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Acute Toxicity of Chlorine-Stabilized Domestic Wastewater Sludges To Fathead Minnows

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Contract Number MDWPC 76-10(3)

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CHAPTER I

INTRODUCTION

Sludge Treatment and Disposal

The treatment and disposal of sludges produced in wastewater treatment processes represent an important segment of treatment plant design and operation. Sludge processing and disposal may perhaps be the most complex problem with which the engineer is faced in the field of wastewater treatment (1). Sludge is produced in primary settling, secondary biological processes and in various other chemical and biological operations used in advanced treatment. The difficulties in dealing with sludge are predominantly due to its large organic content, both in primary and secondary sludge, which readily becomes offensive upon decomposition, and its large water content making volume reduction a difficult task.

The Purifax Process

The BIF Corporation has developed a wet chlorine oxidation process to treat domestic wastewater sludges, some industrial and chemical sludges, and domestic septic tank pumpings. The Purifax process continously oxidizes sludge with high doses of chlorine (about 2000 mg/l) in a closed cylindrical reactor in short periods of time. The resulting product is claimed to be disinfected and have good long term stability, meaning it will not putrefy. There is no significant reduction in sludge solids, though the solids are bleached from a dark gray or brown to a light buff color. The original offensive odor of the sludge is removed and an odor of chlorine is detectable from the treated sludge.

The treated sludge has a lower specific resistance in dewatering operations and can be readily dried on sand drying beds. If it is to be dewatered by vacuum filtration, its low pH (a pH of 2 or less is typical) must be chemically adjusted to at least pH 4.

Potential Chlorination Problems

Due to its very nature, the Purifax treatment results in high concentrations of residual chlorine. Total chlorine residuals of 200 to 350 mg/l are not uncommon in treated sludges immediately after treatment. These high concentrations of free available chlorine and combined chlorine residual pose an immediate threat to aquatic life downstream of wastewater treatment plant discharges. The supernatant of the treated sludge is generally recycled to the headworks of the plant, where it is diluted by passage through the bulk of the treatment system.

This dilution may not always be sufficient to negate the deleterious effects of chlorine residuals produced in the Purifax process. BIF Corporation reports (2) have shown that the ratio of Purifaxed supernatant returned to the headworks of the plant to the total plant influent can range from an average of 0.5 to 1.5 percent up to 17 percent when the Purifax unit is operating only at certain hours of the day. With the high chlorine residuals produced in the Purifax process, this dilution could still result in a significantly raised plant effluent residual chlorine concentration.

A more insidious potential problem with the use of the Purifax system is the production of chlorinated organic compounds, some of which

may be harmful to biological organisms. This problem has drawn recent attention in the fields of both wastewater and water treatment where chlorine is used as a disinfectant. Some of these chloroorganics are toxic, some carcinogenic, and many others have effects as yet unknown. A process such as the Purifax system which uses very high doses of chlorine on a high organic content sludge may very well result in the production of a variety of chlorinated hydrocarbons, some of which are harmful. A recently developed technique will be employed in this research to measure the amount of chlorinated organics present in Purifax-treated sludge.

Use of the Bioassay

Bioassays are being utilized to make evaluations of the toxic effects on aquatic life from water pollution. Chemical measurement of the characteristics of domestic and industrial wastes yields much useful information, but cannot be used to determine the toxicity of these wastes to aquatic life. Often the pollutant is too complex to be effectively characterized with available chemical tests. A number of factors other than concentration may affect the toxicity of a given waste. Synergistic trends in toxicity are sometimes found due to the combination of particular components of the waste, the temperature, and the chemical nature of the receiving water.

Bioassays are now used for many purposes in detection, monitoring and determining extent of toxicity of effluents. Many test organisms are used, from algae to invertebrates to fish. Using specific guidelines established in Standard Methods (3) and other sources (4-10), a

greater degree of uniformity of techniques and better reproducibility of results are possible than ever before in bioassay studies.

A variety of responses over a wide range of time intervals may be tested. The most commonly used response is death of the test organism, but other behavioral patterns such as rapidity of movement, respiratory rate, avoidance reactions and breeding efficiency may be measured. The majority of the bioassays in the past have been short term tests to determine acute toxicity levels of the selected pollutant, but more long term tests are now being conducted to evaluate chronic toxicities. Life cycle bioassays may yield useful information showing at which stage of an organism's life it is most vulnerable to a particular pollutant.

This research will involve the use of short term static bioassays employing fathead minnows (Pimephales promelas) as test organisms, and will examine the toxicities of both primary and activated sludges from a domestic wastewater treatment plant. Acute toxicities will be determined for these sludges at three different stages: prior to any sludge treatment; after Purifax treatment; and after removal of chlorine residual present in the Purifaxed sludge. These toxicities will be expressed as LC50 values (lethal concentration to 50 percent of the fish over a specified time interval). Implications of these results to the use of the Purifax system will be discussed.

CHAPTER II

LITERATURE REVIEW

Residual Chlorine

The use of a chlorine oxidation process such as the Purifax system produces a high chloramine concentration, making the processed sludge difficult to dispose of in the environment (11). BIF reports the following total chlorine residual concentrations in Purifax-treated sludge: 160, 200 and 400 mg/l for chlorine dosages of 1100, 1500 and 2000 mg/l respectively (2).

Feng and Shieh (12) found total residual chlorine (TRC) concentrations of 135-149 mg/l in the centrate of primary sludge treated with 2000 mg/l of chlorine in a lab scale Purifax unit, while the same chlorine dose yielded residual chlorine concentrations of 96-248 mg/l in domestic septage. (Variation due to different mixing times in the reactor, with shorter mixing times giving higher TRC concentrations.) Instructions for the lab scale Purifax unit (13) state that the desired effluent chlorine residual concentration is 200 mg/l from the Purifax reactor, clearly pointing out that there is production of a high concentration of residual chlorine by design.

The toxicity of residual chlorine in wastewater effluents is well documented. Brungs (14) describes results of bioassays with fathead minnows showing 96-hr TL50 values to be 0.05 to 0.16 mg/l total residual chlorine. Seven day TL50's were 0.082 to 0.115 mg/l. The TL notation stands for mean tolerance level of the fish, i.e. the amount of toxicant in which a certain percentage of the fish can survive. The TL50

therefore stands for the toxicant concentration at which 50 percent of the fish can survive. It should be noted that the LC notation which is now used more frequently means just the opposite, i.e. the amount of toxicant at which a certain percentage of the fish will die. However, when both these notations examine the effects on 50 percent of the fish (i.e. TL50 and LC50) the results are the same, because at the 50 percent mark half the fish are killed and half the fish survive. Thus in the literature, results reported as TL50's and LC50's may be directly compared. Both notations should be preceeded by a time interval indicating the length of the bioassay run.

Arthur et al. (15) found that more than 50 percent of their test fish died within seven days in tanks where chlorinated secondary effluent was mixed with dilution water in ratios of 12-35 percent by volume. They also determined 96-hr TL50's of 0.086 and 0.130 mg/l total residual chlorine for fathead minnows. Tests by Ward (16) yielded similar results. Using fathead minnows, Zillich (17) determined 96-hr LC50 values of 80-190 µg/l total residual chlorine, by mixing chlorinated wastewater effluent in ratios of 3-7 percent by volume with dilution water.

In other observations, the Michigan Department of Natural Resources (18) found that caged fathead minnows died within 0.6 miles of a wastewater treatment plant outfall, where chlorine residual was measured to be 0.1 mg/l. Penzes (19) reports that water with chlorine content of 0.15-0.4 mg/l kills native European fish and Doudoroff (20) found in 1950 that concentrations of 0.05 to 0.2 ppm of available chlorine may be critical for more sensitive fishes under average water conditions.

Doudcroff (21) reported that both free and combined chlorine residuals of 0.05-0.3 ppm may be toxic to freshwater fish, concluding that both types of residual chlorine have similar toxicities. Merkens (22) also found that free and combined chlorine residuals have similar toxicities to fish. A master's thesis by Katz, described in a recent literature review by Brungs (23), suggests that ammonia given off by fish gills rapidly converts free chlorine to chloramines, thus exposing fish mainly just to the combined chlorine form.

Brungs (23) has found that the acute toxicity of total residual chlorine is about the same for chlorinated clean water and chlorinated domestic wastewaters, making some comparisons between results obtained using these two types of water possible. Larson et al. (24) point out, however, that acute toxicities may vary considerably among the species, size, and stage of growth of fishes.

More research has been conducted recently in the area of long term bioassays to secure information on the chronic toxicity of chlorine residuals, and its effects on growth, behavior patterns, and reproductive efficiency. Arthur et al. (15) studied toxic effects of several different kinds of disinfection processes and foundthat fish survival was lowest in chlorinated effluents. The authors found that no spawning of fathead minnows occurred in water containing only 10 percent chlorinated secondary effluent, which had an average total residual chlorine concentration of 110 µg/l. As summarized by Brungs (23), Zillich found the lowest mean total residual chlorine concentration with a measurable adverse chronic effect to be 0.042 mg/l for fathead minnows, and also that the largest total residual chlorine concentration with no measurable adverse chronic

effect was 0.014 mg/l.

Ward et al. (16) found that a mean total residual chlorine level of 0.045 mg/l was necessary to exert a lethal effect during the first 60 days of a fathead minnow's life. Older fathead minnows survived mean total residual chlorine concentrations as high as 0.074 mg/l for 180 days. The maximum mean total residual chlorine concentration with no lethal or growth retardation effects was 0.01-0.03 mg/l.

Using life cycle bioassays, Ward and DeGraeve (25) found that fathead minnow growth was retarded at total residual chlorine concentrations as low as 0.033 mg/l. Reduced survival was found in bioassay tanks containing 14 to 20 percent chlorinated effluent, or a chlorine residual concentration of 0.045 mg/l.

Zillich (17) reported that chlorinated effluent diluted to as little as 2-4 percent (i.e. 2-4 parts effluent to 98-96 parts dilution water) was toxic in on-site tests in Michigan treatment plants. Complete kills of fathead minnows were observed at 0.16-0.21 mg/l total residual chlorine, with partial kills resulting from only 0.07 mg/l chlorine residual. He established 0.04-0.05 mg/l of total residual chlorine as a threshold concentration for fathead minnow survival.

Using chlorinated Lake Superior water, Arthur and Eaton (26) found that levels of total residual chlorine up to 43 µg/l caused no effect in growth and survival, but that levels above 16 µg/l caused a decrease in the amount of young produced.

Discharge of chlorinated effluents may cause a drop in fish abundance in streams as well as a reduction in species diversity below outfalls (27) and may lead to modification of fish behavior (23,28). Exposure

to residual chlorine often results in initial increased swimming activity, accompanied by a rise in respiratory activity with gasping and rushes at the surface, followed by periods of sluggish activity (29,30,31).

Most researchers agree that the fish's gills are the key location of the toxic effect of residual chlorine (30). The chlorine apparently destroys the epithelium of the gill lamellae, causing it to slough off and reduce respiratory capability (14,19,21,32). Cairns (32) also claims that large amounts of mucus are produced, which will clog gill lamellae, leading to asphyxia.

Groethe and Eaton (33) contend that fish mortality due to residual chlorine is caused by the oxidation of hemoglobin in red blood cells to methemoglobin (via oxidation of the central iron molecule from Fe^{2+} to Fe^{3+}) as well as a decrease in the rate methemoglobin is broken down. This occurs as red blood cells pass by the gills and leads to anoxia since not enough oxygen can be carried through the fish's bloodstream.

Other possible effects of residual chlorine are a decrease in excretory function (21) and an observed hemorrhaging at the base of the fins (17).

Several authors have suggested criteria for aquatic life to exist in continuously chlorinated effluents. Brungs (14) advises that concentrations not greater than 0.002 mg/l total residual chlorine would protect most aquatic species. He states that concentrations not to exceed 0.01 mg/l may be safe for some species, butthey would not protect trout and salmon and other sensitive fish species. Basch and Truchan (28) propose criteria of 0.005 mg/l total residual chlorine for cold water fish exposed to continuous chlorination and 0.02 mg/l for warm water fish.

One method used to meet these criteria and reduce the toxicity of wastewater effluents is the removal of residual chlorine by one of several methods of dechlorination. Arthur et al. (15), Esvelt et al. (34), Ward et al. (16) and Ward and DeGraeve (25) all report excellent results in removing toxicity of chlorinated effluents by dechlorination. Snoeyink and Markus (35) found chlorine residuals in Illinois wastewater treatment plant effluents in the range of 1-5 mg/l, most of which were being discharged to streams with little dilution; thus pointing out the necessity of dechlorination if stream criteria were to be put into use.

Chlorinated Organics

The production of chlorinated organic compounds resulting from chlorine disinfection of water and wastewater is drawing increased concern. Writing about water treatment, Dowty and Laseter (36) states: "It appears the chlorination step contributes to finished water a large number of organic halogenated hydrocarbons which are not removed before the water leaves the plant." Production of these chloroorganics is not surprising when one studies the chlorination process. In fact, certain chemical pathways of chlorine addition are predictable under conditions normally found in chlorination practices. Morris and McKay (37) describe the two most common types of chlorine reactions with organics in water. It is a serious problem due to the wide use of chlorination, and the authors recommend the removal of precursors or post-chlorination treatment with granular activated carbon as measures to prevent release of these halogenated organics.

Carlson et al. (38) caution that the relatively facile chlorination of the biphenyl nucleus, phenols and aromatic ethers (which resemble many naturally occurring compounds) strongly suggests that there may be a plethora of chlorinated organics produced by aqueous chlorination practices. Ingols et al. (39) concur that substitution of chlorine on phenol and its derivatives is very easy during chlorination.

Jolley (40) found chlorine-containing stable organic constitiuents were present in effluents of domestic treatment plants after chlorination. He separated over 50 compounds and identified 17 of these at the 0.5-4.3 µg/l level. The author used ^{36}Cl tagging in further attempts to identify chloroorganics in prmary and secondary effluents (41). He concluded that "it is reasonable to assume that most of the chlorine-containing constituents in the chlorinated primary effluent were the product of chlorination." Jolley found that chlorine yield (the portion of the chlorine dosage added to the effluent that becomes associated with stable chlorine-containing organic compounds by the end of the chlorination reaction period) was about one percent for a typical chlorinated secondary effluent, and similar for primary effluent.

The problem of discharging potentially harmful chlorinated organic compounds to the environment is worsened by the fact that chlorination of many of these organics makes them more stable, both chemically and biologically (40,42). Chambers et al. (43) found that the chloro-derivatives of benzene compounds were more resistant to oxidation by bacteria than non-chlorinated compounds. The discharge of these stable organic compounds in sewage effluents can seriously affect water quality (44). Jolley (40) estimates 100,000 tons of chlorine are used annually

for chlorination and that 1000 tons of chlorine in the form of stable chlorine-containing organic compounds are discharged to the nation's waterways annually.

A further complication to the problem of chloroorganics is that they tend to accumulate in living organisms (40,45). Kopperman et al. (46) relate this bioaccumulation tendency to partition coefficients, a measure of biological affinity for certain chemicals having to do largely with the compound's lipophilic nature. In a study of chlorinated organics found in New Orleans drinking water and in blood plasma of people who drank this water, Dowty et al. (47) concluded that "due to the lipophilic nature of halogenated hydrocarbons it is not surprising that they might be found accumulating in blood and other body tissues."

Formation of chlorinated organic compounds during treatment of sludge and septage by the Purifax process may pose a greater problem than that with water and wastewater disinfection due to the higher doses of chlorine used. It should be noted that substantial amounts of higher chlorinated isomers are observed at increased chlorine concentrations, which is a serious potential problem for "superchlorination" applications such as sludge solubilization (38). Carlson et al. (38) also report that chlorination of organic compounds is more extensive at lower pH's, as is definitely the case in Purifax sludge processing. Glaze and Henderson (48) examined "superchlorinated" effluent and found that the process "seems to yield significant quantities of new chlorinated organics."

In a study done for the BIF Corporation, Metcalf and Eddy (49) measured the levels of certain chloroorganics in sludges before and

after treatment with the Purifax process. They found the concentrations of these chlorinated organics stayed the same at one treatment plant, and were actually decreased at four other plants using the Purifax system. It is significant that the only chlorinated organics monitored were certain pesticides and compounds within the same general complex chemical class. The report did not investigate simpler chlorinated hydrocarbons or determine what compounds the pesticides were broken down to.

A recent study by Wise et al. (50) has focused specifically on the Purifax system and attempts to characterize the chlorinated organics formed during the process. They examined treated samples of septage and mixed primary and secondary sludges. The amounts of organic chlorine found in the Purifaxed solids were relatively large - about 1 percent of the total solids by weight, confirming earlier speculation as to the dangers of producing these compounds with the Purifax process. Chlorinated organics in the liquid portion were of the same kinds found in contaminated U.S. surface waters, though in slightly higher concentrations. These chloroorganics found in the liquid portion of the treated sludge generally accounted for slightly less than 1 percent of the total organic carbon that was either volatile or extractable with helium. The chlorinated organics in the non-volatile and non-extractable portion of the TOC could not be measured.

The toxicities of chlorinated organics produced by chlorination processes are largely unknown, except for a relatively small number of specific compounds that have been used in toxicological studies. Public fear has mounted recently due to the possible carcenogenic nature of certain chlorinated hydrocarbons, as well as reports linking halogenated

hydrocarbon insecticides to fish and bird deaths.

Katz and Cohen (51) have found that small concentrations of some normally innocuous organic substances become very toxic when exposed to chlorine concentrations of 5-10 ppm. Mattson (52) determined the toxicities of compounds formed from 5 chemical classes and found that chlorinated phenols had far greater toxicities than other substances tested. Pentachlorophenol had a 96-hr LC50 of 0.6 mg/l. Ingols et al. (39) examined stable chlorine-containing organic compounds and found that two of them, 4-chlororesorcinol and 5-chlorouracil, had toxic effects on the hatchability of carp eggs at concentrations as low as 0.001 mg/l. Research by Barnhart and Campbell (53) on chlorination of organics showed that 5 of 14 selected organic compounds from different chemical classes combined with chlorine upon chlorination. These 5 compounds were examined in bioassays and the chloroorganics were found to have 96-hr TL_m limits ranging from 0.01 to 10.0 mg/l.

The production of chlorinated organic compounds is a serious problem. Chloroorganics produced by chlorination processes are highly diverse, complex mixtures that are difficult to separate and identify, and, unlike residual chlorine, cannot be removed by dechlorination. Due to their refractory nature and their capacity to bioaccumulate, even small amounts of chlorinated hydrocarbons discharged to the environment may bring harmful effects to a wide variety of aquatic life, as well as organisms higher on the food chain.

CHAPTER III

OBJECTIVES

The objective of this project is to successfully produce Purifax-treated wastewater sludges and test their toxicities to fish. Both primary sludge and activated sludge from the Amherst, Massachusetts wastewater treatment plant will be examined.

Certain chemical parameters will be measured of the sludge prior to and following the Purifax treatment. Changes in pH, temperature, residual chlorine and total organic chlorine (TOCl) will be measured, as these may be important factors relating to toxicities of the sludges. A new pyrohydrolysis unit for TOCl detection will be built and used to help quantify the effect of the Purifax process on the concentration of chlorinated hydrocarbons in the sludge.

The main focus of this research will be to determine the acute toxicity of Purifaxed sludge to fish. Several short term static bioassays will be conducted using adult Fathead Minnows (Pimephales promelas) as the test organism. Results of these tests will be used to determine 96-hr LC50 values (the concentration that is toxic to 50 percent of the fish over a 96 hour period) for the sludges.

The toxicities of both the primary and activated sludges will be determined at three sequential stages of treatment. In all cases only the liquid portion of the sludge will be tested for its toxicity. The first bioassay will be on raw sludge obtained from the Amherst wastewater treatment plant. Sludge samples will then be treated in a lab-scale Purifax unit and left to settle overnight. This settling time

allows a clear subnatant to form as most of the solids float to the surface buoyed by small gas bubbles. During this period the total residual chlorine concentration of the sludge is reduced from the very high initial readings to a relatively stable level. This clear subnatant with stabilized chlorine residual is the second type of sludge tested in a bioassay, and is referred to as Purifaxed primary sludge or Purifaxed activated sludge as the case may be. The final type of sludge to be tested is dechlorinated Purifaxed sludge. Addition of an excess of sodium thiosulfate will be used to eliminate all of the residual chlorine in the sludge prior to the running of the third set of bioassays. As noted earlier, however, dechlorination of the sludge will not reduce the amount of chlorinated organic compounds present in the sludge.

The results of these toxicity tests will be examined and conclusions drawn on the effect of the Purifax system on the toxicity of wastewater sludges to fish. The dilution factors involved in recycling the liquid portion of the sludge to the headworks of the wastewater treatment plant will be taken into account in making recommendations on how the toxicities determined for Purifaxed sludges will effect fish in the bodies of water receiving effluents from treatment plants using the Purifax process.

It is desirable to make recommendations on what levels of a particular toxicant can be discharged to a receiving body of water with no long term deleterious effects on aquatic life. This "safe concentration" can be roughly predicted from the results of short term or acute bioassays by use of an application factor. The 96-hr LC50 for a particular toxicant is multiplied by this application factor to give the safe concentration of the toxicant in the receiving water. The use of application factors

in the case of Purifaxed sludges will be examined further later in this report.

CHAPTER IV

METHODOLOGY

Source of Sludge

The sludge used in this research will be obtained at the Amherst Wastewater Treatment Plant, a new activated sludge secondary treatment facility treating domestic wastewater. Primary sludge will be collected from the sludge effluent line leaving the primary clarifiers and activated sludge will be obtained from the sludge recirculation line. Grab samples will be taken from collection ports on these pipelines.

Lab Scale Purifax Unit

Sludge will be processed in a lab scale Purifax unit, which is a batch-mode scale model of the complete-mixing system designed by BIF. This Purifax unit consists of the following basic components (13):

Cylindrical Reaction Chamber

Graduated Sludge Reservoir

Chlorine Supply and Regulator

Chlorine Inlet Valve

Vacuum Pump

Vacuum Valve

Reactor Pressure Gauge

Reactor Inlet and Outlet Valves

Recirculation Pump

Selector Valve

Drain Valve and Drain Line

See Figure 1 for an illustration of the lab scale unit.

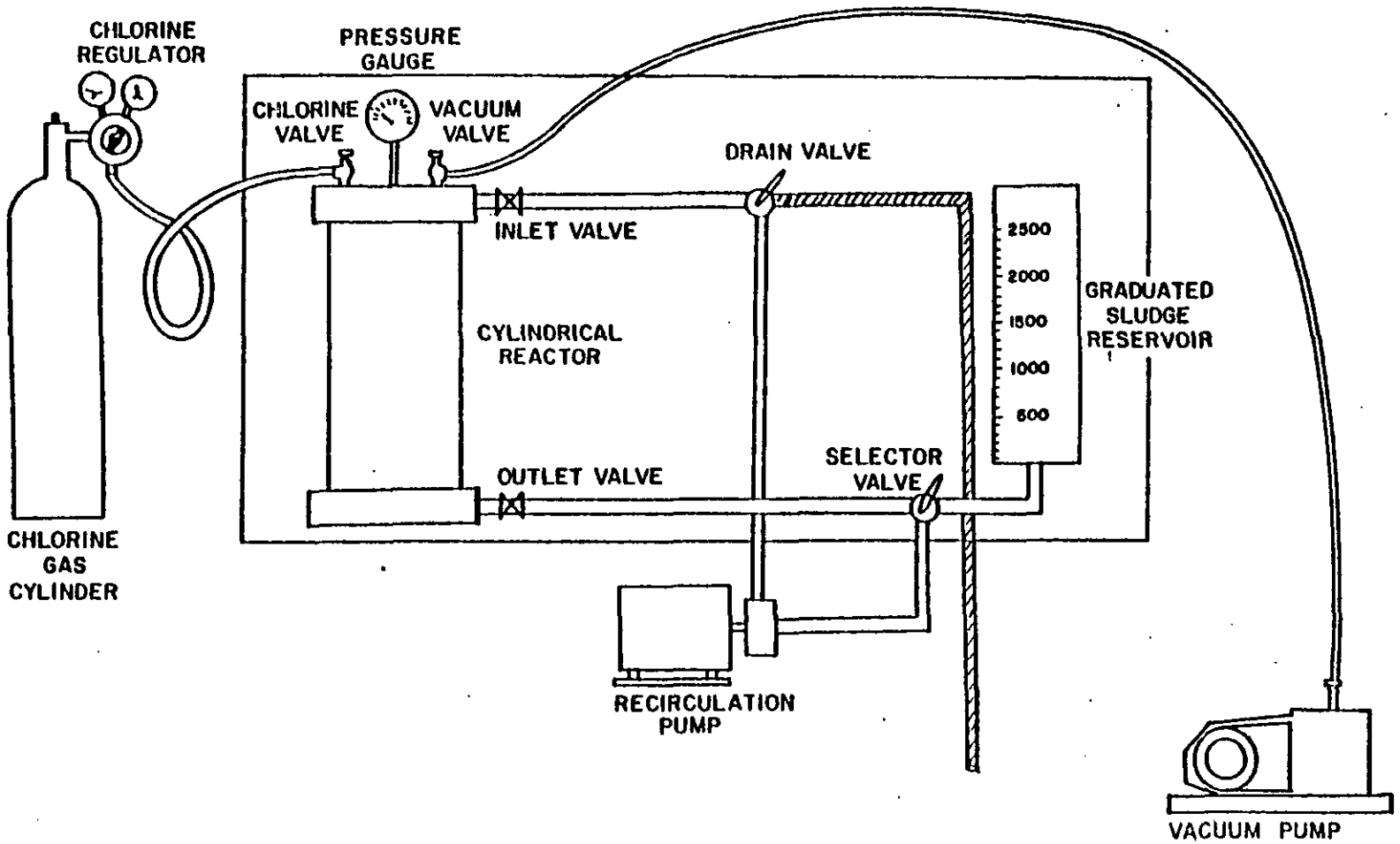

Figure I. Lab Scale Purifax Unit

Vacuum is produced by a belt driven vacuum pump powered by a 1/3 HP electric motor. This pump is connected to the vacuum valve on the reactor by a thick high pressure plastic hose. Chlorine is supplied from an F sized cylinder which is fitted with a two-stage, corrosion resistant regulator and connected to a reactor valve by a 1/2 inch flexible stainless steel hose.

The reactor is a cylindrical non-corrodible plastic chamber capped with plastic end blocks. The top block contains, in addition to the vacuum and chlorine valves, a pressure gauge and an inlet port. The inlet to the reactor is situated so that the influent to the reactor enters tangentially to insure good mixing. The exit port is located in the bottom end block. Both the entrance and exit lines are fitted with valves adjacent to the reactor.

The sludge reservoir has a capacity of 2500 ml, and is marked in 100 ml graduations. It is connected to a selector, which is a three way valve determining whether sludge from the reservoir or sludge from the reactor will be piped to the recirculation pump.

The recirculation pump is a 1/4 HP positive displacement pump with a Viton impeller. The discharge of the pump is connected to a drain valve which determines whether the recirculating sludge will be directed back to the top of the reactor or out a drain line.

The lab scale Purifax unit is operated according to the BIF Instruction Manual (13), with some minor modifications. A brief description of the operating procedure follows.

Place 2500 ml of pretreated sludge into reservoir. After testing unit for leaks, a vacuum of 22 inches of mercury is created in the reactor with the vacuum pump. Chlorine is then introduced into the reactor until the reactor pressure reads 5 psi (for maximum dose). Turn on recirculation pump and turn selector valve handle toward reservoir to introduce sludge into reactor. Turn selector valve to recirculate position when all the sludge is drained from reservoir and start timing mixing stage of process. At end of desired mixing time, turn off pump, switch drain valve to drain position and turn pump back on, collecting treated sludge at end of drain line. Appropriate measures must then be taken to vent chlorine in inlet line and wash out reactor.

Smaller chlorine dosages may be obtained by adding predetermined portions of the total 2500 ml sludge volume to the reactor prior to drawing a vacuum and adding chlorine. Chlorine dosages may be determined from a calibration curve supplied by BIF (13) (see Figure 2).

Sludge Pretreatment

The sludge samples will be treated prior to processing in the Purifax unit. This pretreatment consists of two steps (12). First the sludge must be ground to break up large particles of matter. This is necessary to allow the solids to pass through the pipes, valves, and orifices of this miniaturized unit. A blender is used for this size reduction, with an average mixing time of 3 minutes. Secondly, the primary sludge must be elutriated to reduce the solids content of the sludge to approximately 1-2 percent. This is accomplished by diluting the sludge (2-6 percent solids) with deionized water, at a ratio of 1 part sludge to one part dilution water.

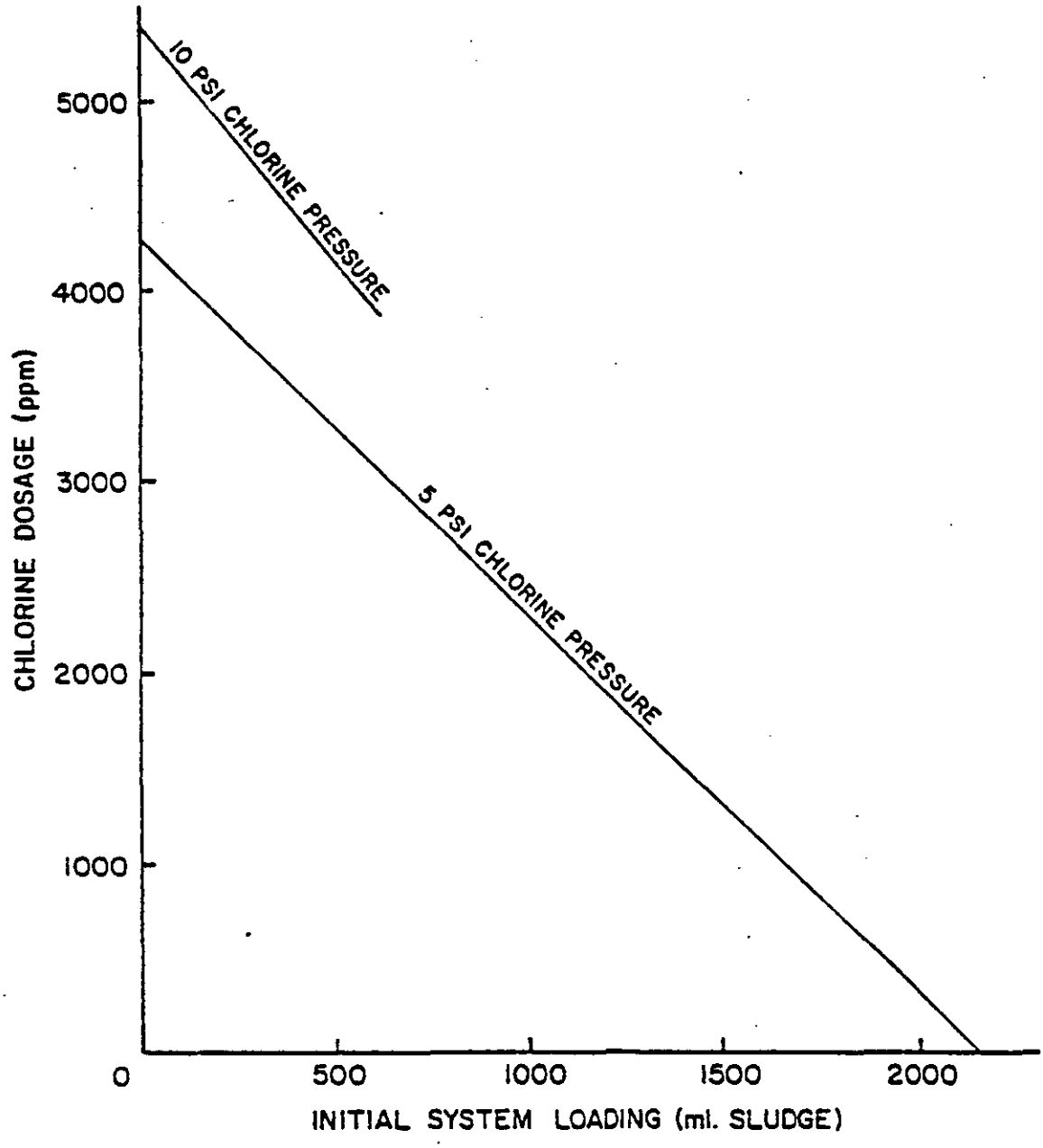

Figure 2. Chlorine Dosage in Lab Scale Purifax Unit

Chemical Testing of Sludge Samples

Purifaxed sludge samples will be allowed to settle so that a relatively clear subnatant is formed under the solid portion of the sludge. In most cases, the great majority of the solids will entrap bubbles of escaping gas and rise to the surface, leaving a clear liquid below. This liquid (referred to as subnatant) will be carefully collected. Raw sludge samples will be centrifuged in a large IEC centrifuge for approximately five minutes at 5000 rpm to remove suspended solids.

The liquid portion of the sludge will then be tested for pH, chlorine residual (free and combined), and total organic chlorine (TOCl). Chlorine residual concentration will be measured by the DPD-Ferrous Titrimetric Method, as described in Standard Methods (3). This EPA-approved method will yield concentrations of free available chlorine, monochloramine, dichloramine, and nitrogen trichloride.

The determination of total organic-bound chlorine in the samples will be conducted using a pyrohydrolysis technique developed by Kühn and Sontheimer (54) and further described by Dressman et al.(55). Refer to Figure 3 for an illustration of this apparatus. This method measures TOCl by the following process:

Organic compounds in the samples are adsorbed on washed ground granular activated carbon for one hour. The carbon sample is placed in the first of two tube furnaces connected in series by a quartz combustion tube. A constant flow of oxygen and steam is passed through the tube at specific rates. In the furnace a rapid rise in temperature

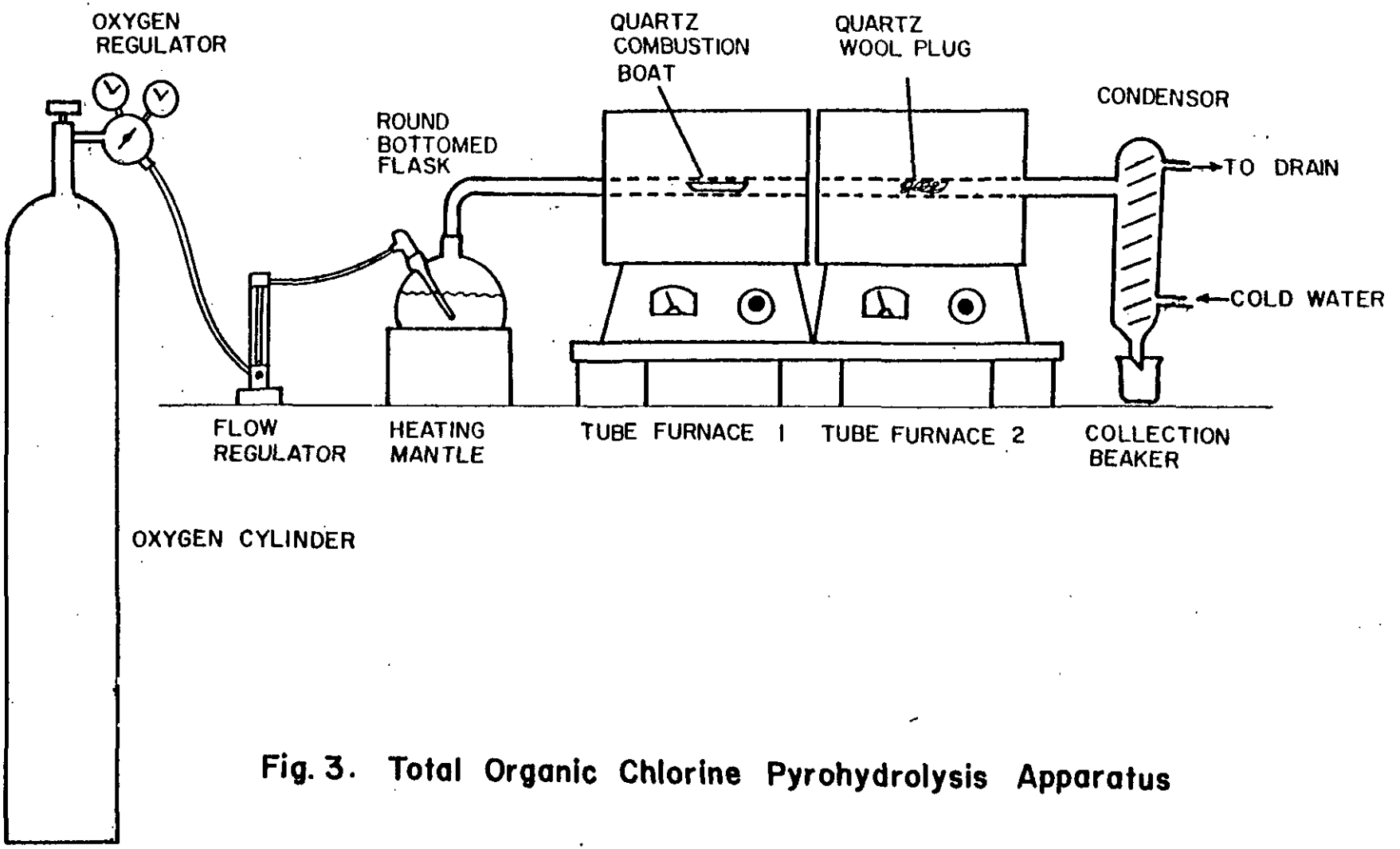

Fig. 3. Total Organic Chlorine Pyrohydrolysis Apparatus

(100°C to 700°C in 15-20 minutes) volatilizes the organochlorine compounds, which pass on to the second furnace, which is heated to a constant temperature of 1000°C. Here the compounds are combusted with steam (pyrohydrolysis) resulting in the production of HCl. The carrier steam is then condensed and the condensate is measured for chlorine ion concentration. The Mercuric Nitrate method for chloride determination from Standard Methods (3) will be used, and TOCl concentration will be calculated from the measured chloride ion concentration.

Interference from chloride ions in the sludge due to the large chloride ion concentrations in Purifaxed sludge must be avoided. Dressman et al. (55) have developed a "direct" method of correcting for inorganic chloride ion presence which has yielded good results and will be used here. The carbon sample will be washed in a nitrate solution prior to placement in the furnace, with the nitrate ion displacing the chloride ion on the carbon. The organic-bound chlorine remains on the carbon and when pyrohydrolyzed, yields an accurate value for TOCl free of inorganic ion interference.

Bioassay Procedure

The acute toxicities of the sludge samples, both raw and after Purifax treatment, will be determined by the use of a short term bioassay using fathead minnows (Pimephales promelas) as a test organism. Fathead minnows are a commonly used fish species in bioassay experiments, and the fish used in this research were supplied by the EPA's Newtown Fish Toxicology Station in Cincinnati. The fish received from Cincinnati are placed in a Frigid Unit's "Living Stream", an acclimation

tank with constant flow of water at constant temperature and with good aeration. The bioassay laboratory is run on a cycle of 15 hours of light and 9 hours of dark each day and is kept at a temperature of 20-22°C.

Dilution water used in these bioassay tests should be of a high quality to provide a healthy environment for the fish, and also should be reproducible. To accomplish these ends, a synthetic dilution water is used, prepared according to a standard formula for a soft water type. The source of raw water will be Amherst tap water, treated to a high degree by a modified Millipore Super-Q system. In this treatment, water passes through a prefilter, then a bed of granular activated carbon, which adsorbs organic contaminants and any remaining chlorine residual. Finally the water passes through a series of two mixed bed strong acid-strong base ion exchange columns to remove ionic contaminants. Water quality is monitored by a direct reading resistivity meter on the Super-Q unit.

The chemicals necessary to produce the desired soft water are added, and the resulting dilution water is stored in a large cylindrical plastic holding tank, which is equipped with a mechanical stirrer to provide thorough mixing. The chemical addition is designed to give the dilution water a hardness of 40-48 mg/l as $CaCO_3$ and an alkalinity of 30-35 mg/l as Ca CO_3. This water is aerated by means of a filtered air supply bubbled into the tank through a large airstone.

Preliminary bioassays will be carried out prior to the full scale tests to determine the general range of toxicant concentration at which 50 percent of the fish die in 96 hours (the 96-hr LC50). These preliminary tests will be conducted in glass jars with 3 ℓ volume,

each containing 2 fish.

The full-scale bioassays will be carried out in stainless steel tanks with a 19 liter capacity, using 8 fish per tank. A series of 4 or 5 toxicant concentrations in addition to a control (no toxicant) will be examined for each test in accordance with accepted bioassay procedure. The dilutions of sludge used in the bioassays will be chosen to provide a logarithmic series of toxicant concentrations (i.e. concentrations whose values are of equal spacing when plotted on a logarithmic scale). This practice is suggested by Standard Methods (3), and this source lists several possible toxicant concentration series in Section 801D.3.b.

Before each bioassay run, the dilution water and toxicant will be analyzed for pH, dissolved oxygen (DO), residual chlorine, temperature, and the toxicant for total organic chlorine. During the tests, pH, DO, temperature and chlorine residual will be monitored daily in each tank to provide a record of the possible changes in the quality of the test solutions over the duration of the bioassay.

A possible problem with the static bioassay as a test method is the depletion of dissolved oxygen in the tanks by fish respiration and the oxygen demand of the toxicant. This problem can be minimized by aerating the test solutions prior to introduction of the fish and by following guidelines established on the recommended loadings of fish to volume of tank (3). It should also be pointed out that fathead minnows can survive at low dissolved oxygen levels (16). Preliminary tests have shown that fathead minnows have survived DO levels less than

0.5 mg/l and can live for at least several weeks in water with DO concentrations around 4 mg/l.

The fish population will be monitored several times a day and counts of fish surviving at 24, 48 and 96 hours will be reported, with the 96 hour results of prime interest, as this time interval has generally been found to be long enough for acute toxicity of the toxicant to be exhibited in tests with freshwater fish (3). From these results the 96 hour LC50 will be determined for each test solution, providing there is good survival of the control fish. The LC50 can be found by several methods.

A commonly used graphical technique employs interpolation between a concentration of toxicant at which more than 50 percent of the fish survived and a concentration at which less than 50 percent survived. This data is plotted on a graph with percent survival on a probability scale and relative toxicant strength on a logarithmic scale. The interpolation of LC50 is made by simply drawing a straight line between the two points representing survival at the two successive concentrations that were lethal to more than half and less than half of the fish respectively. The concentration of toxicant where the interpolated line crosses the 50 percent survival line is the LC50. LC50 values may be determined for various time intervals by this method, but the 96 hour LC50 is most commonly used and will be most useful in comparing the toxicities of various samples tested with other results.

A number of more sophisticated methods of analyzing the results of quantal bioassays are now available. Quantal bioassays are those in which an organism either shows a particular response or does not,

whether the response be death or some sub-lethal reacton (3). (A second type of test is the quantitative bioassay where the organism yields a response that is variable in degree, such as amount of growth (3)). Some of these methods of quantal bioassay analysis are quite complex, requiring the use of a computer.

One recommended technique of estimating the LC50 of a quantal bioassay and confidence limits for this value is the moving average-angle method which employs arcsine transformations and use of a moving average for estimation of the LC50 (3). This method was used by Pickering and Vigor (57) and described in detailed by Harris in 1959 (56). Readers are asked to consult the Harris reference for the method, though a set of sample calculations for estimation of both the LC50 and its confidence limits may be found in Appendix A.

CHAPTER V

RESULTS

Preliminary Bioassays

The first step of the toxicity studies was the performing of a series of preliminary bioassays. These tests were done prior to full scale tests in order to find the general range of concentrations at which the liquid portion of a sludge was toxic. The results of these tests are shown in Table 1, in which the 96-hr LC50 values are expressed as percent sludge by volume. It should be noted the word sludge will be used in this report to mean the liquid portion of a sludge, which is the only part of a sludge tested for toxicity in these bioassays as stated earlier.

Caution must be taken in interpreting these results, as these tests cover wider ranges of toxicant concentrations than those to be used for full scale bioassays, and are used only as indicators of what narrower set of full scale test concentrations should be selected. They are also done using small tank volumes (3 liters of a sample in each glass jar) and small numbers of fish (2 fish per jar), thus lending little statistical reliability to the resulting LC50 values.

Nine toxicant samples (see Table 1) were tested and in most cases the results proved helpful in chosing a narrower range of toxicant concentrations for the full scale tests. The first LC50 reported for raw primary sludge was an exception, as this result had been determined from a test on primary sludge vacuum filtrate from the old Amherst wastewater treatment plant. This LC50 was later mistakenly used to choose concentrations of primary sludge from the new Amherst treatment

Table 1. Results of Preliminary Bioassays

Toxicant	96-hr LC50* (% toxicant)
Raw primary sludge	10
Raw primary sludge	2.8
Purifaxed primary sludge	3.2
Dechlorinated Purifaxed primary sludge	5.6
Raw activated sludge	72.
Raw activated sludge	<50.
Purifaxed activated sludge	<0.5
Purifaxed activated sludge	0.5
Dechlorinated Purifaxed activated sludge	10.3

*96-hr LC50's found by graphical technique

plant tested in the subsequent full scale bioassay. This resulting concentration range was set too high and its high toxicity resulted in the deaths of the test fish in all the tanks. An additional preliminary bioassay was performed on the sludge from the new plant and more meaningful results were then obtained.

While not required for preliminary bioassays, most of these runs included a control jar (no toxicant) and in each of these cases all the fish in these jars survived. As much as possible conditions to be used in the full scale tests were applied in the preliminary runs. The same dilution water was used, sludge sample preparation was very similar, and external conditions such as light cycle and temperature were kept the same in order to give the best possible correlation between the preliminary results and the full scale assays. The jars were monitored for pH, DO and temperature similar to the full scale tests, but on a less frequent schedule.

Full Scale Bioassays

Full scale bioassays were carried out in accordance with procedures detailed earlier. In all six of these tests there were 100 percent survivalsof the fish in the control tanks. In addition the stock of fish kept in the acclimation tank remained quite healthy throughout these tests, with a mortality rate of less than 2 percent per month. The average length of the fathead minnows used was 4.6 cm and the average weight was 1.1 g.

All the bioassays were carried out for 4 days and the resulting fish survivals were used to calculate 96-hr LC50 values for the various sludge samples. These results are presented in Table 2. The LC50's

Table 2. Results of Full Scale Bioassays

Toxicant	96-hr LC50 by graphical procedure (%)	96-hr LC50 by moving average angle method (%)	95% Confidence Limits(%)
Raw primary sludge	3.0	2.7	1.97-4.01
Purifaxed primary sludge	1.0	1.0	0.56-1.78
Dechlorinated Purifaxed primary sludge	8.6	8.8	6.8-12.3
Raw activated sludge	82	82.9	71.2-94.9
Purifaxed activated sludge	0.56	0.49	0.28-0.92
Dechlorinated Purifaxed activated sludge	22.	21.7	15.6-28.7

were found by both the graphical technique and the moving average-angle method, and in addition the latter method was used to calculate 95 percent confidence limits for these LC50 values. The two methods resulted in estimated 96-hr LC50 values that agreed very closely with each other.

In these bioassays the majority of fish deaths (62 percent) that occurred over the 96-hour period were found to happen in the first 24 hours of a run, as is common in biosassay studies. As a check on the progress of the tests and to determine if the 96-hour period was sufficiently long to provide an accurate estimation of acute toxicity of the sludges, several toxicity curves were plotted. These curves show the change in the LC50 over the course of a bioassay run. A ficticious toxicity curve (3) is included here as Figure 4 for illustrative purposes, and the toxicity curve for Purifaxed activated sludge can be found in Appendix A (Figure 7).

On the toxicity curve the termination of acute toxicity is indicated by the curve becoming asymptotic to a line parallel to the time axis. In Figure 4 the curve is becoming asymptotic but may not have reached this stage fully yet. The toxicity curves drawn for the bioassays run on sludge samples have become very steep quite rapidly, indicating that the acute toxicity of the sludges have been fully expressed in the 96-hour period chosen for these bioassays. Also supporting this conclusion is the observation of essentially no change in fish survival in the bioassays occasionally allowed to run several days beyond the 96-hour period of interest.

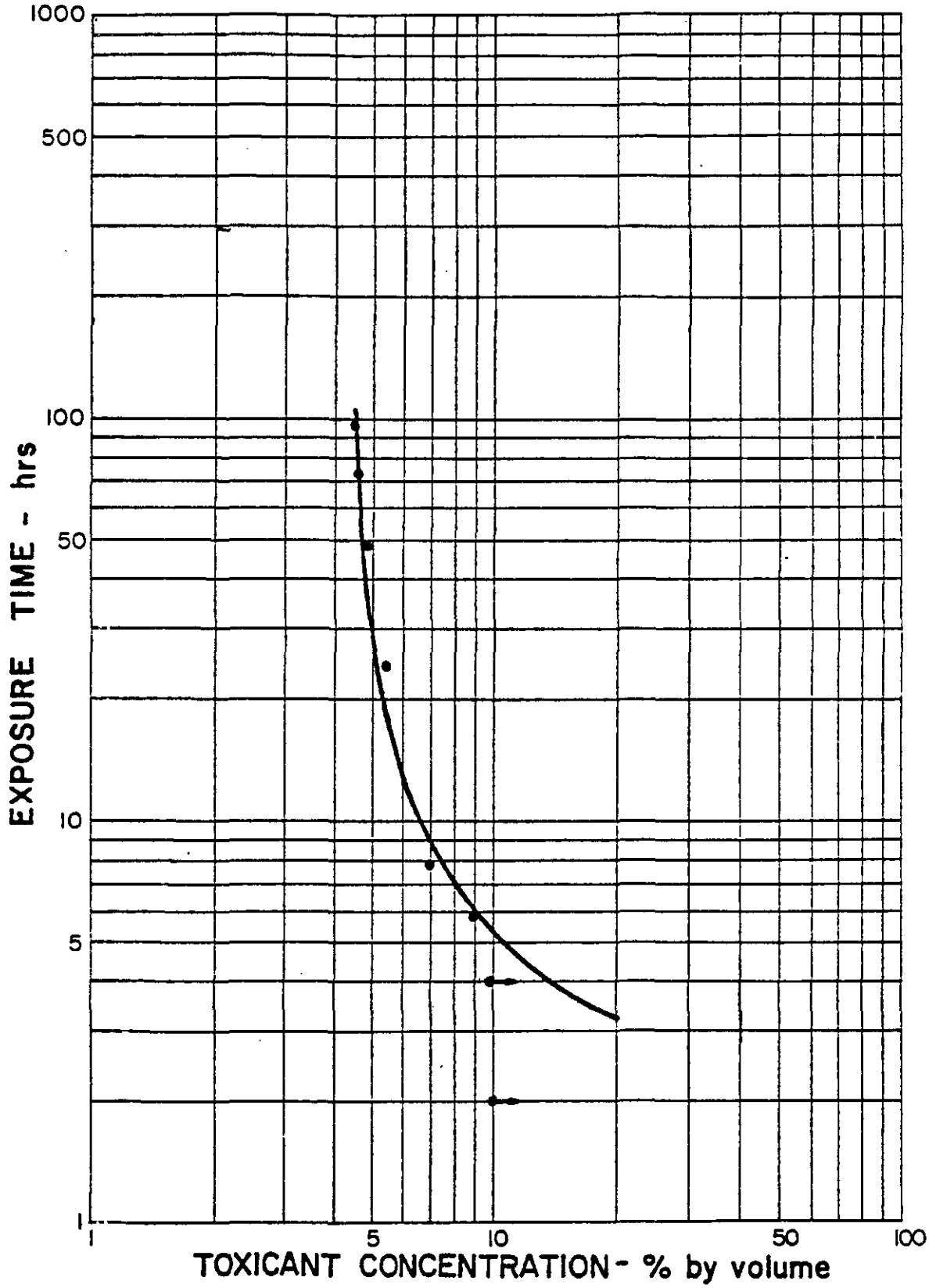

Fig. 4. Toxicity Curve from Standard Methods (3). Points represent LC50's at different exposure times.

Primary sludge. Examination of the 96 hour LC50 values determined for the different stages of treatment of primary sludge reveals that primary sludge is quite toxic to fish in all three forms, especially when it is in the raw form or after it has been Purifaxed. The raw primary sludge LC50 is quite low and maybe due in part to the lowering of dissolved oxygen by the high oxygen demand of this waste. Dissolved oxygen levels dropped to 0.8-1.0 mg/l in all of the tanks, possibly causing additional stress to the fish, although these levels are not necessarily low enough to kill the fish on their own. Observed pH levels ranged from 7.4 down to 6.5 with increasing sludge concentrations, but over the course of the test they all levelled off in the range of 6.9-7.2.

Use of the Purifax process increases the toxicity of the primary sludge over that of the raw sludge, as shown by the 96-hr LC50 value of 1.0 percent (by volume) Purifaxed sludge. The subnatant of the Purifax-treated sludge after being allowed to settle overnight had a relatively stable total residual chlorine (TRC) concentration of 50 mg/l. If it were assumed that the toxicity of the Purifaxed primary sludge is caused solely by the residual chlorine in the treated sludge, an LC50 of slightly below 1.0 percent sludge would be predicted from the literature, as this would correspond to an LC50 of 0.2-0.4 mg/l expressed as total chlorine residual. In fact, this is the value of the total residual chlorine concentration obtained for the critical 1.0 percent Purifaxed primary sludge solution by interpolating between the TRC levels in the sludge dilutions actually used in the bioassay. Chemical monitoring showed that the initial total residual chlorine concentration in the tank containing 0.56 percent Purifaxed primary sludge was 0.10 mg/l and

in the tank containing 1.8 percent treated sludge was 0.65 mg/l. These chlorine concentrations did decrease slightly over the course of the bioassay run.

The pH levels in these tanks ranged from 7.3 in the tank with the lowest sludge concentration down to approximately 6.0 in the 1.8 percent sludge tank, and down to a low of 3.3 in the tank containing 5.6 percent Purifaxed primary sludge. Dissolved oxygen concentrations remained high (above 7.0 mg/l) in all tanks for the duration of the test.

In order to examine the toxicity of the Purifax-treated primary sludge without the residual chlorine already known to be toxic, samples of Purifaxed sludge were dechlorinated using sodium thiosulfate. This dechlorination method was very successful, as no residual chlorine could be detected after the sodium thiosulfate additions.

As shown in Table 2, the toxicity of the dechlorinated Purifaxed primary sludge actually decreased below that of the raw primary sludge. The 96-hr LC50 value of 8.6-8.8 percent dechlorinated sludge is about three times that of the raw primary sludge centrate, indicating that at least on a short term basis the liquid portion of primary sludge may be least harmful to fish when treated with high dosages of chlorine followed by dechlorination to remove all residual chlorine. It should be noted that other studies similar to this one have found that wastewater effluents disinfected with chlorine and then dechlorinated by various chemical methods are less toxic to fish than the original raw effluent (17,34).

Activated sludge. This research has shown that unlike raw primary sludge, raw activated sludge is not very toxic to fathead minnows. An LC50

of 82 percent (by volume) raw activated sludge centrate was calculated, and in this bioassay it was found that the fish lived in tanks containing as high as 56 percent sludge without a single mortaility.

In the raw activated sludge bioassay the pH levels ranged from 6,9-7.6 with a general upward trend over the 96-hour period. The dissolved oxygen concentrations showed a sharp initial drop followed by a levelling off or slight increase after about 36 hours. The DO concentration reached a low of 0.8 mg/l in the tank containing 75 percent sludge, and dropped to a low of 0.25 mg/l in the 100 percent sludge tank. The test solutions containing smaller proportions of sludge had minimum DO level: of 2.8 to 4.0 mg/l.

The toxicity of activated sludge increased dramatically following treatment with the Purifax process. Whereas the 96-hr LC50 value of raw activated sludge was 82 percent (by volume) sludge, the Purifaxed activated sludge had a 96-hr LC50 value as low as 0.47-0.56 percent (by volume).

The subnatant of the Purifax-treated activated sludge allowed to settle for a day was found to have a total residual chlorine concentration of 74 mg/l, higher than that of similarly treated primary sludge. Again it was found that the resulting 96-hr LC50 level of this Purifaxed sludge was such that the total residual chlorine concentration at this sludge dilution was consistant with literature values of 96-hr LC50's expressed as chlorine residual concentration found for chlorinated wastewater effluents. That is to say one would expect a tank containing 0.5 percent treated sludge (at a TRC level of 74 mg/l) to contain roughly: 74 x 0.005 = 0.37 mg/l of residual chlorine. Actual chemical analysis

showed the tank containing 0.56 percent treated sludge had an initial total residual chlorine concentration of 0.30 mg/l. In summary it can be noted that the determination of 0.47-0.56 percent Purifaxed activated sludge as the 96-hr LC50 value corresponds to an LC50 value for this sludge expressed as total residual chlorine concentration of approximately 0.3 mg/l, which is consistant with results reported in the literature.

One may not conclude, however, that the chlorine residual in the Purifaxed sludges is the sole toxic constituent, even though it has been shown for both primary and activated sludges treated with the Purifax system that the acute toxicity of the liquid portion of these sludges is not noticeably higher than the toxicities expected from simply looking at the total residual chlorine concentrations of these treated sludges.

As was the case with dechlorination of treated primary sludge, the chlorine residual in Purifax-treated activated sludge was entirely removed by addition of sodium thiosulfate. The toxicity of the Purifaxed activated sludge was greatly reduced from its previously high lethality by this dechlorination step. The 96-hr LC50 for Purifaxed activated sludge after dechlorination was determined to be 22 percent sludge by volume, a much lower toxicity than the LC50 of 0.56 percent before removal of the chlorine residual. Despite the drop in toxicity, the dechlorination step could not detoxify the treated sludge to a level lower than the raw activated sludge as was the case with primary sludge.

Once again the lack of dissolved oxygen may have been a factor in the toxicity of the dechlorinated sludge. Dissolved oxygen levels

dropped to 0.9 mg/l and 1.55 mg/l in tanks containing 32 percent and 18 percent dechlorinated sludge respectively.

Total Organic Chlorine

Total organic chlorine (TOCl) analyses were first run on distilled water samples to obtain a background value for TOCl. The results showed that distilled water alone indicated a TOCl concentration of 311 µg/l (an average of three results ranging from 280 to 345 µg/l). This value is much higher than that reported by Dressman *et al.* (55) in their extensive study of the technique.

A series of TOCl determinations on known solutions of total organic chlorine was then performed in order to construct a TOCl calibration curve. A series of dilutions of m-chlorobenzoic acid in distilled water was examined and the resulting curve is shown as Figure 8 in Appendix B. A least-squares regression analysis was performed on these points and the resulting equation was used as a means to adjust the observed TOCl concentrations in the following tests. The calculated y-intercept agreed well with the experimentally determined background TOCl level found with distilled water.

Dressman *et al.* (55) explained that the results obtained from their TOCl tests are equal to actual TOCl concentrations (minus their relatively small background value). This was not found to be the case with the m-chlorobenzoic acid tests, i.e. the measured TOCl value minus the background value did not yield the actual TOCl concentration. This necessitated the use of the above calibration curve, and while the relationship between observed and actual values of TOCl was not 1:1, it was not too far off, resulting in a slope of 0.902 for the regression line.

The use of just one chemical compound to generate a calibration curve for such complex solutions as wastewater sludges is questionable, but nevertheless it is a starting point. Further research needs to be conducted in this area, exploring the Dressman et al. (55) claim of a one to one relationship between true and observed TOCl concentrations and also looking at calibration curves for other chlorinated organic compounds.

This project concentrates on the toxicities of the various sludge samples to fish, and further background research into the total organic chlorine measurement technique was not feasible, especially in light of the tedious nature of the experimental procedure. The TOCl concentrations reported herein can best be used for comparison of the relative magnitudes of chloroorganic concentrations in the different sludge samples, rather than for absolute quantification of these concentrations.

The total organic chlorine concentrations determined for the various sludges are listed in Table 3. The reported concentrations are averages of the results from two or three tests performed on each type of sludge. Several observations can be made from these data. First, the TOCl concentrations of the raw sludges seem fairly high. This may result either from chloroorganics present in the raw wastewater entering the treatment plant or from chlorinated organics produced if chlorination is used as a pretreatment for odor control.

The most striking trend in the data is the large increase in total organic chlorine concentration between raw and Purifaxed sludges. In both primary and activated sludge the Purifax process caused an increase in chloroorganics by about a factor of six over the raw sludge. This is

Table 3. Total Organic Chlorine Results

Sample	TOCl Concentration µg/l
Raw primary sludge	965
Purifaxed primary sludge	5446
Dechlorinated Purifaxed primary sludge	5845
Raw activated sludge	557
Purifaxed activated sludge	3464
Dechlorinated Purifaxed activated sludge	5808

not unexpected, due to the large dosage of chlorine used (2000 mg/l) in the process. These values of 3.5 to 5.5 mg/l TOCl are higher than those reported by Wise et al. (50) for the liquid portion of Purifaxed sludges, but in their study only those chloroorganics that were volatile or helium-extractable could be measured in the gas chromatograph/mass spectrophotometer (GC/MS) analyses, and all other chloroorganics were not included.

Dechlorination of the Purifaxed sludges raised the observed TOCl concentrations for both sources of sludge, though the increase in TOCl for primary sludge is insignificant. The increase in chloroorganics in the activated sludge was sizeable, but due in large part to one TOCl reading of 7364 µg/l that was particularly high. No explanation for this high TOCl level is readily apparent. The amount of chloroorganics in the Purifaxed sludge and in the dechlorinated Purifaxed sludge should be the same, as it is expected that dechlorination only removes the chlorine residual of the samples and does not either add or remove any chlorinated hydrocarbons.

CHAPTER VI

DISCUSSION

Toxicity Results

The results of this research project have shown that the liquid portion of domestic wastewater sludges treated with the Purifax process is acutely toxic to fathead minnows at concentrations as low as 0.5-1.0 percent by volume. Solutions prepared using 0.5 percent Purifaxed activated sludge and using 1.0 percent Purifaxed primary sludge were found to have total residual chlorine concentrations in the range of 0.2-0.4 mg/l, which are consistant with the levels generally reported in the literature as 96-hr LC50's for freshwater fish. Furthermore the toxicity of Purifaxed sludges can be greatly reduced by dechlorination with sodium thiosulfate, which eliminates the chlorine residual. In the case of primary sludge it was found that the dechlorinated Purifaxed sludge was actually less toxic than the original raw sludge.

Use of the Purifax process for treating wastewater sludges was also shown to increase the concentration of total organic chlorine in the liquid portion of the sludge. The effect on fish of these added chloroorganics is not yet clear.

It appears that the acute toxicity of the Purifaxed sludges was predominantly due to the chlorine residual in these samples. Both the fact that elimination of the chlorine residual by dechlorination greatly reduced the acute toxicity and the observation that toxic concentrations of sludge could be reasonably well predicted by equating their total residual chlorine concentrations with known 96-hr LC50's from the literature

support this assumption. While this does not prove that total residual chlorine is the only toxic part of Purifaxed sludges, it may possibly be used as a general indicator of the acute toxicities of domestic wastewater sludges treated with the Purifax process. Caution, however, should be taken in applying this type of reasoning to industrial waste sludges, as some highly toxic compounds such as cyanogen chloride can be readily formed when the Purifax process is applied to certain types of wastes (50).

What then is the significance of increased total organic chlorine levels in Purifaxed sludges? While no good correlation between TOCl concentration and acute toxicity to fish could be found, these elevated TOCl levels are still a concern for the health of aquatic life. It is important to remember that this project investigated only acute toxicities, i.e. the bioassays performed were short term tests covering only a segment of the adult stage of fish life. Generally the effects of the organic chlorine compounds are long term ones that are compounded by the bioaccumulation of these chloroorganics in the fish's body. Effects such as suppression of normal growth, reproductive capability and survival of fish eggs and larvae are not measured in short term tests, and may be especially important in cases such as this where the fish are exposed to high levels of chlorinated organic compounds.

Dilution of Purifaxed Sludges

In order to put the toxicity of sludges treated with the Purifax process into better perspective, it would be wise to step back and view the process within the context of the total wastewater treatment plant

in which it is used. This research has determined the acute toxicities of the liquid portions of primary and activated sludges at three stages of treatment, but does not take into account how these toxicities may be expressed in the final effluent of a treatment plant using the Purifax process.

Of primary concern is the effect that returning Purifax-treated sludge to the mainstream of a treatment plant may have on that plant's effluent. Reports by BIF (2) have shown that the liquid portion of the Purifaxed sludge returned to the headworks may amount to 0.5-1.5 percent of the total treatment plant effluent, as an average. If the Purifax system is used only at certain hours of the day this proportion may reach as high as 17 percent of the plant effluent.

If we were for the moment to consider chlorine residual as a conservative quantity throughout the plant and also to assume the total residual chlorine concentration of the Purifaxed sludge to be 200 mg/l, we could assume that the use of the Purifax system would on the average add 1-3 mg/l total residual chlorine to the plant effluent if the Purifaxed sludge liquid was returned at a flowrate amounting to 0.5-1.5 percent of the total effluent flowrate. In actuality the chlorine residual concentration of the liquid portion of Purifaxed sludge after dewatering will be substantially less than 200 mg/l whether sludge drying beds, lagoons, vacuum filters or belt presses are used. Observations in this research have found that the total residual chlorine concentration of the treated sludge subnatant was reduced from 140-170 mg/l to 50-74 mg/l by allowing the sludge to settle overnight. The level of chlorine residual would then be further reduced by mixing it with unchlorinated sewage that

has a relatively high chlorine demand. In fact, BIF recommends (2) one possible method of utilizing the Purifax system to its fullest benefit is to add the Purifaxed sludge liquid to the plant effluent for odor control. This will use up the chlorine residual from the Purifaxed sludge to a large extent and result in a figure for chlorine residual addition to the plant effluent of far less than the 1-3 mg/l as calculated above, though it would be very difficult to determine what this addition might actually be.

If the increase in chlorine residual due to the use of the Purifax system is to be noticeably lower than 1.0 mg/l then one could term its impact on the acute toxicity of the treatment plant effluent as minimal, since the effluent total residual chlorine concentrations are generally between 2 to 4 mg/l in well run new plants and may be 8 mg/l in plants that are less optimally constructed and operated (58). These effluents pose a definite threat to aquatic life at treatment plant outfall locations in their own right, and dechlorination is recommended to prevent this toxic waste from being discharged.

Use of the Purifaxed system at only certain hours of the day may pose a toxicity problem if the recycling of the Purifaxed sludge liquid is not managed properly. From information recorded at one of the wastewater treatment plants using the Purfiax system given in a BIF report (2), it was calculated that the flow of sludge through the Purifax unit could reach a maximum equivalent to 17 percent of the influent flow to the plant. If all of the liquid portion of this treated sludge was to be returned to the headworks in the same limited amount of time the Purifax system was run, then the level of return flow could approach 17 percent

of the influent flow. If once again the total residual chlorine concentration of the Purifaxed sludge liquid was assumed to be 200 mg/l and if it was assumed to be conservative, this would mean the addition of 34 mg/l residual chlorine to the treatment plant effluent during those hours which is certainly a very dangerous level. However, as pointed out earlier chlorine residual is not conservative and will decay, especially when added to wastewater treatment plant influent which has high chlorine demand. It may also be noted that it is likely the Purifax-treated sludge will be retained for some time in the dewatering process, thus increasing the time for decay and effectively increasing the dilution it undergoes at the headworks of the plant by lengthening the time over which it is recycled.

The problem of determining the impact on aquatic life of the increase in chlorinated organics in the sludge due to use of the Purifax process remains unsolved when taken into account as part of the whole wastewater treatment process. Many of these chloroorganics are highly refractory and therefore will not decay for a long time. Their removal by different processes is unknown, but cannot be assumed to be very high. While they will be diluted by the larger volume of flow through the treatment plant, the total mass of chlorinated organics produced in the Purifax treatment of sludge may not be changed much by passage through the whole treatment plant. If chlorination is used as the method of disinfecting the plant effluent, as is often the case, the concentration of chloroorganics in the effluent will be raised even more.

The concentrations of these substances reaching aquatic organisms after discharge to the receiving body of water may seem small, but again

there are the problems of bioaccumulation and of the refractory nature of these compounds to consider. We still do not know a great deal about the long range effects of these chloroorganics on aquatic life and their eventual effects on organisms higher on the food chain.

The Use of Application Factors

The use of application factors to determine what amounts of the liquid separated from Purifaxed sludge can be safely discharged to receiving bodies of water without long term deleterious effects to aquatic life using the results of these short term bioassays is limited for two reasons. The first reason is that application factors are used when a short term LC50 is known for a particular toxicant that is to be directly discharged to an aquatic ecosystem, which is not the case with Purifaxed sludge. Here the 96-hr LC50's have been determined for Purifaxed primary and activated sludges, but the evolution of these sludges through the course of a treatment plant, from a toxicological point of view, is unknown. The application factor would only be appropriate if the liquid portion of the Purifaxed sludges were directly discharged to a body of water, or on the other hand if the acute toxicity of the effluent of a treatment plant using the Purifax process had been determined.

The second reason for not using application factors is that they are arbitrary by nature and traditionally do not account for possible wide variations between acute and chronic toxicities due to the type of toxicant and the different aquatic organisms examined. The application factor generally used for freshwater fish is one tenth, i.e. the safe concentration of a toxicant in long term exposures is one tenth the 96-hr

LC50 calculated for that toxicant.

Brungs (23) summarizes several studies in which both short term LC50's and long term safe concentrations (no effect on growth, survival or reproduction) were determined for chlorinated wastewater effluents using fathead minnows. In these cases an application factor could then be determined and was found to be 0.13 (or about one eighth)times the concentration of total residual chlorine determined to be the acute LC50. This application factor's use for other species of fish or for other toxicants is questionable, however. It might be a good approximation of an application factor for Purifaxed sludge if the effects of recycling the sludge liquid through the treatment plant were better known, but on the other hand this factor was derived for wastes that did not have such high levels of chlorinated organic compounds.

CHAPTER VII

CONCLUSIONS

In summary, the main conclusions of this research are as follows:

1. The liquid portion of wastewater sludges treated with high dosages of chlorine is highly toxic to fathead minnows (Pimephales promelas). 96-hr LC50's were determined to be 1.0 percent by volume Purifaxed primary sludge and 0.5 percent by volume Purifaxed activated sludge.
2. The total residual chlorine concentrations of solutions made up of 1.0 percent Purifaxed primary sludge and of 0.5 percent Purifaxed activated sludge were found to be 0.2 to 0.4 mg/l. These concentrations are consistant with 96-hr LC50 values expressed as total residual chlorine concentrations reported in the literature for wastewater effluents.
3. The acute toxicity of Purifaxed wastewater sludges could be greatly reduced by dechlorination with sodium thiosulfate. In the case of primary sludge the dechlorinated Purifaxed sludge was found to be significantly less toxic than the raw primary sludge.
4. Use of the Purifax process greatly increases the concentration of total organic chlorine in wastewater sludges. For both primary and activated sludge a six-fold increase in TOCl concentration was found between the raw sludge and the Purifaxed sludge.

5. When decay of chlorine residual and dilution of the liquid portion of the Purifaxed sludge recycled to the headworks of a wastewater treatment plant were taken into account, it was found that the use of the Purifax process would have a minimal effect on the acute toxicity of the final effluent of a domestic wastewater treatment plant, as long as the liquid portion of the Purifaxed sludge was released back to the plant at a slow rate, such as over a full 24 hour cycle.
6. The long term effects on fish of exposure to increased concentrations of chlorinated organic compounds are unknown. While there does not seem to be any significant short term toxic effects from the chloroorganics in the liquid portion of the Purifaxed sludges, the long term effects might be significant when the refractory nature of these compounds and their ability to be bioaccumulated are taken into account.

CHAPTER VIII

RECOMMENDATIONS

The following recommendations are made as a result of this research project:

1. Further research should be conducted into the long range effects of Purifaxed wastewater sludges on freshwater aquatic organisms. Specifically the long term effects of the high chlorinated organic concentrations found in these sludges on the growth, survival and reproduction of freshwater fish should be investigated.
2. Further refinements in the methods for measuring total organic chlorine concentration are needed. The carbon adsorption and pyrohydrolysis technique employed in this research should be used to generate additional calibration curves with known chloroorganics to provide better reliability of results.
3. While this research has shown that the use of the Purifax process on domestic wastewater sludges has a minimal effect on the toxicity of the final treatment plant effluent, it is recommended that bioassays similar to those in this project be conducted on industrial sludges before the Purifax process is chosen as the method of treatment. Significant acute toxicities may result from the interaction of the high dosages of chlorine used and some of the constituents of these industrial wastes. Long term effects on aquatic life due to these wastes should also be examined where possible.

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APPENDIX A

BIOASSAY DATA FOR PURIFAXED ACTIVATED SLUDGE

Preliminary Bioassay Results

Toxicant: Purifaxed activated sludge, chlorine dose 4300 mg/l

Starting Date: January 24, 1979 Run #: P3

Test Species: Fathead Minnows

Dilution Water: Reconstituted soft water

Container Size: 3 liters Fish per Container: 2

Toxicant Concentrations Used: 0.5%, 1.0%, 5%, 10%

Resulting 96-hr LC50: <0.5%

Table 4

Preliminary Bioassay Survival Data

Purifaxed Activated Sludge, Chlorine dose 4300 mg/l

Tank Number	Toxicant Concentration	Fish Surviving				
		Start	24 hr	48 hr	72 hr	96 hr
A	Control	2	2	2	2	2
B	0.5%	2	2	0	0	0
C	1.0%	2	0	0	0	0
D	5.0%	2	0	0	0	0
E	10.0%	2	0	0	0	0

Table 5

Preliminary Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, Chlorine dose 4300 mg/l

Time Since Start of Test: 30 minutes

Percent Toxicant	Temp. °C	pH	Dissolved Oxygen (mg/l)	Chlorine Residual, mg/l				
				Free Chlorine	Mono-chloramine	Di-chloramine	Tri-chloramine	Total Chlorine
0	20	7.0	8.3	0	0	0	0	0
0.5	20	6.5	8.5	0	0.05	0.05	0	0.1
1.0	20	6.0	8.7	0	0.2	0.3	0	0.5
5.0	20	2.8	9.1	0.05	0.45	1.8	0	2.3
10.0	20	2.45	9.0	0.05	0.75	1.9	0	2.7

Table 6

Preliminary Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, chlorine dose 4300 mg/l

Time Since Start of Test: 72 hours

Percent Toxicant	pH
0	6.65
0.5	6.7
1.0	6.4
5.0	2.8
10.0	2.8

Preliminary Bioassay Results

Toxicant: Purifaxed activated sludge, chlorine dose 2000 mg/l

Starting Date: January 31, 1979 Run #: P4

Test Species: Fathead Minnows

Dilution Water: Reconstituted soft water

Container Size: 3 liters Fish per Container: 2

Toxicant Concentrations Used: 0.01%, 0.05%, 0.25 %, 1.0%

Resulting 96-hr LC50: 0.5%

Table 7

Preliminary Bioassay Survival Data

Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Tank Number	Toxicant Concentration	Fish Surviving Start	24 hr	48 hr	72 hr	96 hr
A	Control	2	2	2	2	2
B	0.01%	2	2	2	2	2
C	0.05%	2	2	2	2	2
D	0.25%	2	2	2	2	2
E	1.0%	2	0	0	0	0

Table 8

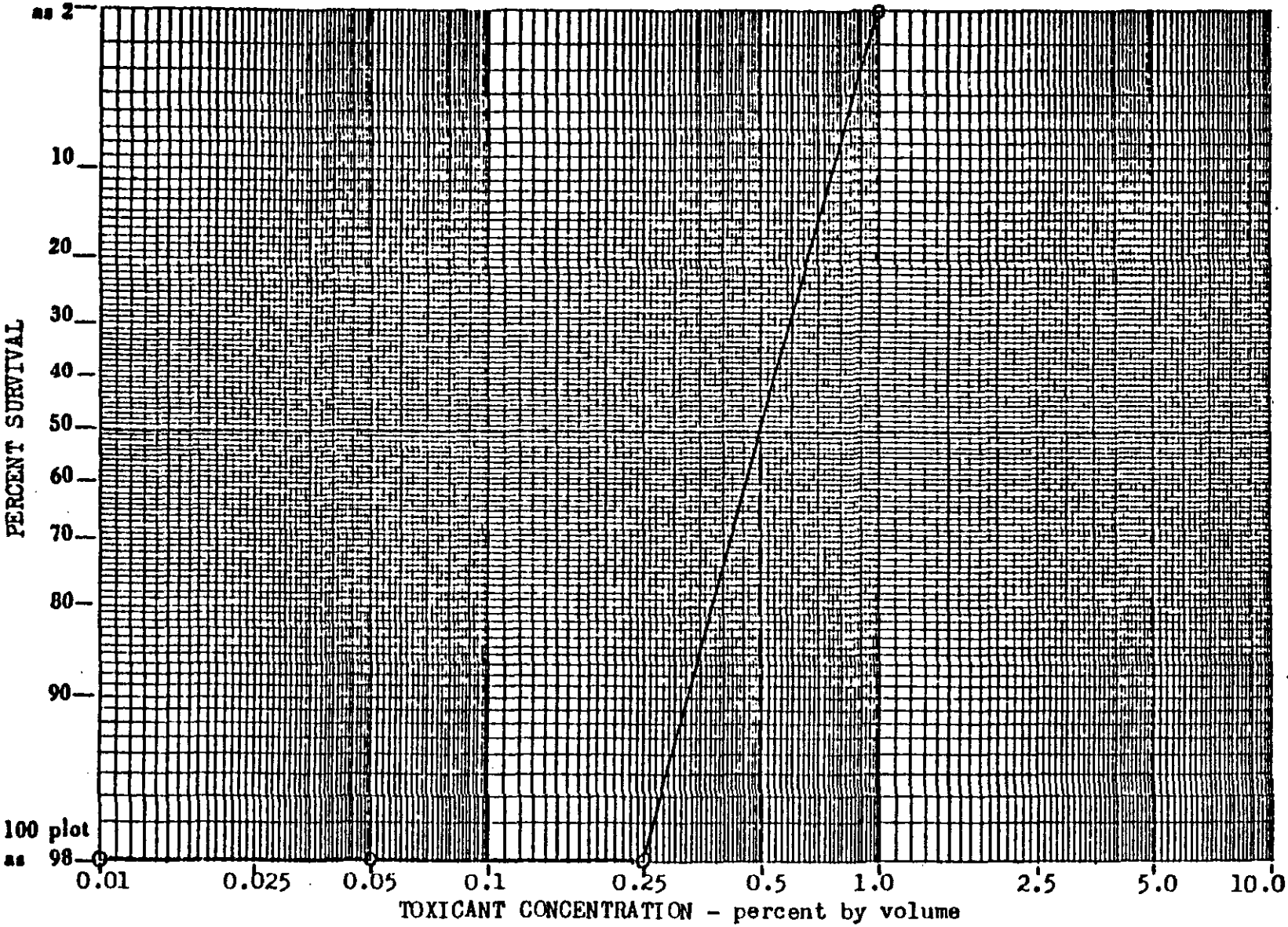
Preliminary Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Time Since Start of Test: 17 hours

Percent Toxicant	Temp. °C	pH	Dissolved Oxygen mg/l	Chlorine Residual, mg/l Free Chlorine	Mono-chloramine	Di-chloramine	Tri chloramine	Total Chlorine
0	20	6.7	7.0	0	0	0	0	0
0.01	20	6.7	5.9	0	0	0	0	0
0.05	20	6.85	7.4	0	0	0	0	0
0.25	20	6.7	6.95	0	0	0.05	0	0.06
1.0	20	6.15	8.6	0	0.05	0.5	0	0.55

Fig. 5. Determination of 96-hr LC50 for Preliminary Bioassay on Purifaxed Activated Sludge by Graphical Technique



Bioassay Results

Toxicant: Purifaxed activated sludge, chlorine dose 2000 mg/l

Starting Date: April 21, 1979 Run #: F5

Test Species: Fathead Minnows

Dilution Water: Reconstituted soft water

Container size: 19 liters Fish per container: 8

Toxicant Concentrations Used: 0.056%, 0.18%, 0.56%, 1.8%

Resulting 96-hr LC50: 0.56%

Table 9

Bioassay Survival Data

Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Tank Number	Toxicant Concentration	Fish surviving Start	24 hr	48 hr	72 hr	96 hr
2a	Control	8	8	8	8	8
3a	0.056%	8	8	8	8	8
4a	0.18%	8	8	7	7	7
5a	0.56%	8	5	5	4	4
6a	1.8%	8	0	0	0	0

Table 10

Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Time Since Start of Test: 45 minutes

Percent Toxicant	Temp. °C	pH	Dissolved Oxygen mg/l	Chlorine Residual, mg/l				
				Free Chlorine	Mono-chloramine	Di-chloramine	Tri-chloramine	Total Chlorine
0	19	7.4	8.75	0	0	0	0	0
0.056	19	7.3	8.75	0	0	0.05	0	0.05
0.18	19	7.1	8.55	0	0	0.1	0	0.1
0.56	18.5	6.6	8.55	0	0.05	0.25	0	0.3
1.8	18.5	5.45	8.65	0.05	0.15	0.7	0	0.9

Table 11

Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Time Since Start of Test: 47 hours

Percent Toxicant	Temp. °C	pH	Dissolved Oxygen mg/l	Chlorine Residual, mg/l				
				Chlorine	Mono-chloramine	Di-chloramine	Tri-chloramine	Total Chlorine
0	19	7.2	7.3	0	0	0	0	0
0.056	19	7.4	8.0	0	0	0	0	0
0.18	19	7.4	8.15	0	0	0.05	0	0.05
0.56	18.5	7.15	8.15	0	0	0.05	0	0.05
1.8	18.5	6.0	8.0	0	0.1	0.2	0	0.3

Table 12

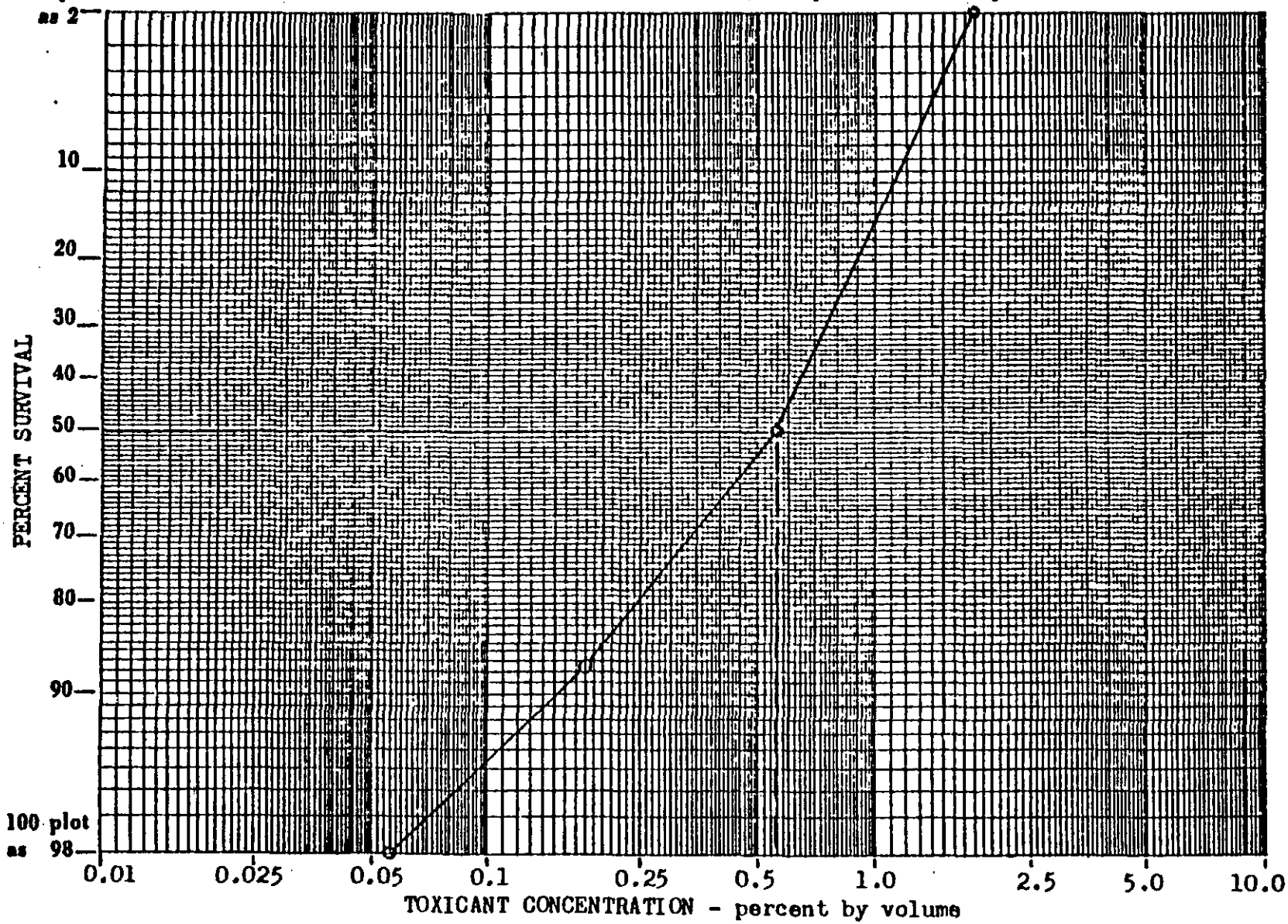
Bioassay Chemical Data

Toxicant: Purifaxed Activated Sludge, Chlorine dose 2000 mg/l

Time Since Start of Test: 96 hours

Percent Toxicant	Temperature °C	pH	Dissolved Oxygen mg/l
0	19	7.2	7.5
0.056	19	7.3	7.9
0.18	19	7.2	7.6
0.56	18.5	7.15	7.6
1.8	18.5	6.4	8.6

Fig. 6. Determination of 96-hr LC50 for Full Scale Bioassay on Purifaxed Activated Sludge by Graphical Technique



Determination of the 96-hr LC50 and Its 95 Percent Confidence Limits for Purifaxed Activated Sludge Using the Moving Average-Angle Method

Dose mg/l x 100	log Dose	Percent Mortality	Angle	3 Point Moving Average
5.6	0.748	0	10.2	--
18	1.255	12.5	20.7	25.3
56	1.748	50	45.0	48.5
180	2.255	100	79.8	--

$$\text{Estimated log LC50} = X + (X'-X)\left[\frac{45-Y}{Y'-Y}\right] ,$$

where: $X = 1.255$ $\quad Y = 25.3$

$X' = 1.748$ $\quad Y' = 48.5$

Therefore:

$$\text{Est. log LC50} + 2 = 1.255 + (1.748-1.255)\left[\frac{45-25.3}{48.5-25.3}\right]$$

$$= 1.674$$

Thus: Estimated 96-hr LC50 = 0.47% Purifaxed Activated Sludge

For 95 percent Confidence Limits (refer to Harris (57)):

$$g = \frac{1641.4\ (1.96)^2}{(3)^2(8)(23.2)^2} = 0.163$$

$$A = \frac{45-25.3}{48.3-25.3} = 0.849$$

$$A' = 1.0 - 0.849 = 0.151$$

From Table 1, Harris (57):

$$A_u' = 0.602, \qquad A_1 = 0.398$$

$$A_1' = 0.440, \qquad A_u = 1.440$$

Confidence limts:

$$X + (X'-X)(A_1) \quad , \quad X + (X'-X)(A_u)$$

In this case the 95 percent Confidence Limits for log LC50 are:

$$1.255 + (1.748-1.255)(0.398) = 1.451$$

$$1.255 + (1.748-1.255)(1.440) = 1.965$$

Therefore the 95 percent Confidence Limits for the 96-hr LC50 are:

0.28-0.92 Percent Purifaxed Activated Sludge

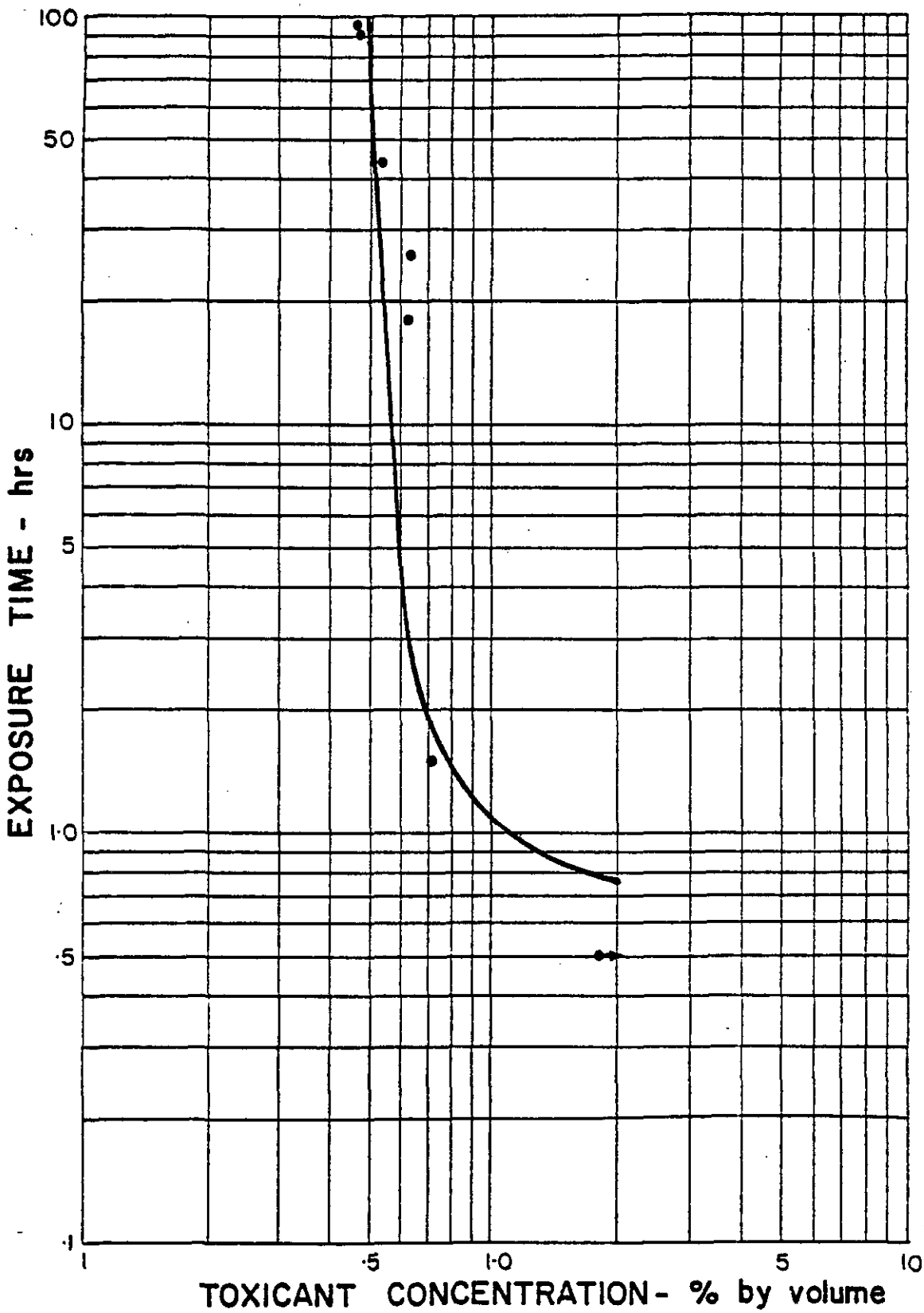


Fig. 7. Toxicity Curve for Purifaxed Activated Sludge. Points represent LC 50's determined at different exposure times

APPENDIX B

CALIBRATION CURVE FOR TOCl MEASUREMENTS

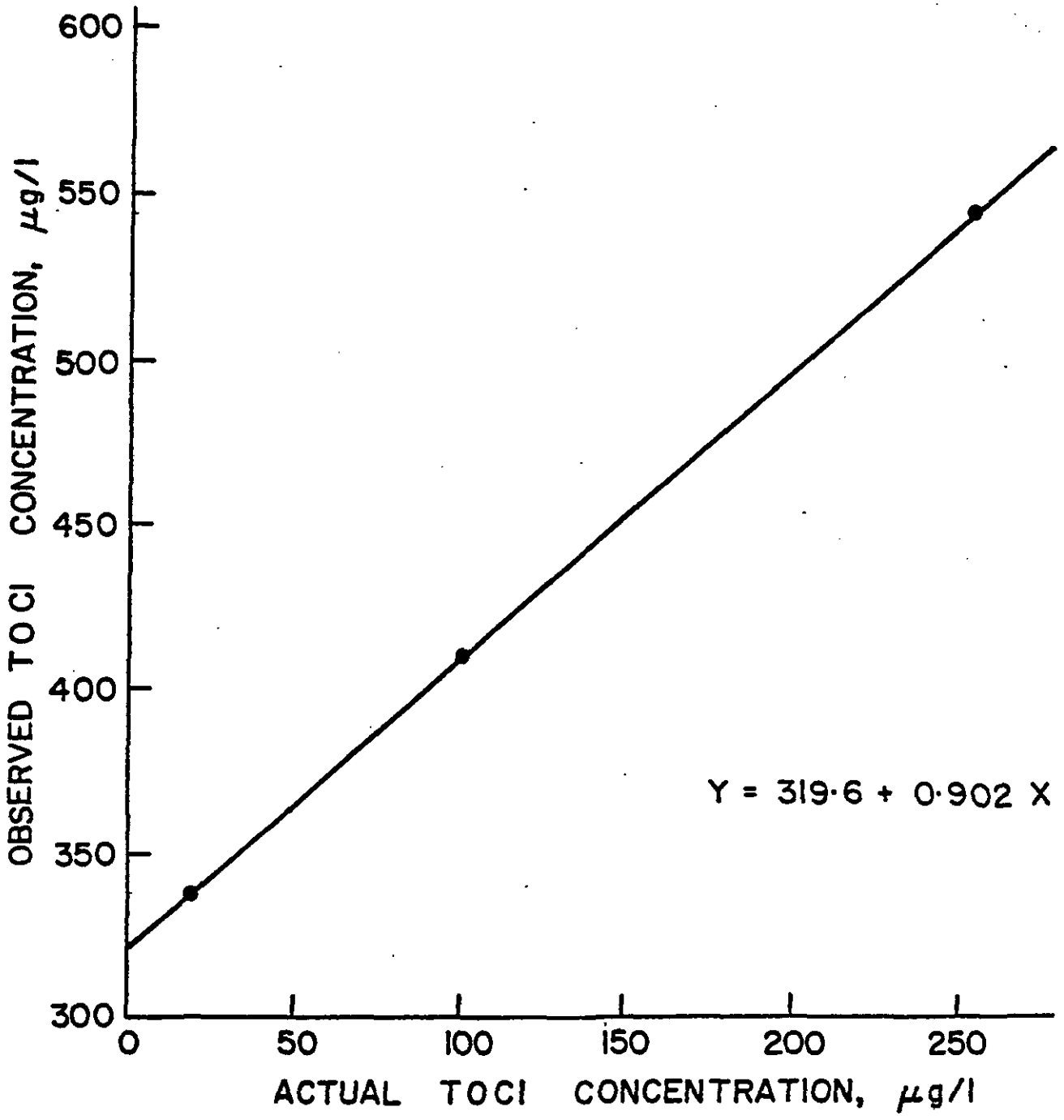


Fig. 8. Calibration Curve for TOCl Measurements

APPENDIX C

BIOASSAY LABORATORY MANUAL

LABORATORY MANUAL

FOR THE

BIOASSAY LABORATORY

Environmental Engineering Program
Department of Civil Engineering
University of Massachusetts/Amherst

PETER H. GROSE

BACKGROUND

Bioassay studies are a valuable tool in the evaluation of the effects of water pollution on aquatic life. Many test organisms are used in bioassays, from algae to invertebrates to fish. A variety of responses may be tested over a large range of time intervals. The most common response tested is death of the test organism, but sub-lethal effects such as decreases in growth and reproductive capacity may also be used. Toxicities can be determined in short term tests (acute toxicities) or long term bioassays (chronic toxicities). Life cycle bioassays may yield useful information showing at which stage of an organism's life it is most vulnerable to a particular pollutant.

The University of Massachusetts Environmental Engineering Bioassay Laboratory is set up primarily for short term bioassay studies with fish. Both static and continuous flow bioassays may be performed. Static tests are those in which the organisms are kept in the same sample of water and toxicant for the duration of the test while in continuous flow tests this water and toxicant solution is continuously replenished.

The laboratory was designed by Larry Smith, a graduate student in the Environmental Engineering Program, and was constructed during the 1976-1977 academic year and the first bioassay runs were performed during the summer of 1977.

DESCRIPTION OF FACILITIES

The following is a brief description of the facilities in the Environmental Engineering Bioassay Laboratory. A floor plan of the laboratory is shown in Figure 1 and may help in establishing the locations of the various components in the room.

High quality distilled, deionized water is available from the Millipore Super-Q water treatment system (EVE #0053) mounted on the back wall of the bioassay laboratory. This unit operates in a recirculation mode whereby only part of the water flow passing through the system is available and the rest is pumped (pump-EVE #0054) back to the beginning of the system to keep water quality high. A flow controller sets the flowrate of finished water at 0.3 gal/min (1.14 l/min), and water quality is monitored by a direct reading resistivity meter (range: 0.5-18 megohms/cm).

The treatment scheme of this system is as follows: Amherst tap water first passes through a prefilter, then a carbon adsorption bed for removal of organics. From there the water goes through a strong acid-strong base ion exchange column for removal of ionic contaminants, and finally through a Millitube membrane filter. This system is presently modified to have a second ion exchange cartridge in place of the Millitube filter. The water quality obtained from this system is excellent (15-18 megohms/cm).

Two large cylindrical plastic tanks near the Super-Q system provide storage for dilution water and a chemical feed solution. The chemical feed is made up using a high strength solution of the dilution water such that the chemical feed solution and the Super-Q water can be

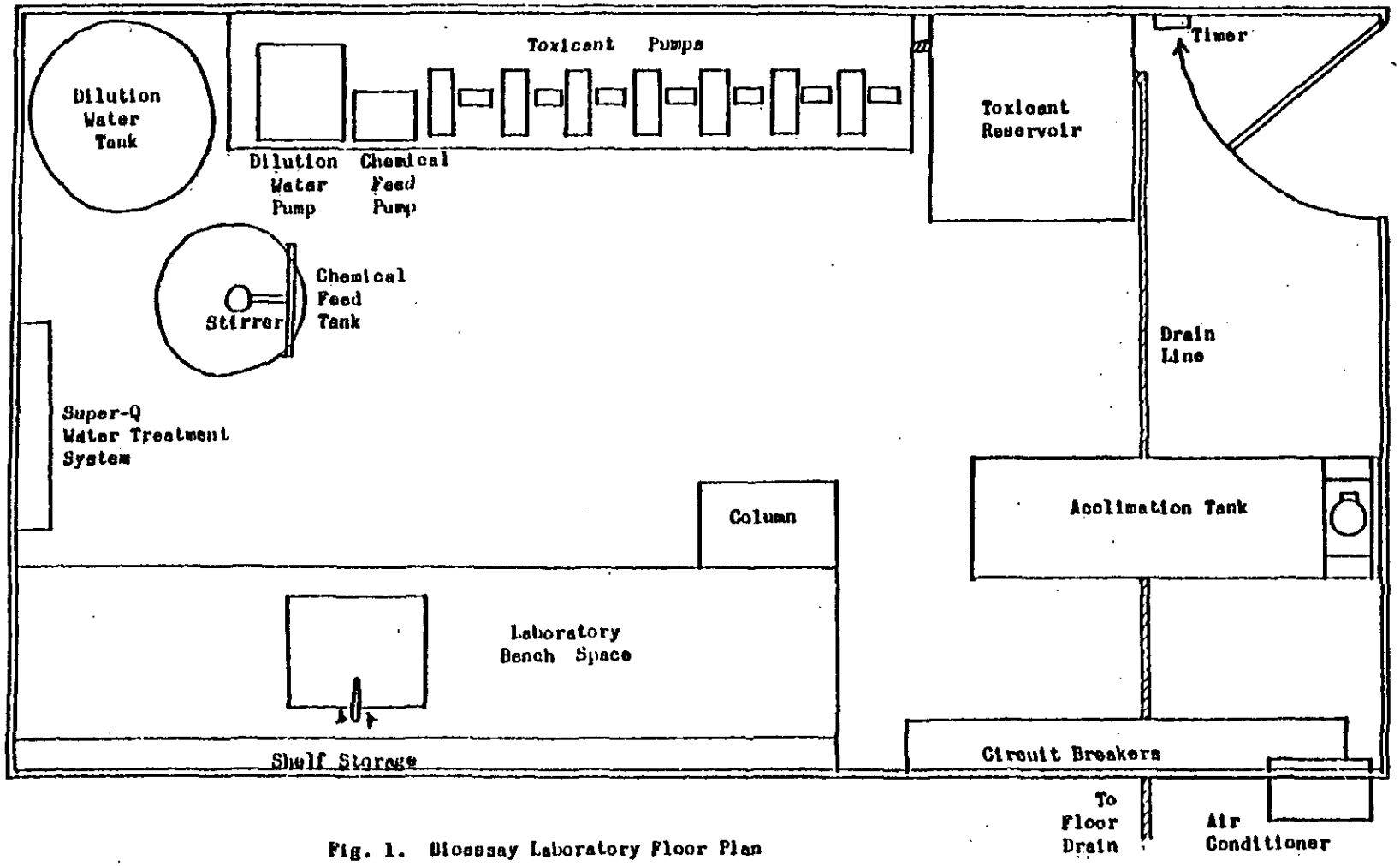


Fig. 1. Bioassay Laboratory Floor Plan

added concurrently to the dilution water tank to yield the concentrations of chemicals desired for the dilution water. The chemical feed tank holds 425 ℓ and is equipped with a mechanical stirrer (EVE #0191) for mixing. The chemical feed is pumped to the dilution tank by a Cole Parmer Console Masterflex Pump (EVE #0062) which provides accurate flowrates over a large range of flows.

The dilution water tank holds 600 ℓ and has a supply of filtered air which can be bubbled into the tank to provide aeration and some agitation. If a continuous flow bioassay is being conducted, a 10 channel drive Masterflex pump (EVE #0061) can be used to supply up to 10 bioassay tanks with dilution water. A dual head variable speed Masterflex pump is available for dilution water pumping if a full complement of 12 tanks are used.

A 400 ℓ rectangular tank is located to the right of the bioassay tanks to provide storage of toxicants used in continuous flow bioassays. The toxicant is pumped to the bioassay tanks at predetermined rates through 5 dual head variable speed Masterflex pumps. These pumps are fitted with a variety of pump head sizes so that a proper range of toxicant concentrations may be tested and still allow the pumps to operate at optimum rotational speeds. Proper plastic tubing sizes should be selected for use with these pump heads. All tubing lines and pumps are coded with color tape for easy tracing and identification of the lines.

The bioassay tanks are made of stainless steel and hold approximately 19 liters of liquid. Twelve of these tanks are mounted on a movable rack in two rows of six tanks each. The top of the rack provides a shelf for the above-mentioned pumps. The tanks are equipped with drain standpipes for continuous flow operation. These drains are

connected by plastic tubing to a larger common drain line which crosses the laboratory floor and goes through the wall to empty into a floor drain in Room 5. Three extra stainless steel tanks are stored on the bottom of this rack.

Prior to use in the bioassays, test fish are kept in a Frigid Units "Living Stream", a 250 ℓ (65 gal) acclimation tank providing constant temperature and continuous water circulation and aeration. A 1/6 HP motor drives the cooling and recirculation unit at the head of the tank which also aerates the water. The water flows through two fish compartments and then is filtered by a sponge rubber filter, and a carbon bed before returning to the head of the tank by a channel under the false bottom of the tank. A dial on the cooling unit provides control in a temperature range of 1.0-21.0°C, though these readings do not exactly correspond with actual temperatures, requiring the use of a thermometer in the tank to monitor temperature accurately.

After use the acclimation tank should be drained, washed with mild detergent and rinsed repeatedly. A final rinse with distilled water is recommended. The acclimation tank should be filled with the same dilution water as that to be used in the bioassays and should be kept at the temperature desired for the bioassays as well. The carbon used in the second filter is aquarium grade bone char (available in 50 lb bags from Frigid Units, Toledo, Ohio) and must be washed prior to use to remove carbon dust.

The ambient air of the bioassay laboratory is cooled to the desired temperature by a White-Westinghouse air conditioner which vents into an exhaust pipe in Room 5. The room temperature should be monitored carefully as this indirectly controls the water temperature of the

bioassay tanks.

The light and dark cycle of the bioassay laboratory is regulated by a timer mounted on the wall behind the door. This seven day cycle timer can be set for different daily light periods as it is wired into the overhead lights (but not the outlets). The wall switch for the lights should be left on for proper operation. The timer is currently set to provide 15 light hours and 5 dark hours daily.

PROCEDURE

The following is a brief outline of the procedures recommended for conducting bioassays in the Environmental Engineering Bioassay Laboratory. The reader is advised to consult other references for further background on bioassay techniques, especially Standard Methods (1).

The researcher must select the species of fish he wishes to use and find a reliable source of healthy specimens before starting his experiments. Good results have been obtained using fathead minnows (Pimephales promelas) from the EPA's Newtown Fish Toxicology Station (3411 Church Street, Cincinnati, Ohio 45244). One should contact Jim Ruwaldt (513-684-8601) several weeks in advance to ask for fish and then call just before the shipping date to confirm the flight number and arrival time at Bradley International Airport. In the past the fish have been free of charge, but the researcher must arrange for payment of the air freight bill. The fish were shipped on Allegheny Airlines to the Bradley International Airport Cargo Terminal, where they could be picked up and driven to the lab. It is difficult to obtain fish in the winter time due to the icing over of holding ponds, so it is advised to plan to run bioassays in the warmer months.

The fish are shipped in a plastic bag containing water and oxygen, which is then placed in a sturdy cardboard box. Survival rates through the shipping procedure have been excellent. Once in the laboratory the fish are carefully poured with some of the water in bag into the acclimation tank. The water temperature of the arriving fish shipments has been about 18°C and the temperature of the acclimation tank should be adjusted to this level ahead of time to prevent temperature shocking of the fish.

It is advisable to treat each new shipment of fish with antibiotics to keep them healthy. The Newtown Fish Toxicology Station recommends applications of Tetracycline or Neomycin at 30 ppm and Dexon at 20 ppm for several days as a preventative treatment. Good results were obtained here with the commercial preparations Maracyn and Maracyn-Two from Mardel Laboratories, Inc. The fish should be monitored periodically for any external signs of disease, fungus or parasites and treated accordingly if problems are detected.

An acclimation period of two weeks is recommended prior to the start of a bioassay run. During this time fish deaths should be recorded and the dead bodies removed as soon as possible. If fish deaths reach 10 percent of the original population during this time, the lot should not be used. The remaining fish should be destroyed and the acclimation tank carefully washed.

The fish should be fed one or two times a day, though they should remain healthy if not fed for a week or more. Tetramin, a commercial fish food mix has been used here as the sole diet of the fish. Besides feeding the fish, the researcher should add additional dilution water to make up for evaporation and should occasionally remove the solids

that accumulate on the floor of the second fish compartment.

The dilution water used in bioassay studies should be made from a reproducible combination of very high quality water and specified amounts of selected chemicals. A number of "recipes" are available in the literature for making up dilution water that is good for the fish and that gives desired hardness and sufficient alkalinity to provide some buffering capacity. The following is a simple formula providing a soft water with hardness of 40-48 mg/ℓ, as $CaCO_3$ and alkalinity of 30-35 mg/ℓ as $CaCO_3$:

$NaHCO_3$	48 mg/ℓ
$CaSO_4 \cdot 2H_2O$	30 mg/ℓ
$MgSO_4$	30 mg/ℓ
KCl	2 mg/ℓ

A concentrated solution of this dilution water should be made up in the chemical feed tank of such strength that it can be pumped to the dilution water tank simultaneously with the flow of 0.3 gal/min of Super-Q water to deliver the desired final dilution water concentrations.

Preliminary bioassays are used prior to full scale bioassays to provide information on the range of toxicant concentrations to be used in the full scale tests. The preliminary tests are small scale (3 liter glass jars containing 2 fish apiece are suggested), but conditions such as toxicant preparation, dilution water and temperature should be as close as possible to those used in the full scale assays. Measurements of temperature, dissolved oxygen (DO) and pH should be taken several times during these tests, as well as measurements of any other substances thought to be toxicologically important in that particular bioassay. A wide range of toxicant concentrations is prepared and survivals noted for the chosen test period length. A control jar

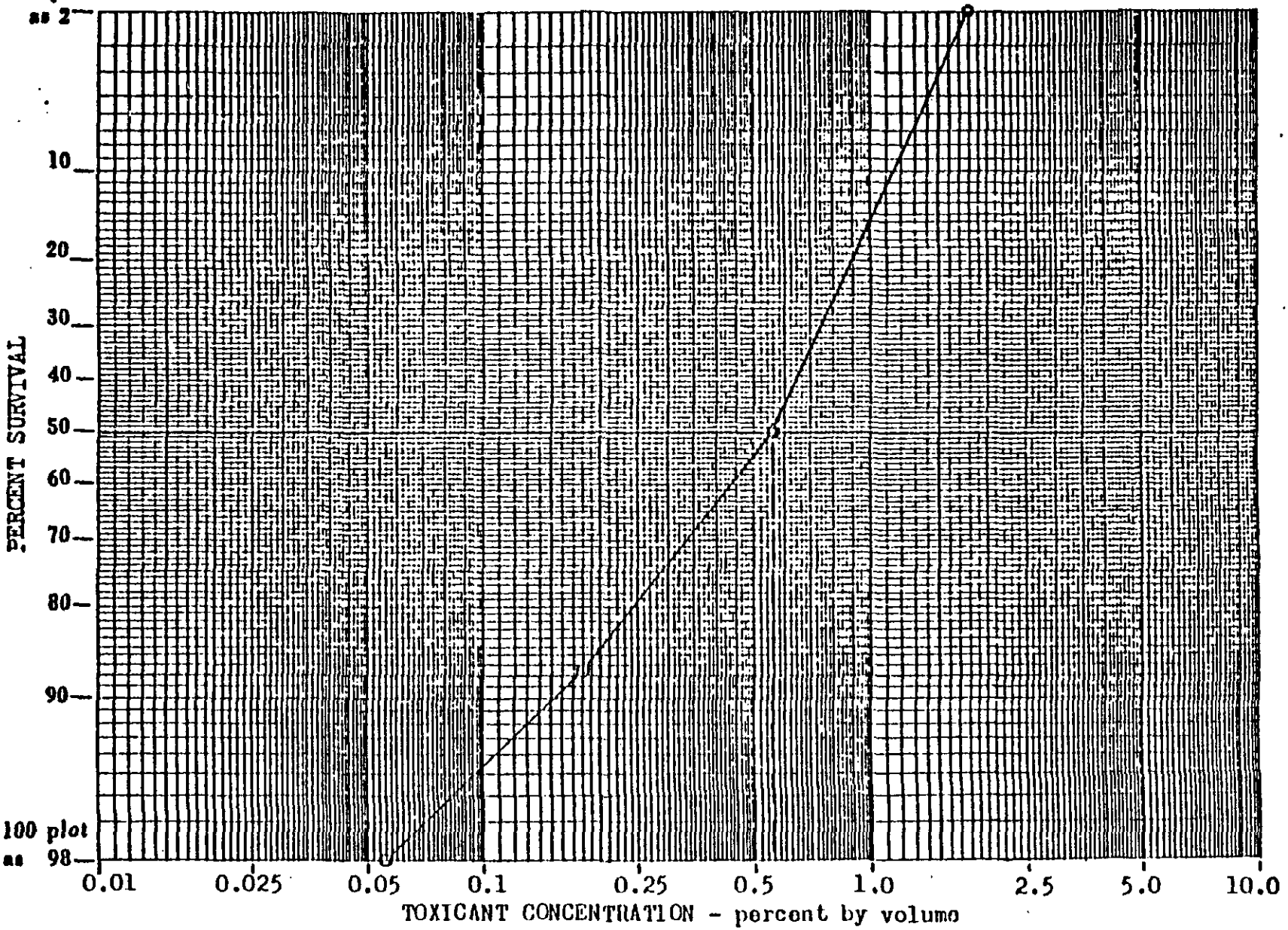
(no toxicant) is not required, but may be desirable as a check on the health of the fish.

Once a preliminary bioassay has been completed, the results may be used to determine a rough LC50 value (LC50 denotes the lethal concentration to 50 percent of the fish) by a graphical technique using log-probability paper (see Figure 2). The fish survivals are plotted (as percents) for the various toxicant concentrations tested, with fish survival on a probability scale and toxicant concentration on a logarithmic scale. A line is drawn connecting successive points and the LC50 is determined from the line drawn between a toxicant concentration in which more than half the fish survived and a concentration at which less than half the fish survived. The toxicant concentration at which this line crosses the 50 percent survival line is reported as the LC50 and is proceeded by the time period of the test (e.g. 96-hr LC50).

The toxicant concentrations to be used in the full scale bioassays are chosen around this LC50 and spaced such that intervals between them are equidistant when plotted on a logarithmic scale. Several toxicant concentration series are recommended in Standard Methods (1). Regardless of whether static or continuous flow tests are to be used, the bioassay tanks should be filled with these solutions just prior to the start of the tests.

The number of fish used should be enough to give good statistical reliability but not so many as to exceed recommended loading rates (1). Duplicate series of the same toxicant concentration are desirable for the most precise results. More fish can be used in the continuous flow tests due to the constant addition of fresh dilution water and toxicant.

Fig. 2. Determination of 96-hr LC50 for Full Scale Bioassay on Purifaxed Activated Sludge by Graphical Technique



Fish selection at the start of the test should be random so that unhealthy fish are not selected first and placed in the same tank. The weight and length of the test fish should be recorded at this time.

Static Bioassays

Once the series of toxicant concentrations is chosen for a bioassay run, conducting a static bioassay is fairly simple. The toxicant solutions are made up and fish added to the tanks as outlined above, marking the start of the test. The number of fish surviving in each tank should be recorded each day, and more frequently at the start of the run. The pH, temperature, dissolved oxygen concentration and any other parameters important to a particular toxicant should be measured at the start of the run and on each subsequent day for as long as the test runs.

At the conclusion of the test the fish survival data can be used to compute an LC50 value for the toxicant tested, either by the graphical method described earlier or by one of a number of statistical methods found in Standard Methods (1).

As a check on the assumption that 96 hours or whatever test length chosen is sufficiently long to measure all the acute toxicity of the toxicant, the researcher may wish to plot a toxicity curve. To construct this curve, LC50 values must be determined for a number of time intervals from start to finish of a bioassay run. These LC50's are plotted on log-log paper with the toxicant concentration on the abscissa and the exposure time on the ordinate (see example in Figure 3). If the toxicity curve has become asymptotic to a line parallel to the time axis, one may conclude that the acute toxicity of the sample has terminated.

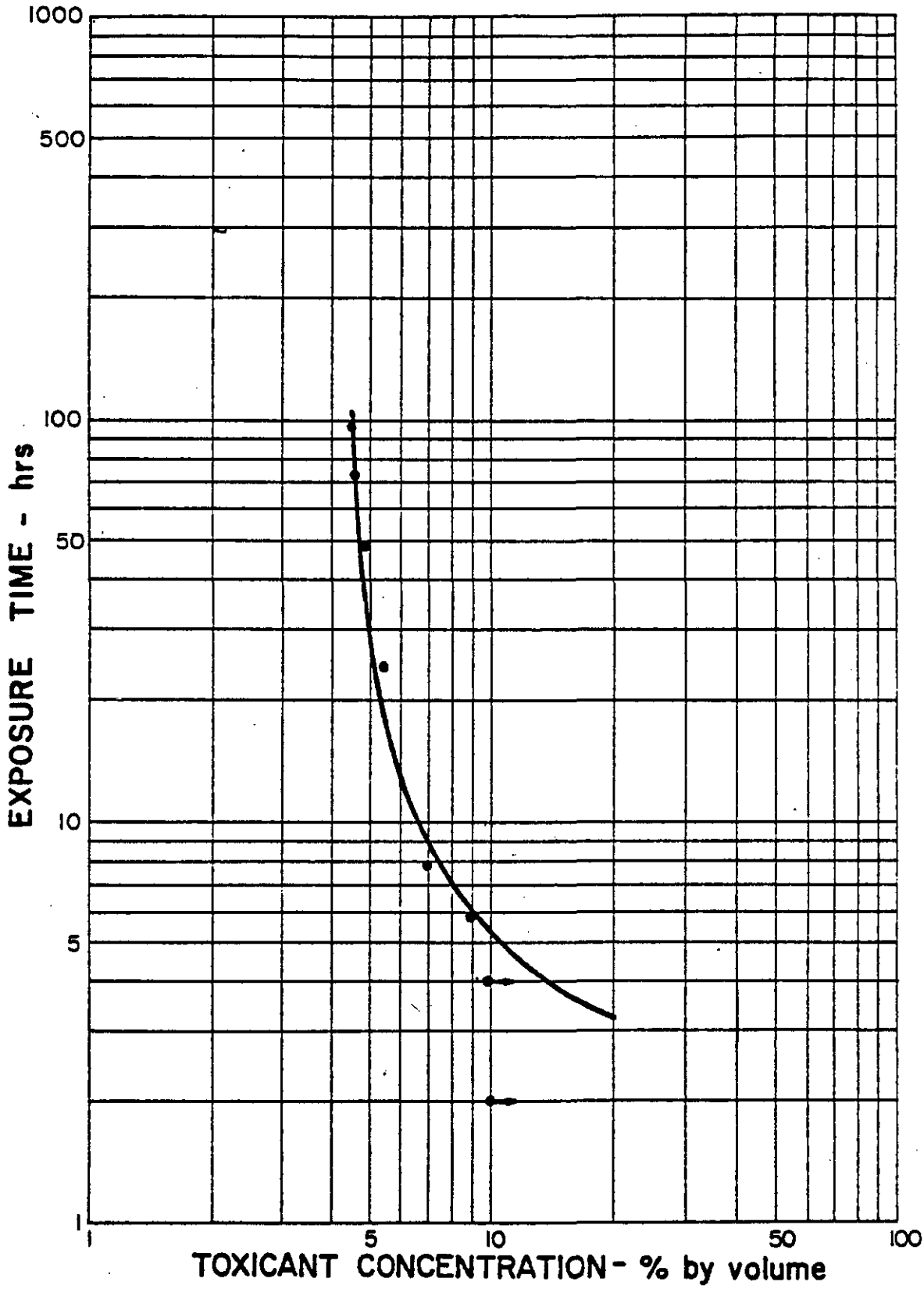


Fig. 3. Toxicity Curve from Standard Methods (3). Points represent LC50's at different exposure times.

Continuous Flow Bioassays

Continuous flow bioassays require constant addition of dilution water and toxicant for the duration of a bioassay run. A helpful guideline is that the volume of the bioassay tanks should be replaced six times a day. (i.e. in a 19ℓ tank the desired flowrate to the tank is 6 x 19 = 114 ℓ/day.) Care must be taken to insure the flowrates of dilution water and toxicant are set to provide the desired toxicant concentration over the test period.

The dilution water is supplied from the dilution water tank and the volume of dilution water should be monitored carefully so that the Super-Q system and chemical feed pump may be turned on to deliver additional dilution water when required. The toxicant is supplied from the toxicant reservoir and should be stirred occasionally to give a homogeneous toxicant sample. Addition of a mechanical mixer to this tank is recommended.

The flowrates of dilution water and toxicant entering each bioassay tank should be recorded periodically and adjusted if necessary. Problems with inconsistant flowrates and occasional clogged toxicant delivery lines demand frequent attention if proper toxicant concentrations are to be maintained in the bioassay tanks. These flows should be combined at the conclusion of a bioassay to give average flowrates which can then be used to find actual toxicant concentrations in the tanks. (These calculated concentrations should not be very different from original desired concentrations.)

The dilution water and toxicant lines should empty into a trough or mixing chamber mounted inside the edge of the bioassay tanks to provide some mixing between the two streams prior to spilling into the tanks. This premixing eliminates the problem of full strength toxicant plumes

coming in contact with the fish when only weaker dilutions of toxicant were intended to be tested.

Fish survival data and chemical measurements should be carried out in these bioassays as outlined for static tests, and the determination of LC50 values is the same, once actual toxicant concentrations are calculated. The researcher is urged to pay close attention to the flowrates of both dilution water and toxicant, because significant disruptions in these flows for any sizeable period of time may necessitate termination of the bioassay run due to gross variations in the toxicant concentrations the fish are exposed to.

REFERENCES

1. American Public Health Association, Standard Methods for the Evaluation of Water and Wastewater, 14th Ed., Washington, D.C. (1975).

APPENDIX D

TOTAL ORGANIC CHLORINE APPARATUS LABORATORY MANUAL

LABORATORY MANUAL

FOR THE

TOTAL ORGANIC CHLORINE APPARATUS

Environmental Engineering Program
Department of Civil Engineering
University of Massachusetts/Amherst

PETER H. GROSE

TOTAL ORGANIC CHLORINE MANUAL

PURPOSE

Total organic chlorine (TOCl) is a composite parameter that is a measure of the chlorinated organic compounds in a water sample. While it does not identify specific compounds, TOCl measurement may provide a valuable tool to be used in detecting chlorinated organics, many of which have detrimental health effects.

The pyrohydrolysis method discussed here was developed by Kuhn, Sontheimer and Fuchs (1) in the Federal Republic of Germany in an effort to determine the performance of granular activated carbon filters in removing organic contaminants in water treatment facilities. This technique was modified by Dressman, McFarren and Symons (2) so that it could be used to test grab samples of water, as this apparatus is set up to do.

THEORY

Organic chlorine compounds in the water sample are adsorbed onto granular activated carbon in a completely mixed reactor over a period of one hour. The carbon sludge is allowed to settle, collected and put in a combustion boat for pyrohydrolysis. A series of two tube furnaces is used in the pyrohydrolysis step, connected by a quartz tube and supplied with a carrier stream of oxygen and steam. In the first furnace the temperature is increased rapidly from under 100^{o}C to 700^{o}C, causing the chloroorganics to be volatilized and passed on to the second furance, where the 1000^{o}C temperature combusts these compounds to HCl. The HCl is carried out to a condensor where the pyrohydrolyzate is collected and the chloride ion concentration is then measured by one of a number of methods. From this measurement, the TOCl concentration can be determined.

Care must be taken to prevent interference from inorganic chloride ions present in the water sample. Dressman, McFarren and Symons outline two methods of dealing with this problem. They recommend the "direct" method of TOCl measurement in which two steps are taken to exclude inorganic Cl^- from being measured. First sodium nitrate is added to the water sample prior to introduction of the carbon in order to reduce or prevent adsorption of inorganic Cl^-. Secondly the collected carbon sludge is mixed with a sodium nitrate solution so that the nitrate will displace the chloride ions that were adsorbed on the carbon.

LABORATORY SETUP

Carbon Preparation

The carbon used in this procedure is ground Filtrasorb 400 granular carbon. Dressman et al. (2) suggest the carbon be ground for 90 seconds in a laboratory mill, but do not specify the size distribution of this resulting ground carbon. If an automatic grinder is not available the carbon may be ground by hand with a mortar and pestle and graded in standard soil sieves. The selection of the size distribution of the carbon used for adsorption is left to the researcher. The initial work done with this technique used carbon particles between 30 and 60 sieve sizes. The carbon must be washed with high quality distilled, deionized water such as that treated with the Millipore Super-Q system to remove the carbon dust on the particles, and then dried in an oven.

Contactor

The carbon will be mixed with the water sample in a contactor. The container is a cylindrical glass jar with 13 liter capacity (Fisher 2-525D) and is set in a circular stand with mounting bars. Mixing is provided by a mechanical stirrer ("Lightnin" Model L mixer, EVE #0192) mounted on this stand and connected to a rheostat (EVE #0177) so that speed of rotation may be controlled.

If small carbon grain sizes are used in the adsorption step of this procedure, flocculent aides may be needed to settle the carbon to the bottom of the contactor. Two solutions are suggested by Dressman et al. (2). The first is an aluminium sulfate solution (1 g Al/l) and the second is a solution of a commercial polyacrylamide floc aide (Magnifloc 985N, 200 mg/l) which has been obtained from the American Cyanimid Company.

Filtering Apparatus

The carbon sludge collected after adsorption will need to be filtered through a Millipore 8μ pore size cellulose acetate filter (47 mm diameter, #SCWRP04700). An all glass filtering apparatus is recommended and a vacuum pump is required.

Pyrohydrolysis Unit

The basic components of the pyrohydrolysis unit are two tube furnaces, an oxygen and steam supply and a condensor, as shown in Figure 1. The furnaces are Lindberg model #55035 split tube furnaces (VWR #30760-058; EVE #0345,0346) that can be heated to 1000°C. There is a temperature indicator and a control dial to set the temperature, though there is not a direct correlation between the two and the dial settings must be calibrated before use. The two furnaces are connected by means of a quartz combustion tube that has ground glass connections fused on its ends (20 mm ID, 23 mm OD quartz tube stock, Heraeus Amersil #0810230E36, modified with ♀ 24/40 connections by the University of Massachusetts Glass Shop).

Oxygen is supplied from a T size cylinder of extra dry oxygen (from Merriam Graves) and passed through a two-stage regulator (Airco model 8401). The regulator is connected by Tygon tubing to a flow regulator and flow meter. The flow regulator currently in use is somewhat too large and a smaller flowmeter has been secured for determination of more accurate flowrates. The new device is a Brooks Instruments type 1555-00A1AAN flowmeter with tube size R-2-15-AAA. This has now been modified with a carballoy float ball for measurement of the proper range of O_2 flowrates

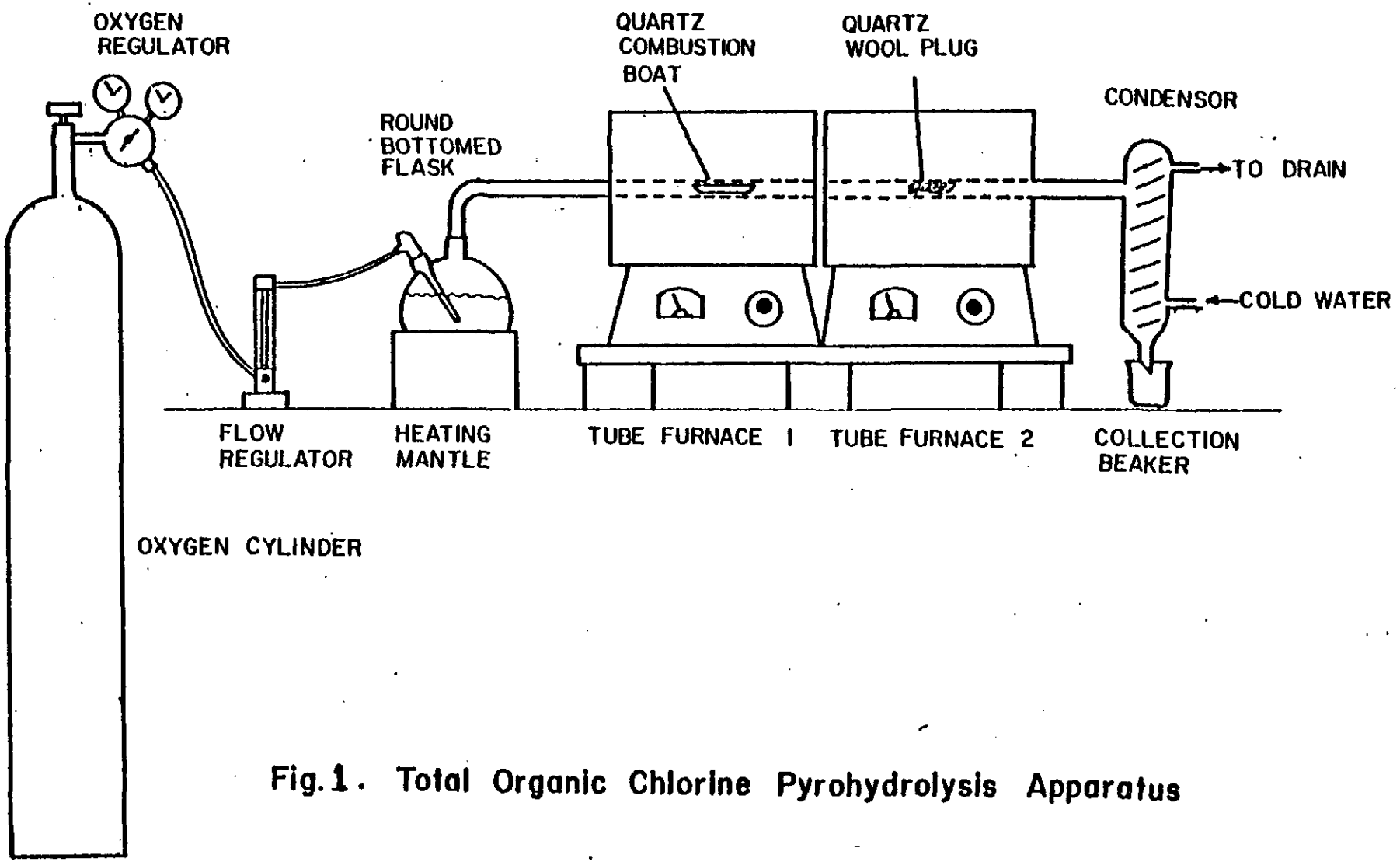


Fig. 1. Total Organic Chlorine Pyrohydrolysis Apparatus

required for the pyrohydrolysis tests. Calibration data for this meter is included as Table 1.

Water vapor is added to the system by heating a 1000 ml round bottomed flask containing distilled deionized water. This flask (Lab Glass LG-7291-254) has two ℔ 24/40 necks, which fits in an appropriately sized heating mantle (VWR #33752-125; EVE #0347). This mantle is connected to a rheostat (EVE #0118) for control of heating temperature. The metered oxygen is bubbled into the water in the flask by means of a gas inlet adapter (Lab Glass LG-1972-102) placed in the side neck of the flask. Oxygen and steam leave the flask through the center neck and enter the combustion tube via a 90^{0} glass bend (with two ℔ 24/40 female connections).

After passing through the furnaces, the pyrohydrolyzate is condensed in a COD-type condensor (Fisher #7-744-5) and collected in a beaker (250 ml). The condensor is supplied with cooling water from a nearby tap and drains into a sink.

Before using the pyrohydrolysis unit a plug of quartz wool is inserted into the second (high temperature) furnace. A large quantity of quartz wool is on hand and was obtained from Heraeus-Amersil, Inc. (#09WC08).

The quartz combustion boats used for holding the carbon samples placed into the furnace were made by the UMASS Glass Shop from quartz tube stock (14 mm ID, 16 mm OD, Heraeus-Amersil #08T0160C36) and are approximately 12 cm long. These may be placed in the combustion tube and slid into the first furnace with a metal rod and may be pulled out later by means of a wire with a small hook in its end.

ROTAMETER CALIBRATION DATA

BROOKS INSTRUMENT DIVISION
EMERSON ELECTRIC CO
HATFIELD, PENNSYLVANIA 19440
EMERSON

CUSTOMER	CUSTOMER ORDER NUMBER	BROOKS SERIAL NUMBER
University of Massachusetts	B 50436	7904 H73521

MAX. FLOW	MIN. FLOW	UNITS	METERED FLUID	DATE
0.224	0.012	STD. LIT./MIN.	OXYGEN	05 JAN 1979

MAX. PRESS. DROP			ATMOS. PRESSURE		
MODEL NUMBER			METERING TEMPERATURE	70.00	DEG.F
TUBE NUMBER	R-2-15-4AA		METERING PRESSURE	14.70	PSI A
FLOAT NUMBER	SPHERICAL		METERING DENSITY	0.001330	GMS/CC
FLOAT MATERIAL	CARBOLOY		FLOAT DENSITY	14.95	GMS/CC
STD. CONDITIONS	1 ATMOS. & 70°F		DENSITY AT STD. COND.	0.001330	GMS/CC
PERF. CURVE NO.	0010		METERING VISCOSITY	0.02045	CP.

STD. LIT./MIN.

INSCRIPTION

SCALE READING	FLOW	SCALE READING	FLOW	SCALE READING	FLOW	SCALE READING	FLOW	SCALE READING	FLOW
148.9	0.224	104.2		52.8					
147.6		103.1		51.2					
146.3	0.22	102.0	0.14	49.5	0.06				
145.0		100.8		47.8					
143.7		99.7		46.2					
142.5		98.5		44.5					
141.2		97.4		42.8					
139.9	0.21	96.3	0.13	41.0	0.05				
138.6		95.1		39.3					
137.3		94.0		37.6					
136.0		92.8		35.9					
134.8		91.7		34.1					
133.8	0.20	90.6	0.12	32.4	0.04				
132.8		89.3		30.7					
131.8		87.9		28.6					
130.8		86.6		26.3					
129.8		85.2		24.0					
128.8	0.19	83.9	0.11	21.6	0.03				
127.9		82.5		19.3					
126.9		81.1		17.0					
125.9		79.8		14.7					
124.9		78.4		12.6					
123.9	0.18	77.0	0.10	10.6	0.02				
122.9		75.7		8.5					
121.9		74.4		6.4					
120.9		73.1		4.3					
119.9		71.9		2.2	0.012				
118.8	0.17	70.7	0.09						
117.7		69.5							
116.5		68.2							
115.4		67.0							
114.3		65.8							
113.2	0.16	64.5	0.08						
112.1		63.3							
111.0		62.1							
109.8		60.9							
108.7		59.5							
107.6	0.15	57.8	0.07						
106.5		56.2							
105.4		54.5							

Table 1. Flowmeter Calibration Data.

PROCEDURE

The TOCl analysis is divided into three main parts: extraction of chloroorganics from the water sample; pyrohydrolysis; and measurement of chlorine ions. A brief description of the procedure used in the initial experiments with this apparatus follows below. The reader may also wish to consult the Dressman *et al.* article (2) which served as a basis for a large part of this procedure.

Extraction

Add 10 liters of water sample to the contractor. The sample should previously have been treated to remove suspended solids (by filtration, centrifugation or settling). Smaller sample sizes may be used, but mixing is poor if the sample is much less than 5 liters. Turn on stirrer (a rheostat setting of 40 is good), add 685 mg of sodium nitrate for every liter of sample (i.e. 6.85 g for a 10 liter sample), and allow this to dissolve. Add 1 g of carbon prepared as detailed earlier and mix for one hour.

Turn off stirrer and allow the carbon to settle. (If small carbon particles are used, the flocculation aids must be added at this time, a slow mixing period provided, followed by a long settling time). For larger carbon sizes several minutes will be sufficient. Pour or siphon off the supernatant of the carbon sludge, collecting it carefully in another container, leaving about 200 ml behind with the sludge. Collect sludge quantitatively in a 500-1000 ml beaker.

Filter this sludge through a 8μ pore size cellulose acetate filter, washing it onto the filter with distilled deionized water. Return the filter cake to the same beaker and add 200 ml of 685 mg/l sodium nitrate

solution (500 mg/l NO_3^-). Stir this at slow speed for one hour on a magnetic stirrer. At the end of this mixing period, refilter the sludge as before, allowing it to dry well on the filter paper. Carefully transfer this filter cake into a quartz boat. It is advisable to place the quartz boat on a sheet of paper so that stray carbon particles can be collected and returned to the boat.

This entire sequence is then repeated with the supernatant of the original extraction. More $NaNO_3$ is added, followed by a second gram of carbon. The carbon is collected, filtered, mixed with sodium nitrate solution for one hour, refiltered and then added to the first gram of carbon in the quartz boat.

Pyrohydrolysis

The first task of the pyrohydrolysis step is to preheat the furnaces and the water in the round-bottomed flask. The second furnace should be set at 6.8-7.0 about 3 hours before the pyrohydrolysis run is scheduled to obtain a temperature of 1000°C and a half hour before the start of a run the first furnace should be set to LOW and the heating mantle turned on high (rheostat at 100). The high temperature furnace can be preheated in a shorter period of time by setting the control on HIGH, but care should be taken to turn the dial down to about 7.0-7.4 when 1000°C is reached. This is the maximum temperature rating of the furnace and higher temperatures should be avoided.

Just before the start of a pyrohydrolysis run the first furnace should be 50-90°C and the second furnace should be 1000°C. The water in the round bottomed flask should be boiling or steaming heavily. Turn the

rheostat connected to the heating mantle down to 60 so that a more moderate temperature will be achieved. (This should be done about 5 minutes before starting time.) Place the collection beaker under the condensor and start water flowing to the condensor.

Turn on the oxygen valve on top of the cylinder and then open the regulator so the exit pressure reads 2 psi. Set flowmeter so that the required 200 ml/min flowrate is obtained. (This flowrate can be checked ahead of time by bubbling the oxygen into a large graduated cylinder full of water inverted in a basin of water.) On the old flow regulator this setting is such that the top of the float ball is 1/8 inch below the top of the letters SCFH near the bottom of the flowmeter. Place gas inlet adapter into flask so that oxygen is now bubbling into the hot water.

Place quartz boat containing the two grams of carbon into the first furnace and then slide the heating mantle and round bottomed flask assembly toward the furnaces, making sure the 90^{o} elbow and the combustion tube fit securely together. Now change the setting of the control dial on the first furnace to HIGH.

It should take approximately 15 minutes for the first furnace to reach $700^{o}C$, at which time the dial should be turned down to 4.6-4.7 in order to keep the temperature at $700^{o}C$ in this furnace. Allow the test to run a full hour from the starting time, checking the furnaces periodically to make sure the proper temperatures are maintained.

At the end of the hour remove the collection beaker from under the condensor and cover it for protection. Turn off both furnaces, the heating mantle, the oxygen tank valve and the water flow to the condensor. Allow the furnaces to cool for several hours or overnight before removing the quartz boat.

The volume of the pyrohydrolyzate should be carefully measured, especially if the chloride ion measurement method measures Cl^- concentration rather than the total amount of chloride ions in the pyrohydrolyzate.

Chloride Ion Measurement

Chloride ion measurement may be done one of three ways: bench titration; chloride ion probe; or by microcoulometry. Dressman et al. (2) found microcoulometry to be the method of choice and its use is urged in the future for the best possible results. Early TOCl tests were done using the Mercuric Nitrate Titration method described in Standard Methods (3). This technique was found to be adequate but more precision at very low chloride ion levels is desired.

DATA ANALYSIS

A number of TOCl runs should first be conducted on distilled water samples to obtain a background value for this test. Dressman et al. (2) found a fairly consistant background value of 10 µg/l TOCl with distilled water, but early experiments here resulted in an average of 320 µg/l TOCl. The TOCl concentration of the sample may be calculated by one of the following formulas:

1. If the total amount of chloride ions present in the pyrohydrolyzate was measured (such as by titration) the following formula holds:

$$\text{TOCl } (\mu g/l) = \frac{X}{V_1},$$

where: X = total µg of Cl^- in pyrohydrolyzate

V_1 = volume of water sample (liter)

2. If the concentration of Cl^- in the pyrohydrolyzate is measured (such as by microcoulometry) the following formula is used:

$$\text{TOCl } (\mu g/l) = \frac{C\ V_2}{V_1}$$

where: C = concentration of Cl^- in pyrohydrolyzate (mg/l)

V_1 = volume of sample (liter)

V_2 - volume of pyrolydrolyzate (ml)

The total organic chlorine concentration for the water sample is then reported as this calculated value minus the background TOCl value reported for distilled water. Results from earlier experiments done here using solutions of m-chlorobenzoic acid showed that this was not exactly the case, but rather the actual TOCl value was approximately 1.108 times the

observed TOCl concentration minus a background value. The researcher may wish to run a series of calibration curves prior to conducting the bulk of his research in order to better determine this relationship between real and observed TOCl values.

REFERENCES

1. Kuhn, W., et al., "Use of Ozone and Chlorine in Water Utilities in the Federal Republic of Germany", Jour. Amer. Water Works Assoc., 70, 326 (1978).

2. Dressman, R. C., et al., "An Evaluation of the Determination of Total Organic Chlorine (TOCl) in Water by Adsorption onto Ground Granular Activated Carbon, Pyrohydrolysis, and Chloride Ion Measurement," Presented at Water Quality Technology Conference, Kansas City, Missouri (December 5-6, 1977).

3. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 14th ed., Washington, D.C. (1975).