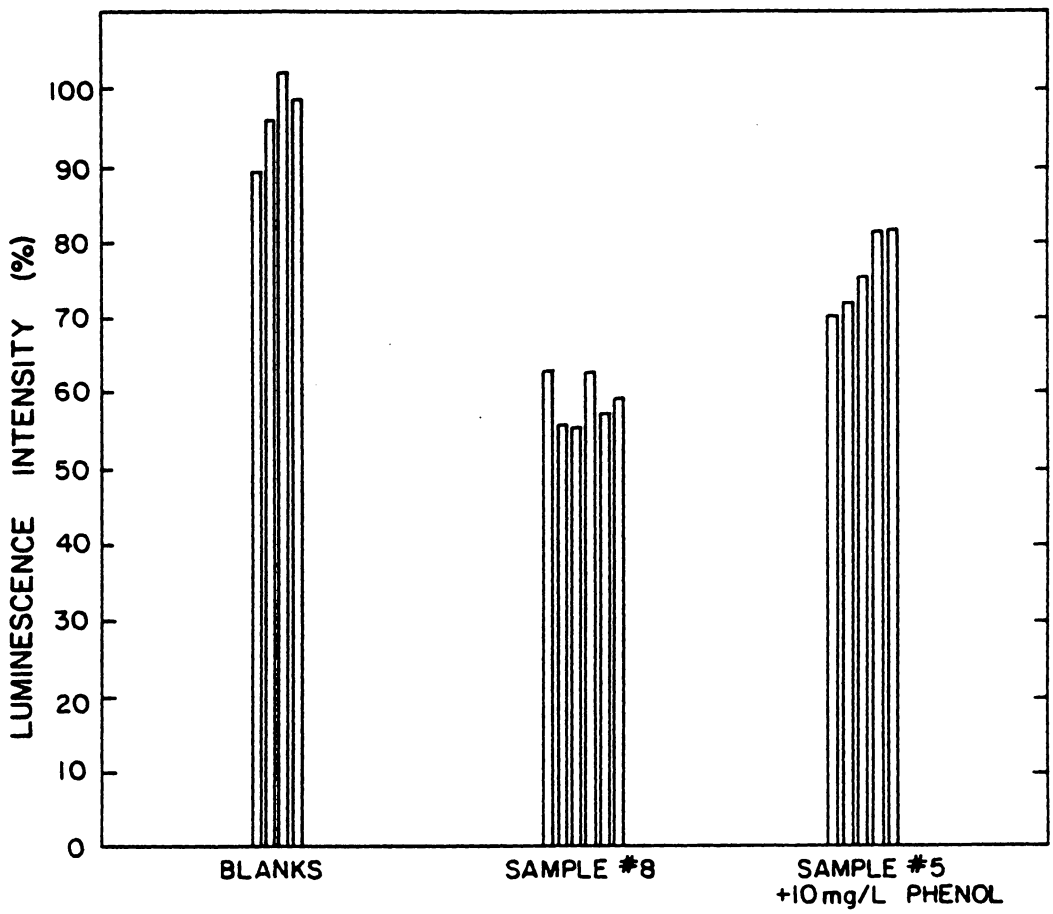

FIGURE 5. Example of Microtox Bioassay Strip Chart Recording of Field Sampling. (Each bar represents an individual well.)

Microtox Testing of Field Samples

Toxicity screening of groundwater samples was accomplished by analyzing undiluted samples in comparison to an average of four diluent blanks (See Appendix B for results of field testing). Figure 5 shows an example of the strip chart recorded graphs for this procedure. The procedure for analyzing field samples was identical to that described earlier, except that a single response was reported for each sample based on five replicates. Each peak depicted in Figure 5 represents the results obtained from tubes in separate wells. Because the objective was to maximize the sensitivity of the test, the procedure was slightly modified for the field samples. Field samples of 1.0 mL were dosed with 10 uL of reagent. Multiple replicates (five) were then analyzed to obtain an average response in comparison to the average response of four diluent blanks. The pH was adjusted to 6.0 to 7.0, as needed.

Absorbance correction method 8.5 in the Microtox Manual was followed in the analyses of samples 7 (Vulcan Materials) and 8 (Dixie Caverns Landfill) because they were somewhat turbid and colored. This test procedure consisted of using a special cuvette equipped with an inner and an outer well. A response was recorded with diluent in the inner well, and then a reponse was recorded with the sample in the outer well. No interference was noted.

The samples were allowed to stabilize for 15 minutes prior to spectrophotometric analysis, thus making the initial time of analysis 15 minutes after injection of reagent. Light intensity was then recorded at 5, 20, and 30 minutes after the reagent was introduced. All samples

were adjusted to 2 per cent salinity with sodium chloride. This procedure produced a response that was compared to the diluent blank average. The response was then expressed as a positive or negative percentage.

IV. RESULTS

Results of Total Organic Halide Testing

Table 1 lists the TOX data generated from experiments with parachlorophenol and trichlorophenol. Data is also included for sodium chloride injected directly onto the quartz boat prior to pyrolysis. Direct injection of sodium chloride onto the quartz boat provides a check of the accuracy of the microcoulometric titrator. As the data presented indicate, the accuracy of the titrator (measured by per cent recovery of organic halides from solutions of known concentration) was excellent: 94 to 97 per cent. The average per cent recovery of the organic halides was 87 per cent which is somewhat less than the 97 per cent reported by Dressman (13). The TOX data for all field samples are given in sections concerning the field data and in Appendix C.

Results of Laboratory Microtox Bioassay Study

The results of the laboratory testing provided information concerning the sensitivity of the Microtox bioassay to para- chlorophenol, phenol, and benzene, 2,4,6-trichlorophenol, and trichloroethane. A field sample (number 5) was spiked with the first three compounds, thus providing for a comparison between the toxicities of the compounds in MilliQ water to their toxicities in a field sample.

TABLE 1 - Recovery of Organic Halides on the TOX

Source	Conc. ppb	TOX ppb	Per Cent Recovery
NaCl	50	48	96
NaCl	30	29	97
NaCl	20	21	97
NaCl	10	11	94
TCP	50	42	85
TCP	50	41	81
TCP	50	46	94
TCP	50	44	89
TCP	25	23	93
TCP	25	16	65
TCP	25	18	74
TCP	25	26	95
TCP	25	20	81
TCP	25	25	100
TCP	25	27	93
pCP	10	9.5	95
pCP	10	8.6	86
pCP	5	3.8	77
pCP	5	3.3	65

Per cent recovery of NaCl injected directly onto quartz boat = 96
Per Cent recovery of Organic Halides Tested = 85
Per Cent Recovery of Organic Halides Tested greater than 10 = 87
Per Cent Recovery of Organic Halides Tested Equal to 50 = 87
Average TOX of tap water : 145 ug/L

TCP = 2,4,6-trichlorophenol
pCP = parachlorophenol

Table 2 lists data generated in laboratory studies with the Microtox bioassay. The responses noted are reported as EC50s and/or EC20s. If the concentration tested did not cause a level of response equal to 20 or 50 per cent, then the result was recorded as greater than (>) that concentration. The results of the organic assays are displayed in Figure 6 in bar chart form. It is apparent from Figure 6 that the EC50s were lower at lower temperatures of assay, except for the 20 C test with trichlorophenol.

Variability of the Microtox Bioassay for Identical Tests

Figure 7 displays the average per cent difference between identical tests (side by side wells for each assay). The average variabilities were 9.1 per cent at 10 C, 10.8 per cent at 15 C, 8.1 per cent at 20 C, and 16.7 per cent at 25 C. These numbers are comparable to those recorded in the literature (3, 4). It was apparent from Figure 7 that the variability associated with parachlorophenol was less than that for phenol and benzene at 15 and 20 C. Parachlorophenol exhibited lower variability at higher temperatures and benzene and phenol exhibited higher variability at higher temperatures. The variabilities for all compounds were found to be very high at 25 C and therefore testing at 25 C was discontinued. The erratic character of benzene solutions was established in the Literature Review and this behavior was also noted in this work.

Results of Microtox Bioassays on Mixed and Hardness Solutions

TABLE 2 - Microtox In Laboratory Data

Chemical	10 C EC50	15 C EC50	20 EC50	10 C EC20	15 C EC20	20 C EC20
A	0.7	2.0	2.4	<0.1	0.2	0.4
B	16.2	26.0	>5.0	2.7	5.4	6.9
C	20.0	74.0	>12.5	4.4	9.6	12.5
H	>150	>150	>150	>150	>150	>150
AH	1.5	1.4	2.4	0.2	0.2	0.5
BH	13.0	26.0	>5.0	1.8	6.6	>10.0
CH	28.0	74.0	>50.0	4.4	21.0	40.0
AB	0.7	1.1	4.4	1.2	1.4	1.0
AC	0.8	1.5	3.2	<0.1	0.2	0.7
BC	35.0	46.0	>5.0	2.5	9.6	>5.0
ABH	0.8	1.4	>5.0	<0.1	1.9	0.7
ACH	0.5	1.1	3.3	<0.1	0.2	0.7
BCH	9.0	>20.0	>5.0	<1.0	6.4	>5.0
ABC	1.2	1.8	3.5	<0.1	0.4	0.7
ABCH	0.6	1.5	4.2	<0.1	0.2	0.8
TCE	4.7	9.4	46.0	1.0	1.6	3.2
TCP	11.5	17.0	14.5	4.2	5.4	3.1
5 A	0.8	1.7	4.4	<0.1	0.2	0.8
5 B	28.5	33.0	68.0	2.0	5.8	13.0
5 C	25.0	49.0	145.0	4.1	13.0	34.0

Key: A = parachlorophenol; B = phenol;
C = benzene; H = Hardness standard 150 mg/l as CaCO3 (80 % calcium carbonate and 20 % magnesium carbonate);
TCE = trichloroethane; TCP = 2,4,6-trichlorophenol; 5 = springhouse water (Lehr residence)
note: EC20s for all compounds except parachlorophenol are estimates extrapolated from EC plots

All concentrations are in mg/L.

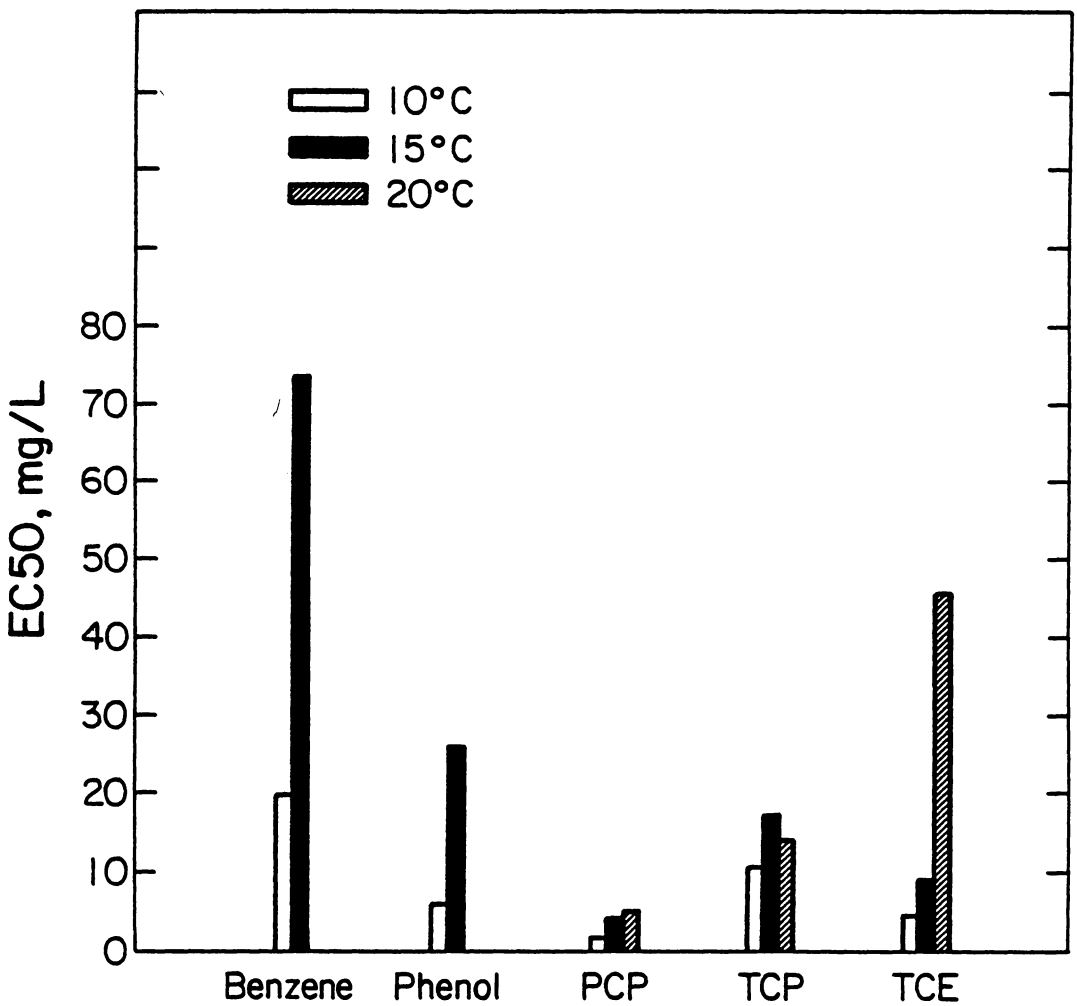

FIGURE 6. EC50s of Toxic Organics Assayed at Varying Temperatures. (PCP = parachlorophenol; TCP = 2,4,6-trichlorophenol; TCE = trichloroethane.)

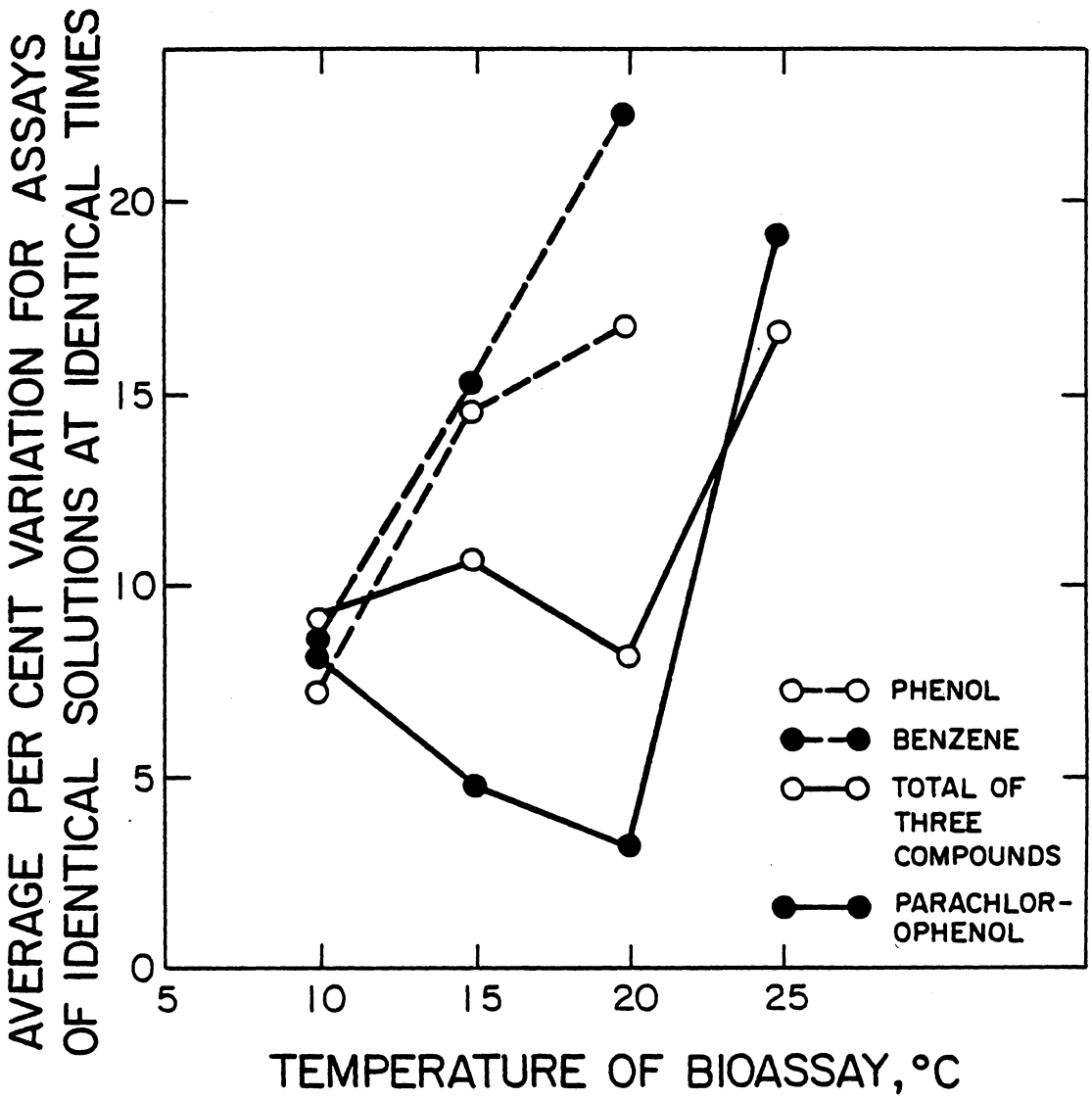

FIGURE 7. Average Per Cent Variation of Microtox Bioassays for Identical Tests

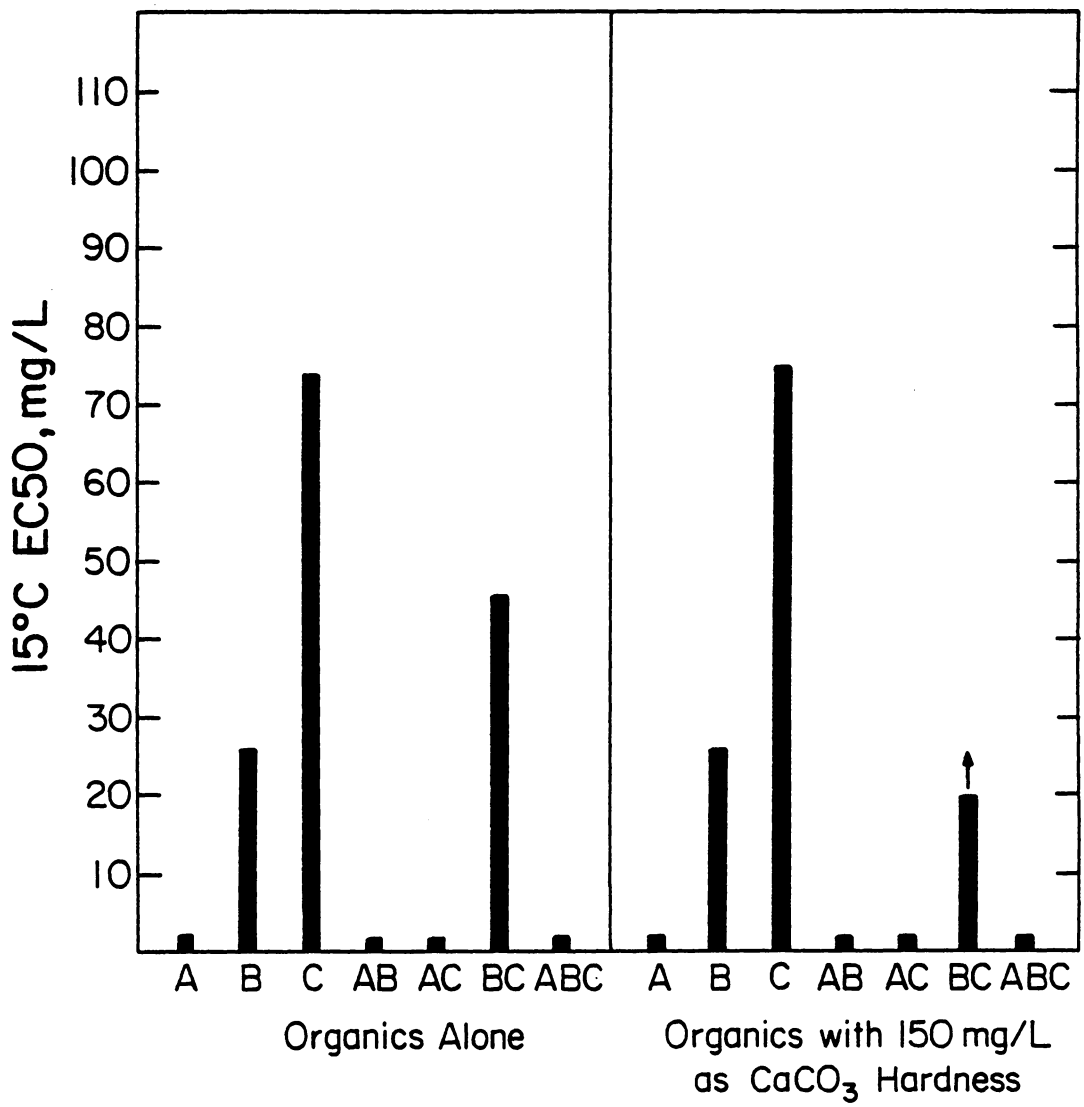

FIGURE 8. EC50s of Toxic Organics (5 minutes, 15 C) With and Without Hardness Added (A = parachlorophenol; B = phenol; C = benzene)

Figure 8 displays EC50 data generated on mixed solutions of compounds with and without hardness (150 mg/L as CaCO3). Comparing solution A (parachlorophenol) to the same solution with hardness added shows that the EC50s were nearly identical. In the same way, comparing solution A to solution AB (parachlorophenol plus an equal concentration of phenol) shows that the EC50s of these were nearly identical. Hardness solutions of 150 mg/L as CaCO3 assayed alone also showed no substantial light diminuition except at 10 C, where the response recorded was -13 per cent.

Microtox Bioassay Response to Free Residual Chlorine

A study was performed to evaluate the effect of free residual chlorine on the Microtox bioassay response. Testing of tap water showed that chlorine caused a toxic response. Later evaluation of the the field data showed that chlorinated samples often caused a 100 per cent light diminuition. Tap water taken from a Virginia Tech laboratory produced a Microtox bioassay response of -99 per cent. The tap water was found to have a TOX concentration of 100 to 150 ug/L. Addition of sodium thiosulfate to the chlorinated tap water sample decreased the toxic response to -15 per cent. A chlorine standard containing a free chlorine residual of 0.80 mg/L provided a 5 minute 15 C Microtox bioassay response of -100 per cent. Addition of sodium thiosulfate reduced the toxic response to -7 per cent. Under identical conditions, for tap water containing 0.55 mg/L free chlorine residual, the Microtox bioassay response was -100 per cent. Addition of sodium thiosulfate reduced the toxic response to -15 per cent.

Establishing the Microtox Bioassay Response of Pure Samples

The Microtox bioassay response to pure samples was investigated prior to field sampling. 'Pure' samples refers to distilled or MilliQ waters. It was discovered that 'pure' water sources can not be assumed to produce an identical reponse to that of the specially prepared Microtox diluent. The Microtox bioassay response of MilliQ water was -9 per cent at pH 6.3 and 20 C. The response of distilled water was -6 per cent at pH 6.1 and 15 C. The negative response of pure water in comparison to the diluent blanks indicated the tendency of the Microtox bioassay to be extremely sensitive to factors that are not known. The sensitivity or variability from pure water source to pure water source was a major reason why it was not possible to report EC05s or EC10s and that the light decrease considered to represent a toxic response must be -20 per cent.

Studies performed with MilliQ and deionized water showed that if the solution pH were not within the range of 6.0 to 7.0, the resulting light output could not be compared to the diluent blank. This finding is crucial to the use of the Microtox as a groundwater screening test. The pH effect was established in the Literature Review. The pH data is given under the Results of Field Analysis section. The pH of the Microtox diluent is 6.2.

Results of Field Analyses

The results of field analyses are detailed in Appendix C. Table 3 lists all major parameters reported for each field sample. Table 3 includes an indication of whether or not that sample was considered as contaminated. The level of TOX deemed to be associated with contaminated samples was established by plotting each parameter versus the per cent Microtox bioassay response as shown in Figure 9. Figure 9 shows that 7 of 9 samples that had a TOX concentration of greater than 50 ug/L produced a toxic Microtox bioassay response. One of these samples (9a) had a borderline TOX concentration of 51 ug/L and a borderline Microtox bioassay response of -15 per cent. Sample 13a had a TOX concentration of 160 ug/L and a 5 minute, 20 C Microtox bioassay response of +21 per cent. Thirteen of nineteen samples that had a TOX concentration of less than 50 ug/L had a Microtox bioassay response of less than 20 per cent. Sample 20a had a Microtox bioassay response of -84 per cent and a TOX concentration of less than 10 ug/L. Sample 20a had a TOC concentration of 4.5 mg/L. Sample 7 had a Microtox bioassay response of -64 per cent and a TOX concentration of less than 10 ug/L. This sample had a zinc concentration of 1.146 mg/L that may have caused the negative response. Sample 18a had a Microtox bioassay response of -43 per cent. No contamination was found for sample 18a. No direct relationship between the TOX concentration and the Microtox bioassay response can be drawn from the data presented due to the effects of other groundwater parameters.

Figure 10 shows that fourteen of twenty one samples that had a TOC concentration of less than 3.0 mg/L had a Microtox bioassay response that

Table 3 - Summary of Field Data

Sample	20 C Microtox % Response	15 C Microtox % Response	TOX (ug/L)	TOC (mg/L)	Other Contaminants (mg/L)
1a!	-70	nd	78	<1.0	Fl=4.5; Cu=0.18
2a	-7	nd	<10	<1.0	Fl=3.9; Ba=0.23 Cu=0.11
3a	+24	nd	<10	<1.0	Fl=6.7; Cu=0.11
5a	-1	nd	17	<1.0	Fl=5.0; SO4=21.1 Ar=0.001; Ba=0.32
6a	+9	nd	<10	<1.0	Fl=6.2; Al=0.15
7a	-3	nd	<10	<1.0	Fl=5.3; NO3=1.5 Zn=0.12; Cu=0.29
8a	+27	nd	<10	<1.0	Fl=6.7; SO4=18.1 NO3=1.7; Al=0.15
9a!	-15	nd	51	<1.0	Fl=7.2; NO3=2.3 Hg=0.016
11a*	-52	nd	205	<1.0	Fl=1.15; Al=0.10 Co=0.12; Zn=0.21 chloroform= 0.06
12a	+16	-4	28	<1.0	Fl=1.9; SO4=34.6 Co=0.15 Ar=0.0012
13a	+21	0	160	<1.0	Fl=1.4; SO4=27.3 NO3=0.77; Ar=0.003 Zn=0.114 1,1,1-TCE=0.015
14a	+2	-3	<10	<1.0	Fl=1.3; SO4=16.7 NO3=0.51 Ar=0.015
15a*	-82	-68	125	2.3	Fl=1.31; Al=0.10 Co=0.10; Cu=0.10 chloroform= 0.033
16a*	-100	-100	95	<1.0	Fl=0.70; NO3=0.87 Ar= 0.004 1,1,1-TCE=0.069 1,1,2-TCE=0.021
17a	-1	+2	16	<1.0	Fl=0.52; SO4=28.7
18a	-43	-49	<10	<1.0	Fl=1.15; NO3=0.53 Cu=0.18; Co=0.17
19a*	-100	-100	220	<1.0	Fl=1.3; SO4=13.2 Al=0.10; Co=0.11 chloroform=0.094
20a!	-92	-84	<10	4.5	Fl=1.06; Co=0.29 Cu=0.60
21a*	-44	-24	250	<1.0	Fl=0.81; Al=0.20 Co=0.08; Cu=0.15 Chloroform= 0.155

Table 3 - Summary of Field Data (Cont'd)

Sample	20 C Microtox % Response	15 C Microtox % Response	TOX (ug/L)	TOC (mg/L)	Other Contaminants (mg/L)
22a	-10	-3	<10	<1.0	Fl=0.44; NO3=0.8 Zn=0.19; Al=0.10 Co=0.15
2	-4	nd	<10	0.214	NO3=2.8
5	+4	nd	<10	1.13	
6	+6	nd	<10	0.523	NO3=6.2
7!	-64	nd	<10	0.721	Zn=1.146
8!	-37	nd	376	>20	Zn=0.203; Cl2=416

KEY: Fl=fluoride; Cl2=chloride; NO3=nitrate as N; SO4=sulfate; Zn=zinc; Ar=arsenic; Co=cobalt; Cu=copper; Al=aluminum; Ba=barium; Hg=mercury; *=chlorinated; !=contaminated according to defined limits; nd=no data

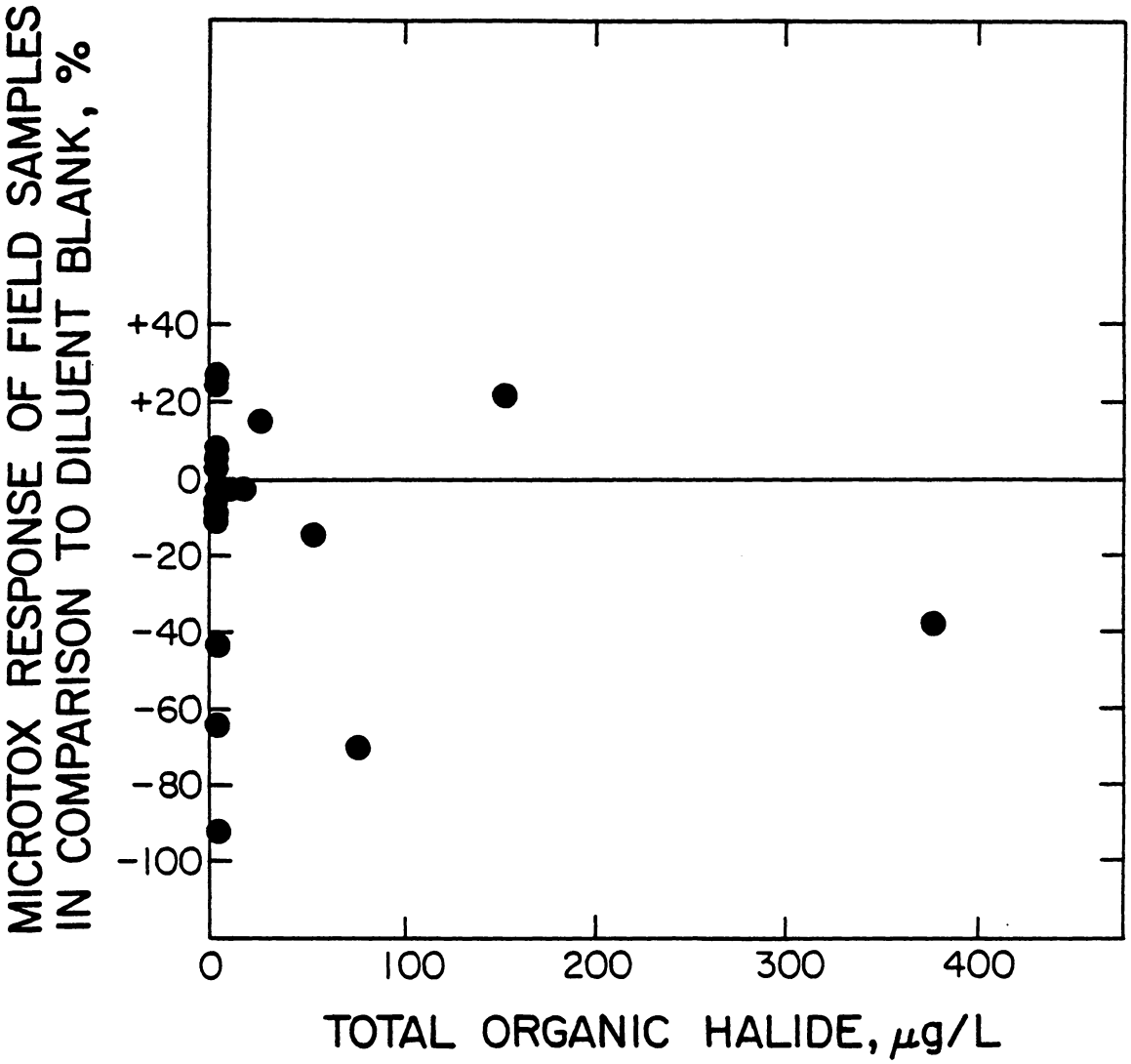

FIGURE 9. The Effect of the TOX Parameter on the Microtox Response of Field Samples

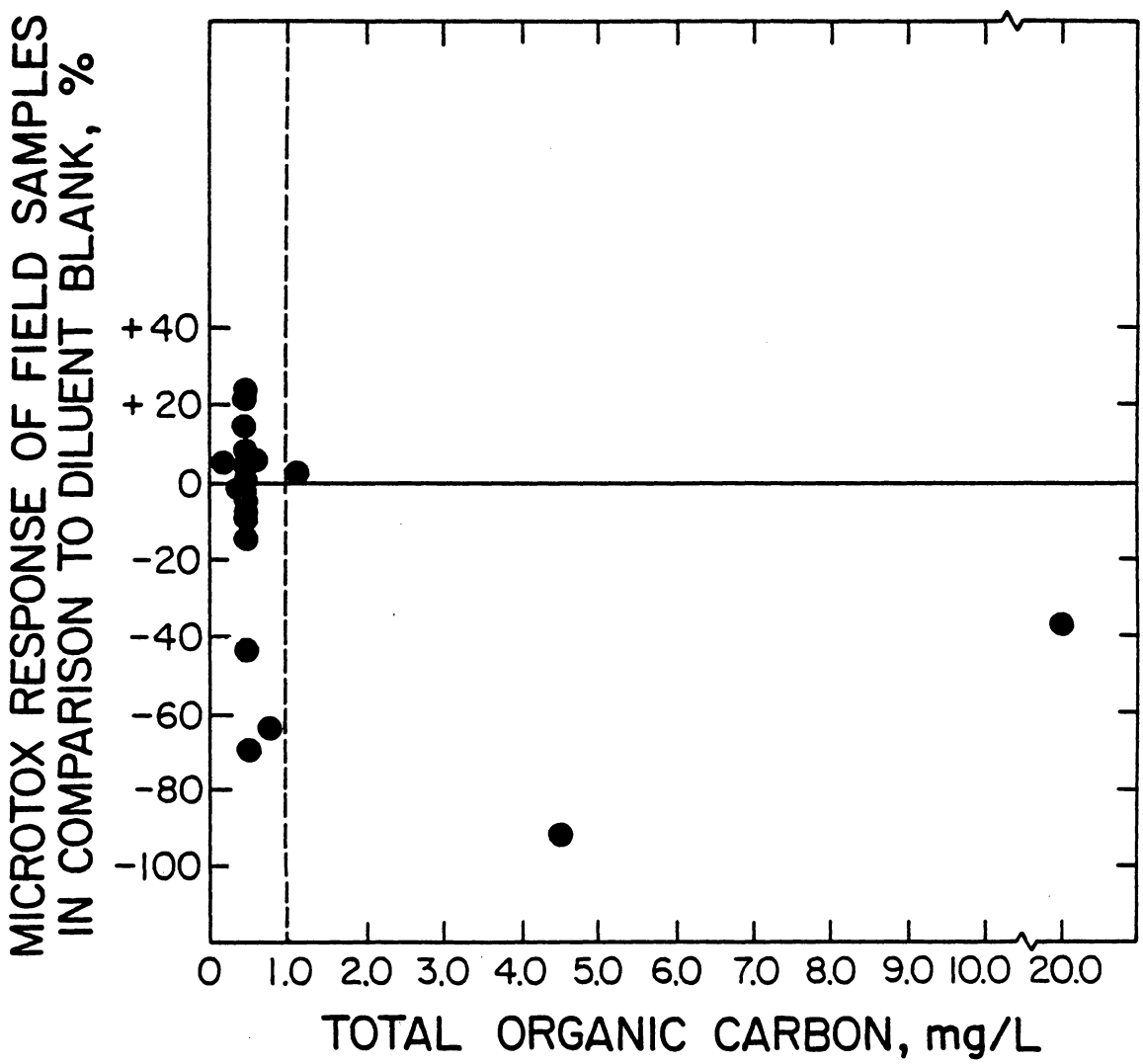

FIGURE 10. The Effect of the TOC Parameter on the Microtox Response of Field Samples

was not in the toxic range. Four of the seven samples that had a toxic response were chlorinated samples. Of the remaining three samples that had a TOC concentration of less than 1.0 mg/L, sample 1a had a TOX concentration or 78 ug/L, and samples 7 and 18a were discussed in the above section concerning Figure 9.

The correlation between contamination (according to predefined limits) and Microtox bioassay responses of 20 per cent or greater was 88 per cent or 22 of 25 samples (Table 3). The percentage reported includes the chlorinated and field samples that were not included in the USDA contract. The correlation also included sample 7, with a zinc concentration of 1.146 mg/L that may or may not have accounted for the toxic response. The correlation between Microtox bioassay response and contamination was 85 per cent, or 17 of 20 samples, if the chlorinatd samples were not included.

Hardness Effects

The findings of the laboratory Microtox testing showed that moderate concentrations of hardness (75 mg/L or less) caused no significant effect on the bioassays. A hardness concentration of 150 mg/L caused a decrease of 13 per cent at 10 C only. Figure 11 shows hardness concentrations of field samples in mg/L as CaCO3 versus the Microtox bioassay response. These data show no clear tendency. If the darkened data points are considered as outliers, then a general trend becomes apparent indicating slightly more negative Microtox bioassay responses at higher

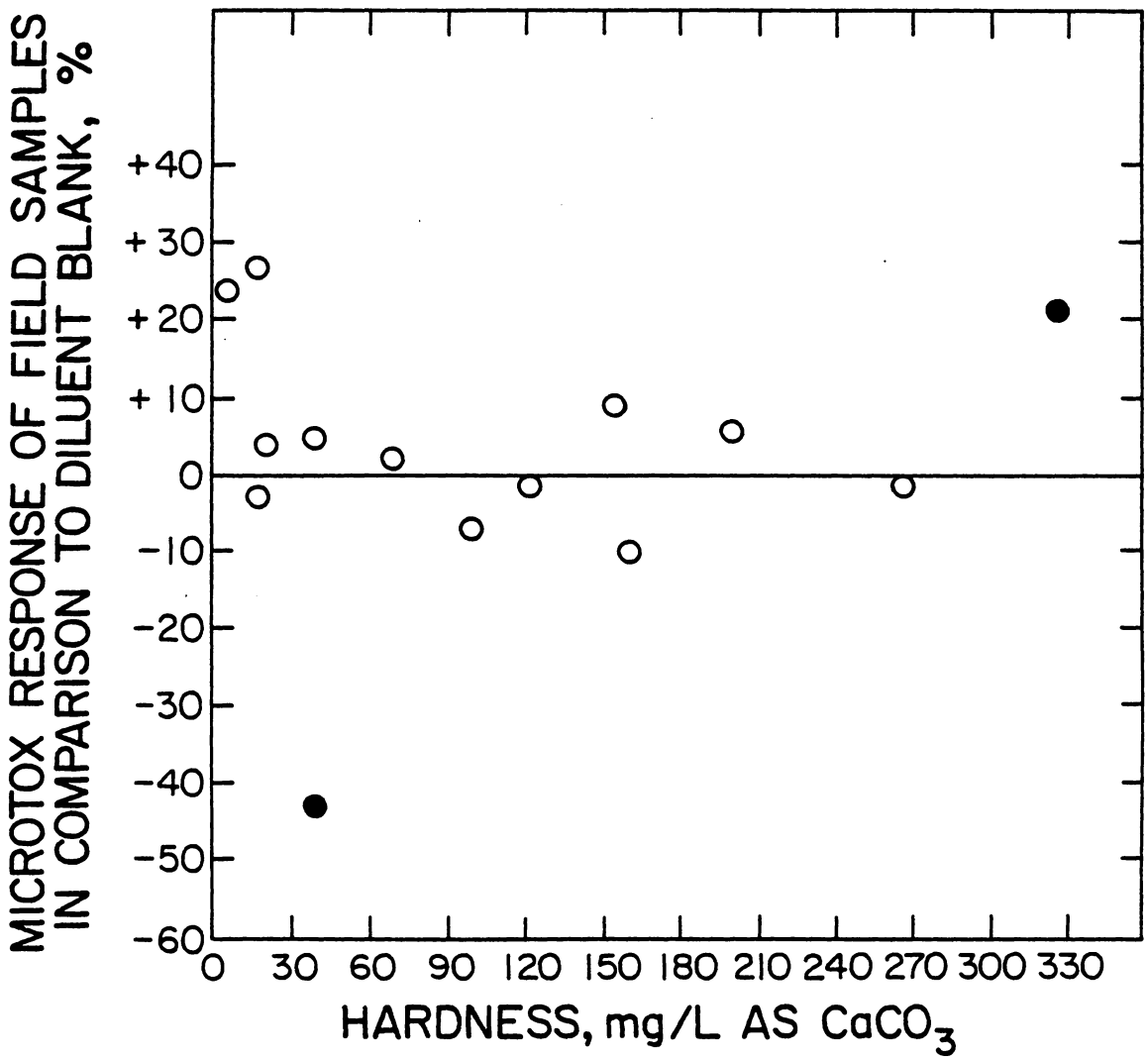

FIGURE 11. The Effect of the Hardness Parameter on the Microtox Response of Field Samples. (Figure does not include samples that had a TOX concentration of greater than 50 ug/L or sample 20a.)

hardness concentrations. Only one of the data points (18a) showed a toxic Microtox bioassay response.

pH Effects

Figure 12 shows pH versus the Microtox bioassay response for all field samples that had a TOX concentration of less than 50 ug/L, except for the darkened point (9a) that had a TOX concentration of 51 ug/L. Figure 12 shows that three samples had a toxic Microtox bioassay response. One was sample 9a. The other two were 18a and 20a, which have been reported earlier as being possible exceptions to correlation. The figure shows a general correlation between the 6.0 to 7.0 pH range and non-toxic Microtox responses.

Temperature effects.

The effect of temperature on the Microtox bioassay was found to be important. Figure 13 shows the EC50s of parachlorophenol and sample 5 spiked with parachlorophenol versus temperature of Microtox bioassay. Figure 13 also shows the line of best fit determined from linear regression analyis of all solutions containing parachlorophenol. The coeficient of correlation was 0.854. Figure 13 indicates a nearly linear relationship between temperature of Microtox bioassay and the sensitivity of the Microtox bioassay. Table 2 and Figure 6 also indicate this relationship.

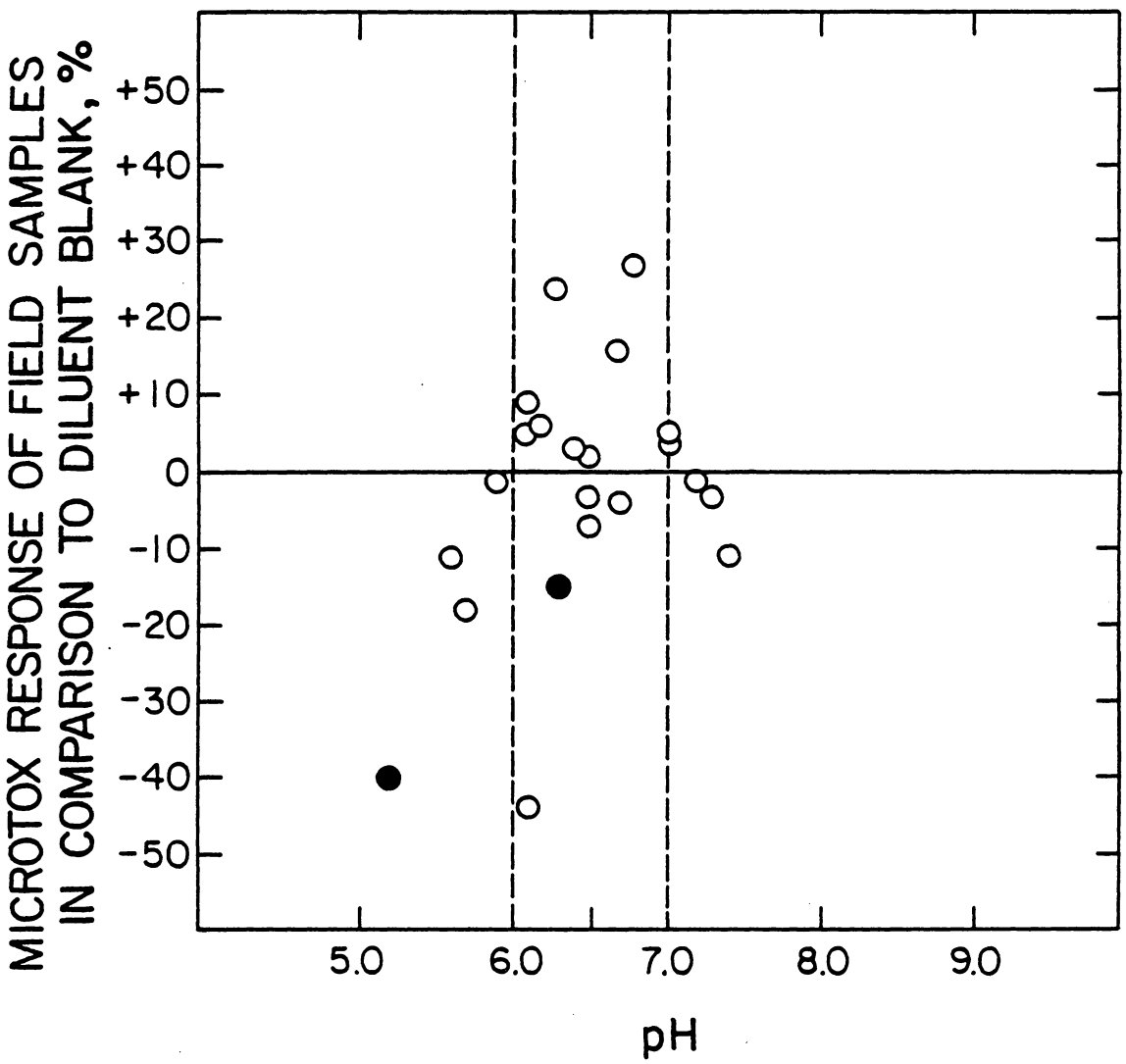

FIGURE 12. The Effect of the pH Parameter on the Microtox Response of Field Samples (The Figure does not include samples that had a TOX concentration of greater than 50 ug/L , except for the darkened points that represent sample # 9a that had a TOX concentration of 51 ug/L. Figure also excludes sample 20a).

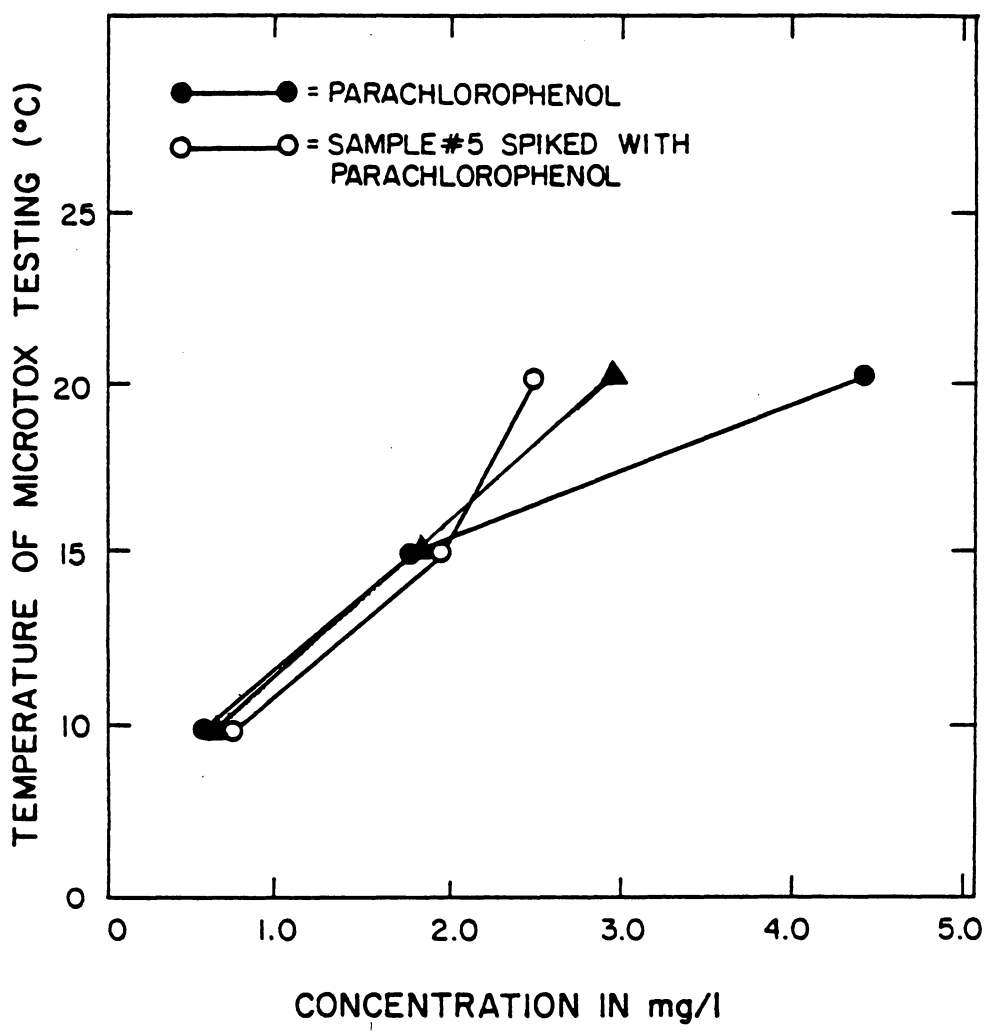

FIGURE 13. The Relationship Between Temperature of Bioassay and Sensitivity of the Bioassay (The triangular line represents the line of best fit for all parachlorophenol solutions as determined by linear regression analysis).

V. DISCUSSION

Possible Microtox Bioassay Interferences

The effect of groundwater hardness on the Microtox Bioassay was established in the Results section. Laboratory testing showed that 150 mg/L hardness solutions caused no interference. No interference was noted from field samples with up to a 206 mg/L hardness concentration. Results from the field sampling showed that chloride (61 mg/L), sulfate (28 mg/L), and fluoride (6.7 mg/L) caused no important interference.

pH Effect

The effect of pH on the Microtox bioassay was discussed in the Literature Review and Results sections. The pH effect is particularly important for groundwaters due to low buffering capacity of many groundwaters.

Temperature Effect

The effect of temperature on the sensitivity of the Microtox bioassay was established in the Results section (See Figure 5). It was apparent that the bacterial reagent (Photobacterium phosphoreum) reacted differently to toxicants at different temperatures. The sensitivity of the bioassay appeared to be greatest at 10 C. The optimum life of the

bacterial reagent appeared to be greatest at 15 C. The developers of the Microtox bioassay related that the Microtox bioassay was most sensitive at temperatures between 10 and 15 C.

Sensitivity of the Microtox Bioassay

The minimum detectable amount of parachlorophenol was considered to be equivalent to EC20 values from Table 2. The EC20s for for the remaining four compounds are estimates extrapolated from EC50 plots due to the fact that the lower concentrations assayed did not produce at least a 20 per cent light reduction. The 10 C EC20 concentration for parachlorophenol was 0.10 mg/L. For the remaining compounds, the values are as follows: for phenol, 2.7 mg/L, for benzene, 4.4 mg/L, for trichloroethane, 1.0 mg/L, and for trichlorophenol, 4.2 mg/L. Figure 14 shows an example of an effective concentration plot. This figure indicates that the relationship between log Gamma and log concentration may be curved for some compounds. The curved effect indicates that there is a range for which the relationship between toxicant concentration and light intensity is linear. The estimated lower limit of detection for specific toxicants is therefore actually somewhere between the estimated lower point and the linear range. In addition, the sensitivity of the Microtox bioassay will be different for different conditions (temperature of assay, etc.) and different compounds.

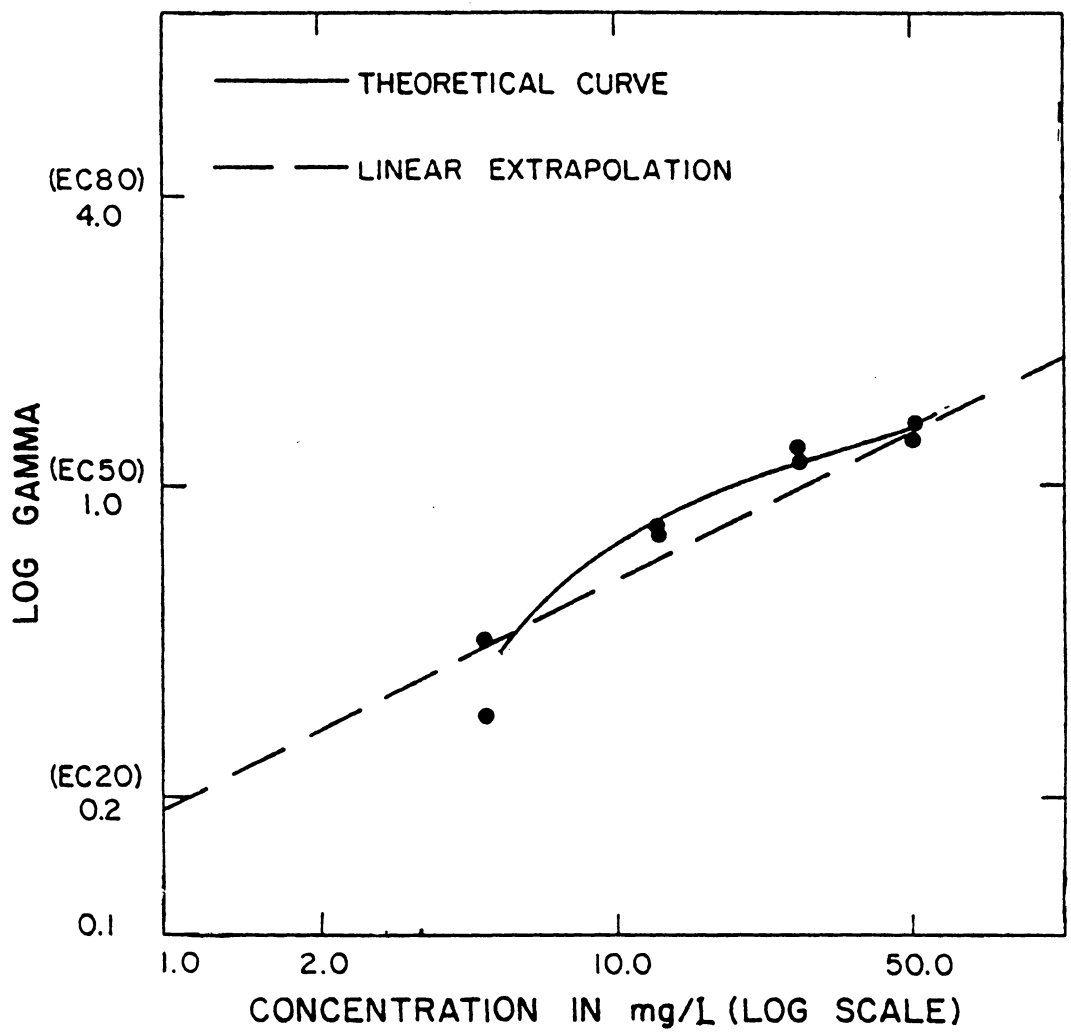

FIGURE 14. EC50 Plot Showing Possible Non-Linear Effect

Table 4 - Comparison of EC50s from Literature to Laboratory Data

Compound	Literature Value*	Laboratory Value
parachlorophenol	8.6 mg/L (44)	2.0 mg/L
phenol	29 (47)*	26
	25 (27)	
	39 (34)	
benzene	2 (24)	74
	4 (34)	
	214 (11)	
trichlorophenol	6 (47)	17
trichloroethane	no data	9

Comparison of EC50s Reported to Literature Reports

The effective concentrations for the organics assayed here generally were found to be comparable to those reported in the literature. Table 4 provides a list of EC50s determined in this study (Table 3) values reported in the literature. The only EC50 reported which was extremely different from the literature value is that for benzene. The EC50 for benzene was reported to range from 2.0 mg/L (24) to 214 mg/L (29). The variability of benzene is possibly related to this compounds high volatility. Part of the variance of EC50s from literature values can be attributed to differences in organic stock solution age and/or purity.

Discussion of Field Samples 2,5,6,7, & 8

Samples 2,5,6,7, and 8 are discussed separately because they were not included in the USDA contract were not analyzed for organics. Samples 2 and 5 were chosen as examples of uncontaminated groundwaters on the basis of the author's knowledge of the history of the wells. Both were located on homesteads in rural areas and have been used for drinking water sources for several years without any known taste or odor problems, or negative health effects. Sample 2 was collected from a well water source located in a rural area of Maryland. Sample 5 was collected from a springhouse located at approximately 3,000 feet elevation and more than one mile from the nearest house in West Virginia. Neither water source had any apparent possible source of contamination except for agricultural croplands that could have been treated with pesticides. Both of these

samples produced positive Microtox bioassay reponses. Sample 2 was a good example of a water that produced a negative Microtox bioassay response (-19 per cent) at a low pH (5.7) and a positive response (+5 per cent) at a pH adjusted to within the designated range.

Sample 6 was collected from a water source that had been alledgedly contaminated with benzene, as reported by Virginia State Water Control Board technicians. This water had been carbon filtered and the odor of benzene, that had been easily detected, had dissipated. The carbon filtered sample had a 5 minute, 20 C Microtox bioassay reponse of +6 per cent.

Sample 7 is discussed under the following section concerning the correlation of field sampling to bioassay reponse. This sample had a foul odor and the employees at Vulcan Materials would not drink it prior to filtering.

Sample 8 was chosen as an example of a contaminated groundwater. As reported in Table 3, this sample was highly contaminanted with chlorides, total organic carbon, and total organic halides. Sample 8 was a leachate from Dixie Caverns Landfill. Other industries in the area included Atlas Chemicals, Mid Atlantic Explosives, South Star, and Meddon Quarry. The water had a reddish color and a very foul odor.

The five samples mentioned above showed that the Microtox bioassay could effectively be used to indicate whether or not additional testing of a groundwater sample would be needed.

Correlation of Field Sampling to Microtox Bioassay Response

In order to quantitatively assess the correlation between chemical analyses and Microtox response, specific limits for contamination had to be chosen. Figure 9 was used to establish the contamination limit for TOX of 50 ug/L. The relationship between TOX Microtox response was difficult to quantify because of the number of groundwater parameters that may have had an effect on the Microtox bioassay response, and because TOX is a parameter that measures a general group of compounds. The toxicity of organic halides is not in question. The questions are at what level will TOX be detected as toxic to the Microtox bacterial reagent, and how does this level translate to human toxicity?

There really can be no direct association between TOC concentration and toxicity because of the fact that the TOC parameter measures all organic compounds, including those that are commonly found in nature and are considered as non-toxic. In the case of sample 20a, the Microtox bioassay indicated that there was some contaminant present in this sample but no contamination was found by any of the analyses performed here, except for a high level of total organic carbon. For these reasons, the correlation percentage was computed without sample 20a as correlating with the Microtox bioassay. There was probably some contaminant that was not measured that caused the acutely negative Microtox bioassay response for sample 20a. The TOC parameter was included as a screening parameter because it detects organics that the TOX does not. For this reason, the TOC and TOX could be seen as complementary parameters. The TOC will not indicate groundwater contamination of itself, but could indicate the

presence of an organic contaminant if the Microtox bioassay response indicated toxicity, as was possibly the case for sample 20a. A correlation was also computed with sample 7, that had a zinc concentration of 1.146 mg/L. The 45 minute, 15 C EC50 for zinc was reported as 0.98 mg/L. Because the Microtox bioassay response was recorded at a 5 minute time of exposure, it is questionable whether or not the level of zinc reported was responsible for the Microtox bioassay response of -64 per cent at 20 C and pH 6.2. It must be noted that the actual time of exposure of the Microtox bacterial reagent was 20 minutes, including the 15 minute stabilization period. Table 5 shows background data on the Vulcan Materials sample obtained from the State Water Control Board. The average concentration of zinc over 1977 to 1984 was 14.1 mg/L. This number is 9.1 mg/L over the secondary Maximum Contaminant Level set by the USEPA (44). It was apparent that sample 7 was a sample that was justifiably termed 'contaminated'. It is possible that a combination of metals present in this sample caused the toxic Microtox bioassay response.

The question that must be answered concerning the usefulness of the Microtox bioassay as a screening test must be, "If the Microtox were used to screen the groundwater samples without extensive and costly chemical analyses, would it have identified those samples for which chemical analyses were needed?". The answer appears to be yes, in every case except sample 13a, that had a TOX concentration of 160 ug/L. Samples 20a and 18a may have been false positives for contamination, but these two may have been contaminanted by constituents that were not measured. Overall, the correlation was considered as 'good' in comparison to other biological monitoring systems (6).

Table 5 - Background Data for Sample 7 - Vulcan Materials

Parameter	Mean	Standard Deviation
pH	6.9	0.31
alkalinity (mg/L as CaCO3)	157	9.8
T.D.S. (mg/L)	195	23.0
Chloride (mg/L)	3.1	0.9
Hardness, EDTA	130	16.2
TKN (mg/L)	0.2	0.5
Phosphorous (mg/l)	0.1	0.1
Ammonia (mg/L as N)	0.02	0.04
Nitrate (mg/L as N)	0	0
Nitrite (mg/L as N)	0	0
Sulphate (mg/L)	3.7	3.1
COD (mg/L)	4.7	4.5
T.O.C. (mg/L)	3.7	3.8
Conductivity	317	43
Total Calcium (mg/L)	31.2	6.5
Total Copper (mg/L)	0.01	0.02
Total Iron	12.3	13.2
Total Lead (mg/L)	0.01	0.01
Total Mg (mg/L)	8.2	0.9
Total Mn (mg/L)	0.5	0.2
Total Zinc (mg/L)	14.1	9.3
Total Na (mg/L)	16.0	4.7
Total K (mg/L)	0.5	0.14
Total Ni (mg/L)	0.01	0.01

Means are averages of 15 tests taken over the period 1980 to 1984.

The fact that the Microtox bioassay can not replace gas chromatographic analyzation techniques is not questioned. The Microtox bioassay definitely can not be used as a replacement for specific drinking water standards, because the assay is not specific and had a tendency to be too sensitive. The final evaluation of the Microtox bioassay must be expressed in terms of comparison to the screening tests now available. The Microtox bioassay could provide a means to screen large numbers of samples for contamination more rapidly, more economically, and provide a clearer idea of groundwater quality than any screening test now available. Of course, there are really no groundwater screening tests presently in use, except for general chemical parameters such as COD and TOX. It must also be realized that the Microtox bioassay might be performed from time to time to detect important changes in the groundwater quality. Consider for example the State Water Control Board groundwater study that was mentioned earlier in the Introduction and Literature Review. The information obtained from Microtox bioassays would have provided a better means to evaluate numerous groundwater samples than the parameters that were recorded for that study.

It appears that the Microtox bioassay could be used in conjunction with the TOX and TOC parameters to gain a better measure of groundwater quality than is now being obtained in a variety of circumstances. The organic analyses reported here demonstrate that even fairly extensive organic analyses will always leave open the possibility that an organic contaminant was present that was not specifically analyzed for.

The original purpose of this research was to evaluate the possible use of the Microtox bioassay, TOX, and TOC for screening over 600 water

sources throughout the U.S. that were identified as being within a 5 mile radius of National Priorty List waste sites. These water sources are used to wash meats and mix in with meat products such as sausage. The major problem with the Microtox test seemed to be that it was too sensitive and would have indicated poor groundwater quality for samples that would not be considered as a health hazard. The fact that the Microtox bioassay was very sensitive could be seen as providing a safety factor. It was apparent that had any of samples been grossly contaminated, the Microtox bioassay would have recorded a toxic response. Had the Microtox bioassay been used with TOX and TOC, the amount of money and time spent on chemical analyses could have been drastically reduced, and that was the original goal of the USDA.

The determination of health hazard is a subject of much complexity, particularly in this case where drinking water standards are not directly applicable. The Maximum Contaminant Level (MCL) for trihalogenated methanes (THMs) is 0.10 mg/L (17). This standard includes chloroform that was detected in samples 11a, 15a, 19a, and 21a. These four samples were all chlorinated. Several of the TOX concentrations were well above the THM standard, but there is no legal limit for TOX. It is the author's opinion, based on the research reported here and the literature, that the TOX parameter is superior to THMs as a measure of organic halide formation in drinking water.

The MCL of 2.4 mg/L (44) for fluoride was exceeded in samples 1a, 3a, 5a, 6a, 7a, 8a, and 9a. The fact that the Microtox bioassay did not detect fluoride was seen as a positive factor because if it had been sensitive to fluoride, more toxic factors may have been masked.

The MCL for mercury is 0.002 mg/L (44). Sample 9a had a mercury concentration of 0.016 mg/L. Sample 9a had a Microtox bioassay response of -15 per cent after pH adjustment. This sample was considered as a borderline case considering the TOX concentration of 51 ug/L and the borderline Microtox bioassay response.

The MCL for benzene has been established by the EPA at 0.005 mg/L (19). The MCL for 1,1,1-trichloroethane is 0.20 mg/L (19). Trichlroroethane was detected in sample 16a at 0.069 mg/L along with 0.021 mg/L 1,1,2-trichloroethane. The MCL concentrations for benzene and trichloroethane are well below the maximum sensitivity level of the Microtox bioassay. The TOX would detect the trichloroethane. The inability of the Microtox bioassay to detect benzene and phenol at low levels must be seen as a drawback to using the Microtox bioassay as a screening test for groundwater contamination.

No conclusions about the mechanisms responsible for the toxicities of the various compounds is offered, but there does appear to be some important trend that might be gleaned from Figure 15, i.e. substitution in the aromatic ring increased toxicity. More data of this type was warranted for a better understanding of the Microtox response.

General Comments

It was encouraging to learn that the groundwater sources sampled in areas that had been known to have groundwater contamination problems were fairly clean. Some contamination was found, but the levels were low. This statement must be qualified in three ways by remembering that any

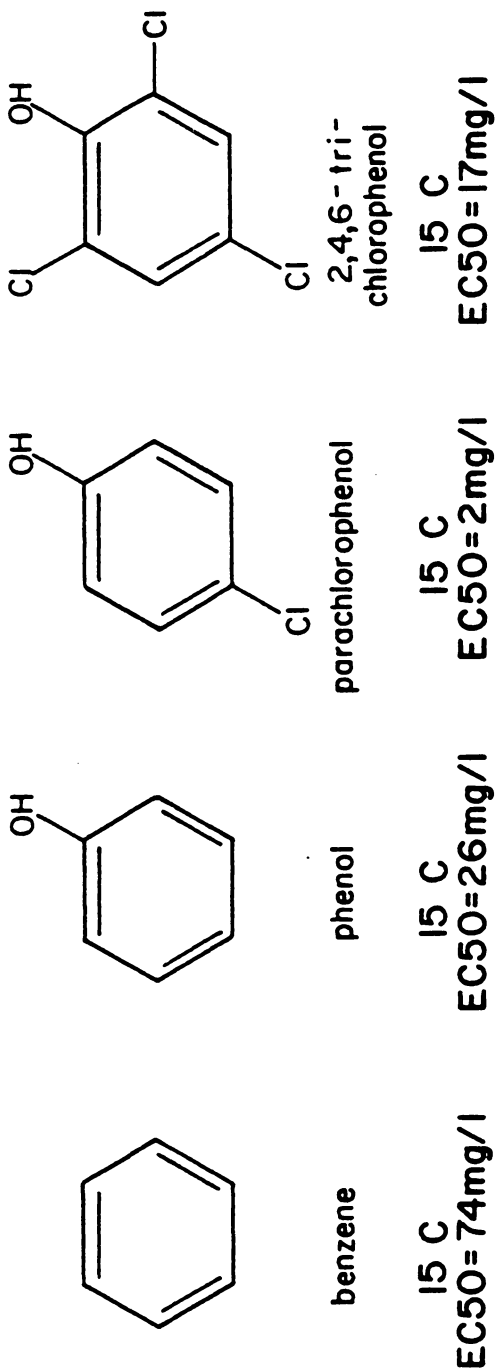

FIGURE 15. Illustration of Molecular Structures of the Compounds Assayed with EC50s

level of contamination by synthetic chemicals represents a problem that is persistantly getting worse and that "low" levels may cause chronic effects. Also, it must be stated that single point sampling (in time and geographic space) does not preclude significant contamination in the area. It is well known that contamination of groundwater aquifers can be highly variable with respect to time and spacial configuration. It is hoped that with greatly improved methods, including screening tests such as those studied here, groundwater contamination can be more accurately identified and more extensively remedied. Most importantly, it is hoped that projects such as this will help to bring to bear the scientific, legislative, and economic forces to bear that will reverse the present trend of waste.

VI. CONCLUSIONS

Conclusions that can be drawn from the data gathered are:

1) The Microtox bioassay could be a useful tool to evaluate groundwater quality.

2) TOC and TOX data can complement Microtox results and the three tests together are recommended as a series of screening tests.

3) The Microtox bioassay was most sensitive to the organics tested at 10 C.

4) Hardness, sulfate, chloride, and fluoride did not interfere with the Microtox tests at concentrations of 150 mg/L, 28 mg/L, 61 mg/L, and 6.7 mg/L, respectively.

5) The maximum sensitivity of the Microtox bioassay varied widely with the toxicity of the compound tested, ranging from 0.1 mg/L for parachlorophenol to 2.7 mg/L for phenol and 4.1 mg/L for benzene.

7) The sensitivity of the Microtox screening test for parachlorophenol, pehnol, benzene, trichlorophenol, and trichloroethane was greatest at a 5 minute time of exposure.

8) Parachlorophenol, phenol, and benzene acted neutrally in mixed solutions, and the toxicity of the mixed solution was controlled by the most toxic compound. In this case, toxicity responses were a function of the parachlorophenol level.

9) The Microtox bioassay should not be used to screen chlorinated samples unless the samples are first dechlorinated.

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APPENDIX A - RESULTS OF LABORATORY MICROTOX ANALYSIS

sample	conc	pH	temp	%	conc	pH	temp	%
A	5.0	5.0	15	-70	5.0	6.0	15	-71
A	5.0	6.4	20	-68	5.0	6.5	10	-69
A	5.0	6.5	10	-76	5.0	5.8	25	-29
B	50	6.2	10	-67	50	6.2	15	-72
B	50	6.5	15	-48	5	6.3	20	-6
B	10	6.1	15	-32				
C	50	6.3	10	-67	50	5.3	15	-37
C	50	6.2	15	-34	10	5.6	15	0
C	12	6.3	20	-20				
H	150	7.2	10	-13	150	6.3	15	- 4
H	150	6.9	20	0	150	6.9	25	0
AH	5.0	6.7	10	-67	5.0	6.5	15	-72
AH	5.0	7.6	25	-61	5.0	6.0	25	-26
BH	50	6.4	10	-74	50	6.4	15	-62
BH	10	6.1	15	-25	50	6.0	20	-11
CH	50	10	60	-50	50	6.0	15	-62
CH	50	5.9	20	-50	5.9	20	0	
AB	5.0	6.1	10	-84	5.0	6.3	15	-74
AB	5.0	5.8	20	-49				
AC	5.0	5.9	10	-72	5.0	6.0	15	-73
AC	5.0	5.2	20	-60				
ABH	5.0	6.4	10	-71	5.0	6.3	15	-72
ABH	5.0	6.0	20	-52				
ACH	5.0	7.3	10	-71	5.0	5.9	15	-79
ACH	5.0	6.3	20	-56				
BCH	50	6.4	10	-70	20	6.0	15	- 4
BC	50	6.2	10	-50	20	6.0	15	-20
BC	5.0	6.4	20	>0				
BCH	50	6.4	10	-70	10	6.0	15	-29
BCH	5.0	6.1	20	0				
BC	5.0	5.5	20	0				
ABC	5.0	6.3	10	-70	5.0	6.5	15	-67
ABC	5.0	6.4	20	-55				
ABCH	5.0	6.2	10	-82	5.0	6.4	15	-70
ABCH	5.0	6.3	20	-53				
TCE	12.5	5.9	10	-73	12.5	5.9	15	-58
TCE	12.5	nd	20	-50				
TCP	12.5	5.7	10	-53	12.5	5.7	15	-40
TCP	12.5	nd	20	-45				
5+A	5.0	6.7	10	-75	5.0	6.7	15	-69
5+A	5.0	6.7	20	-63				
5+B	50	6.7	10	-75	50	6.7	15	-57
5+B	50	6.7	20	-43				
5+C	50	6.8	10	-57	50	6.8	15	-52
5+C	50	6.8	20	-23				

All mixtures are equal concentrations of each chemical. All concentrations are in mg/l.

KEY A = parachlorophenol; B = phenol; C = benzene; H = hardness standard (80% calcium carbonate & 20% magnesium carbonate); TCE = trichloroethane; TCP = 2,4,6, trichlorophenol; 5 = springhouse water (Lehr residence)

APPENDIX B - MICROTOX RESULTS OF FIELD TESTING

sample	pH	temp	%	pH	temp	%	pH	temp	%
tap	8.6	15	-100	6.6	15	-99			
tap+Na.	8.7	-15	15	6.6	15	-10			
Cl	6.4	15	-100						
Cl+Na	6.6	15	-7						
19a	7.4	15	-100	7.3	20	-100			
19a+Na	8.1	15	-12	6.5	15	-5	6.8	20	-3
16a	7.3	15	-100	7.3	20	-100			
16a+Na	5.8	15	-45	6.4	15	-4	7.0	20	+4
17a	7.4	15	+2	6.5	15	0	7.2	20	-1
22a	7.3	15	-1	6.5	15	-3	7.1	20	-9
21a	7.0	15	-24	7.1	20	-44			
21a+Na	6.7	15	-3	6.9	20	+9			
12a	6.7	15	-4	6.7	20	+14			
13a	6.6	15	0	6.6	20	+18			
11a	6.3	15	-91	6.3	20	-52			
11a+Na	7.6	15	-91	7.6	20	-52	7.6	20	-40
14a	6.5	15	-3	6.5	20	-2			
15a	6.9	15	-80	6.9	20	-82			
15a+Na	7.2	15	-50	6.8	20	-64			
18a	6.1	15	-49	6.1	20	-45	6.1	20	-43
20a	6.2	15	-91	6.2	20	-96	6.1	20	-92
1a	6.2	20	-31	6.0	20	-70			
2a	6.5	20	-7	5.6	20	-11			
5a	6.5	20	-2	5.9	20	-1			
9a	6.3	20	-15	5.2	20	-40			
7a	7.3	20	-3						
8a	6.8	20	+27						
8	6.5	20	-36	6.1	20	-3	6.1	20	-37
7	6.7	20	-34	6.2	20	-64			
NaS O 250mg/l	nd	15	0						
5	6.3	20	+4						
2	5.7	20	-18	5.7	20	-22	7.0	20	+5
2	6.7	20	-4	7.0	20	-1			
5	6.4	20	+5	7.1	20	+5			
MQ H O	7.0	20	-10						
dein.+H O	nd	20	-1						
5+ B 5.0 mg/l	6.3	20	-53						
5+ A 1.0 mg/l	6.4	20	-14						
5+ D 50 mg/l	6.4	20	-41						

APPENDIX C - USDA GROUNDWATER PROJECT FIELD DATA

Sample number: 1a
Location: PA
Description of area: rural
Date of Sampling: 8-8-85

pH: 6.0
TOX: 78 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

water source: well
notes: owner relates that waste dump is 1 mile away

Anions -
hardness: 92 mg/L
carbonate: <10
bicarbonate: 31.8
chloride: 32.8
fluoride: 5.8
sulfate: <10
nitrate: 2.05
Trace elements -
aluminum: 0.2 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 25.7
chromium: <0.05
cobalt: <0.05
iron: 0.03
lead: <0.1
magnesium: 6.7
manganese: 0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.04
copper: 0.18
Additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C:
20 C: -70 per cent

Sample number: 2a
Location: PA.
Description of area: rural
Date of Sampling: 8-7-85

pH: 5.6
TOX: <10 ug/L
TOC: <1.0 mg/L

water source: well
notes:

Anions -
hardness: 100 mg/L
carbonate: <10
bicarbonate: <10

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

chloride: 38.3
fluoride: 3.9
sulfate: nd
nitrate: 0.91
Trace elements -
aluminum: <0.1mg/L
arsenic: <1.0 ug/L
barium: 0.23 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 25.2
chromium: <0.05
cobalt: <0.05
iron: 0.11
5ead: <0.1
magnesium: 8.9
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: <0.005
copper: 0.11
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
20 C: -11 per cent (pH 5.6)
20 C: -7 per cent (pH 6.5)

Sample number: 3a
Location: N.J.
Description of area: rural, flat
Date of Sampling: 8-6-85

pH: 6.3
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L

water source: well
notes: Industrial odor in air
Maurice aquifer

Anions -
hardness: 7 mg/L
carbonate: <10
bicarbonate: >10
chloride: 9.3
fluoride: 6.7
sulfate: <10
nitrate: 0.38
Trace elements -
aluminum: <0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 0.90
chromium: <0.05

arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

cobalt: 0.07
iron: 1.0
lead: <0.1
magnesium: 1.3
manganese: 0.04
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.06
copper: 0.15
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C:
20 C: +24 per cent

Sample number: 5a
Location: PA.
Description of area: very rural
Date of Sampling: 8-8-85

water source: well
notes: 20 miles out of Philadelphia

pH: 5.9
TOX: 17 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -

Anions -
hardness: 268 mg/L
carbonate: <10
bicarbonate: 225
chloride: 11.2
fluoride: 5.0
sulfate: 21.1
nitrate: <0.01
Trace elements -
aluminum: 0.1 mg/L
arsenic: 10.8 ug/L
barium: 0.32 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 76.3
chromium: <0.05
cobalt: <0.05
iron: 0.08
lead: <0.1
magnesium: 19.0
manganese: 0.03
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.18
copper: <0.02
additional notes: No odor detectable. Whitish turbidity.
Taste is good

phenol: <10 ug/L
pentachlorophenol: <10

Microtox response -
15 C:
20 C: -1 per cent

Sample number: 6a
Location: DE.
Description of area: very rural
Date of Sampling: 8-5-85

water source: well
notes: Metals Corp. 6 miles away.

pH: 6.1
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

Anions -
hardness: 156 mg/L
carbonate: <10
bicarbonate: 170
chloride: 3.2
fluoride: 6.2
sulfate: <10
nitrate: <0.01
Trace elements -
aluminum: 0.15 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 51.8
chromium: <0.05
cobalt: <0.05
iron: 1.54
lead: <0.1
magnesium: 6.6
manganese: 0.22
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.017
copper: <0.02
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C:
20 C: +9 per cent

Sample number: 7a
Location: N.J.
Description of area: rural
Date of Sampling: 8-7-85

water source: well
notes:

pH: 7.3

Anions -

TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

hardness: 18 mg/L
carbonate: <10
bicarbonate: 31.0
chloride: 8.76
fluoride: 5.28
sulfate: <10
nitrate: 1.52
Trace elements -
aluminum: 0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 2.98
chromium: <0.05
cobalt: <0.05
iron: 0.25
lead: <0.1
magnesium: 3.1
manganese: 0.04
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.115
copper: 0.29
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C:
20 C: -3 per cent

Sample number: 9a
Location: N.J.
Description of area: rural
Date of Sampling: 8-6-85

pH: 5.2
TOX: 51 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

water source: well
notes: Canary grass waste treatment field adjacent

Anions -
hardness: 35 mg/L
carbonate: <10
bicarbonate: <10
chloride: 17.6
fluoride: 7.2
sulfate: nd
nitrate: 2.3
Trace elements -
aluminum: <0.5 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

cadmium: <0.005
calcium: 7.3
chromium: <0.05
cobalt: <0.05
iron: 0.10
lead: <0.1
magnesium: 4.2
manganese: 0.09
mercury: 1.16 ug/L
nickel: <0.04 mg/L
zinc: 0.03
copper: 0.07
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
20 C: -40 per cent (pH 5.2)
20 C: -15 per cent (pH 6.3)

Sample number: 8a
Location: N.J.
Description of area: rural
Date of Sampling: 8-6-85

pH: 6.8
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L

water source: well
notes: Vineland City Landfill is across the street (100 yds)

Anions -
hardness: 21 mg/L
carbonate: <10
bicarbonate: <10
chloride: 9.01
fluoride: 6.7
sulfate: 18.1
nitrate: 1.7
Trace elements -
aluminum: 0.15 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 4.3
chromium: <0.05
cobalt: <0.05
iron: 0.05
lead: <0.1
magnesium: 2.4
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: <0.005
copper: <.02

toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

additional notes: No odor or turbidity detectable taste is good
Microtox response -
20 C: +27 per cent (pH 6.8)
20 C: +5 per cent (pH 6.1)

Sample number: 11a
Location: PA.
Description of area: suburban
Date of Sampling: 9-10-85

pH: 6.3
TOX: 205 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: 69 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

water source: city water
notes: sodium thiosulfate added as needed

Anions -
hardness: 23 mg/L
carbonate: <10
bicarbonate: 5.7
chloride: 9.4
fluoride: 1.15
sulfate: <10
nitrate: 0.055
Trace elements -
aluminum: 0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 7.4
chromium: <0.05
cobalt: 0.12
iron: 1.34
lead: <0.1
magnesium: 1.17
manganese: 0.27
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.209
copper: 0.07
additional notes: No odor or turbidity detectable

Microtox response -
15 C: -44 per cent
20 C: -52 per cent
15 C: -91 per cent ($Na_2S_2O_3$ added)
20 C: -39 per cent "

Sample number: 12a
Location: N.J.
Description of area: urban

water source: well
notes:

Date of Sampling: 9-9-85

pH: 6.7
TOX: 28 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

Anions -
hardness: 329 mg/L
carbonate: <10
bicarbonate: 197
chloride: 189
fluoride: 1.9
sulfate: 34.6
ntrate: 0.34
Trace elements -
aluminum: <0.1 mg/L
arsenic: 1.2 ug/L
barium: 0.35 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 92.2
chromium: <0.05
cobalt: 0.15
iron: 0.11
lead: <0.1
magnesium: 24.0
manganese: 0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.059
copper: 0.03
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -4 per cent
20 C: +16 per cent

Sample number: 13a
Location: N.J.
Description of area: urban
Date of Sampling: 9-9-85

water source: well
notes: well is more than 200 ft. deep

pH: 6.6
TOX: 160 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10

Anions -
hardness: 206 mg/L
carbonate: <10
bicarbonate: 131
chloride: 61.0
fluoride: 1.4
sulfate: 27.3
nitrate: 0.770
Trace elements -
aluminum: 0.1 mg/L

1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: 15

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

arsenic: 2.87 ug/L
barium: 0.1 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 51.2
chromium: <0.05
cobalt: 0.18
iron: 0.10
lead: <0.1
magnesium: 19.0
manganese: 0.02
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.114
copper: 0.02
additional notes: No odor detectable. Slightly turbid
Taste is good
Microtox response -
15 C: 0 per cent
20 C: +21 per cent

Sample number: 14a
Location: Conn.
Description of area: very rural
Date of Sampling: 9-10-85

water source: well
notes: Local says that water problems are common

pH: 6.5
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

Anions -
hardness: 72 mg/L
carbonate: <10
bicarbonate: 43.4
chloride: 11.1
fluoride: 1.3
sulfate: 16.7
nitrate: 0.508
Trace elements -
aluminum: <0.1 mg/L
arsenic: 1.49 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 23.5
chromium: <0.05
cobalt: 0.12
iron: 0.04
lead: <0.1
magnesium: 3.2
manganese: 0.01
mercury: <1.0 ug/L

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

nickel: <0.04 mg/L
zinc: 0.046
copper: 0.03
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -3 per cent
20 C: +2 per cent

Sample number: 15a
Location: Conn.
Description of area: very rural
Date of Sampling: 9-10-85

pH: 6.9
TOX: 125 ug/L
TOC: 2.3 mg/L

purgeable halocarbons -
chloroform: 33 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

water source: city water
notes: Inspector relates that several wells in the area were closed down due to high nitrate concentrations
Anions -
hardness: 13 mg/L
carbonate: <10
bicarbonate: 5.82
chloride: 9.10
fluoride: 1.31
sulfate: <10
nitrate: 0.074
Trace elements -
aluminum: 0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 3.5
chromium: <0.05
cobalt: 0.10
iron: 0.23
lead: <0.1
magnesium: 0.99
manganese: 0.03
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.035
copper: 0.10
additional notes: No odor or turbidity detectable
Chlorinated taste
Microtox response -
15 C: -68 per cent
20 C: -82 per cent
15 C: -45 per cent ($Na_2S_2O_3$)
20 C: -64 per cent "

Sample number: 16a
Location: PA.
Description of area: rural
Date of Sampling: 9-28-85

pH: 7.3
TOX: 95 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: 69
1,1,2-trichloroethane: 21
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

water source: well
notes: chlorinated water industrial compound behind establishment

Anions -
hardness: 87 mg/L
carbonate: <10
bicarbonate: 45.0
chloride: 63.4
fluoride: 0.70
sulfate: <10
nitrate: 0.87
Trace elements -
aluminum: <0.1 mg/L
arsenic: 4.0 ug/L
barium: 0.3 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 22.7
chromium: <0.05
cobalt: 0.14
iron: 0.08
lead: <0.1
magnesium: 7.2
manganese: 0.02
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: <0.005
copper: 0.47
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -100 per cent (pH 7.3)
20 C: -100 per cent (pH 7.30
15 C: -45 per cent (pH 5.8)
15 C: -3 per cent (pH 6.4)
20 C: +4 per cent (pH 7.0)

Sample number: 17a
Location: N.Y.
Description of area: rural
Date of Sampling: 10-1-85

pH: 7.4
TOX: 16 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L

water source: well
notes: obtained water from well field prior to chlorination

Anions -
hardness: 121 mg/L
carbonate: <10
bicarbonate: 36.0
chloride: 108
fluoride: 0.52

1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

sulfate: 28.7
nitrate: 0.300
Trace elements -
aluminum: <0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 37.8
chromium: <0.05
cobalt: <0.05
iron: 0.06
lead: <0.1
magnesium: 6.6
manganese: 0.04
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.016
copper: <0.02
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: +2 per cent (pH 7.4)
20 C: -1 per cent (pH 7.3)
15 C: 0 per cent (pH 6.5)
20 C: -1 per cent (pH 7.2)

Sample number: 18a
Location: Conn.
Description of area: very rural
Date of Sampling: 9-11-85

water source: well
notes:

pH: 6.1
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0

Anions -
hardness: 41 mg/L
carbonate: <10
bicarbonate: 23.6
chloride: 17.5
fluoride: 1.15
sulfate: <10
nitrate: 0.525
Trace elements -
aluminum: <0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 11.8
chromium: <0.05
cobalt: 0.17
iron: <0.03

arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

lead: <0.1
magnesium: 2.8
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.011
copper: 0.18
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -49 per cent
20 C: -43 per cent

Sample number: 19a
Location: PA.
Description of area: industrial
Date of Sampling: 9-28-85

water source: city water
notes: Used to operate on a well but it is closed down

pH: 7.4
TOX: 220 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: 94 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

Anions -
hardness: 100 mg/L
carbonate: <10
bicarbonate: 37.0
chloride: 42.7
fluoride: 1.3
sulfate: 13.2
nitrate: 0.371
Trace elements -
aluminum: 0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 32.8
chromium: <0.05
cobalt: 0.11
iron: 0.09
lead: <0.1
magnesium: 4.5
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.012
copper: <0.02
additional notes: No odor or turbidity detectable
Metallic taste
Microtox response -
15 C: -100 per cent (pH 7.4)

20 C: -100 per cent (pH 7.3)
15 C: -12 per cent (pH 8.1)
15 C: -5 per cent (pH 6.5)
20 C: -3 per cent (pH 6.8)

Sample number: 20a
Location: Conn.
Description of area: very rural
Date of Sampling: 9-11-85

water source: well
notes:

pH: 6.2
TOX: <10 ug/L
TOC: 4.5 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

Anions -
hardness: 19 mg/L
carbonate: <10
bicarbonate: 13.5
chloride: 14.3
fluoride: 1.06
sulfate: <10
nitrate: 0.076
Trace elements -
aluminum: <0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 5.5
chromium: <0.05
cobalt: 0.29
iron: 0.13
lead: <0.1
magnesium: 1.3
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.052
copper: 0.60
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -84 per cent
20 C: -92 per cent

Sample number: 21a
Location: N.Y.
Description of area: rural
Date of Sampling: 10-1-85

water source: city water
notes: Local says that there is a warning concerning chemical contamination on water bills

pH: 7.0
TOX: 250 ug/L

Anions -
hardness: 39 mg/L

TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: 155 ug/L
1,1-dichloroethane: <10
1,2-dichloroethane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

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Sample number: 22a
Location: N.Y.
Description of area: rural
Date of Sampling: 10-2-85

pH: 7.3
TOX: <10 ug/L
TOC: <1.0 mg/L

purgeable halocarbons -
chloroform: <10 ug/L
1,1-dichloroethane: <10
1,2-dichloroehtane: <10
1,1-dichloroethene: <10
trans-1,2-dichloroethene: <10
1,1,1-trichloroethane: <10
1,1,2-trichloroethane: <10
trichloroethene: <10

carbonate: <10
bicarbonate: 39.5
chloride: 9.0
fluoride: 0.81
sulfate: <10
nitrate: 0.07
Trace elements -
aluminum: 0.2 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005
calcium: 14.2
chromium: <0.05
cobalt: 0.08
iron: 0.18
lead: <0.1
magnesium: 0.9
manganese: 0.02
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.03
copper: 0.15
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -24 per cent (pH 7.0)
20 C: -44 per cent (pH 7.06)
15 C: -3 per cent (pH 6.7)
20 C: +9 per cent (pH 6.9)

water source: well
notes: obtained samples from prior to chlorination

Anions -
hardness: 163 mg/L
carbonate: <10
bicarbonate: 35.5
chloride: 24.1
fluoride: 0.44
sulfate: <10
nitrate: 0.890
Trace elements -
aluminum: 0.1 mg/L
arsenic: <1.0 ug/L
barium: <0.2 mg/L
berylium: <0.005
cadmium: <0.005

PCB's -
arachlor 1016: <1.0 ug/L
arachlor 1221: <1.0
arachlor 1232: <1.0
arachlor 1242: <1.0
arachlor 1248: <1.0
arachlor 1254: <1.0
arachlor 1260: <1.0

purgeable aromatics -
benzene: <10 ug/L
toluene: <10

phenols -
phenol: <10 ug/L
pentachlorophenol: <10

calcium: 48.6
chromium: <0.05
cobalt: 0.15
iron: 0.06
lead: <0.1
magnesium: 10.2
manganese: <0.01
mercury: <1.0 ug/L
nickel: <0.04 mg/L
zinc: 0.186
copper: 0.03
additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
15 C: -1 per cent (pH 7.3)
20 C: -10 per cent (pH 7.2)
15 C: - 3 per cent (pH 6.5)

Sample number: 2 (Jackson House)
Location: Mt. Airy, Md.
Description of area: very rural
Date of Sampling: 4-7-85

water source: well
notes: No evident possible source of contamination

pH: 5.7
TOX: <10 ug/L
TOC: 214 ug/L

purgeable halocarbons -
chloroform: nd
1,1-dichloroethane: nd
1,2-dichloroethane: nd
1,1-dichloroethene: nd
trans-1,2-dichloroethene: nd
1,1,1-trichloroethane: nd
1,1,2-trichloroethane: nd
trichloroethene: nd

PCB's -
arachlor 1016: nd ug/L
arachlor 1221: nd
arachlor 1232: nd
arachlor 1242: nd
arachlor 1248: nd
arachlor 1254: nd
arachlor 1260: nd

purgeable aromatics -
benzene: nd ug/L
toluene: nd

Anions -
hardness: 40 mg/L
total alkalinity: 20
bicarbonate: nd
chloride: 3.7
fluoride: nd nitrate: 2.8
sulfate: nd
turbidity: 0.60 ntu
Trace elements -
aluminum: nd mg/L
arsenic: nd ug/L
barium: nd mg/L
berylium: nd
cadmium: nd
calcium: nd
chromium: nd
cobalt: nd
iron: 0.03
lead: nd
magnesium: 0.039
manganese: nd
mercury: nd ug/L
nickel: nd mg/L
zinc: 0.017 mg/L

additional notes: No odor

phenols -
phenol: nd ug/L
pentachlorophenol: nd

or turbidity detectable
Taste is good
Microtox response -
20 C: -18 per cent (pH 5.7)
20 C: -4 per cent (pH 6.7)
20 C: +5 per cent (pH 7.0)

Sample number: 5 (Lehr Residence)
Location: Grassy Meadows, WVa.
Description of area: very rural
Date of Sampling: 2-10-85

pH: 5.6
TOX: <10 ug/L
TOC: 1.13 mg/L

purgeable halocarbons -
chloroform: nd ug/L
1,1-dichloroethane: nd
1,2-dichloroethane: nd
1,1-dichloroethene: nd
trans-1,2-dichloroethene: nd
1,1,1-trichloroethane: nd
1,1,2-trichloroethane: nd
trichloroethene: nd

PCB's -
arachlor 1016: nd ug/L
arachlor 1221: nd
arachlor 1232: nd
arachlor 1242: nd
arachlor 1248: nd
arachlor 1254: nd
arachlor 1260: nd

purgeable aromatics -
benzene: nd ug/L
toluene: nd

phenols -
phenol: nd ug/L
pentachlorophenol: nd

water source: springhouse
notes: nearest house is one mile away. Only possible source of contamination is corn field treated with pesticides
Anions -
hardness: 21 mg/L
carbonate: nd
bicarbonate: nd
chloride: 0.50
fluoride: <0.20 nitrate: 0.90
sulfate: 1.70 bromine: <0.50
turbidity: 2.0 ntu phosphate: <0.5
Trace elements -
aluminum: nd mg/L
arsenic: nd ug/L
barium: nd mg/L
berylium: nd
cadmium: <0.005
calcium: 7.13
chromium: <0.05
cobalt: <0.05
iron: 0.20
lead: <0.05
magnesium: 0.825
manganese: 0.032
mercury: nd ug/L
nickel: <0.04 mg/L
zinc: 0.02

additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
20 C: +3 per cent (pH 6.4)
20 C: +4 per cent (pH 7.0)

Sample number: 6 (Newcomb House)
Location: Roanoke, Va.
Description of area: suburban
Date of Sampling: 7-19-85

water source: well
notes: Contamination with benzene reported two weeks prior to sampling. Odor was strong.

pH: 6.7
TOX: <10 ug/L
TOC: 0.523 mg/L

purgeable halocarbons -
chloroform: nd ug/L
1,1-dichloroethane: nd
1,2-dichloroethane: nd
1,1-dichloroethene: nd
trans-1,2-dichloroethene: nd
1,1,1-trichloroethane: nd
1,1,2-trichloroethane: nd
trichloroethene: nd

PCB's -
arachlor 1016: nd ug/L
arachlor 1221: nd
arachlor 1232: nd
arachlor 1242: nd
arachlor 1248: nd
arachlor 1254: nd
arachlor 1260: nd

purgeable aromatics -
benzene: nd ug/L
toluene: nd

phenols -
phenol: nd ug/L
pentachlorophenol: nd

Odor now gone.
Anions -
hardness: 200 mg/L
carbonate: nd
bicarbonate: nd
chloride: 7.6
fluoride: 0.40 turbidity: 0.7ntu
sulfate: 3.4 phosphate: <0.5
nitrate: 6.2 bromine: <0.5
Trace elements -
aluminum: nd mg/L
arsenic: nd ug/L
barium: nd mg/L
berylium: nd
cadmium: <0.005
calcium: 41.1
chromium: <0.05
cobalt: <0.05
iron: 0.02
lead: <0.05
magnesium: 22.3
manganese: 0.013
mercury: nd ug/L
nickel: <0.04 mg/L
zinc: 0.036
copper: <0.02
additional notes: No odor or turbidity detectable
Sample was filtered with G.A.C.
Microtox response -
20 C: -11 per cent (pH 7.4)
20 C: +6 per cent (pH 6.2)

Sample number: 7(Vulcan Materials)water source: well
Location: Chatham, Va.
Description of area: rural
Date of Sampling: 7-19-85
notes: High metals concentrations reported by State Water Control Board. Quarry area

pH: 6.7
TOX: <10 ug/L
TOC: 0.721 mg/L

purgeable halocarbons -
chloroform: nd ug/L
1,1-dichloroethane: nd
1,2-dichloroethane: nd
1,1-dichloroethene: nd
trans-1,2-dichloroethene: nd
1,1,1-trichloroethane: nd

Anions -
hardness: 125 mg/L
carbonate: nd
bicarbonate: nd
chloride: 2.6 turbidity: 15 ntu
fluoride: 0.20 phosphate: <0.5
sulfate: 5.9 bromine: <0.5
nitrate: <0.5
Trace elements -
aluminum: nd mg/L
arsenic: nd ug/L

1,1,2-trichloroethane: nd
trichloroethene: nd

PCB's -
arachlor 1016: nd ug/L
arachlor 1221: nd
arachlor 1232: nd
arachlor 1242: nd
arachlor 1248: nd
arachlor 1254: nd
arachlor 1260: nd

purgeable aromatics -
benzene: nd ug/L
toluene: nd

phenols -
phenol: nd ug/L
pentachlorophenol: nd

barium: nd mg/L
berylium: nd
cadmium: <0.005
calcium: 35.4
chromium: <0.05
cobalt: <0.05
iron: 5.85
lead: <0.1
magnesium: 8.8
manganese: 0.415
mercury: nd ug/L
nickel: <0.04 mg/L
zinc: 1.146

additional notes: No odor or turbidity detectable
Taste is good
Microtox response -
20 C: -64 per cent (pH 6.2)
20 C: -42 per cent (pH 6.7)

Sample number: 8 (Dixie Caverns)
Location: Dixie Caverns, Va.
Description of area: industrial
Date of Sampling: 8-11-85

pH: 6.1
TOX: 376 ug/L
TOC: >20 mg/L

purgeable halocarbons -
chloroform: nd ug/L
1,1-dichloroethane: nd
1,2-dichloroethane: nd
1,1-dichloroethene: nd
trans-1,2-dichloroethene: nd
1,1,1-trichloroethane: nd
1,1,2-trichloroethane: nd
trichloroethene: nd

PCB's -
arachlor 1016: nd ug/L
arachlor 1221: nd
arachlor 1232: nd
arachlor 1242: nd
arachlor 1248: nd
arachlor 1254: nd
arachlor 1260: nd

water source: leachate
notes: Landfill leachate. Nearby Industries include: Atlas Chemical Mid Atlantic Explosives, South Star, and Meddon Quarry
Anions -
hardness: 336 mg/L
carbonate: nd
bicarbonate: nd
chloride: 416 phosphate: <0.50
fluoride: 0.70 bromine: 3.2
sulfate: 0.60
nitrate: <0.5 (as N)
Trace elements -
aluminum: nd mg/L
arsenic: nd ug/L
barium: nd mg/L
berylium: nd
cadmium: 0.013
calcium: 52.2
chromium: <0.03
cobalt: <0.05
iron: 32.7
lead: 0.05
magnesium: 50.2
manganese: 0.598
mercury: nd ug/L
nickel: 0.18 mg/L

purgeable aromatics -
benzene: nd ug/L
toluene: nd

phenols -
phenol: nd ug/L
pentachlorophenol: nd

zinc: 0.202
copper: 0.05
additional notes: Foul odor
Reddish color
Taste is good
Microtox response -
20 C: -37 per cent
20 C:

2 APPENDIX D - Details of USDA Contract

a. Purgeable Halocarbons

The primary compounds are:

chloroform

1,1-dichloroethane

1,2-dichloroethane

1,1-dichloroethene

trans-1,2-dichloroethene

1,1,1-trichloroethane

1,1,2-trichloroethane

trichloroethene

The analytical method to be used shall be Method 601, published as a proposed rule in the Federal Register (Vol. 49, No. 209, 10/26/84). (See Attachment I)

NOTE: All samples shall be analyzed within seven (7) days of receipt at the Contractor's Laboratory.

b. Purgeable Aromatics

The primary compounds of interest are:

benzene

toluene

The method to be used for the analysis of the aromatics shall be Method 602, published as a proposed rule in the Federal Register (Vol. 49, No. 209, 10/26/84). (See Attachment I)

NOTE: All samples shall be analyzed within seven (7) days of collection.

c. Phenols

The compounds of interest are:

pentachlorophenol

phenol

The method to be used for the analysis of the phenols shall be Method 604, published as a proposed rule in the Federal Register (Vol. 49, No. 209, 10/26/84). (See Attachment I)

NOTE: All samples shall be extracted within seven (7) days and analyzed within thirty (30) days of collection.

d. Organochlorine Pesticides and PCB's

The primary compounds of interest are:

Arachlor 1016

Arachlor 1221

Arachlor 1232

Arachlor 1242

Arachlor 1248

Arachlor 1254

Arachlor 1260

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The recommended methods to be used shall be either the Interim Inductively Coupled Plasma (ICP) Optical Emission Spectrometric Method (proposed in the Federal Register, Vol. 49, No. 209, 10/26/84) or the Atomic Absorption procedures proposed by the EPA (EPA 600/4-79-020; March, 1983).

The contractor shall specify which method he will use on Schedule, page 3.

f. Anions

The anions of interest are:

carbonate/bicarbonate

chloride

fluoride

nitrite/nitrate

sulfate

Carbonate/bicarbonate levels shall be measured in accordance with the procedures specified in Section 403 of Standard Methods for the Examination of Water and Wastewater. The recommended analytical procedure for the remaining anions is EPA's Test Method 300 (EPA 600/4-84-017; March, 1984).

The samples shall be preserved and tested in accordance with the procedures specified by the EPA (EPA 600/4-79-020; March, 1983).

g. Total Organic Halides (TOX) and Total Organic Carbon (TOC)

The procedures recommended for TOX and TOC analysis are referred to as Methods 450.1 (Interim; November, 1980 and 415.2 (Test; December, 1982), respectively, by the EPA.

The method to be used for the analysis shall be Method 608, published as a proposed rule in the Federal Register (Vol. 49, No. 209, 10/26/84). (See Attachment I)

Note: All samples shall be extracted within seven (7) days and analyzed within thirty (30) days of collection.

e. Trace Elements

The elements of interest are:

aluminum

arsenic

barium

berylium

cadmium

calcium

chromium

cobalt

copper

iron

lead

magnesium

manganese

mercury

nickel

zinc