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Pyrogenic Activity of Carbon-Filtered Waters

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PYROGENIC ACTIVITY OF CARBON-FILTERED WATERS

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes the endotoxin content and pyrogenic response found in granular activated carbon filtered waters. With a better understanding of any health effects, measures can be developed to reduce exposure to potentially harmful materials.

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ABSTRACT

The endotoxin content and pyrogenic response of granular activated carbon (GAC) filtered waters were studied. GAC-filtered secondary effluent from an activated sludge pilot plant contained free endotoxins in the range 6-250 μg/l yielding positive pyrogenic responses in 18 of 20 trials. Samples obtained from 27 different water supplies in the U.S. that utilize GAC adsorption contained free endotoxin ranging from 1.2-25 μg/l but none gave a pyrogenic response. No relationship was discernible between endotoxin content and pyrogenic response.

Small removals of total organic carbon (TOC) by GAC beds which had been in operation in water treatment plants without regeneration for as long as 110 months were observed. However, 5 of 28 samples showed an increase in TOC through GAC and 8 of 28 samples showed an increase in standard plate count. One of 25 samples yielded pseudomonads, but none of the 28 samples contained coliforms.

Good correlations were observed on non-disinfected AWT effluent samples between standard plate count and total endotoxin ($r = 0.945$), standard plate count and free endotoxin ($r = 0.932$), and total coliforms and free endotoxin ($r = 0.939$). Lack of good correlations, however, were observed in assaying AWT samples that had been subject to the disinfecting procedures of chlorination, ozonation, pH or UV irradiation.

This report was submitted in fulfillment of Grant No. R-804420 by Texas A&M University, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period March 9, 1976 to July 31, 1978, and work was completed as of August 29, 1978.

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SECTION 1

INTRODUCTION

The efforts of U.S. Environmental Protection Agency (EPA) programs relating to water pollution control and drinking water safety are resulting in increased emphases on conservation of the water resource and its reuse--but with an over-riding and dominant concern for protection of the public health. Among the agents in water of health concern are some of the organic chemicals -- particularly those commonly grouped as trihalomethanes (THMS) and as synthetics (chemical products produced by man). The unit process employed in water and waste water treatment that appears most promising in providing for a greater degree of control of these unwanted organic chemical substances is activated carbon adsorption.

Activated carbon has a long history of use in both water and waste water treatment. It is available in powdered and granular forms. The powdered form has found its greatest use in water treatment. Granular activated carbon (GAC) has been the form most used in waste water treatment, but this use has been largely restricted to pilot studies. Full-scale plant use of GAC is rare in waste water treatment, Garland, Texas, being a notable example.

Although the GAC function is primarily adsorptive, the observation of a concomitant biological activity has long been observed. In fact, the observation of some denitrification occurring in GAC adsorption columns at the Pomona, California, advanced waste treatment (AWT) pilot plant gave rise to successful experiments designed to enhance this biological process by providing a cheap carbon source via the addition of stoichiometric amounts of methanol. The use of biological processes to treat waste water is common, but the use of such processes to treat drinking water (i.e., water for drinking purposes) raises many apprehensions. A whole generation of engineers schooled in the physical-chemical treatment of water has been produced; few have aquaintance with the former use of slow sand filters in water treatment which process is to a great extent biological.

In addition to this general apprehension, there is the caution expressed by Dr. Joshua Lederberg, Nobel laureate geneticist from Stanford University, who reminded the National Drinking Water Advisory Council of the EPA that man still does not have a complete understanding of the carbon cycle. Nevertheless, he also encouraged the thorough research and application of adsorptive processes as the most promising tools available to obtain better control of unwanted organic substances in our drinking water supplies.

The observation of substantial biological activity in GAC treatment of waste water does not prove that a similar degree of such activity will occur

when treating drinking water. Source waters used for potable supplies have considerably smaller amounts of biodegradable materials than do biologically treated waste waters. Also, water treatment processes frequently employ preliminary disinfection (prechlorination) as well as coagulation, flocculation, and sedimentation techniques with the result that the waters that would reach a GAC process would be of considerably higher quality than in a waste water application. Nonetheless, some degree of biological development in a water treatment application of GAC would be certain.

One of the main concerns about biological growth in GAC filters or beds is the potential for generation of unwanted microorganisms and/or their toxic by-products. Among the microorganisms that might become established are the pseudomonads. They are known for their ubiquity, growth in minimal media, and their importance in denitrification (1). Also, some strains are opportunistic pathogens of man.

Among the toxic by-products to be aware of are the gram-negative endotoxins. Gram-negative endotoxins are lipopolysaccharides which are all thought to cause a pyrogenic response (fever) when injected into animals. The rabbit is used in the standard United States Pharmacopeia (USP) pyrogen test (2), but man can reportedly be 100-fold more sensitive to endotoxins than is the rabbit (3).

Although some lipopolysaccharides will elicit a toxic response in some individual animals when ingested in sufficient amounts, it is supposed to occur only as a result of an increased permeability of the gastrointestinal tract. Contention apparently exists about this point since it is reported that the observation of absorption of endotoxin by the normal bowel is disputed (4). Certainly, the normal bowel of most mammals contains a generous supply of endotoxins (5).

Just as different animals possess different sensitivities to endotoxin, so do different bacteria produce toxins of different toxicity. The recently developed Limulus Amebocyte Lysate (LAL) assay for gram-negative endotoxins includes within its measure lipopolysaccharides from organisms which are not particularly endotoxic. Originally described by Levin and Bang (6), the assay can detect as little as 1 nanogram of bacterial endotoxin per milliliter (or 1 μg/l) in a period of less than two hours. The test is reportedly simple, specific, and rapid, and inexpensive when compared to the USP rabbit pyrogen test (2). It has found use in detecting clinical endotoxemia as well as for the detection of bacterial endotoxins (pyrogens) in biological products and other drugs or fluids for parenteral administration to man (7,8).

The LAL assay was first described in an environmental application by DiLuzio and Friedmann (9). They had observed gram-negative endotoxin contamination of their dionized water, then found endotoxins in New Orleans tap water, and -- curiosity getting the better of them -- went on to examine a host of different waters. Their results are reproduced in Table 1. Additionally, they reported Mexico City tap water at 800 μg/ml, a commercially bottled water from Mexico City as negative, random samples of beer,

TABLE 1. ENDOTOXIN CONTENT OF CERTAIN MUNICIPAL DRINKING WATERS, MISSISSIPPI RIVER WATER, THE GULF OF MEXICO, AND ADJACENT BAYS (9)

Sample tested	Water source	Endotoxin	Endotoxin concentration ($\mu g\ ml^{-1}$)
Drinking water			
Baltimore, Md.	Reservoirs	+	
Chicago, Ill.	Lake Michigan	+	
Denver, Colo.	South Platt River and streams from Bear Creek	+	10
Galveston, Texas	Artesian wells	+	
Harrisburg, Pa.	Reservoir	+	
Hazelton, Pa.	Reservoir	+	
Kalamazoo, Mich.	Artesian well	—	
Knoxville, Tenn.	Fort Loudoun Lake	+	
Las Vegas, Nev.	Lake Mead (60%)	+	
	Wells (40%)	+	
Little Rock, Ark.	Lake Maumelle	+	
	Lake Winnoa		
Los Angeles, Cal.	Wells (12%)		
	Colorado River (10%)		
	Owen's River aqueduct from Mammouth Lake (77.7%)	+	
Memphis, Tenn.	Artesian well	—	
Mobile, Ala.	Reservoir	+	1
Nashville, Tenn.	Cumberland River	+	
New Orleans, La.	Mississippi River	+	1
Riverside, Ill.	Chicago water supply and 2 wells	+	
San Francisco, Cal.	Hetchy Reservoir	+	10
Washington, DC	Potomac River	+	
Surface water			
Barataria Bay, La.			20
Gulf of Mexico*			200
Little Dauphin Island Bay, Ala.			20
Mississippi River (surface levels)			130
Mississippi River (deep levels)			400
Mobile, Ala. (reservoir)			80

Samples were obtained between June and September 1972.
* Gulf of Mexico sample obtained off Grant Isle, La.

cola drinks, and wine as negative, one brand of local commercial bottled water as positive, another brand derived from a 610-M (2000-ft) deep artesian well as negative, and milk at 30 to 130 μg/ml which rose sixteen-fold after 24 hours at room temperature but which showed no rise when refrigerated. The authors predicted value in use of the procedure for monitoring water, milk and other beverages, and biological solutions.

Jorgensen, Lee, and Pahren (10) applied the LAL procedure to drinking waters and to AWT effluents. Their tabular results are also reproduced herein, drinking water samples in Table 2, and AWT water samples in Table 3. Sodium thiosulfate was not added to any of the drinking water samples. The lowest endotoxin activity shown for drinking water samples is from Miami which also was the only ground water supply sampled.

All AWT samples were frozen prior to mailing. The only AWT samples in Table 3 free of endotoxin are from Escondido which uses reverse osmosis as a final treatment. Blue Plains uses breakpoint chlorination following GAC filtration and had relatively low endotoxin levels. Samples from Pomona treated by ozone did not differ substantially from samples treated by "normal" chlorination. The highest endotoxin-containing samples -- both from the Lake Tahoe plant -- were mailed to Cincinnati instead of to San Antonio and because of the lengthy time in transit getting to the proper lab, the results are not comparable with the other sample results.

The authors described variability in the potency of the amebocyte lysate preparation, problems of sample shipment, failure to perform bacteria plate counts, and failure to discriminate between bound and free endotoxin as their greatest shortcomings. Bound endotoxin is described as endotoxin remaining in association with the cell wall of viable bacteria, and free endotoxin is endotoxin that has been solubilized without autolysis or disruption of the cells. Further, the authors state that bound endotoxin can be used as a means of quantifying the number of bacteria present. A linear relationship exists between the number of cells and bound endotoxin over the range 10^3 to 10^6 bacteria per ml. Two of the most recent papers published utilize endotoxin methods to describe biomass and bacterial counts (11,12).

The study reported herein attempts to fill in some of the shortcomings while striving for additional objectives, viz:

1) To quantify pyrogenic activity of waters following GAC filtration in terms of capacity to initiate a fever via injection, ingestion, and inhalation in experimental animals, and

2) To observe the relationships of pyrogenicity with bacterial counts and the endotoxin assay.

3) To determine the effects of treatments on the pyrogenic or endotoxin results and

TABLE 2. RESULTS OF LIMULUS TESTS ON DRINKING WATER SAMPLES (10)

Municipal water plant	Treatment systems	Typical free chlorine (mg/liter)[α]	Sample frozen	Endotoxin equivalents (ng/ml)
Cincinnati, Ohio	Prechlorination, sedimentation, coagulation-filtration, postchlorination	1.4	Yes	1.25
Grand Forks, Iowa	Prechlorination, coagulation-filtration, softening, powdered activated carbon, postchlorination	0	No	12.5
Seattle, Wash.	Chlorination	0	No	12.5
New York, N.Y.	Chlorination	0.05	No	12.5
Miami, Fla.	Prechlorination, softening, sedimentation recarbonation, breakpoint chlorination, filter	2.1	No	<0.625
Boston, Mass.	Chlorination	0	Yes	500
Lawrence, Mass.	Prechlorination, granular-activated carbon, coagulation-filtration, postchlorination	0.8	Yes	125
Philadelphia, Pa.	Prechlorination, sedimentation, coagulation-filtration, postchlorination	1.5	No	10
Terrebonne Parish, La.	Prechlorination, granular-activated carbon, coagulation-filtration, postchlorination	0	No	2.5
Ottumwa, Iowa	Prechlorination, powdered-activated carbon, coagulation-filtration, postchlorination	1.9	No	2.5

α Free chlorine values are those obtained during the National Organics Reconnaissance Survey of 1975.

TABLE 3. RESULTS OF LIMULUS TESTS ON AWT WATER SAMPLES (10)

AWT plant	Treatment system	Sodium thiosulfate added	Endotoxin equivalents (ng/ml)
Blue Plains pilot plant, Washington, D.C.	Screening + lime + nitrifying activated sludge + denitrification + carbon + multimedia filter + breakpoint chlorination	Yes	2.5
		No	12.5
Lake Tahoe, Calif.	Activated sludge + lime + NH_3 strip + CO_2 + multimedia filter + carbon + chlorination	Yes	1,250
		No	1,250
Orange County, Calif.	Trickling filter + lime + settling	Yes	0.78
		No	0.78
Escondido, Calif.	Activated sludge + sand filter + reverse osmosis	Yes	<0.313
		No	<0.313
EPA AWT pilot plant, Cincinnati, Ohio	Trickling filter + settling + lime + flocculation + settling + CO_2 + multimedia filter + chlorination + carbon	No	125
Pomona, Calif., Process 1	Activated sludge + carbon + chlorination	Yes	125
		No	500
Pomona Process 2	Activated sludge + carbon + chlorination + carbon	Yes	250
		No	125
Pomona Process 3	Activated sludge + carbon + ozone + carbon	Yes	250
		No	250

4) To observe potential effects of i.v. injection on the animals' hemodynamic system.

The study commenced with AWT waters and then progressed to the examination of drinking water samples obtained from water treatment plants that utilize GAC filtration.

SECTION 2

CONCLUSIONS

1. The injection of drinking waters of varying endotoxin content (1.2 to 25 μg/l assay) failed to demonstrate pyrogenic activity in any of the 28 rabbits tested.

2. The injection of AWT effluents of varying free-endotoxin content (6 to 250 μg/l assay) into rabbits yielded positive pyrogenic results in 18 of 20 trials. There was, however, no discernible relationship between endotoxin content and the increase in body temperature.

3. Although the ingestion of a membrane-filtered wastewater effluent (3000 μg/l endotoxin activity) resulted in an elevated temperature in five of eight animals tested, the increases were too small to satisfy the requirements of the USP test for pyrogenicity.

4. The inhalation of a membrane-filtered waste water effluent aerosol (1560 μg/l free-endotoxin activity) failed to show a pyrogenic response in 18 rabbits exposed.

5. Blood morphological changes as a result of the injection of pyrogen-containing waters were observed, but the changes were within the normal variations for the test animals.

6. That a potential exists for Pseudomonas proliferation in a GAC bed was supported by one positive observation out of 25 examinations.

7. The numbers of bacteria found to be present in GAC product waters from water treatment plants during this study were very few and certainly would constitute no major health concern as long as a disinfection process is subsequently applied.

8. The four disinfection processes employed (ultraviolet, chlorine, high pH, and ozone) all appeared to decrease endotoxin content.

9. Good correlations were observed on non-disinfected AWT effluent samples between standard plate count and total endotoxin ($r=0.945$), standard plate count and free endotoxin ($r=0.932$), and total coliforms and free endotoxin ($r=0.939$). Lack of good correlations, however, were observed in assaying AWT samples that had been subject to the disinfecting procedures of chlorination, ozonation, high pH, or UV irradiation.

10. Free endotoxin appears refractory to GAC adsorption. Bound endotoxin

appears removed via a filtration mechanism. Hence, total endotoxin is reduced through GAC only by the amount of bound endotoxin filtered out.

11. Using two carbon columns in series on a filtered, nitrified activated sludge effluent, the first column reduced COD 20 percent and total, bound, and free endotoxins 64, 77, and 41 percent respectively. Additional reductions in COD, total, and bound endotoxin of 35, 20, and 42 percent, respectively, were observed in the second column (longer empty-bed contact), but free endotoxin increased by 12 percent.

12. Small but substantial removals of TOC were observed after as long as 110 months of GAC operation. In five of 28 samples, however, an increased TOC resulted. The lack of relationship of TOC removal to empty-bed contact time suggests that adsorption kinetics are not descriptive of the data collected.

13. The array of methods used to monitor the operation of GAC beds is bewildering. The TOC procedure used only by a single plant and suggested by the proposed regulations for the control of organic contaminants requires expensive, sophisticated instrumentation. The chemical oxygen demand procedure (COD) can be used in lieu of TOC, yet not a single use of COD was found in practice.

SECTION 3

RECOMMENDATIONS

Pyrogenicity was demonstrated for AWT effluents but not for GAC-processed drinking waters. Although endotoxin levels were generally lower for drinking waters, many samples possessed higher endotoxin activity than some of the AWT samples that resulted in a pyrogenic response. This suggests some basic differences in water quality which must be further researched.

The drinking water samples were of higher microbiological quality prior to GAC contact than were the AWT waters. Twenty-seven of the 28 drinking water samples had been subjected to a prechlorination procedure, and the microbiological mileau of the carbon beds could almost certainly be altered as a consequence of this application. For example, coliforms were found to be present in every sample of the GAC product water of the AWT application whereas none were found in the drinking water samples. Such a difference in microbiological mileau might explain the greater potency of AWT endotoxins to elicit a pyrogenic response.

Since endotoxins appear to be refractory to adsorption processes but possibly amenable to disinfection procedures, some kinetic studies of disinfection procedures on endotoxins are needed.

A general biological impairment of water by the GAC process was not observed in this study with the possible exception of the Pseudomonas detected in one sample. A different array of data might have been observed, however, had prechlorination not been so broadly employed. Since prechlorination practice might in the future be minimized to control trihalomethane content, it is strongly recommended that studies of GAC and its impact on the microbiological quality of processed waters be implemented on a variety of waters when a prechlorination process is not employed.

A serious information gap appears to exist between the fields of research and practice in the application of GAC in water treatment. The suggestion is that training activities must be vastly improved if there is to be any improvement in the nation's drinking water quality by virtue of increased application of GAC.

SECTION 4

EXPERIMENTAL PROCEDURE

AWT Plant and Operation

A detailed description of the Dallas Demonstration Plant has been previously published (13). The unit processes involved in this study were:

1) No. 1 Activated Sludge Unit - this complete-mix unit treated primary effluent from Dallas' White Rock Plant and was operated to nitrify. An average COD value for the influent was about 270 mg/l, and aeration tank volume is about 560 M^3 (6000 ft^3). Using a COD:BOD ratio of 2.2 for this waste water, the resulting loading/day on the unit in terms of BOD_5 was about 0.5 kg BOD_5/M^3 (31 lbs/1000/ft^3). These values apply to a flow of 7.9 lps (125 gpm). In the winter months, flow was dropped to 6.3 lps (100 gpm) since nitrification proceeds more slowly at colder temperatures. Average activated sludge effluent values for COD were in the range 25-60 mg/l.

2) Activated sludge effluent was passed through a mixed-media filter. The anthracite-sand-garnet filtration depth is 99 cm (39 in), and it operated at a flow of 2.4 lps (38 gpm) throughout the study, the rate being approximately 175 M/day (3 gpm/ft^2). Effluent COD values were in the range 11-36 mg/l.

3) The water was lastly processed through two activated carbon contactors operating in series. Flow through the first column was consistently at 1.9 lps (30 gpm) and through the second, 1.3 lps (20 gpm). Both were charged with Calgon Filtrasorb 400, 8x30 mesh; the second column in the series had the newer carbon. Empty-bed contact time was about 30 minutes for the first column and about 45 minutes for the second column, a total of approximately 75 minutes. Extreme COD values for the product water ranged over 2.4-23 mg/l, with an average value around 10 mg/l.

The microbiological quality of the final effluent waters were quite high considering that the original water was sewage and that no disinfecting agent was used. For example, the six samples assayed in August and September on the first samples to be run at the Demonstration Plant showed the following results:

Assay	Range
Total Plate Count/ml	100-4900
Total Coliforms/100 ml	60-1300
Coliphages, pfu/ml	<2-6

Such microbiological qualities are more descriptive of a surface water than of a non-disinfected waste water effluent.

AWT Sampling

Large samples of water were collected at the Demonstration Plant, mixed, and divided into three smaller samples. One sample was retained at the plant for characterization analyses (see Appendix pp A-1 to A-8), and the other two samples were shipped, one to the University of Texas Health Science Center in San Antonio and the other to the Environmental Engineering laboratories of Texas A&M University in College Station. Since Environmental Engineering personnel had no previous experience with the LAL assay, a sub-contract was made with the experienced personnel at the Health Science Center to perform the LAL assays during the first year of effort.

The first samples were shipped wholly by bus. Straight-through bus service from Dallas to San Antonio was fairly reliable. Samples to College Station, however, had to change buses in Waco, and the delays sometimes occasioned by this transfer (the next several buses might even be missed) resulted in a change to air freight. Air freight, too, proved an unreliable means of shipping samples. Only one set of samples shipped by air arrived in the Environmental Engineering laboratories in College Station at the proper temperature -- the very last samples to be shipped from Dallas.

Water Treatment Plant Sampling

The exasperating experiences with sample shipment over the relatively short distance between Dallas and College Station demanded some rather detailed planning if success was to be achieved with water samples shipped from widely scattered points throughout the United States to College Station. A minimum sample size was calculated to be 500 mls and two were required, one before and one after GAC. Hence, two 500-ml glass bottles were placed in a styrofoam insulated container (later, four 500-ml bottles were used.) The container had cover latches on each end, but nylon web straps with buckles and a handle were added for additional security (see Figure 1). Gel paks were placed in a freezer overnight and inserted with the sample bottles (see Figure 2). Such a kit was tested. The sample bottles were filled with 27°C tap water at 7:30 in the morning. Three gel paks which had been placed in a home freezer at 5:30 the previous evening were added. The kit was closed and placed in the sun. Outdoor temperature reached 37°C. The kit remained outdoors all through a warm night. The following morning at 8:40 the kit was opened and the temperature of the sample bottles at mid-depth was 4.5°C. Hence, we felt that if we could get 24-hour delivery,

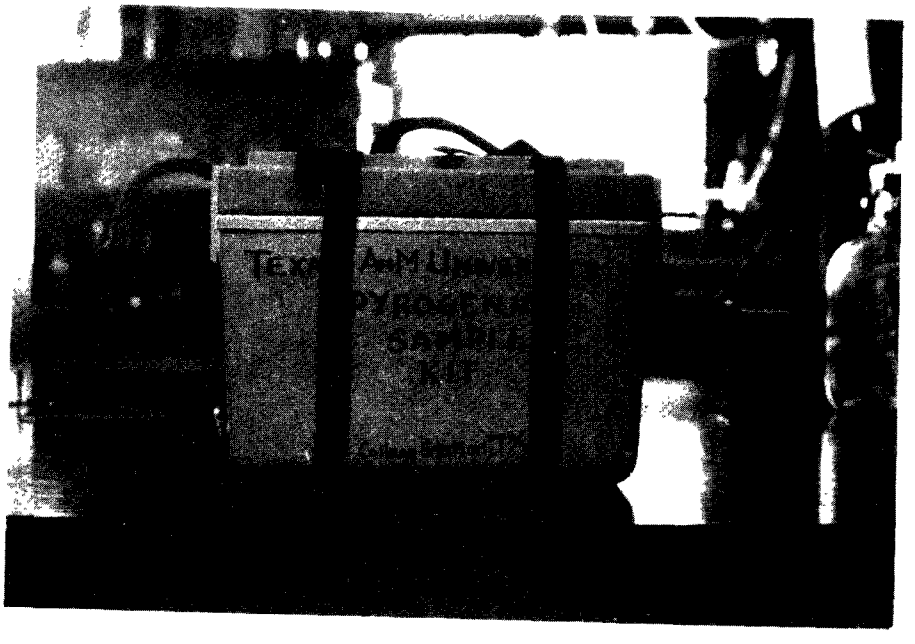
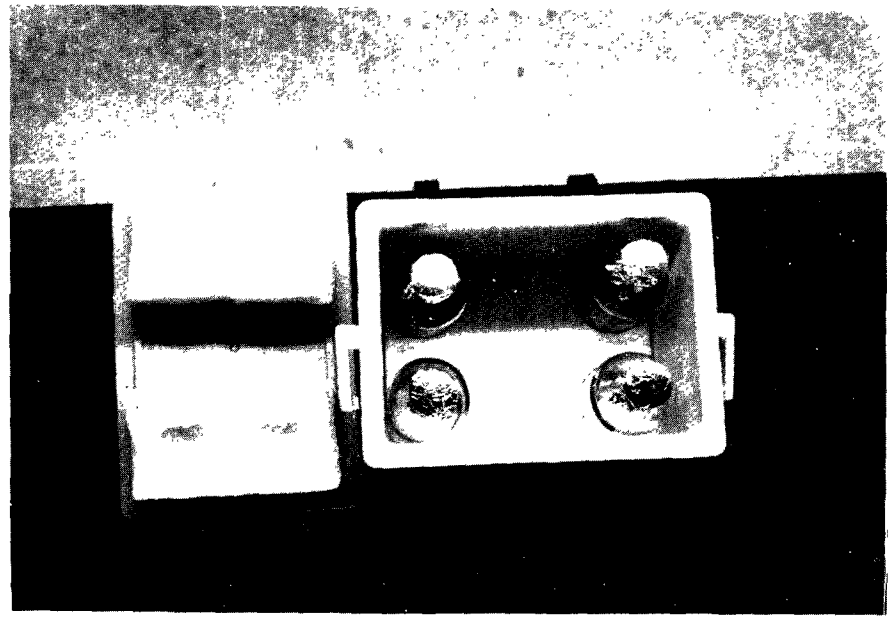


FIGURE 1. EXTERIOR VIEW OF SAMPLE KIT



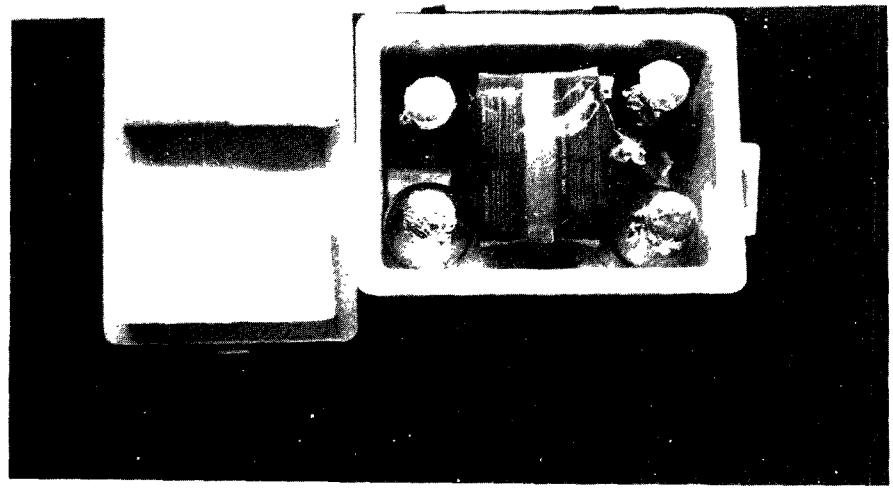


FIGURE 2. INTERIOR VIEWS OF SAMPLE KIT ARRANGED TO HOLD FOUR 500-ML SAMPLE BOTTLES

the samples would arrive in good condition.

The municipal supplies selected for sampling were obtained from a list furnished by EPA. The list contained 45 supplies that utilize GAC filtration either: a) in routine use, b) on a full plant scale, c) in experimental use, or d) on a partial plant use basis. The supply we elected to sample was contacted by telephone, the study, sampling, and analytical program explained, and their help sought. All supplies contacted agreed to help; there were no turn-downs. Having agreed, the sampling kit was shipped to them. It contained sterile sampling bottles, gel paks, sampling and shipping instructions, a data sheet to be filled out,and a reimbursement statement for $10.00 to help defray costs in getting the sample kits to and from airports. Air freight was first utilized with disastrous results. The lid clasps on the very first kit were knocked off and the kit was lost for 10 days. A repeat of this experience occurred with the second kit. Then Emery Air Freight was utilized. This service commenced in excellent fashion, providing 24-hour service. But for those locations where Emery had no facilities, severe problems still occurred.

Laboratory personnel often wished to double-check an analysis and the 500-ml sample volumes were often inadequate. Hence, during the winter months, the kit was shipped with four 500-ml sample bottles, fewer gel paks, and "peanut styrofoam" filler. This was satisfactory but would not have been so in warm weather.

Six of the 45 water treatment plants are located in a localized area of New England. These plants were sampled last by project personnel. The only dissolved oxygen values obtained during the study were from these six plants.

SECTION 5

ANALYTICAL PROCEDURES

The analytical procedures used in this study followed Standard Methods (14) procedures unless otherwise noted. Additional details:

Chlorine residual - Method 409 E, DPD Ferrous Titrimetric Method. Duplicate tests were run on each sample.

Dissolved oxygen - DO was measured using a YSI model 51 A oxygen meter and a YSI 5720 A BOD probe. The DO meter was calibrated for temperature and pressure before measurements were taken. BOD bottles (300 mls) were filled with water, the probe inserted, and the DO read.

Standard plate count - One ml of sample was mixed with approximately 12 mls of Difco Plate Count Agar (at about 45°C) in 15x100 mm Pyrex petri dishes. The plates were allowed to solidify, and were then inverted and incubated at 35°C ± 0.5° for 48 hours. Duplicate tests were run on each sample. The number recorded was an average of the two counts.

Total coliforms - The MPN procedure (5-tube series) was used with waste water samples and both MPN and MF procedures were used with drinking water samples. The MPN procedure is Method 908 A, Standard Total Coliform MPN Test. A dilution scheme of 10 ml, 1 ml, and 0.1 ml for drinking water (10^0 to 10^{-5} for waste water, and 10^0 to 10^{-2} for disinfected waste water) was used with Difco Lauryl Tryptose Broth in the presumptive test and Difco Brilliant Green Bile Broth in the confirmed test. The MF procedure is Method 909 A, Standard Total Coliform Membrane Filter Procedure. A 100-ml sample was used with Millipore 0.45 μm filters and Difco Ends Agar.

Pseudomonas aeruginosa - Method (tentative) 914 E, Multiple-Tube Technic for Pseudomonas aeruginosa.

Pseudomonas fluorescens - From a pour plate that had been incubated for 24 hrs, 10 representative colonies were aseptically transferred to sterile Pseudomonas Agar F agar slants. The slants were incubated for 48 hours at 35°C ± 0.5°. A green pigment formation would indicate the presence of Pseudomonas fluorescens.

Gram stain - From a pour plate that had been incubated for 24 hrs, 10 representative colonies were aseptically transferred to individual Corning Micro Slides. The colonies were dispersed with sterile dilution water, fixed, and stained. The number of individual types of colonies stained depended upon the ratio of each morphological group to the whole

plate count. For example, if 4 colonies are similar among 20 colonies on a plate, then 1 of the 4 colonies would be selected for staining.

Coliphages - To 10 mls of sample was added 0.5 ml of chloroform. The solution was shaken 25 times and stored overnight in a refrigerator at about 9°C. On the same day that the chloroform was added to the sample, 5 mls of tryptone was inoculated with Difco 15597 E. coli. The inoculated solution was placed in an incubator (35°C ± 0.5°) overnight. The E. coli solution was then mixed on a vortex mixer and 0.1 ml was extracted and added to 10 mls of tryptone broth. This solution provides the indicator cells for the coliphage. Then 0.5 ml of the chloroformed sample was mixed with 2.0 mls of indicator solution and 2.5 mls of tryptone overlay agar (47°C) in a sterile 16x125 mm pyrex test tube. The tube and contents were mixed on a vortex mixer and poured onto a petri dish containing 20 mls of solidified tryptone agar. The plate was swirled to evenly distribute the mixture over the surface, and placed upright in an incubator (35°C ± 0.5°) for 24 hrs. Duplicate tests were run for each sample and the number recorded is the average titer (pfu/ml) calculated for each test.

Total organic carbon - This TOC procedure used Oceanography International (O.I.) equipment and methods. The procedure consists of two parts, preparation of the ampule, and testing procedure.

Ampule preparation - A 5.0-ml sample is volumetrically pipetted into a precombusted ampule covered with aluminum foil. The ampule with sample is placed in a holder attached to the O.I. ampule sealing unit. The ampule sealing unit consists of a purging unit in which purified oxygen is bubbled through the sample and an oxygen-propane microburner that seals the ampules. Then 0.25 ml of 6% phosphoric acid is added to the ampule with sample just before purging. The sample is purged with purified O_2 for 4 min. After 3 min. of purging, 1 ml of saturated persulfate solution is added to the ampule. The ampule is sealed by the oxygen-propane microburner. A purified oxygen atmosphere is maintained inside the ampule during the sealing process. After all the ampules have been sealed, they are placed in a holding rack. The rack fits into a metal pressure vessel. Approximately 1 liter of distilled water is added to the pressure vessel. The vessel is sealed by a metal top that bolts on. The pressure vessel is placed in an oven at 170°C for 24 hours. The pressure vessel is allowed to cool to room temperature before the ampules are removed. The ampules are stored at room temperature until analyzed.

Testing procedure - The samples are analyzed on an O.I. ampule analyzing unit. Standard TOC samples (10.0 ppm, 7.5 ppm, 5.0 ppm, and 2.5 ppm) are run prior to the GAC samples. A linear curve is established relating an integrated machine number with the respective TOC standard. Boiled distilled water is used as dilution water for the TOC standards. The dilution water is analyzed on the ampule analyzing unit and the integrated machine number is subtracted from each of the TOC standards before the linear curve is plotted. A minimum of 5 samples are analyzed to obtain an average value. Once an average integrated machine number is found for a GAC sample, the respective TOC value is taken from the standard TOC curve.

Limulus amobocyte lysate assay - Two different sources of Limulus lysate were used during the second half of the project. Prior to December 1977, lysate from Difco was used. After that time, lysate was obtained from Associates of Cape Cod.

Difco procedure -

I. Standardization of Stock (stock in this description refers to Difco Co. products)

A. Reconstitute the contents of the Pyrotrol Positive Control, 0.5 ng/.2 ml, with 4 mls pyrogen-free water.

B. Vortex contents for 1 min.

C. Preparation of dilutions.

1. Dilute positive control (0.5 ng/.2 ml) 1:1 giving 0.25 ng/.2 ml.
2. Vortex 1 min.
3. Draw 0.2 ml and add to Pyrotest vial. Swirl very slightly. Do not agitate violently.
4. Dilute the 0.25 ng/.2 ml solution 1:1 giving 0.125 ng/0.2 ml and repeat steps 2 and 3.
5. Continue the 1:1 dilution scheme repeating steps 2 and 3 for sequential 1:1 dilutions of 0.0625 ng/0.2 ml, 0.03125 ng/0.2 ml and 0.01565 ng/0.2 ml.
6. Add 0.2 ml of pure dilution water to a Pyrotest vial as a control.

D. All prepared vials are incubated at 35°C for 75 min.

E. After incubation, the vials are examined for the formation of a gel and are graded from 1+ to 4+ (1+ is opaque without any gel and 4+ is a solid gel.)

F. The sensitivity of the stock is the highest dilution of the standard giving a positive <u>Limulus</u> test (3+ or 4+).

For example:

0.3125 ng/ml	0.156 ng/ml	0.0781 ng/ml
4+	3+	1+

The sensitivity of this stock would be 0.156 ng/ml.

II. Method of Testing

A. Agitate sample thoroughly for 1 min.

B. Make dilutions

1. Dilute sample 1:100 and vortex for 1 min.
2. Draw 0.2 ml and add to one Pyrotest vial. Do not agitate violently.
3. From the 1:100 dilution make a 1:1000 dilution and vortex for 1 min.
4. Draw 0.2 ml of dilution and add to Pyrotest vial. Do not agitate violently.

C. Incubate vials for 75 min. at 35°C. After incubation record the formation of any gel as 1+ to 4+.

D. Once the range of endotoxin equivalents is found, subsequent dilutions are made to find the approximate endotoxin equivalent number. For example, if the range is found to be above 1:100 but below 1:1000, subsequent dilutions of 1:200, 1:400, and 1:800 can be made and tested. If the end point turns out to be 1:400, the endotoxin equivalents would be 400 x 0.156 ng/ml (endotoxin sensitivity) = 62.4 ng/ml or 62 ng/ml endotoxin equivalents.

III. Materials Used

A. Difco Pyrotest kit.

B. McGaw sterile water for irrigation or 0.9% sodium chloride sterile irrigation solution.

C. Falcon 16x125 mm disposable tissue culture tubes.

D. Scientific Products disposable serological pipets; 1 and 10 ml, individually wrapped.

Associates of Cape Cod procedure -

A. Agitate sample thoroughly for 1 min.

B. Make dilutions.

1. Dilute sample 1:100 and vortex for 1 min.
2. Draw 0.1 ml and add to one pyrogen-free 10x75 mm culture tube.
3. From the 1:100 dilution make a 1:1000 dilution and vortex for 1 min.
4. Draw 0.1 ml of dilution and add to one pyrogen-free 10x75 mm culture tube.

C. Remove LAL from freezer and add 1 ml of pyrogen-free water to the vial and slowly swirl until all the LAL

has gone into solution. Add 0.1 ml LAL to each pyrogen-free culture tube with sample. Place the 10x75 mm culture tubes in a 37°C water bath and incubate for 75 min. After 75 min. of incubation, slowly and carefully remove the culture tubes singly, directly from the water bath. Invert the culture tube 180°. A solution is considered positive if the gel can be inverted twice without breaking. If the gel breaks, the test is considered negative.

D. Once the range of endotoxin equivalents is found, subsequent dilutions are made to find the approximate endotoxin equivalent number. The sensitivity of the LAL is determined by Associates of Cape Cod and each vial is labeled with its sensitivity.

Pyrogenic assay -

The amount of bacterial pyrogen in an unknown sample can be quantitatively determined by injecting the test sample into rabbits and measuring changes in body temperature. The factors that influence the rabbits response to pyrogens are normal temperature, body weight, individual sensitivity and excitation (15).

Male New Zealand albino rabbits were selected for the assay. Individual rabbits were selected on the basis of a normal temperature between 37.8° and 39.5°C, and a weight of 2 to 4 kg. Each rabbit was injected with 1 ml of pyrogen-free water under assay test conditions. If their temperature response was greater than 0.3°C, they were eliminated from the program. Seven out of fourteen rabbits, selected at random, were given a known dose of pyrogens and checked for a pyrogen response. All rabbits injected with the known quantity of pyrogens had a fever response. To avoid the effect of tolerance the rabbits were rested for two weeks between injections. At the end of the project period, all rabbits were given a standard pyrogen dose of 2 ng/kg and monitored for a pyrogen response. All the rabbits in the colony had a fever response to the 2 ng/kg dose.

Three rabbits were used for each assay. The rabbits were placed in wooden stanchions one hour before being injected. Temperatures were monitored using a YSI Tele-Thermometer and a small animal thermistor probe. The probe was inserted 7-10cm into the rectum. Temperatures were recorded every half-hour during the assay. The rabbits were injected in the marginal vein with 1 ml 0.45 μ - filtered sample. Their temperatures were monitored for 2 hours or more after the injection.

USP Pyrogen Test (15) -

The pyrogen test is designed to limit to an acceptable level the risks of a febrile reaction in the patient to the administration, by injection, of the product concerned. The dose specified for the test

is related to that generally given to the patient, but for practical reasons, it does not exceed 10 ml per kg. of body weight of the test animal, injected in a brief period of time. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph.

Apparatus - Render the syringes, needles, and glassware free from pyrogens by heating at 250°C for not less than 30 minutes or by any other suitable method. Just prior to injecting it, warm the product to be tested to approximately 37°C.

Test Animals - Use healthy, mature rabbits each weighing not less than 1500 g. House the animals individually in an area of uniform temperature [±3°C(±5°F)] and free from disturbances likely to excite them. Before using an animal for the first time in a pyrogen test, condition it by a sham test that includes all of the steps as directed under *Procedure* except the injection of the test dose. Do not use animals for pyrogen tests more frequently than once every 48 hours, nor prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic.

Note - Perform the test under environmental conditions similar to those under which the animals are housed. During the test, withhold all food from the animals being used. Access to water may be allowed.

Temperature Recording - Use an accurate clinical thermometer for which the time necessary to reach the maximum reading is known, or any other temperature-recording device of equal sensitivity. Insert the thermometer into the rectum of the test animal to a depth of not less than 7.5 cm., and, after a period of time not less than that previously determined as sufficient, record the animal's body temperature.

Procedure - Not more than 40 minutes prior to the injection of the test dose, determine the "control temperature" of each animal; this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one test use only those animals the control temperatures of which do not vary by more than 1°C from each other, and do not use any animal with a temperature exceeding 39.8°C.

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 ml of the product per kg. of body weight within 40 minutes. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

Interpretation and Retest - If no rabbit shows an individual rise in temperature of 0.6°C or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4°C, the product meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6°C or

more, or if the sum of the temperature rises exceeds 1.4°C, repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6°C or more, and if the sum of the eight temperature rises does not exceed 3.7°C, the material under examination meets the requirements for the absence of pyrogens.

SECTION 6

AWT PLANT RESULTS

The Demonstration Plant of the Henry J. Graeser Environmental Research and Training Facility, Dallas, Texas was operated for most of the first-year study period as depicted in Figure 3. Large grab samples of product waters were split into three subsamples; one was retained at the Demonstration Plant and the other two were shipped to laboratories in San Antonio and College Station, Texas. Analytical characterization of the samples was performed at the Demonstration Plant; the data are in the Appendices, pp A-1 to A-18. The University of Texas Health Science Center at San Antonio directed its effort at in vitro approaches, separating the endotoxin analyses into the bound and free forms, and observing the relationships of all forms with microbiological analytical results. Texas A&M University in College Station directed its effort at in vivo tests subjecting the test animals to endotoxin administration via i.v. injection, ingestion, and inhalation.

Comparison of Endotoxins with Viable Bacteriological Assays

A total of 48 AWT product water samples were examined for endotoxin, standard plate count, and total coliforms (Table 4). Standard plate counts varied from 0/ml to 5.5×10^4/ml plus several indeterminate values of TNTC (too numerous to count). Total coliform counts by the membrane filter (MF) method ranged from less than 2/100 ml to 1.4×10^6/100 ml. The total endotoxin content (unfiltered samples) ranged from 6-600 μg/l endotoxin equivalents, free endotoxin levels (filtered samples) ranged from 3-480 μg/l endotoxin equivalents, and the bound endotoxin content ranged from 0-250 μg/l endotoxin equivalents. Note in Table 4 the apparent increase in endotoxin content with time. The treatments of UV, Cl_2, O_3, and pH are explained on page 31.

The gross data for the finite values of Table 4 are displayed in Figure 4. Figure 4a compares the relationship between total endotoxin content and total number of bacteria determined by the standard plate count procedures (three replicates). The correlation coefficient was determined to be 0.726. Figure 4b examines the relationship of bound endotoxin content to standard plate count (correlation coefficient = 0.736). Figure 4c compares free endotoxin content with the standard plate count (correlation coefficient = 0.620). Figure 4d compares total endotoxin content with total coliform count (correlation coefficient = 0.822) and Figure 4e compares bound endotoxin content with total coliform count (correlation coefficient = 0.472). Figure 4f relates the free endotoxin content to the total coliform count (correlation coefficient = 0.525).

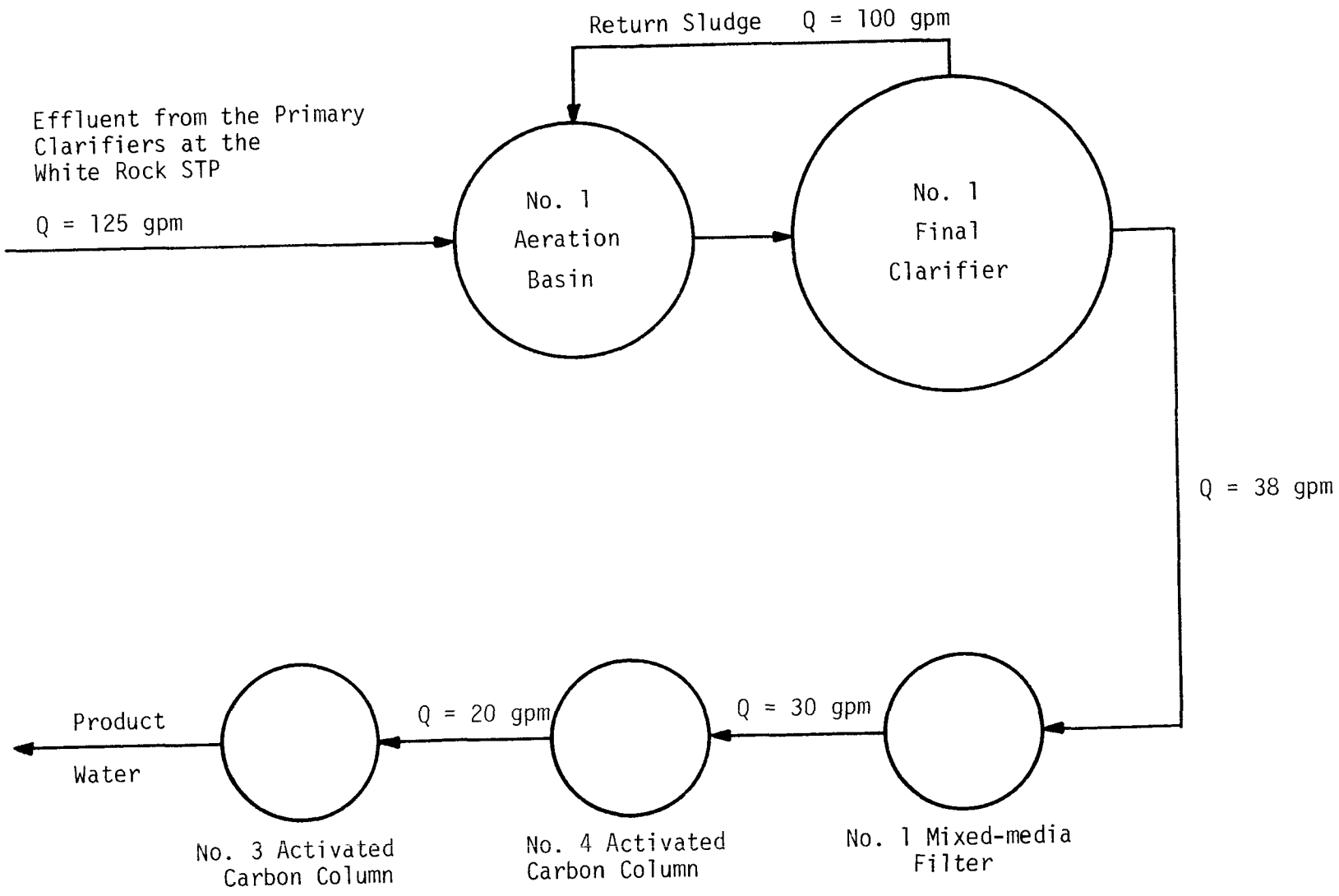


FIGURE 3. FLOW DIAGRAM OF THE DEMONSTRATION PLANT AT THE HENRY J. GRAESER ENVIRONMENTAL RESEARCH AND TRAINING FACILITY, DALLAS, TEXAS

TABLE 4. RESULTS OF STANDARD PLATE COUNTS, TOTAL COLIFORM COUNTS, AND ENDOTOXIN DETERMINATIONS ON AWT SAMPLES

Sample No.	Sample Date	Treatment	Standard Plate Count (#/ml)	MF Coliform Count (#/100 ml)	Endotoxin Equivalents (μg/l)		
					Total	Free	Bound
1	8-5-76	-	8×10^2	6.5×10^3	48	24	24
2	8-12-76	-	7.0×10^2	1.3×10^3	24	12	12
3	8-19-76	-	1.4×10^3	3.8×10^3	48	24	24
4	9-9-76	-	1.4×10^3	5.0×10^3	60	48	12
5	9-14-76	-	1.2×10^3	4.9×10^3	48	12	36
6	9-21-76	-	1.9×10^2	4.8×10^3	48	24	24
7	9-28-76	-	3.0×10^1	3.2×10^2	24	12	12
8	10-5-76	-	8.0×10^2	6.8×10^3	48	24	24
9	10-12-76	-	5.6×10^2	1.7×10^3	12	6	6
10	10-19-76	-	1.5×10^3	3.2×10^4	36	24	12
11	10-26-76	-	3.7×10^3	4.3×10^4	48	12	36
12	11-9-76	-	2.7×10^2	3.9×10^3	48	12	36
13	11-16-76	-	3.3×10^3	1.4×10^3	96	48	48
14	11-23-76	-	5.9×10^2	3.8×10^3	96	48	48
15	12-7-76	-	5.4×10^3	2.1×10^4	96	48	48
16	12-15-76	-	5.5×10^4	1.4×10^6	600	480	120
17	12-15-76	-	2.5×10^3	1.6×10^4	192	48	144
18	12-15-76	-	1.2×10^3	5.9×10^3	192	96	96

(continued)

TABLE 4. (continued)

Sample No.	Sample Date	Treatment	Standard Plate Count (#/ml)	MF Coliform Count (#/100 ml)	Endotoxin Equivalents (μg/l)		
					Total	Free	Bound
19	12-21-76	-	5.0×10^4	2.7×10^5	500	250	250
20	12-21-76	-	2.7×10^3	2.9×10^4	100	50	50
21	12-21-76	-	6.0×10^3	1.0×10^5	200	100	100
22	1-25-77	-	1.5×10^4	2.2×10^5	250	125	125
23	1-25-77	-	1.8×10^3	2.3×10^4	100	50	50
24	1-25-77	-	2.8×10^2	2.0×10^3	100	75	25
25	2-8-77	UV	1.0×10^3	1.0×10^2	60	15	45
26	2-8-77	Cl_2	3.8×10^1	4.0×10^2	60	15	45
27	2-8-77	O_3	0	0	12	6	6
28	2-8-77	pH	1.6×10^0	0	24	6	18
29	3-1-77	UV	9.0×10^2	2.8×10^2	ND	ND	ND
30	3-1-77	Cl_2	1.6×10^1	3.2×10^1	ND	ND	ND
31	3-1-77	O_3	2.9×10^1	0	ND	ND	ND
32	3-1-77	pH	2.5×10^1	2.0×10^0	ND	ND	ND
33	3-16-77	UV	1.8×10^2	2.6×10^1	96	24	72

(continued)

TABLE 4. (continued)

Sample No.	Sample Date	Treatment	Standard Plate Count (#/ml)	MF Coliform Count (#/100 ml)	Endotoxin Equivalents (μg/l)		
					Total	Free	Bound
34	3-16-77	Cl_2	1.0×10^1	0	48	24	24
35	3-16-77	O_3	0	0	48	12	36
36	3-16-77	pH	5.9×10^1	0	48	24	24
37	3-30-77	UV	8.5×10^1	1.8×10^1	72	12	60
38	3-30-77	Cl_2	3.6×10^0	0	48	48	0
39	3-30-77	O_3	2.3×10^0	0	24	6	18
40	3-30-77	pH	3.0×10^3	0	12	3	9
41	4-6-77	UV	1.1×10^2	2.6×10^1	96	48	48
42	4-6-77	Cl_2	TNTC	0	48	24	24
43	4-6-77	O_3	TNTC	0	24	6	18
44	4-6-77	pH	TNTC	0	12	3	9
45	4-13-77	UV	9.4×10^1	5	24	6	18
46	4-13-77	Cl_2	2	0	48	12	36
47	4-13-77	O_3	1	0	24	6	18
48	4-13-77	pH	1.8×10^1	0	6	3	3

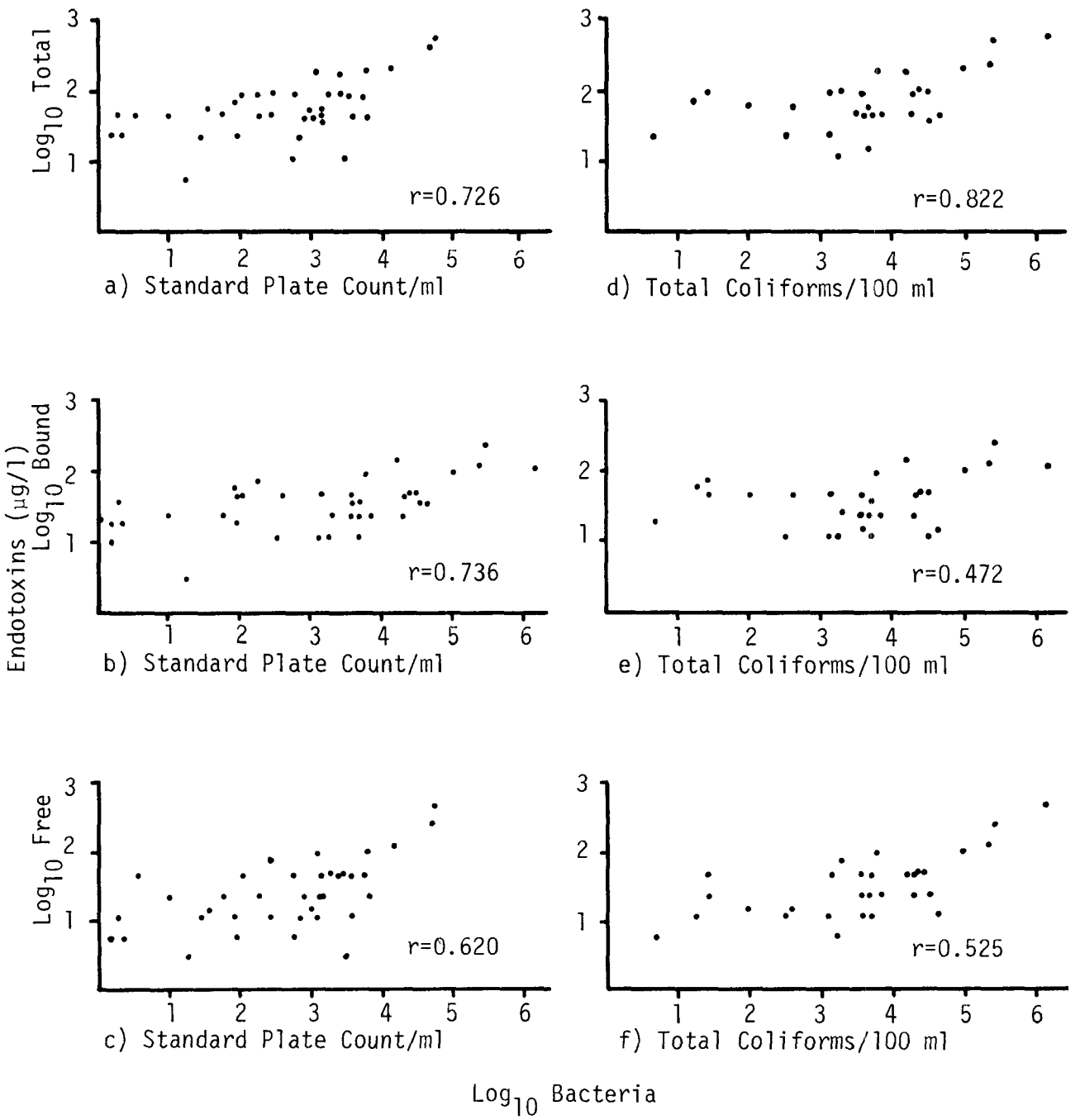


FIGURE 4. ENDOTOXIN EQUIVALENTS vs. BACTERIOLOGICAL ASSAYS

A previous preliminary investigation demonstrated the feasibility of testing water samples by the Limulus assay for the presence of endotoxin due to gram-negative bacteria (10). However, in this present study using highly treated waste waters which had been subjected to a biological process, the results of Limulus assays did not correlate extremely well with the two established techniques for assessing microbiological water quality, i.e., the standard plate count and total coliform count. Levels of total, bound, or free endotoxin activities seemed unable to reliably predict the microbial content of the water samples examined if disinfection treatments of Cl_2, O_3, high pH, or UV had been performed.

Examination of the first 24 samples from Table 4 which had not been exposed to disinfection processes yielded mixed correlations: standard plate count and total endotoxin ($r = 0.945$), standard plate count and free endotoxin ($r = 0.932$), and total coliforms and free endotoxin ($r = 0.939$), all quite good. However, the correlations between standard plate count and bound endotoxin ($r = 0.745$), total coliforms and total endotoxin ($r = 0.822$), and total coliforms and bound endotoxin ($r = 0.419$) were less encouraging. In the study by Evans, et al. (12), better correlations between endotoxin content and bacterial numbers were achieved by use of the spectrophotometric modification of the Limulus assay than by the clot formation end-point method utilized in the present study. Evans, et al. found that the bound endotoxin component (which was most predictive of bacterial numbers) was decreased following chlorination procedures. However, it has also been previously demonstrated that agitation of gram-negative bacteria in a liquid environment serves to release additional amounts of cell-associated endotoxin (16). Thus, it is reasoned that the poor correlation between endotoxin content and densities of viable microorganisms observed in this study result from a combination of partial destruction of Gram-negative bacteria and varying degrees of solubilization of cell-associated endotoxin. Therefore, the endotoxin content of highly treated waste water effluent, unlike stream (12) or sea water (11), probably reflects the remnants of pre-existant bacterial growth rather than continued proliferation of Gram-negative microorganisms.

Endotoxin and Organic (COD) Removal

The operation of the two GAC columns in series afforded a comparative observation of the endotoxin activity of the waters at different points in the adsorption process. The points examined were: just prior to the first contactor (influent), between the contactors (midpoint), and after passing through the second contactor (effluent.) Perhaps more suitable terminology would be expressed by: samples after 0, 30, and 75 minutes of empty-bed carbon contact. These samples were coded and the type of sample being analyzed was not known by laboratory personnel. Table 5 presents the results of three such samples for both chemical oxygen demand (COD) and endotoxin activity. The first column removed 6 mg/l (26%) of COD, 286 μg/l (64%) of total endotoxin activity, 67 μg/l (41%) of bound endotoxin activity, and 219 μg/l (77%) of free endotoxin activity. The suggestion is that the first column does better in removing free endotoxin (presumably by adsorption) than it does in removing the cells (as indicated by the bound

TABLE 5. COD CONCENTRATIONS AND ENDOTOXIN ACTIVITIES OF AWT WATERS BEFORE, AT THE MIDPOINT AND AFTER GAC FILTRATION

Sample No.	Influent (Before)	Midpoint (Between)	Effluent (After)	Overall
		COD, mg/l		
12-14	23.4	17.7	9.4	
12-21	22.2	17.8	14.3	
1-25	22.5	14.7	9.0	
$\bar{X}$	22.7	16.7	10.9	
% Removed		26	35	52
		Total Endotoxin Activity, μg/l		
12-14	600	192	192	
12-21	500	200	100	
1-25	250	100	100	
$\bar{X}$	450	164	131	
% Removed		64	20	71
		Free Endotoxin Activity, μg/l		
12-14	480	48	96	
12-21	250	100	50	
1-25	125	50	75	
$\bar{X}$	285	66	74	
% Removed		77	+12	74
		Bound Endotoxin Activity, μg/l		
12-14	120	144	96	
12-21	250	100	50	
1-25	125	50	25	
$\bar{X}$	165	98	57	
% Removed		41	42	65

endotoxin). The second column removed an additional 5.8 mg/l (35%) of COD, 33 μg/l (20%) of total endotoxin activity, and 41 μg/l (42%) of bound endotoxin activity, but produced an additional 8 μg/l (12%) of free endotoxin activity. A mass balance indicates that the first column in the series was removing 0.96 kg/day (2.16 lb/day) of COD, whereas the second column was removing only 0.62 kg/day (1.39 lbs/day). Since both columns contained the same amount of carbon, the increased free endotoxin value in the effluent from the second column is likely not attributable to an increased bacterial growth (assuming the same biodegradability of the COD retained by both columns), and therefore is more likely due to the death and lysis of cells thus releasing more free endotoxin -- or to analytical insensitivity. Free endotoxin is defined by Jorgensen (see page 4) as endotoxin that has been solubilized without autolysis or disruption of the cells. However, in the event of cell death and lysis, it would be impossible to separate the free endotoxins into those coming from living cells and those coming from lysed cells; both would be discernible only as free endotoxin.

Evaluation of Endotoxins with Four Disinfection Procedures

The endotoxin activity of samples subjected to four different disinfection procedures was determined in six sets of samples. The disinfection procedures were conducted at the Demonstration Plant as follows:

UV - GAC product water was passed through a Kelly-Purdy UV unit at a depth of 5 cm (2 in) and detention time of 3.9 minutes.

Cl_2 - chlorine solution was added to a grab sample until a free residual of approximately 1 mg/l was achieved. It was held for 30 minutes after which autoclaved sodium thiosulfate was added.

pH - the pH of a grab sample was raised to approximately 11.5 by the addition of CaO and held for 2.5 hours. It was then neutralized with the careful addition of a strong acid. The CaO was not sterilized since plates of the stock were negative.

O_3 - GAC product water at 1.58 lps (25 gpm) was passed through a Union Carbide injection ozone contactor which provided an O_3 dose of approximately 7.5 mg/l. The system provides a contact time at the flow rate used of 1.6 minutes. The sample was not "deozonated."

The chemical and microbiological assays and the actual disinfecting parameters achieved for each sample are shown in the Appendix, pp A-12 to A-18. The disinfecting procedures could not all be employed on the same parcel of water at the same time; both UV and O_3 were in-line unit processes and operated at different times, and the Cl_2 and pH treatments were also performed at different times on grab samples. Hence, the results of the individual tests are not specifically comparable with each other.

Table 6 shows the endotoxin activity of the individually disinfected samples. Average values were determined for the measured total endotoxin and free endotoxin assays, and from these averages the bound endotoxin values were determined. The percent of the total endotoxin that was free and bound are also shown. Differences between disinfection procedures cannot be considered of significance because of the manner in which the tests were conducted. The greater power of ozone to oxidize the free endotoxin as compared with UV may be a valid observation, as might be the lesser effect of ozone on the bound (cellular) endotoxin compared to the results of other procedures.

The relatively high endotoxin values noted for the UV and Cl_2 samples of 2/8 should not be construed as errors. It had been observed that the GAC product water quality had been increasing in endotoxin activity with time. The decision was made -- wrongly it turns out -- to not run endotoxin assays on samples prior to applying a disinfection procedure because it was believed that sufficient such data were already available. However, Table 7 shows the rapidity of the general increase in product water endotoxin activity with respect to time. Plotting the mean values on semi-log paper, a minimum projected value for the period Jan 26 - Apr 18 when the disinfection samples were processed would be about 600 μg/l -- somewhat lower for the earlier samples and higher for the later samples. Hence, there is little question but that the disinfection processes considerably reduce the endotoxin activity, but we cannot quantify the decrease nor can we prove it.

Impacts of Sample Shipment

Of the entire disinfection series, only three samples -- the last three -- which were shipped by air arrived in a cool condition, and only the last at a temperature which we had hoped to achieve for all. The more important variables of the disinfection process are collected in Table 8 to show the effects of shipment on these variables. The chlorine residuals shown are for free chlorine, but the "after" values are likely the background scatter of the technique used. The high-pH treated samples showed both the highest average turbidity and highest average COD.

Table 9 is a presentation of the bacteriological results on the various disinfected samples, the before shipment values were determined before samples were shipped from the Dallas site and the after shipment values were determined after the samples were received at College Station. It should be noted that the treatments were not quite as effective as was anticipated. No coliforms were found in the ozonated or pH-treated waters before the samples were shipped, but some were recovered after shipment. The chlorination procedure used was not quite as effective as the ozone or pH treatments in spite of the higher turbidity and COD of the high-pH treated samples and the extremely short contact time for ozone (although the contact continued in the sampling vessel). Rather extensive growth occurred during shipment as determined by the plate count procedures. We had expected the regrowth of the ozonated samples to be greater because ozone increases the amount of biodegradable material present -- but the results appear to be of the same order of magnitude as the other disinfected samples.

TABLE 6. TOTAL AND FREE ENDOTOXIN ACTIVITIES OF DIFFERENT WATER SAMPLES SUBJECTED TO FOUR DISINFECTION PROCEDURES

Sample Date	Disinfection Procedure UV	Cl_2	pH	O_3
	Endotoxin Equivalents, μg/l, Total (Free)			
2/8	320(320)	160(80)	32(16)	32(8)
2/28	—	—	—	—
3/14	80(48)	80(8)	80(8)	80(8)
3/20	30(22)	30(22)	9(2)	30(3)
4/4	90(30)	30(30)	12(6)	15(12)
4/11	30(22)	47(25)	5(3)	30(6)
4/18	47(25)	47(25)	47(25)	30(6)
$\overline{X}$	100(78)	66(32)	31(10)	36(7)
% Free	78	48	32	19
Bound Endotoxin (by difference, μg/l)	22	34	21	29
% Bound	22	52	68	81

TABLE 7. INCREASE IN TOTAL ENDOTOXIN ACTIVITY IN GAC PRODUCT WATER WITH TIME

Time Interval of Sampling	Total Endotoxin Equivalents μg/l			
	Max	Min	Mean	Number of Samples
Aug. - Sept.	60	24	43	7
Oct. - Dec. 6	96	12	60	8
Dec. 14 - Jan. 25	200	100	164	3
Jan. 26 - Apr. 18 (estimated)	—	—	600	0

TABLE 8. EFFECTS OF SHIPMENT ON SOME OF THE IMPORTANT VARIABLES OF THE DISINFECTION PROCESS

Date of Sample	Method of Shipment	Time in Transit hrs.	Temp. on Arrival °C	pH Samples Before	pH Samples After	Cl_2 Samples Before mg/l	Cl_2 Samples After mg/l	Turbidity UV	Turbidity Cl_2	Turbidity pH	Turbidity O_3	COD mg/l UV	COD mg/l Cl_2	COD mg/l pH	COD mg/l O_3
2/8	bus	24	15	11.6	6.6	0.9	0.05	.35	.35	.23	.25	12.0	11.7	23.2	9.4
2/28	bus	24	22	11.5	6.7	1.0	0.02	.4	.4	.5	.4	11.2	-	11.8	9.3
3/14	bus	24	19	11.4	6.7	1.2	0.03	.65	.65	1.8	.45	12.6	-	13.3	2.6
3/28	bus	48	19	11.8	7.2	1.4	0.03	.28	.48	1.4	.37	7.8	11.0	7.0	7.3
4/4	air	8	10	12.5	6.8	1.5	0.01	.45	.45	2.0	.35	9	9.4	8.6	9.2
4/11	air	8	8	11.5	5.9	2.0	0.04	.3	.3	.85	.25	10.7	12	18	9.1
4/18	air	8	5	11.3	6.7	1.6	0.02	.5	.4	.6	.3	10.4	12	11.1	11.9
							$\overline{X}$	.42	.43	1.05	.34	10.5	11.2	13.3	8.4

TABLE 9. EFFECTS OF SHIPMENT ON BACTERIOLOGICAL QUALITIES OF WATER SAMPLES

Date of Sample (1977)		Standard Plate Count/ml				Total Coliforms/100 ml			
		UV	Cl_2	pH	O_3	UV	Cl_2	pH	O_3
2/8	before	112	43	1	0	0	0	0	0
	after	1,820	475	500	310	23	0	0	0
2/28	"	24	4	0	0	2	0	0	0
	"	125	35,000	44,000	213	49	23	5	0
3/14	"	81	8	27	3	7	0	0	0
	"	50	10	25	-	23	0	0	2
3/28	"	280	3	13	3	4	1	0	0
	"	235	11	59	379	23	0	0	0
4/4	"	140	26	26	3	14	0	0	0
	"	5,540	3,800	9,340	11,800	23	0	0	0
4/11	"	240	5	17	8	6	1	0	0
	"	63	14	13	0	2	0	0	0
4/18	"	79	65	60	50	15	1	0	0
	"	7	128	96	8	2	2	2	0
$\bar{X}$	Before Shipment	137	22	21	10	7	<1	0	0
$\bar{X}$	After Receipt	1,120	5,634	7,719	2,118	21	4	1	<1

0 = <1/ml for SPC, <2/100 mls for TC.

Coliphages were run on every after-sample with negative results, <2 pfu/ml.

Coliphage assays were run on all samples after receipt of the samples at College Station. Table 10 displays the individual coliphage results along with the coliform MPN values for the various water samples. We cannot explain the initial visibility of coliphages and subsequently the disappearance.

Pyrogenic Studies

The pyrogenic response of experimental animals exposed to various waste water samples by ingestion, inhalation, and injection was studied. We did not expect to find a pyrogenic response due to ingestion because the literature clearly indicates that there is no danger of endotoxin shock as long as the intestinal wall is normal, i.e. does not have an increased permeability (4), and the animals that were used in these studies were held under veterinary care and consequently were quite healthy. We could not find many references in the literature concerning the inhalation route of endotoxin exposure. Several years ago, a federal office building in Dallas, Texas was evacuated because of a high rate of illness among office workers, the cause of which was suspected to be air borne. Extensive study failed to reveal an etiologic agent and there was some speculation about the possibility of air-borne endotoxins. Rylander et al. (18) published a clinical investigation of sewage treatment plant workers and their exposure to aerosols of sewage sludge and its dusts. They reported that the workers' symptoms and the results of the blood studies performed were consistent with the expected effects of exposure to endotoxins. However, air-borne endotoxins were not measured. Since there is such a void on this topic in the literature and since we were set up to measure pyrogenic activity, a subexperiment was included to evaluate the potential for response to endotoxins transmitted by the inhalation route. The main pyrogenic studies in this report, however, concern the classical i.v. route of endotoxin administration.

Ingestion --

The test water was obtained from the College Station municipal waste water treatment plant. This plant utilizes a trickling filter and a contact stabilization unit operating in parallel. The combined product water prior to clarification was sampled and after passage through a 0.45 μm membrane assayed 3,000 μg/l of endotoxin activity. Drinking water was withheld from the animals for a prior period of 18-22 1/2 hours. Rectal temperatures were determined and then each animal was provided 200 mls of test effluent from which to drink freely over the exposure period. Then their temperatures were again monitored. The exposure period and the before, after, and peak temperatures observed are shown in Table 11. As can be seen, there were 5 temperature increases, 1 decrease, and 2 unchanged. None of the increases satisfies the requirements of a 0.6°C increase according to the USP test for pyrogenicity (15), but a pyrogenic response was nonetheless observed in a majority of the test animals.

Inhalation --

A simple aerosol exposure chamber was built that was large enough to hold three rabbits in individual stanchions -- Figure 5. The stanchions

TABLE 10. COLIPHAGE AND COLIFORM ASSAYS OF AWT WATER SAMPLES AFTER RECEIPT IN COLLEGE STATION

Sample No.	Coliphages pfu/ml	Total Coliforms MPN/100 ml	Type of Sample
8-12	4	$.17 \times 10^3$	GAC product water
8-19	6	1.3×10^3	"
9-9	2	1.7×10^3	"
9-14	4	9.2×10^3	"
9-21	2	$.49 \times 10^3$	"
9-28	<2	$.27 \times 10^3$	"
10-5	<2	7.0×10^3	"
10-12	<2	$.33 \times 10^3$	"
10-19	2	5.4×10^3	"
10-26	<2	5.4×10^3	"
11-9	<2	$.33 \times 10^3$	"
11-16	<2	$.33 \times 10^3$	"
11-23	<2	$.79 \times 10^3$	"
12-6	<2	3.5×10^3	"
12-14 A	<2	350×10^3	GAC influent
12-14 B	<2	2.2×10^3	GAC midpoint
12-14 C	<2	$.7 \times 10^3$	GAC product water
12-21 A	<2	$.17 \times 10^3$	GAC influent
12-21 B	<2	3.5×10^3	GAC product water
12-21 C	<2	49×10^3	GAC midpoint
1-25 A	<2	92×10^3	GAC influent
1-25 B	<2	7.9×10^3	GAC midpoint
1-25 C	<2	3.3×10^3	GAC product water

TABLE 11. RESULTS OF INGESTION EXPOSURE

Type Animal	Period Without Water hrs	Waste Water Consumed mls	Period of Exposure hrs	Endotoxin Consumed µg	Temperature °C Before Exposure	After Exposure	Peak	Hours* After Exposure
Rabbit	18	103	1	309	39.4	39.3	39.7	2
Rabbit	18	78	1	234	39.0	39.2	39.4	3
Guinea pig	18	130	1	390	39.3	39.3	38.9	3
Guinea pig	18	101	1	303	39.7	39.7	39.7	3
Rabbit	22 ½	82	½	246	38.9	38.7	39.4	4
Rabbit	21 ½	70	3	210	39.2	39.2	39.2	3
Rabbit	22 ½	87	½	261	38.9	38.3	39.4	6
Rabbit	21 ½	40	3	120	39.2	39.2	39.3	2

* Hours after termination of exposure to reach the peak temperature observed.

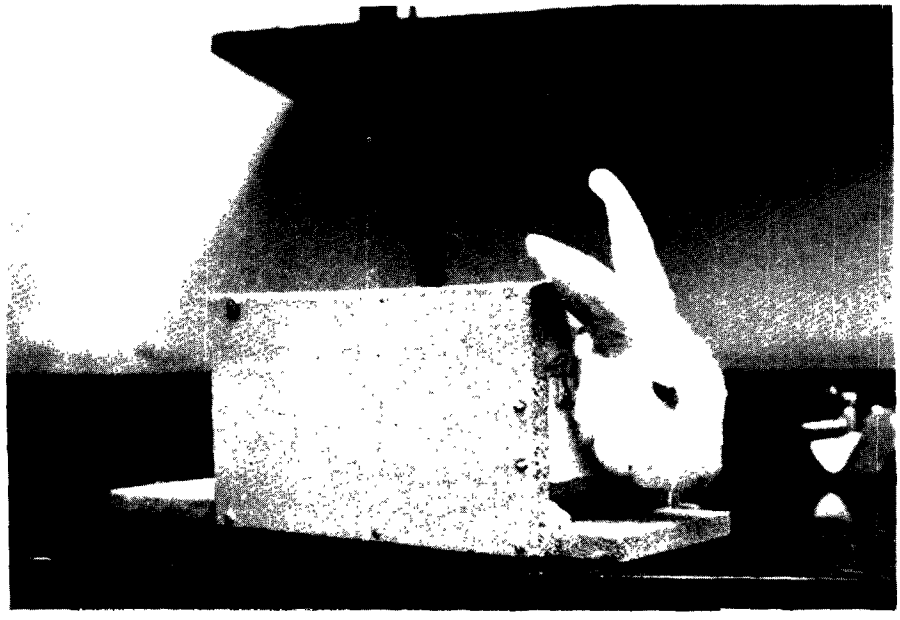


FIGURE 5. RABBIT IN STANCHION

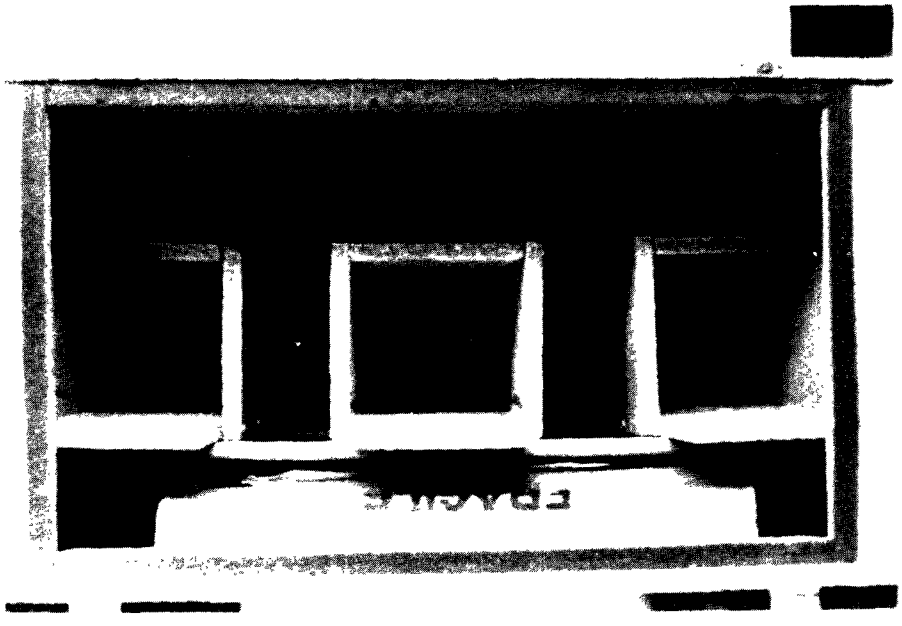


FIGURE 6. REAR OF THREE STANCHIONS SHOWN MOUNTED IN INHALATION CHAMBER WHICH ALSO SHOWS PLASTIC AEROSOL EXIT PIPES

were open in the rear -- Figure 6. Aerosols were generated by two DeVilbiss No. 15 atomizers operating at 20 psi. Each atomizer outlet was directed downward into a 500 ml beaker, each initially containing a reservoir of 300 mls of sample to be atomized. Atomizer suction lines were connected to withdraw from these reservoirs. Each beaker was placed under a 3.8 cm (1-1/2 in) ID plastic pipe about 13 cm (5 in) long which exhausted into the chamber -- Figure 7. A simple corrugated paper-board deflector was located between the inlets and the rabbits. Three exhaust pipes of the same type as the inlet pipes were connected to a box-manifold which was connected to the chemical hood by a corrugated plastic pipe -- Figure 8. Figure 9 shows the termistor probes in the chamber ready to receive rabbits.

A Climet 280 particle-size analyzer was used to evaluate an aerosol of a membrane-filtered final clarifier effluent from the College Station waste water treatment plant, since it was essential that respirable particles of less than 5 μm be produced. In two tests, particle size numbers in the 0.3-0.5, 0.5-1.0, 1.0-3.0, and 3.0-5.0 μm ranges were observed. There was an abundance of particles less than 3.0 μm but very few in the 3.0-5.0 μm range, and the system was considered satisfactory for producing respirable-sized particles.

Two aerosol sampling impingers, Ace Glass Incorporated -- 30 ml glass, were used at a flow of 1 lpm with a sampling medium of 20 ml of 0.9% NaCl irrigation solution which is pyrogen-free (McGaw Laboratories). One l pm is the approximate breathing rate of a rabbit (19). Both sampling impingers were used to determine the background endotoxin levels of the sampled chamber aerosols when pyrogen-free distilled water was atomized as well as during each test exposure. The 30-minute distilled water exposures demonstrated that the background air was free of endotoxin down to the sensitivity of the assay for the impinger fluid -- 0.06 μg/l.

All rabbits subsequently tested were first exposed to a pyrogen-free aerosol control. We stipulated that we would not use an animal for test if it showed an increase of 0.3°C or more in its rectal temperature after a control exposure (17), but none showed such a response.

In the actual test exposures, which duplicated the control exposure except for the substitution of endotoxin-containing water for the pyrogen-free water, the animals were placed in the chamber one hour before exposure and their temperatures recorded. They were then exposed for 30-minutes to an aerosol of dilutions of 0.45 μm filtered final effluent from the College Station plant which demonstrated an endotoxin activity of 1560 μg/l. The content of endotoxin per liter of air to which the animals were exposed was determined to be 0.6, 1.2, 1.25, and 25.0 ng/l by the all-glass impingers. The resulting calculated quantities of inhaled endotoxins was 18, 36, 37.5, and 720 ng, respectively. Not a single animal of the 18 exposed demonstrated a pyrogenic response. Since the last sample was undiluted final effluent, it was considered not necessary to conduct any further tests.

Injection --

A total of 26 1-ml injections to 24 rabbits were made with carbon-filtered waters from samples of 12-21 and 1-25 which were taken from different

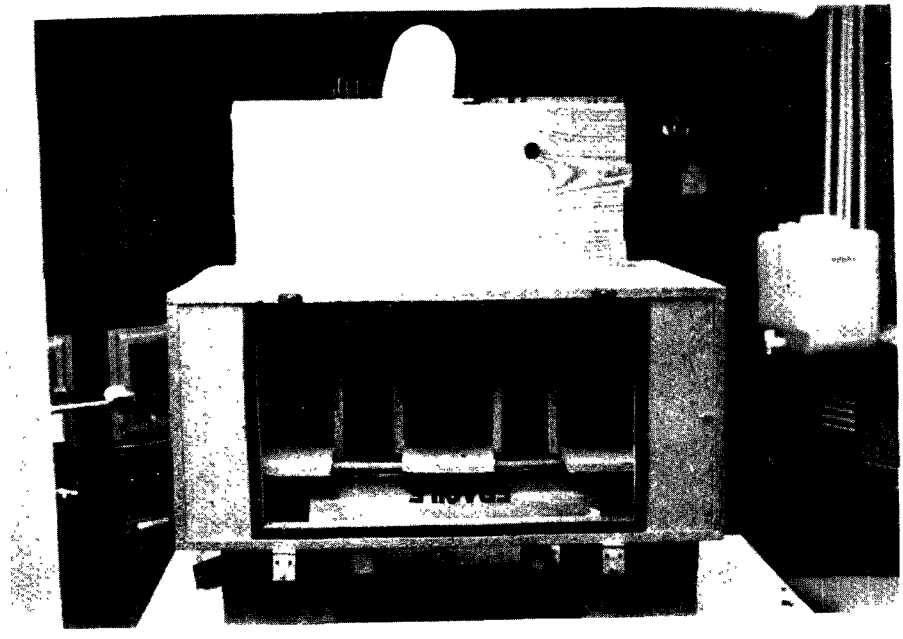


FIGURE 7. TWO PLASTIC AEROSOL ENTRANCE PIPES BELOW PAPERBOARD DEFLECTOR

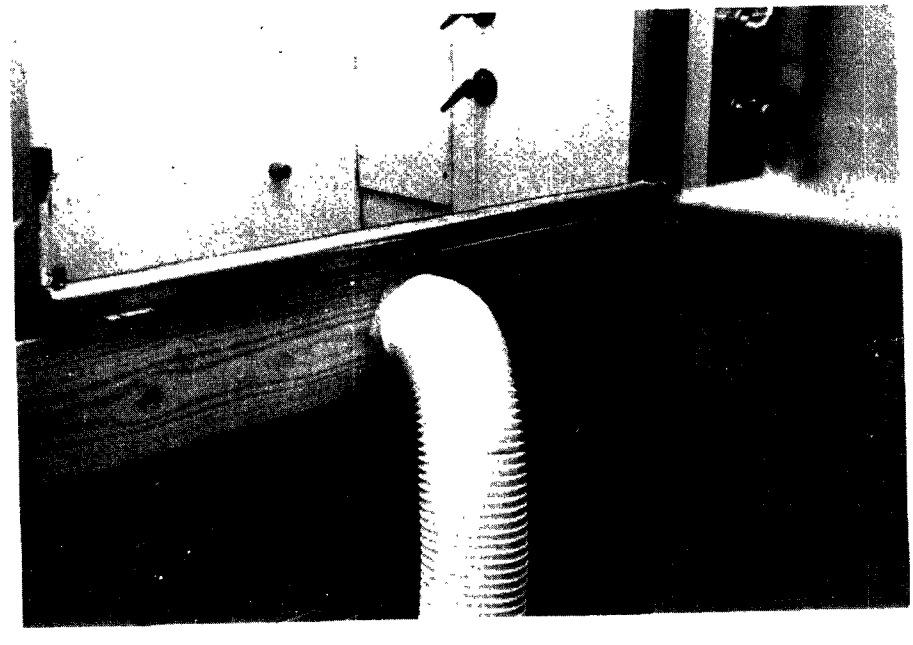


FIGURE 8. EXHAUST PIPE CONNECTED TO CHEMICAL HOOD

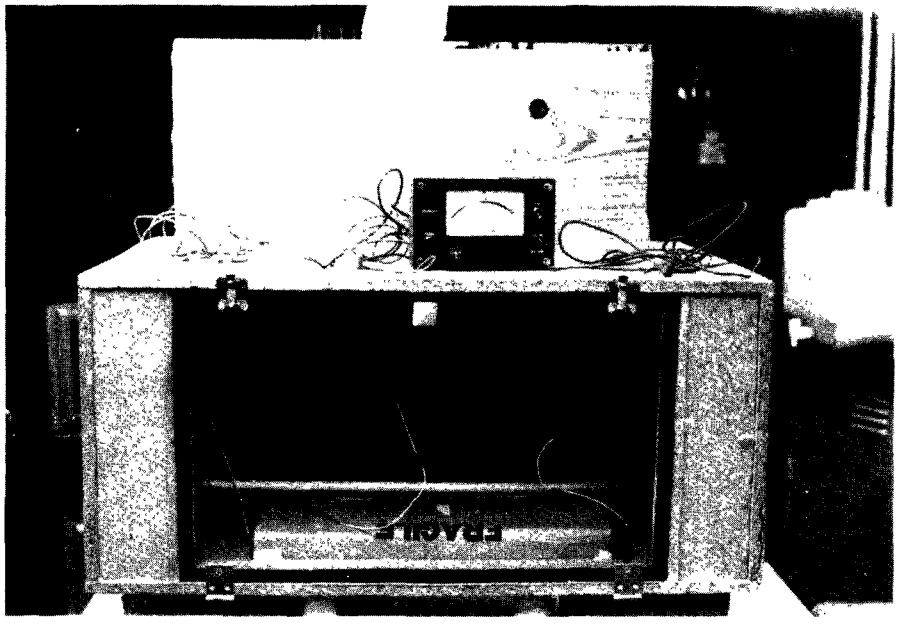


FIGURE 9. AEROSOL CHAMBER WITH THREE THERMISTORS READY TO RECEIVE RABBITS

points in the adsorption process (before, in the middle, and after) and of 2-8 and 3-1 which were samples that had been disinfected by UV, O_3, Cl_2, and high pH. Initially, we had wanted to use guinea pigs along with rabbits to determine the magnitude of pyrogenic response. However, the small blood vessels of the guinea pigs proved to be too difficult for our student researchers to locate and the animals were obviously uncomfortable with the students' fumbling probes and consequently we obtained permission to eliminate guinea pigs from the study.

Twenty rabbits were followed for changes in blood morphology that might be relatable to the injection of samples. This was done because we desired to use as few animals as possible in these studies and were concerned about maintaining their good health. Table 12 presents the pre-injection blood counts and Table 13 the post-injection blood counts for methematocrit (MHCT), red blood cells (RBC), white blood cells (WBC), monocytes (MONO), basophiles (BASO), eosinophles (EOSIN), lymphocytes (LYMPH), and neutrophiles (NEUTRO). It appeared to us that there were some startling changes in the lymphocytes and neutrophiles as well, perhaps, as in the white blood cells and monocytes. Some of the changes were contrary to expectations, but when the literature was checked it appeared that all observed changes are within normally expected variations. After noting this, blood testing was discontinued.

The individual responses of the 26 injections are displayed in Table 14. A pyrogenic response was observed following all 26 injections, ranging from a minimum of 0.1°C to a maximum of 1.7°C and averaging 1.0°C. Of the 26 responses, three were of insufficient magnitude to qualify the injected water as pyrogenic according to the USP test for pyrogenicity (15). Of these three, one had not been disinfected, one had been treated by high pH, and one by UV. Obviously, individual variability or sample variability are the likely explanations.

Twenty of the 26 injection samples were analyzed for endotoxin content. The temperature rise exhibited by each sample and its free endotoxin titer are collected in Table 15. A plot (not included) of the two sets of data showed that a relationship does not exist. It has been suggested that the lack of a relationship should be expected because of the wide response in individual animals (18).

The filtration of samples through 0.45 μm membrane filters does not positively exclude viruses. Hence, in order to check on viruses, coliphage analyses were routinely conducted on all membrane-filtered samples as well as on the samples prior to filtration but none was found.

TABLE 12. HEMATOLOGICAL ASSAY VALUES OF INDIVIDUAL RABBITS PRIOR TO I.V. INJECTION WITH MEMBRANE-FILTERED AWT SAMPLES

Animal	Date	Sample	Pre-injection							
			MHCT	RBC	WBC	MONO	BASO	EOSIN	LYMPH	NEUTRO
Fu	12-21	A	40	6,860,000	8,500	1	2	1	75	21
Joe	12-21	A	42	5,720,000	6,200	6	2	4	71	18
Bruce	12-21	B	45	6,640,000	5,400	4	4	3	56	33
Luke	12-21	B	39	6,040,000	10,505	5	1	0	62	32
Zulu	12-21	C	40	6,740,000	8,400	1	2	0	80	16
Ron	12-21	C	42	7,310,000	7,850	1	4	2	57	36
Zero	1-25	A	40	6,670,000	10,000	2	2	2	65	29
Fred	1-25	A	48	6,500,000	7,000	5	3	2	71	18
Jackass	1-25	B	37	5,960,000	7,800	4	2	2	73	19
Rufus	1-25	B	H	6,750,000	6,300	2	5	2	65	26
Duke	1-25	C	36	5,150,000	6,400	4	4	2	75	15
Lenny	1-25	C	40	5,800,000	7,000	4	2	1	81	12
Zonker	2-8	O_3	H	5,820,000	11,600	4	1	0	88	7
Funky	2-8	O_3	35	5,760,000	7,500	3	3	2	78	14
Jobe	2-8	pH	41	6,540,000	9,300	4	2	2	75	17
Billy	2-8	pH	39	6,380,000	8,900	1	3	4	65	27
Zek	2-8	Cl_2	39	6,640,000	13,400	4	2	2	63	29
Boyd	2-8	Cl_2	34H	6,120,000	7,400	5	3	1	67	24
Like	2-8	UV	41	6,940,000	7,900	3	2	2	75	18
Ferrus	2-8	UV	39	6,210,000	8,600	4	2	3	68	23

TABLE 13. HEMATOLOGICAL ASSAY VALUES OF INDIVIDUAL RABBITS FOLLOWING I.V. INJECTION WITH MEMBRANE-FILTERED AWT SAMPLES

Animal	Date	Sample	Post-injection							
			MHCT	RBC	WBC	MONO	BASO	EOSIN	LYMPH	NEUTRO
Fu	12-21	A	H	5,400,000	5,200	-	-	-	-	-
Joe	12-21	A	45	6,430,000	5,300	-	-	-	-	-
Bruce	12-21	B	40	6,200,000	4,000	4	2	2	63	29
Luke	12-21	B	38	5,770,000	5,400	2	3	1	76	16
Zulu	12-21	C	H	5,460,000	8,250	2	2	2	66	26
Ron	12-21	C	40	5,480,000	6,150	3	4	4	45	44
Zero	1-25	A	40	6,210,000	11,700	0	3	1	47	49
Fred	1-25	A	43	6,400,000	7,050	2	3	1	45	49
Jackass	1-25	B	40	6,300,000	6,300	3	2	2	62	31
Rufus	1-25	B	42	6,250,000	6,500	1	2	1	36	60
Duke	1-25	C	36	-	7,000	2	2	4	37	55
Lenny	1-25	C	35	-	7,000	8	4	3	54	31
Zonker	2-8	O_3	33	6,020,000	12,600	2	1	2	75	20
Funky	2-8	O_3	35	5,550,000	7,000	2	2	1	74	21
Jobe	2-8	pH	36H	7,020,000	8,500	3	2	4	63	28
Billy	2-8	pH	39	5,570,000	7,500	3	3	2	74	18
Zek	2-8	Cl_2	40	6,170,000	15,800	5	1	1	40	53
Boyd	2-8	Cl_2	40	6,450,000	7,400	5	2	3	43	47
Like	2-8	UV	42	7,010,000	7,200	1	1	3	71	24
Ferrus	2-8	UV	41	6,510,000	6,100	3	2	1	71	23

TABLE 14. PYROGENIC RESPONSE OF INDIVIDUAL RABBITS FOLLOWING I.V. INJECTION WITH MEMBRANE-FILTERED AWT SAMPLES

Animal	Date	Sample	Temperature °C (Elapsed Time)									ΔT
			0 HR	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	PEAK	°C
Fu	12-21	A	39.8	40.6						40.0	40.6	0.8
Joe	12-21	A	39.2	39.9						39.3	39.9	0.7
Bruce	12-21	B	39.9	41.2							41.2	1.3
Luke	12-21	B	39.8	41.0							41.0	1.2
Zulu	12-21	C	39.6	40.6							40.6	1.0
Ron	12-21	C	40.2	41.3							41.3	1.1
Zero	1-25	A	39.1	40.4				39.4			40.4	1.3
Fred	1-25	A	39.1	40.3				39.6			40.3	1.2
Jackass	1-25	B	39.6	40.0		39.7					40.0*	0.4
Rufus	1-25	B	39.3	40.7		40.1					40.7	1.4
Duke	1-25	C	39.0		40.2						40.2	1.2
Lenny	1-25	C	39.2		40.4						40.4	1.2
Zonker	2-8	O_3	39.7	40.7	40.4	40.1	39.8				40.7	1.0
Funky	2-8	O_3	39.7	40.4	40.5	40.0	40.1	39.5			40.5	0.8
Jobe	2-8	pH	39.2	39.1	39.3	39.2					39.3*	0.1
Billy	2-8	pH	39.2	39.9	39.9	39.8					39.9	0.7
Zek	2-8	Cl_2	39.4		40.7	40.3	39.9	39.5	39.1		40.7	1.3
Boyd	2-8	Cl_2	39.3	40.5	40.1	39.5	39.5	39.4			40.5	1.2
Like	2-8	UV	39.1	40.7	40.8	40.4	40.1	39.8	39.8		40.8	1.7
Ferrus	2-8	UV	39.5	40.2	40.5	40.4	39.7	39.5	39.3		40.5	1.0

(continued)

TABLE 14. (continued)

Animal	Date	Sample	Temperature °C (Elapsed Time)									ΔT
			0 HR	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	PEAK	°C
Zek	3-1	O_3	38.7	39.5	40.2	39.8	39.2	39.2			40.2	1.5
Jack	3-1	O_3	39.1	39.5	39.9	39.7	39.4	39.2	39.0		39.9	0.8
Boyd	3-1	Cl_2	39.2	40.7	40.0	39.8	39.4				40.7	1.5
Gab	3-1	Cl_2	39.5	40.7	40.4	40.1	39.7				40.7	1.2
Ripe	3-1	UV	39.5	40.0	39.9	39.6					40.0*	0.5
Job	3-1	UV	39.0	39.5	39.7	39.5					39.7	0.7
		Min	38.7								39.3	0.1
		Max	40.2								41.3	1.7
		Mean	39.4								40.4	1.0

* $\Delta T < 0.6°C$

ΔT = Peak minus 0 Hour

TABLE 15. THE PYROGENIC RESULTS OF FREE ENDOTOXIN-CONTAINING AWT SAMPLES

Sample	Temp. Rise °C	Free Endotoxin, ng administered*
12-21-A	0.8	250
"	0.7	250
B	1.3	50
"	1.2	50
C	1.0	100
"	1.1	100
1-25-A	1.3	125
"	1.2	125
B	0.4	50
"	1.4	50
C	1.2	75
"	1.2	75
2-8-O_3	1.0	6
"	0.8	6
pH	0.1	6
"	0.7	6
Cl_2	1.3	15
"	1.2	15
UV	1.7	15
"	1.0	15

* ng/kg dose ∿ ng administered ÷ 3

SECTION 7

WATER TREATMENT PLANT RESULTS

A total of 28 water treatment plant GAC systems were sampled. Several plants whose samples were lost in shipment were resampled; one plant was sampled and analyzed twice (Sample Nos. 11 and T4). The specific analytical results and operational data relating to each sample are contained in the Appendices, pp A-19 to A-70.

Microbiological and Endotoxin Observations

Table 16 is a compilation of the bacteriological results on each sample pair -- one sample taken prior to GAC contact and the other after GAC contact. The table shows only the standard plate count and total coliform results -- the latter by both MF and MPN procedures. Coliphage analyses were also run on each sample; no coliphages were found either before or after GAC treatment. In only two instances were coliforms found, and these were both before GAC contact. They were detected by MF and MPN procedures and in both cases the MPN procedure estimated a higher number than counted by MF. No coliforms were found after GAC.

The standard plate count data of Table 16 show that 14 of the 28 samples decreased thru GAC, 8 increased, and 6 were unchanged. Although the average of the before-GAC samples of 18.9/ml increased to 61.4/ml after GAC, the increase was caused by a minority of samples. If the two high after-GAC sample values of 320 and 980 are deleted, the remaining average would be only 16.1 -- essentially unchanged from the before-GAC average.

The two highest after-GAC standard plate count values were from recently-installed carbon beds (15 days of operation at time of sampling) at the same plant. Sample 26 A-16 is also from that plant -- a third carbon bed. This plant is experimenting with three different brands of activated carbon and all three beds were placed in operation at the same time, were being back-washed at the same frequency, etc. The disparate standard plate count results are explained by the accidental loss of thiosulfate from the sample bottle of 26 A-16 during sampling. This does not, of course, explain the higher plate count samples.

We should expect that the application of chlorine prior to GAC would influence the microbiology of the water both prior to and following GAC. However, only one of the 28 samples (No.33) in Table 16 came from a plant that does not prechlorinate. Three samples showed no Cl_2 residual (see data in Appendices) prior to GAC even though prechlorination was practiced, these being Nos. 24, 88, and T2. Sample 24 was one of two that contained coliforms

TABLE 16. BACTERIOLOGICAL RESULTS OF WATER TREATMENT PLANT SAMPLES BEFORE AND AFTER GAC FILTRATION

Sample No.	Standard Plate Count per ml	Before GAC Total Coliforms/100 ml MF	Before GAC Total Coliforms/100 ml MPN	Standard Plate Count per ml	After GAC Total Coliforms/100 ml MF	After GAC Total Coliforms/100 ml MPN
77	74	0	<2	34	0	<2
22	18	0	<2	11	0	<2
11	25	0	<2	5	0	<2
55	0	0	<2	0	0	<2
33	39	0	<2	4	0	<2
44 A	0	0	<2	0	0	<2
44 B	0	0	<2	1	0	<2
88	88	0	<2	8	0	<2
66	32	9	46	2	0	<2
99	0	0	<2	0	0	<2
12	14	0	<2	1	0	<2
14	86	0	<2	20	0	<2
16	15	0	<2	0	0	<2
18-4	1	0	<2	0	0	<2
18-5	3	0	<2	1	0	<2
24	48	4	49	5	0	<2
26 A-12	0	0	<2	320	0	<2
26 A-14	0	0	<2	980	0	<2
26 A-16	0	0	<2	0	0	<2
28	0	0	<2	0	0	<2
30 A-1	1	0	<2	64	0	<2
30 A-2	64	0	<2	241	0	<2
T1	3	0	<2	3	0	<2
T2	8	0	<2	11	0	<2
T3	1	0	<2	2	0	<2
T4	3	0	<2	1	0	<2
T5	3	0	<2	1	0	<2
T6	2	0	<2	3	0	<2
$\bar{X}$	18.9			61.4		

Standard Plate Count of 0 = <0.5/ml.

MF Coliform Value of 0 = <1.0/100 ml.

prior to GAC.

A most important relationship is the potential for impact of the organic material (TOC) adsorbed onto the carbon to impact on the bacteriological quality of the product water. Table 17 shows the TOC both before and after carbon contact as well as the endotoxin results. The average of all TOC analyses shows a removal through GAC from a level of 3.9 mg/l before to 3.2 mg/l after, a removal of 18 percent. Interestingly, the percent decrease in total endotoxin through GAC also averages 18 percent.

A plot of the standard plate count of the after-GAC samples from Table 16 vs. the change in TOC through the GAC from Table 17 shows in Figure 10 a rather scattered relationship favoring the hypothesis of an increased standard plate count with an increased removal of TOC by the GAC bed. In support of such a relationship is the nonparametric observation that the 7 plants with highest TOC removals include 5 of the 8 plants with the highest plate counts:

Sample	TOC Removed mg/l	Sample	SPC after GAC #/ml
14	2.9	26 A-14	980
77	2.4	26 A-12	320
55	2.3	30 A-2	241
30 A-2	1.8	30 A-1	64
T1	1.6	77	34
26 A-12	1.6	14	20
26 A-14	1.6	22	11
		T2	11

Table 17 also shows that the averages of total endotoxin activity were decreased through GAC from 21.2 to 17.3 μg/l, and the free endotoxin activity also decreased -- but only slightly -- from an average of 9.0 to 8.7 μg/l. By difference, the averages of bound endotoxin activity was decreased from 12.2 to 8.6 μg/l. The percent decreases for the three forms of endotoxin, total, free, and bound, were 18, 3, and 30 percent, respectively. The suggestion is that the carbon was functioning as a filter by removing bound endotoxin and that essentially no adsorption of free endotoxin was occurring. These results are compatible with the performance of the second (downstream) GAC column in the AWT studies which show comparable removal values of 20, +12, and 42 percent for the three forms of endotoxin, respectively. The increased filtration ability of the AWT column should be expected since that carbon was 3.05 M (10 ft) deep compared to the maximum of 1.5 M (5 ft) or average of 0.76 M (30 in.) for the water treatment plant installations (Table 18). Empty-bed contact time for the two applications differs considerably also, averaging 8.75 minutes (Table 18) for the water treatment applications vs. 45 minutes for the second GAC column in the AWT studies. The increase in free endotoxin through the AWT carbon was discussed in Section 6, and in context with the slight removal observed in the drinking

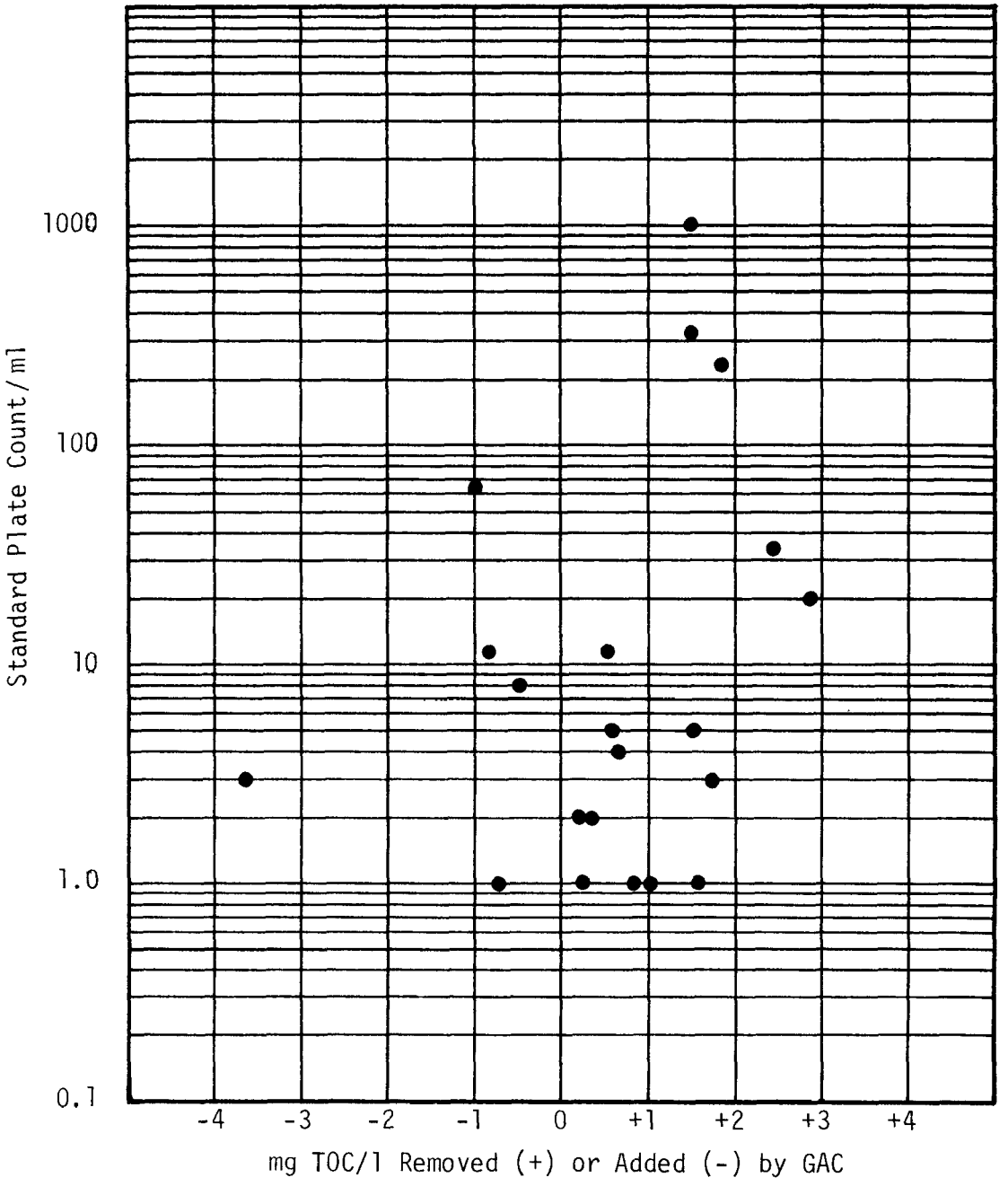


FIGURE 10. STANDARD PLATE COUNT AFTER GAC vs. CHANGE IN TOC

TABLE 17. TOC CONCENTRATIONS AND ENDOTOXIN ACTIVITIES OF WATER TREATMENT PLANT SAMPLES BEFORE AND AFTER GAC FILTRATION

Sample No.	Before				After			
	TOC mg/l	Endotoxin Equivalents, μg/l Total	Free	Bound	TOC mg/l	Endotoxin Equivalents, μg/l Total	Free	Bound
77	6.4	3.1	3.1	0.0	4.0	3.1	3.1	0
22	4.4	3.1	1.6	1.5	3.8	1.6	1.6	0
11	4.8	15.6	3.1	12.5	3.3	3.1	3.1	0
55	3.6	12.5	6.3	6.2	1.3	6.3	6.3	0
33	8.1	31.3	12.5	18.8	7.5	31.3	12.5	18.8
44 A	5.3	6	2.4	3.6	4.4	6	2.4	3.6
44 B	4.8	6	2.4	3.6	4.0	6	2.4	3.6
88	5.9	120	24	96	6.4	60	24	36
66	3.2	36	18	18	2.9	18	12	6
99	3.0	6	0.6	5.4	2.3	6	2.4	3.6
12	2.2	3.6	0.06	3.54	1.2	2.4	1.2	1.2
14	5.2	60	12	48	2.3	60	18	42
16	1.8	18	12	6	1.7	12	12	0
18-4	2.7	5	1.3	3.7	2.1	2.5	1.3	1.2
18-5	2.5	5	1.3	3.7	2.2	1.3	1.3	0
24	3.9	37.5	5	32.5	3.3	37.5	5	32.5
26 A-12	3.1	12.5	12.5	0	1.5	25	12.5	12.5
26 A-14	3.1	12.5	12.5	0	1.5	25	12.5	12.5
26 A-16	3.1	12.5	12.5	0	1.6	12.5	12.5	0
28	3.5	25	12.5	12.5	2.4	25	12.5	12.5
30 A-1	3.5	25	12.5	12.5	4.5	25	12.5	12.5
30 A-2	4.5	25	12.5	12.5	2.7	25	12.5	12.5
T1	5.2	25	12.5	12.5	3.6	25	12.5	12.5
T2	2.9	12.5	2.5	10	3.7	10	2.5	7.5
T3	3.7	12.5	7.5	5	3.5	10	7.5	2.5
T4	3.5	25	12.5	12.5	2.0	10	7.5	2.5
T5	3.0	12.5	10	2.5	3.7	10	5	5
T6	3.3	25	25	0	6.7	25	25	0
$\bar{X}$	3.9	21.2	9.0	12.2	3.2	17.3	8.7	8.6

water studies, supports the observation of a lack of carbon's ability to adsorb free endotoxins.

Pseudomonas aeruginosa assays were conducted on 25 pair of before-and-after GAC samples. Only 3 were positive. Plants 33 and 24 had 30 and 10 percent positives for P. aeruginosa in before-GAC samples; both were negative in the after-GAC samples. Only plant 14 yielded P. aeruginosa in the product water after GAC, but this sample had a 70% yield. P. fluorescens was determined on 21 pair of samples with completely negative results. All three samples yielding pseudomonads prior to GAC showed total chlorine residual values <0.05 mg/l.

Gram-stain results of 14 before-GAC samples showed from 22 to 100% to be gram-negative organisms and of 14 after-GAC samples from 30 to 100% gram negatives. Five samples of before-GAC water yielded 100% gram negative organisms, and 10 samples of after-GAC water yielded 100% gram negatives. Only 10 of the 14 samples were pairs taken from the same GAC beds.

Pyrogenic Observations

Every after-GAC water treatment plant sample was passed through a 0.45 μm membrane filter and one ml was injected into the marginal vein of rabbits. The free endotoxin dose administered to each rabbit is shown in Table 17 and ranged from 1.6 ng to 25 ng (1 μg/l = 1 ng/ml). Since the weight of each rabbit was close to the 3 kg average of all rabbits, the dose administered ranged from approximately 0.5 ng/kg to 8.0 ng/kg. Not a single rabbit developed a fever. At the end of the project, every rabbit used was given an injection of a 2 ng/kg pyrogen standard and every one developed a fever. These results are markedly different from those observed in the AWT studies (Table 15) where all 20 rabbits injected showed a temperature rise, 18 of which rises were sufficient by USP Pyrogen testing criteria to classify the injected waters as pyrogenic. Although the free endotoxin levels were considerably higher for the AWT waters, eight of the AWT samples contained free endotoxins at levels observed in the water treatment plant studies.

Operation of the GAC Beds

The water treatment plants sampled yielded a considerable range in the length of time that the GAC beds had been in operation. Plant No. 26,as has been mentioned, had just installed three fresh beds of carbon -- each from a different proprietor -- with the intention of comparing them. These three GAC beds were the newest encountered in the study. Plant No. 22 had the longest operational run of any -- 110 months! Based upon a single pair of samples, it was still removing 14 percent of TOC and about 50% of the total endotoxins at the time of sampling. Table 19 shows the months of operation, TOC removal in mg/l and percent, and bound endotoxin removed in μg/l for each GAC bed sampled. Average removal of TOC for all data is 0.7 mg/l and percent removal, 16.4.

TABLE 18. OPERATING DATA FOR GAC IN WATER TREATMENT PLANTS SAMPLED

Sample No.	Flow Rate $\frac{gpm}{ft^2}$	Carbon Depth in.	Empty Bed Contact min.	Installation Date	Last Regeneration Date	Temp. at Sampling °C	Cl_2 Residual Before GAC mg/l Free	Total
77	5.5	36	4.1	10/70	8/77	23.3	—	1.0
22	2.0	24	7.5	9/68	9/68	17.2	2.4	4.1
11	2.5	30	7.5	9/71	5/77	11.7	0.2	0.8
55	2.0	18	5.6	8/71	8/71	11.1	—	0.3-0.8
33	2.0	15	4.7	10/71	10/71	5.0	0.0	0.0
44 A	2.8	48	10.7	Fall 74	Fall 74	5.0	<0.1	0.1
44 B	2.8	48	10.7	Fall 74	Fall 74	5.0	<0.1	0.1
88	3.0	30	6.2	1/76	1/76	4.4	—	—
66	2.0	12	3.7	4/77	4/77	1.0	0.1-0.4	0.2-0.6
99	2.3	16	4.4	1973	4/75	2.0	3.2	3.6
12	1.5	31	12.9	1/76	1/76	2.2	0.7	0.8
14	2.0	14	4.4	1972	9/77	6.1	0.4	—
16	2.8	30	2.4	1972	7/77	1.7	1.0	1.7
18-4	2.4	30	7.8	6/73	6/73	2.8	0.2	0.35
18-5	2.4	30	7.8	6/75	6/75	2.8	0.2	0.35
24	1.5	18	7.5	1971	11/77	1.1	0.0	0.0
26 A-12	2.2	33	9.3	3/78	3/78	4.0	1.0	1.25
26 A-14	2.2	33	9.3	3/78	3/78	4.0	1.0	1.25
26 A-16	2.2	33	9.3	3/78	3/78	4.0	1.0	1.25
28	1.9	28	9.2	1972	10/77	14.0	0.15	0.4
30 A-1	4.0	60	9.3	1962	1962	16.7	1.5	1.7
30 A-2	4.0	60	9.3	1962	12/77	16.7	<0.1	0.1
T1	0.75	48	39.9	1973	7/76	13.0	0.35	—
T2	0.64	11	10.7	9/74	9/74	13.0	0.0	0.0
T3	1.6	48	18.7	3/77	3/77	10.0	—	0.3
T4	2.7	33	7.6	9/71	5/77	12.0	0.5	1.0
T5	3.0	11	2.3	1971	6/77	10.0	0.1	0.7
T6	2.8	10	2.2	7/68	3/75	11.0	0.15	0.45
$\bar{X}$			8.75					

TABLE 19. TOC AND ENDOTOXIN PERFORMANCE OF GAC WITH TIME

Sample No.	Month Sampled	Months of Operation	TOC Removed mg/l	TOC Removed %	Bound Endotoxin Removed µg/l
77	9/77	1	2.4	38	0
22	9/77	110	0.6	14	1.5
11	10/77	5	1.5	31	12.5
55	10/77	74	2.3	64	6.2
33	11/77	73	0.6	7	0
44 A	12/77	38	0.9	17	0
44 B	12/77	38	0.8	17	0
88	12/77	23	+0.5	+8	60
66	1/78	9	0.3	9	12
99	1/78	33	0.7	23	1.8
12	2/78	25	1.0	45	2.3
14	2/78	5	2.9	56	6
16	2/78	7	0.1	6	6
18-4	2/78	56	0.6	22	2.5
18-5	2/78	32	0.3	12	3.7
24	3/78	4	0.6	15	0
26 A-12	3/78	0	1.6	52	+12.5
26 A-14	3/78	0	1.6	52	+12.5
26 A-16	3/78	0	1.5	48	0
28	4/78	6	1.1	31	0
30 A-1	4/78	12	+1.0	+29	0
30 A-2	4/78	4	1.8	40	0
T1	4/78	21	1.6	31	0
T2	4/78	43	+0.8	+28	2.5
T3	4/78	13	0.2	5	2.5
T4	4/78	11	1.5	43	10
T5	4/78	10	+0.7	+23	+2.5
T6	4/78	37	+3.4	+103	0
$\bar{X}$			0.7	16.4	

Sample Nos. 11 and T4 are from the same plant and GAC bed. The two samplings yielded virtually the same results in spite of the carbon being 6-months older during the second sampling. Plant No. 44 had two identical GAC beds in parallel operation; the sampling results showed them to be performing essentially identically. Plant No. 18 had 2 GAC beds operated identically, but the carbon in one bed (18-4) was 24 months older than the carbon in the other (18-5) bed. The data show the older bed to be removing more of the TOC but less of the bound endotoxin. However, the TOC levels are quite low; only two samples of the 28 contained lower TOC concentrations. Plant No. 30 utilized two GAC beds in series operation. Water passed through 30 A-1 (the primary bed) first which contained spent carbon and then passed through 30 A-2 (the secondary bed) which contained the newer carbon. Of interest is that the primary bed was the second sample that we observed up to that time which showed an increase in TOC through the GAC bed. And it was a substantial increase of 29 percent vs. the other sample's increase of only 8 percent. Three more samplings among the last five samples taken during the project were also to yield substantial increases. However, in the case of Plant No. 30, the secondary bed removed 40 percent of the applied TOC so that an overall removal of TOC occurred through the plant. Endotoxin levels were unchanged through both beds.

The decreases (or increases) of TOC through the GAC beds in terms of mg/l and percent are plotted vs. months of operation in Figures 11 and 12, respectively. Figure 13 shows a curve of percent TOC removals with time through a GAC bed observed in a pilot-scale study by EPA in Cincinnati, Ohio using Ohio River water (20). Empty-bed contact time was almost the same, approximately 9 minutes for the pilot plant vs. our 8.75-minute average. The percent reductions observed in our study for those beds in operation for less than 10 months are plotted as dots on the same figure. The data seem to show that better removals were consistently observed for Ohio River water, so subsequently the individual empty-bed contact time in minutes for each dot was added to the figure. Viewed along with the contact time, the data do not seem to be as disparate with the main exception being the three clustered data points at one-half month which are from the three beds (samples 26 A-12, 26 A-14, and 26 A-16) in the plant that was previously mentioned as experimenting with 3 different carbons.

The relatively common occurrence of an increase in TOC through a GAC bed -- 5 times out of 28 samplings -- might give cause for some concern. However, it must be remembered that these results are based upon single samples, and on that basis some dispersion of results should be expected. We wondered about the likelihood of a temperature-change effect in going from winter to spring. The average temperature at the time of sampling (Table 18) for all samples collected in the winter months -- December through March -- was 3.92°C and for all samples collected in the one spring month -- April -- was 12.93°C. The increase of 9°C might well be sufficient to stir some biological activity. Endotoxin data are of no help in estimating this likelihood (Table 17), but this should be expected in view of the AWT-water findings (Section 6) that endotoxins appear unable to reliably predict the microbial content of water samples which have been subjected to disinfection (Cl_2, O_3, high pH, or UV) treatments. All but one of the water treatment plants practice prechlorination.

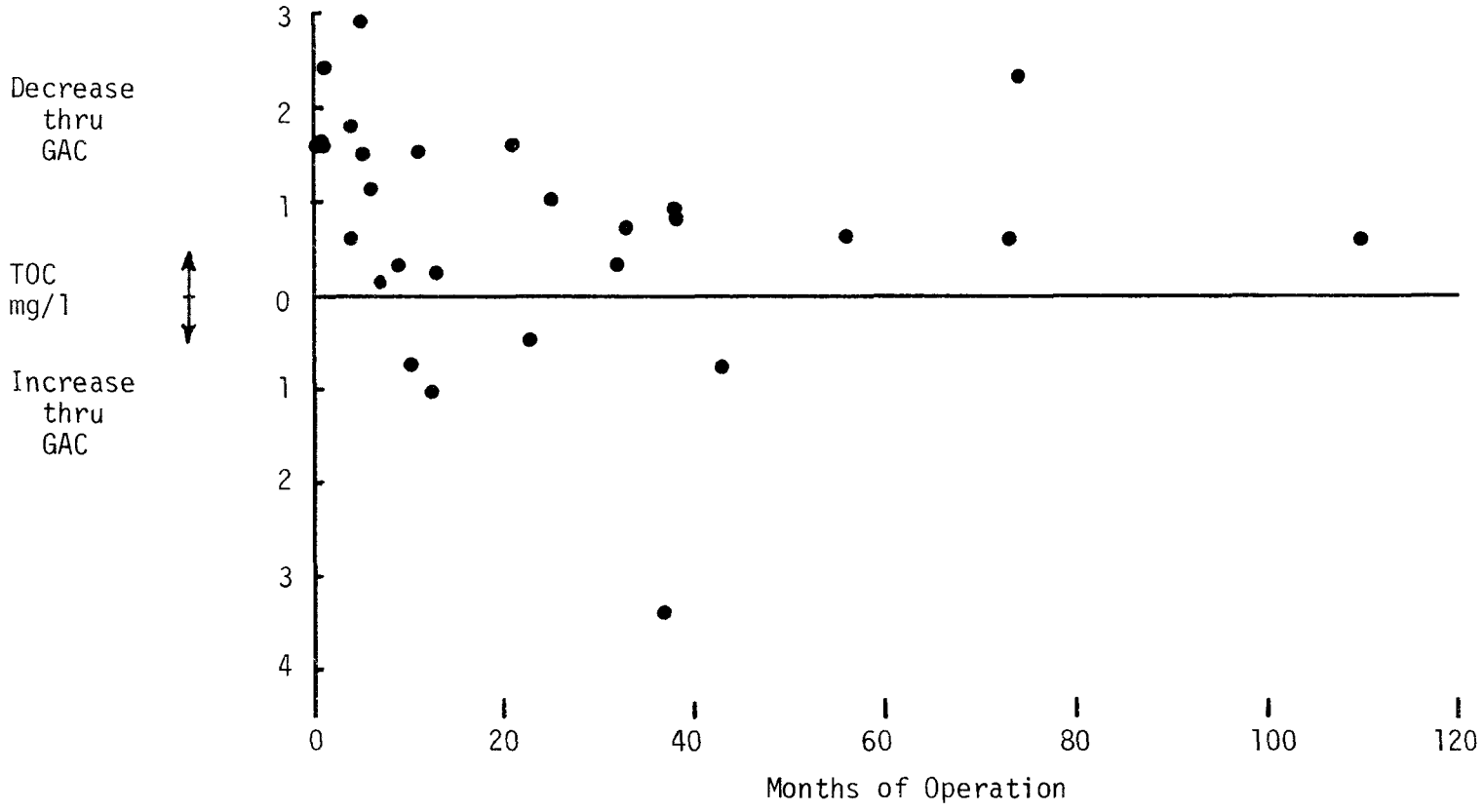


FIGURE 11. TOC REMOVAL IN MG/L vs. MONTHS OF OPERATION OF GAC

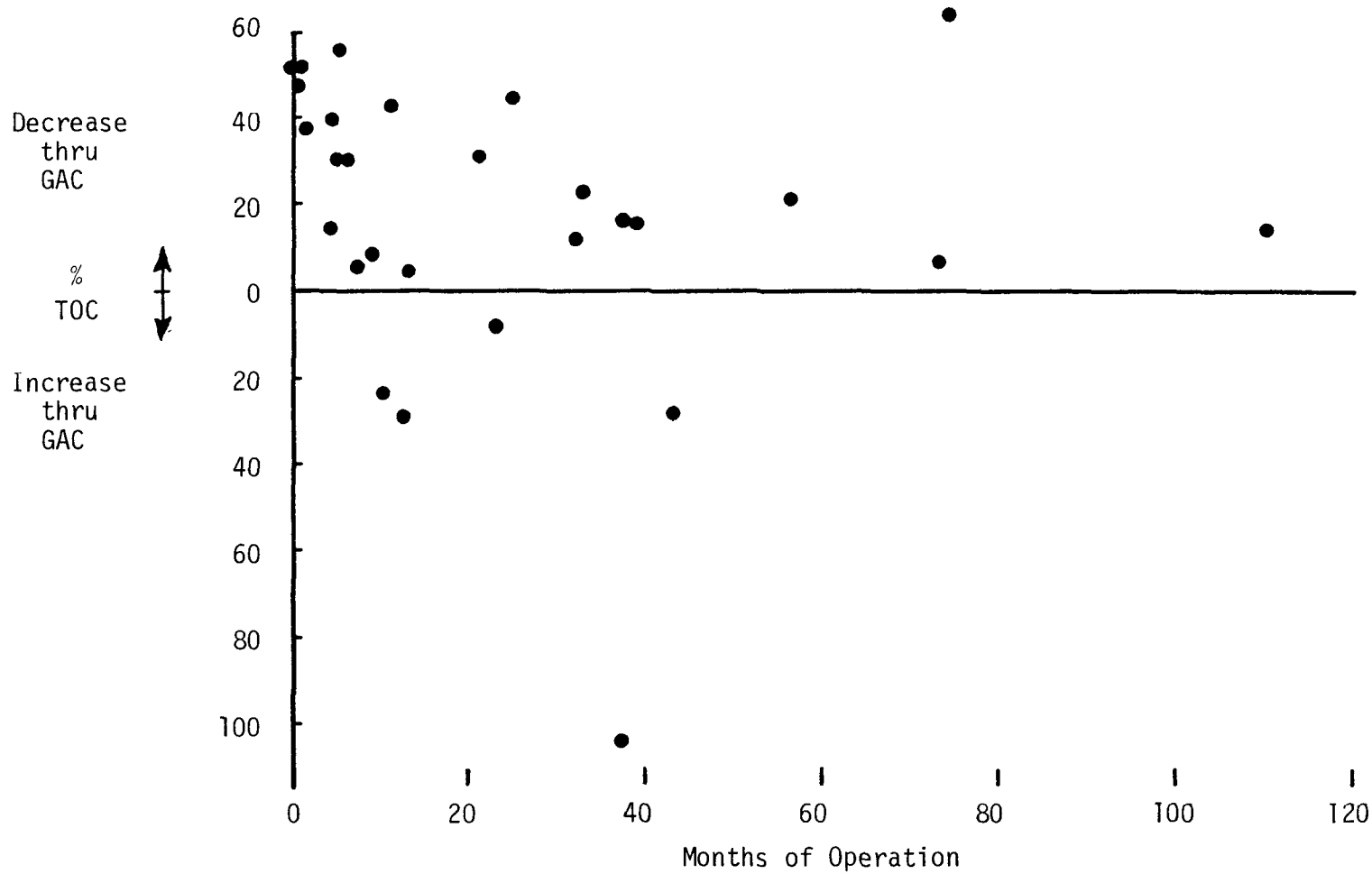


FIGURE 12. TOC REMOVAL IN PERCENT vs. MONTHS OF OPERATION OF GAC

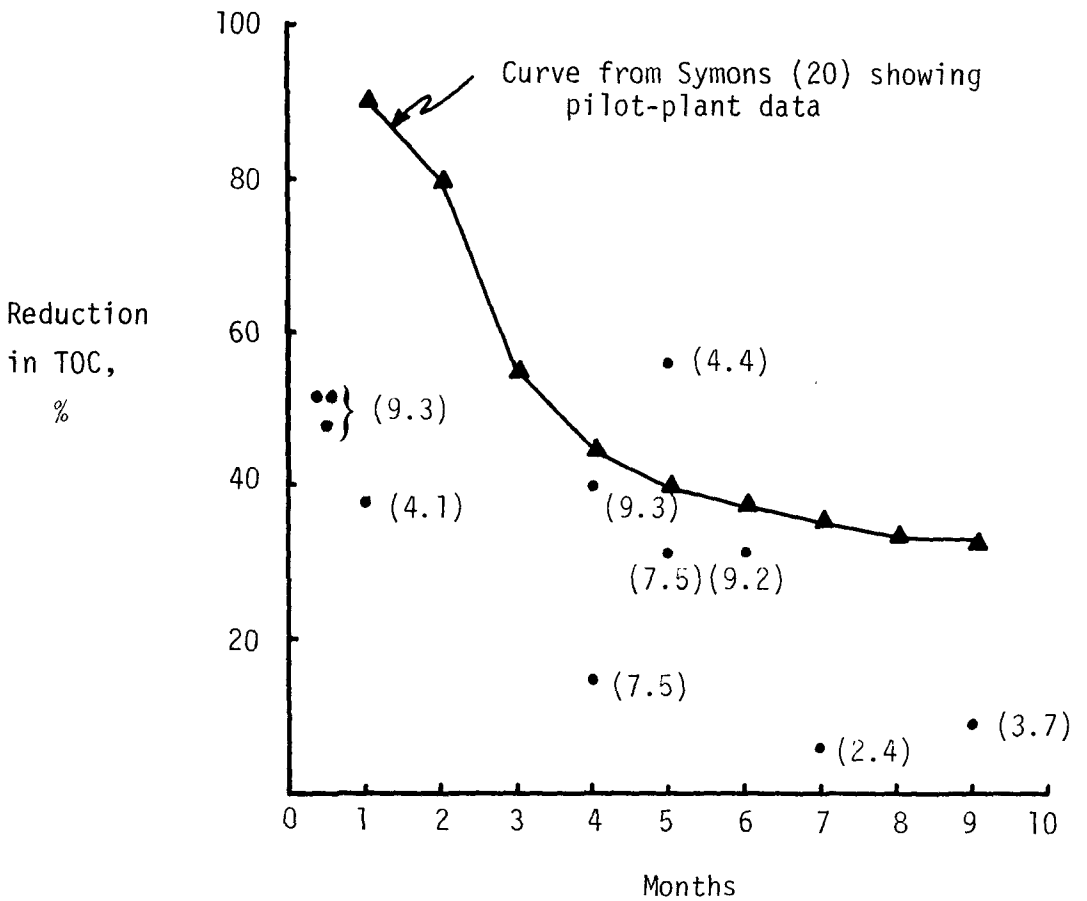


FIGURE 13. COMPARISON OF TOC REMOVALS OBSERVED WITH CINCINNATI PILOT-PLANT DATA

The last six carbon beds were sampled by study-team members and dissolved oxygen (DO) determinations were therefore made. The before-and after-GAC values determined were:

Sample No.	DO content, Before GAC	mg/l After GAC
T1	9.4	9.3
T2	10.8	10.7
T3	11.0	10.6
T4	10.9	10.7
T5	11.3	11.2
T6	11.8	11.8
$\bar{X}$	10.87	10.72

The very slight drop reinforces the observation of only a very slight degree of biological activity in the water treatment plant applications of GAC.

Figure 14 is a plot of percent TOC removed vs. empty-bed contact time in minutes. Negative removals, that is, data showing increases of TOC through GAC, were not included in this plot. Obviously, a near vertical line is apparent which suggests that the two parameters are unrelated. The months of operation of the GAC associated with each data point is also shown in the figure, and once again a non-parametric observation reveals that the average time of operation of the 11 highest (% TOC removed) data points is 13.3 months whereas the average time of operation of the 11 lowest % removed points is 37.7 months. The water treatment plant data assembled in this report, therefore, are better described by the duration of operation of the carbon than by empty-bed contact time. We believe this simply reflects that the adsorptive capacity of the carbon beds which processed the waters sampled in this study was quite generally exhausted, and that the removals -- or increases -- were functions primarily of a low-level of residual adsorptive activity in the beds coupled with an occasional mechanical sloughing.

The project inquired of all locations what tests were used to evaluate the effectiveness of GAC operation. Table 20 lists those tests that were cited by two or more plants. Not listed are single votes for TOC, standard plate count, Fe, Mn, purgeable organic carbon, non-purgeable organic carbon, alkalinity, hardness, CO_2, phenol no., fluoride, core samples, DO, and abrasion no. The scatter strongly suggests the need for a rather massive training effort to accompany any program designed to promote more widespread application of GAC.

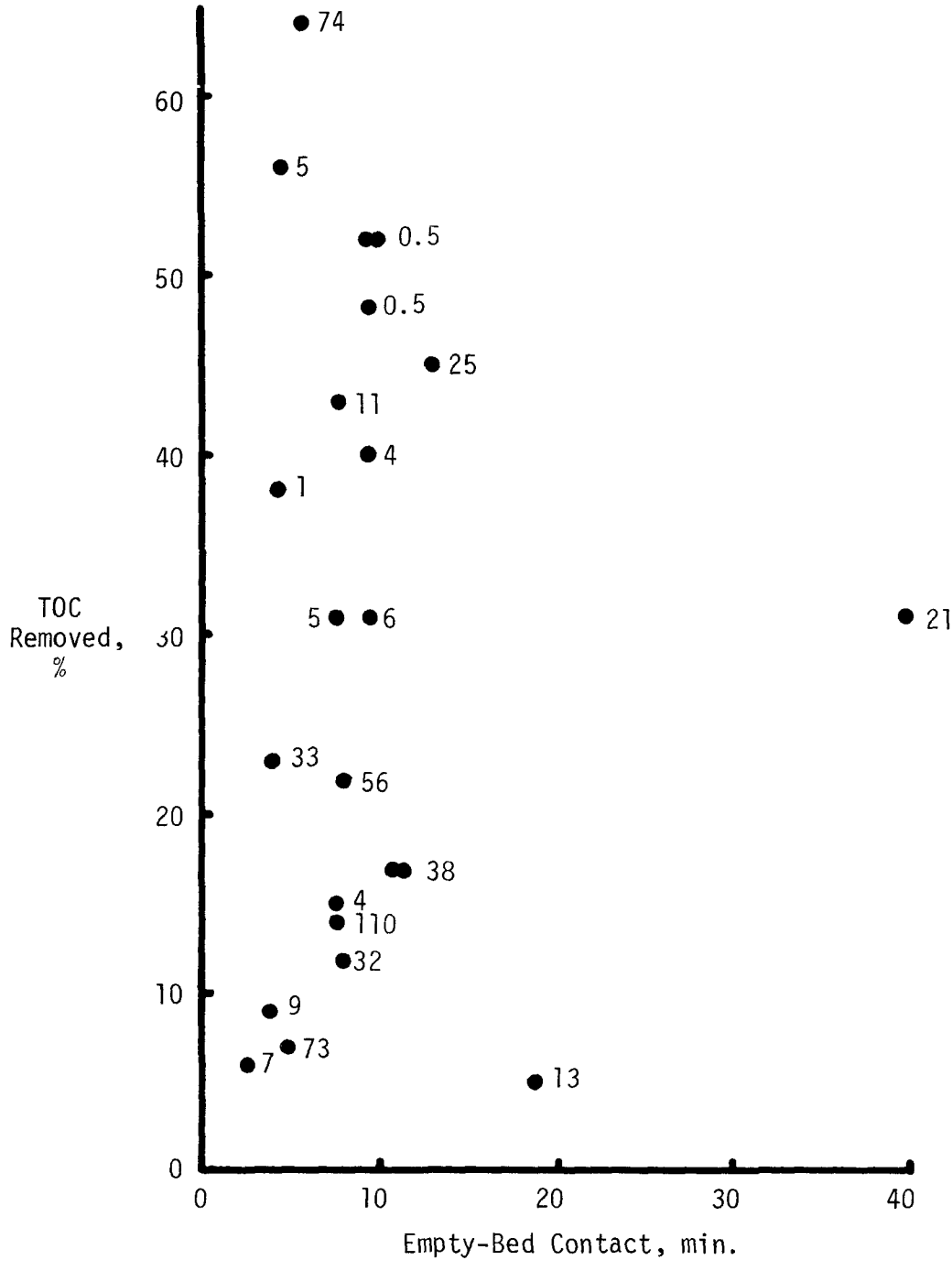


FIGURE 14. PERCENT TOC REMOVED vs. EMPTY-BED CONTACT TIME

TABLE 20. MONITORING METHODS UTILIZED FOR GAC CONTROL

Water Treatment Plant	Color	Turbidity	Cl_2 Residual	pH	Odor	Total Coliforms	Total Tri-halomethanes	Iodine No.	Apparent Density	% Ash	Fluorescence Scan
22			X								
77			X								
11			X		X						X
55	X	X	X		X	X					
33											
44			X				X				X
88					X	X		X	X	X	
66			X								
99			X					X	X		
12			X								
14			X		X						
16			X		X			X			
24			X		X						
18		X	X					X			
26	X	X	X	X	X		X			X	
28			X								
30			X		X						
T1	X	X	X	X							
T2			X								
T3	X	X	X	X							
T4		X	X		X						
T5	X	X	X								
T6			X								

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APPENDIX A

AWT EFFLUENT SAMPLES, BEFORE SHIPMENT

Sample No. 10-5

pH, units	6.8
Spec. cond., μmhos at 25°C	660
Total alkalinity, $CaCO_3$	73
Total hardness, $CaCO_3$	120
NH_3 -N as N	0.4
Org-N as N	0.2
NO_3 as N	12.6
NO_2 as N	.007
Total P	5.8
Cl^- as NaCl	69.7
COD	10.6
Turbidity, NTU	.31
Color, units	0
TSS	1
TDS	466
Standard plate count per ml	29×10^1
Total coliforms per 100 ml	3×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample 10-12

pH, units	6.7
Spec. cond., μmhos at 25°C	650
Total alkalinity, $CaCO_3$	46
Total hardness, $CaCO_3$	120
NH_3-N as N	0
Org-N as N	0.4
NO_3 as N	12.8
NO_2 as N	.07
Total P	5.6
Cl^- as NaCl	67.2
COD	10.0
Turbidity, NTU	0.5
Color, units	0
TSS	0
TDS	496
Standard plate count per ml	33×10^1
Total coliforms per 100 ml	8×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 10-19

pH, units	6.6
Spec. cond., μmhos at 25°C	630
Total alkalinity, $CaCO_3$	49
Total hardness, $CaCO_3$	150
NH_3 -N as N	0
Org-N as N	1.1
NO_3 as N	12.7
NO^2 as N	0.0
Total P	5.6
Cl^- as NaCl	85.0
COD	9.1
Turbidity, NTU	0.5
Color, units	0
TSS	0
TDS	502
Standard plate count per ml	63×10^1
Total coliforms per 100 ml	47×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 10-26

pH, units	6.8
Spec. cond., μmhos at 25°C	590
Total alkalinity, $CaCO_3$	51
Total hardness, $CaCO_3$	120
NH_3 -N as N	0
Org-N as N	0.7
NO_3 as N	12.0
NO_2 as N	0.04
Total P	5.6
Cl^- as NaCl	72.0
COD	7.0
Turbidity, NTU	0.5
Color, units	0
TSS	0
TDS	488
Standard plate count per ml	79×10^1
Total coliforms per 100 ml	40×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 11-9

pH, units	6.9
Spec. cond., μmhos at 25°C	530
Total alkalinity, $CaCO_3$	79
Total hardness, $CaCO_3$	136
NH_3 -N as N	0.0
Org-N as N	1.1
NO_3 as N	13.2
NO_2 as N	0.07
Total P	5.8
Cl^- as NaCl	72.5
COD	11.4
Turbidity, NTU	0.4
Color, units	5
TSS	1
TDS	492
Standard plate count per ml	29×10^1
Total coliforms per 100 ml	75×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 11-16

pH, units	6.5
Spec. cond., μmhos at 25°C	520
Total alkalinity, $CaCO_3$	66
Total hardness, $CaCO_3$	120
NH_3 -N as N	0
Org-N as N	0.4
NO_3 as N	18.2
NO_2 as N	0.7
Total P	4.4
Cl^- as NaCl	69.6
COD	6.5
Turbidity, NTU	0.4
Color, units	0
TSS	1
TDS	464
Standard plate count per ml	82×10^2
Total coliforms per ml	49×10^2

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 11-23

pH, units	6.7
Spec. cond., μmhos at 25°C	610
Total alkalinity, $CaCO_3$	53
Total hardness, $CaCO_3$	118
NH_3 -N as N	0.0
Org-N as N	0.6
NO_3 as N	15.4
NO_2 as N	0.01
Total P	5.8
Cl^- as NaCl	76.8
COD	11.6
Turbidity, NTU	0.4
Color, units	5
TSS	0
TDS	496
Standard plate count per ml	62×10^2
Total coliforms per 100 ml	49×10^1

All values are in mg/l unless otherwise noted.

AWT EFFLUENT SAMPLES,
BEFORE SHIPMENT

Sample No. 12-7

pH, units	6.8
Spec. cond., μmhos at 25°C	550
Total alkalinity, $CaCO_3$	89
Total hardness, $CaCO_3$	154
NH_3 -N as N	0.0
Org-N as N	1.0
NO_3 as N	14.7
NO_2 as N	0.1
Total P	7.0
Cl^- as NaCl	96.2
COD	9.5
Turbidity, NTU	0.5
Color, units	0
TSS	0
TDS	479
Standard plate count per ml	35×10^2
Total coliforms per 100 ml	36×10^1

All values are in mg/l unless otherwise noted.

APPENDIX B

CARBON COLUMN SAMPLES, BEFORE SHIPMENT

Sample No. 12-14	Influent	Midpoint	Effluent
pH, units	7.1	7.0	6.0
Spec. cond., μmhos at 25°C	670	670	650
Total alkalinity, $CaCO_3$	108	98	94
Total hardness, $CaCO_3$	172	170	168
NH_3 -N as N	0	0	0
Org-N as N	2.0	1.5	1.3
NO_3 as N	11.9	12.4	12.8
NO_2 as N	0	0	0
Total P	5.3	4.9	4.8
Cl^- as NaCl	89.0	89.0	91.4
COD	23.4	17.7	9.4
Turbidity, NTU	1.5	.68	.62
Color, units	30	10	5
TSS	4	1	1
TDS	490	478	460
Standard plate count per ml	22×10^3	29×10^1	34×10^1
Total coliforms per 100 ml	60×10^4	49×10^2	11×10^3

All values are in mg/l unless otherwise noted.

CARBON COLUMN SAMPLES,
BEFORE SHIPMENT

Sample No. 12-21	Influent	Midpoint	Effluent
pH, units	6.8	6.7	6.9
Spec. cond., μmhos at 25°C	670	650	650
Total alkalinity, $CaCO_3$	92	87	92
Total hardness, $CaCO_3$	150	146	156
NH_3-N as N	0	0	0
Org-N as N	2.9	2.2	1.4
NO_3 as N	14.2	14.0	14.8
NO_2 as N	0	0	0
Total P	5.3	4.8	4.8
Cl^- as NaCl	74.4	72	72
COD	22.2	17.8	14.3
Turbidity, NTU	1.2	0.5	0.4
Color, units	50	15	10
TSS	2	1	0
TDS	562	498	520
Standard plate count per ml	34×10^3	24×10^3	75×10^2
Total coliforms per 100 ml	60×10^4	90×10^2	96×10^2

All values are in mg/l unless otherwise noted.

CARBON COLUMN SAMPLES,
BEFORE SHIPMENT

Sample No. 1-25	Influent	Midpoint	Effluent
pH, units	7.1	7.0	6.8
Spec. cond., μmhos at 25°C			
Total alkalinity, $CaCO_3$	105	79	93
Total hardness, $CaCO_3$	146	98	142
NH_3-N as N	0	0	0
Org-N as N	2.0	1.4	0.7
NO_3 as N	6.8	5.9	9.6
NO_2 as N	.00	0	.01
Total P	4.3	3.2	4.4
Cl^- as NaCl	69.7	55.3	72.1
COD	22.5	14.7	9.0
Turbidity, NTU	2.0	1.0	0.6
Color, units	30	20	5
TSS	8	1	1
TDS	540	562	498
Standard plate count per ml	38×10^3	21×10^2	39×10^2
Total coliforms per 100 ml	49×10^4	14×10^2	64×10^1

All values are in mg/l unless otherwise noted.

APPENDIX C

AWT EFFLUENT DISINFECTION SAMPLES, BEFORE SHIPMENT

Sample No. 2-8	pH	UV	O_3	Cl_2
pH, units	6.9	7.3	7.4	7.3
Spec. cond., μmhos at 25°C				
Total alkalinity, $CaCO_3$	32	116	105	117
Total hardness, $CaCO_3$	700	180	156	180
NH_3-N as N	0	0	0	0
Org-N as N	.84	.56	.35	0
NO_3 as N	5.8	6.1	6.0	6.4
NO_2 as N	.01	.01	.00	.01
Total P	.02	3.6	4.0	4.0
Cl^- as NaCl	348	55.3	65	288
COD	23.2	12.0	9.4	11.7
Turbidity, NTU	.23	.35	.25	.35
Color, units	5	5	5	5
TSS	0	0	0	0
TDS	1322	444	382	440
Standard plate count per ml	$1x10^0$	$112x10^0$	$43x10^0$	$0x10^0$
Total coliforms per 100 ml	0	0	0	0

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 0.9 mg/l.

O_3 = ozone dose applied, 8.4 mg/l.

pH = raised to 11.56, then neutralized to 6.9.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 2-28	pH	UV	O_3	Cl_2
pH, units	6.9	7.2	7.0	7.0
Spec. cond., µmhos at 25°C				
Total alkalinity, $CaCO_3$	49	95	88	89
Total hardness, $CaCO_3$	300	142	140	132
NH_3-N as N	0	0	0	0
Org-N as N	2.3	1.5	1.6	1.5
NO_3 as N	5.8	6.0	7.8	5.8
NO_2 as N	.02	.01	0	.01
Total P	1.1	3.2	3.0	3.2
Cl^- as NaCl	69.7	75.0	65	67
COD	11.8	11.2	9.3	-
Turbidity, NTU	0.5	0.4	0.4	0.4
Color, units	5	5	0	5
TSS	4	0	0	1
TDS	1240	490	508	504
Standard plate count per ml	0	0	0	$4x10^0$
Total coliforms per 100 ml	0	$2x10^0$	0	0

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 1.02 mg/l.

O_3 = ozone dose applied, 8.3 mg/l.

pH = raised to 11.53, then neutralized to 6.9.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 3-14	pH	UV	O_3	Cl_2
pH, units	6.9	7.0	7.0	6.7
Spec. cond., μmhos at 25°C				
Total alkalinity, $CaCO_3$	149	120	97	97
Total hardness, $CaCO_3$	356	204	194	200
NH_3-N as N	0	0	0	0
Org-N as N	1.1	2.0	0.6	1.2
NO_3 as N	9.6	5.6	4.4	4.8
NO_3 as N	0.03	0.11	0.01	0.04
Total P	.62	4.0	3.9	4.2
Cl^- as NaCl	84	77	75	-
COD	13.3	12.6	2.6	-
Turbidity, NTU	1.8	.65	.45	.65
Color, units	10	15	5	10
TSS	2	1	0	1
TDS	1400	450	420	480
Standard plate count per ml	27×10^0	81×10^0	3×10^0	8×10^0
Total coliforms per 100 ml	0	7×10^0	0	0

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 1.2 mg/l.

O_3 = ozone dose applied, 7.5 mg/l.

pH = raised to 11.4, then neutralized to 6.9.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 3-28	pH	UV	O_3	Cl_2
pH, units	6.2	7.0	7.0	6.9
Spec. cond., μmhos at 25°C				
Total alkalinity, $CaCO_3$	29	119	112	100
Total hardness, $CaCO_3$	398	210	192	160
NH_3-N as N	0	0	0	0
Org-N as N	0	1.5	0	.08
NO_3 as N	7.2	7.2	7.1	7.0
NO_2 as N	0.008	0.008	0.002	0.002
Total P	2.6	2.4	2.6	3.4
Cl^- as NaCl	48.1	89.0	86.6	96.2
COD	7.0	7.8	7.3	11.0
Turbidity, NTU	1.4	0.28	0.37	0.48
Color, units	5	5	5	5
TSS	0	1	0	0
TDS	914	386	448	408
Standard plate count per ml	$13x10^0$	$280x10^0$	$3x10^0$	$3x10^0$
Total coliforms per 100	$0x10^0$	$4x10^0$	$1x10^0$	$0x10^0$

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine = 1.4 mg/l.

O_3 = ozone dose applied, 4.9 mg/l.

pH = raised to 11.8, the neutralized to 6.2.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 4-4	pH	UV	O_3	Cl_2
pH, units	7.1	7.2	7.2	7.0
Spec. cond., μmhos at 25°C	-	-	-	-
Total alkalinity, $CaCO_3$	49	149	141	141
Total hardness, $CaCO_3$	416	204	198	204
NH_3-N as N	0	0	0	0
Org-N as N	0.9	0.8	0.8	0.7
NO_3 as N	7.8	6.4	5.6	6.0
NO_2 as N	0.06	0.02	0.03	0.02
Total P	1.5	3.7	3.3	3.8
Cl^- as NaCl	84	98	94	106
COD	8.6	9	9.2	9.4
Turbidity, NTU	2.0	.45	.35	0.45
Color, units	5	10	5	5
TSS	2	1	1	0
TDS	814	506	480	586
Standard plate count per ml	26	140	3	26
Total coliforms per 100 ml	0	14	0	0

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 1.5 mg/l.

O_3 = ozone dose applied, 8.9 mg/l.

pH = achieved, 12.5.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 4-11	pH	UV	O_3	Cl_2
pH, units	8.3	7.3	7.3	7.2
Spec. cond., µmhos at 25°C	-	-	-	-
Total alkalinity, $CaCO_3$	39	91	68	71
Total hardness, $CaCO_3$	354	192	154	196
NH_3-N as N	0	0	0	0
Org-N ad N	-	-	-	-
NO_3 as N	15.2	10.8	14.4	15.2
NO_2 as N	-	-	-	-
Total P	-	-	-	-
Cl^- as NaCl	90	84	54	62
COD	18	10.7	9.1	12
Turbidity, NTU	0.85	0.3	0.25	0.3
Color, units	20	10	5	10
TSS	8	3	4	5
TDS	724	562	462	476
Standard plate count per ml	17	240	8	5
Total coliforms per 100 ml	0	6	0	1

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 2.0 mg/l.

O^3 = ozone dose applied, 5.58 mg/l.

pH = achieved, 11.5.

AWT EFFLUENT DISINFECTION SAMPLES,
BEFORE SHIPMENT

Sample No. 4-18	pH	UV	O_3	Cl_2
pH, units	7.9	6.9	7.1	7.3
Spec. cond., μmhos at 25°C	-	-	-	-
Total alkalinity, $CaCO_3$	63	140	136	138
Total hardness, $CaCO_3$	258	230	218	232
NH_3-N as N	0	0	0	0
Org-N as N	1.5	0.6	0.6	1.3
NO_3 as N	9.6	9.6	9.2	9.3
NO_2 as N	-	-	-	-
Total P	0.3	5.2	4.2	4.6
Cl^- as NaCl	84	66	74	82
COD	11.1	10.4	11.9	12
Turbidity, NTU	0.6	0.5	0.3	0.4
Color, units	15	25	10	15
TSS	2	1	6	0
TDS	748	540	474	456
Standard plate count per ml	60	79	50	65
Total coliforms per 100 ml	0	15	0	0

All values in mg/l unless otherwise noted.

Cl_2 = free residual chlorine, 1.6 mg/l.

O_3 = ozone dose applied, 9. mg/l.

pH = achieved, 11.3.

APPENDIX D

WATER TREATMENT PLANT SAMPLES

Sample # 77

Treatment prior to GAC:
Prechlorination, Alum-lime Coagulation, Sedimentation
GAC installation date: Oct. 1970
Last regeneration: New carbon Sept. 1977
Depth of GAC, in.: 36
Flow rate, gpm/ft^2: 5.5
Empty bed contact time, min: 4.1
Cl_2 residual before GAC, mg/l:
0.0 mg/l free, 1.0 mg/l total

	Before-*	After-*
pH value	6.7	6.6
TOC, mg/l	6.4	4.0
Endotoxins (LAL) Unfiltered, ng/ml	3.1	3.1
Endotoxins (LAL) Filtered, ng/ml	3.1	3.1
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	23.3	23.3
Temperature on arrival, °C	10.0	12.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	74	34
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*		

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 22

Treatment prior to GAC:
Prechlorination,Lime-softening, Sedimentation
GAC installation date: September 1968
Last regeneration: None
Depth of GAC, in.: 24
Flow rate, gpm/ft^2: 2
Empty bed contact time, min: 7.5
Cl_2 residual before GAC, mg/l:
2.4 mg/l free, 4.1 mg/l total

	Before-*	After-*
pH value	7.7	7.7
TOC, mg/l	4.4	3.8
Endotoxins (LAL) Unfiltered, ng/ml	3.1	1.6
Endotoxins (LAL) Filtered, ng/ml	1.6	1.6
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	17.2	17.2
Temperature on arrival, °C	11.0	11.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	18	11
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*		

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 11

Treatment prior to GAC:
Prechlorination, Alum-lime Coagulation, Sedimentation
GAC installation date: 1971
Last regeneration: 24 inches Virgin carbon, May 1977
Depth of GAC, in.: 30
Flow rate, gpm/ft^2: 2.5
Empty bed contact time, min: 7.5
Cl_2 residual before GAC, mg/l:
0.20 mg/l free, 0.80 mg/l total

	Before-*	After-*
pH value	6.7	6.6
TOC, mg/l		
Endotoxins (LAL) Unfiltered, ng/ml	15.6	3.1
Endotoxins (LAL) Filtered, ng/ml	3.1	3.1
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	11.7	11.7
Temperature on arrival, °C	9.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	25	5
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*		

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 55

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: 1971
Last regeneration: None
Depth of GAC, in.: 18
Flow rate, gpm/ft^2: 1.75
Empty bed contact time, min: 6.4
Cl_2 residual before GAC, mg/l:
0.0 mg/l free, 0.3-0.8 mg/l total

	Before-*	After-*
pH value	6.7	6.7
TOC, mg/l	3.6	1.3
Endotoxins (LAL) Unfiltered, ng/ml	12.5	6.3
Endotoxins (LAL) Filtered, ng/ml	6.3	6.3
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2.0	<2.0
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	11.1	11.1
Temperature on arrival, °C	8.0	8.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*		

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 33

Treatment prior to GAC:
Powdered GAC, Aeration, Alum-Nalco 607 Coagulation, Sedimentation
GAC installation date: 1971
Last regeneration: None
Depth of GAC, in.: 15
Flow rate, gpm/ft^2: 2
Empty bed contact time, min: 4.7
Cl_2 residual before GAC, mg/l:
0.0 mg/l free,0.0 mg/l total

(no prechlorination)

	Before-*	After-*
pH value	7.2	7.2
TOC, mg/l		
Endotoxins (LAL) Unfiltered, ng/ml	31.3	31.3
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	5.0	5.0
Temperature on arrival, °C	13.0	13.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	39	4
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	90	100
Percent of total plate count *Pseudomonas aeruginosa*	30	0
Percent of total plate count *P. fluorescens*		

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 44 A

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation, Sand Filtration
GAC installation date: Fall 1974
Last regeneration: None
Depth of GAC, in.: 48
Flow rate, gpm/ft^2: 2.8
Empty bed contact time, min: 10.7
Cl_2 residual before GAC, mg/l:
<0.1 mg/l free, 0.1 mg/l total

	Before-*	After-*
pH value	6.6	6.7
TOC, mg/l	5.3	4.4
Endotoxins (LAL) Unfiltered, ng/ml	6.0	6.0
Endotoxins (LAL) Filtered, ng/ml	2.4	2.4
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	5.0	5.0
Temperature on arrival, °C	6.0	6.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	-	-

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 44 B

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation, Sand Filtration
GAC installation date: Fall 1974
Last regeneration: None
Depth of GAC, in.: 48
Flow rate, gpm/ft^2: 2.8
Empty bed contact time, min: 10.7
Cl_2 residual before GAC, mg/l:
<0.1 mg/l free, 0.1 mg/l total

	Before-*	After-*
pH value	-	-
TOC, mg/l	4.8	4.0
Endotoxins (LAL) Unfiltered, ng/ml	6.0	6.0
Endotoxins (LAL) Filtered, ng/ml	2.4	2.4
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	5.0	5.0
Temperature on arrival, °C	6.0	6.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	1
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	-	-

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 88

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: January 1976
Last regeneration: None
Depth of GAC, in.: 30
Flow rate, gpm/ft^2: 3
Empty bed contact time, min: 6.2
Cl_2 residual before GAC, mg/l:
0.0 mg/l free, 0.0 mg/l total

	Before-*	After-*
pH value	7.9	7.9
TOC, mg/l	5.9	6.4
Endotoxins (LAL) Unfiltered, ng/ml	120	60
Endotoxins (LAL) Filtered, ng/ml	24	24
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	4.4	4.4
Temperature on arrival, °C	10.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	88	8
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	22	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 66

Treatment prior to GAC:
Prechlorination, Coagulation, Sedimentation
GAC installation date: April 1977
Last regeneration: None
Depth of GAC, in.: 12
Flow rate, gpm/ft^2: 2
Empty bed contact time, min: 3.7
Cl_2 residual before GAC, mg/l:
0.1-0.4 mg/l free, 0.2-0.6 mg/l total

	Before-*	After-*
pH value	7.8	7.5
TOC, mg/l	3.2	2.9
Endotoxins (LAL) Unfiltered, ng/ml	36	18
Endotoxins (LAL) Filtered, ng/ml	18	12
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	1.0	1.0
Temperature on arrival, °C	3.5	3.5
Dissolved oxygen, mg/l		
Total plate count organisms per ml	32	2
Coliforms per 100 ml, Millipore	9	0
Coliforms per 100 ml, MPN	46	<2
Fecal coliforms per 100 ml, MPN	12	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	30	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 99

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: 1973
Last regeneration: April 1975
Depth of GAC, in.: 16
Flow rate, gpm/ft^2: 2.29
Empty bed contact time, min: 4.4
Cl_2 residual before GAC, mg/l:
3.25 mg/l free, 3.60 mg/l total

	Before-*	After-*
pH value	8.0	8.0
TOC, mg/l	3.0	2.3
Endotoxins (LAL) Unfiltered, ng/ml	6.0	6.0
Endotoxins (LAL) Filtered, ng/ml	0.6	2.4
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	1.75	<0.05
Temperature at sampling, °C	2.0	2.0
Temperature on arrival, °C	5.0	6.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 12

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: January 1976
Last regeneration: None
Depth of GAC, in.: 31
Flow rate, gpm/ft^2: 1.5
Empty bed contact time, min: 12.9
Cl_2 residual before GAC, mg/l:
0.67 mg/l free, 0.82 mg/l total

	Before-*	After-*
pH value	7.2	7.2
TOC, mg/l	2.2	1.2
Endotoxins (LAL) Unfiltered, ng/ml	3.6	2.4
Endotoxins (LAL) Filtered, ng/ml	0.06	1.2
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	2.2	2.2
Temperature on arrival, °C	7.0	7.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	14	1
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	40	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 14

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: 1972
Last regeneration: Sept. 1977
Depth of GAC, in.: 14
Flow rate, gpm/ft^2: 2
Empty bed contact time, min: 4.4
Cl_2 residual before GAC, mg/l:
0.4 mg/l free, - mg/l total

	Before-*	After-*
pH value	7.7	7.7
TOC, mg/l	5.2	2.3
Endotoxins (LAL) Unfiltered, ng/ml	60	60
Endotoxins (LAL) Filtered, ng/ml	12	18
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	6.1	6.1
Temperature on arrival, °C	7.0	7.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	86	20
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	50	30
Percent of total plate count *Pseudomonas aeruginosa*	0	70
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 16

Treatment prior to GAC:
Prechlorination, Ferric Sulfate Coagulation, Sedimentation
GAC installation date: 1972
Last regeneration: July 1977
Depth of GAC, in.: 30
Flow rate, gpm/ft^2: 2.8
Empty bed contact time, min: 2.44
Cl_2 residual before GAC, mg/l:
1.0 mg/l free, 1.7 mg/l total

	Before-*	After-*
pH value	7.5	7.4
TOC, mg/l	1.8	1.7
Endotoxins (LAL) Unfiltered, ng/ml	18	12
Endotoxins (LAL) Filtered, ng/ml	12	12
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	1.7	1.7
Temperature on arrival, °C	9.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	15	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	60	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 18-4

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: June 1973
Last regeneration: None
Depth of GAC, in.: 30
Flow rate, gpm/ft^2: 2.4
Empty bed contact time, min: 7.79
Cl_2 residual before GAC, mg/l:
0.2 mg/l free, 0.35 mg/l total

	Before-*	After-*
pH value	6.4	6.4
TOC, mg/l	2.7	2.1
Endotoxins (LAL) Unfiltered, ng/ml	5.0	2.4
Endotoxins (LAL) Filtered, ng/ml	1.3	1.3
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	2.8	2.8
Temperature on arrival, °C	13.0	13.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	1	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	100	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 18-5

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: June 1975
Last regeneration: None
Depth of GAC, in.: 30
Flow rate, gpm/ft^2: 2.4
Empty bed contact time, min: 7.79
Cl_2 residual before GAC, mg/l:
0.2 mg/l free, 0.35 mg/l total

	Before-*	After-*
pH value	6.9	6.9
TOC, mg/l	2.5	2.2
Endotoxins (LAL) Unfiltered, ng/ml	5.0	1.3
Endotoxins (LAL) Filtered, ng/ml	1.3	1.3
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	2.8	2.8
Temperature on arrival, °C	13.0	13.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	3	1
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	100	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 24

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: 1971
Last regeneration: Nov. 1977
Depth of GAC, in.: 18
Flow rate, gpm/ft^2: 1.5
Empty bed contact time, min: 7.48
Cl_2 residual before GAC, mg/l:
0.0 mg/l free, 0.0 mg/l total

	Before-*	After-*
pH value	6.9	6.8
TOC, mg/l	3.9	3.3
Endotoxins (LAL) Unfiltered, ng/ml	37.5	37.5
Endotoxins (LAL) Filtered, ng/ml	5.0	5.0
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	1.1	1.1
Temperature on arrival, °C	8.0	8.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	48	5
Coliforms per 100 ml, Millipore	4	0
Coliforms per 100 ml, MPN	49	<2
Fecal coliforms per 100 ml, MPN	2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	90	100
Percent of total plate count *Pseudomonas aeruginosa*	10	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 26 A-12

Treatment prior to GAC: Prechlorination, Precaustic (NaOH), Alum-coagulation, Sedimentation
GAC installation date: March 1978 (15-days operation)
Last regeneration: None
Depth of GAC, in.: 33
Flow rate, gpm/ft^2: 2.15
Empty bed contact time, min: 9.35
Cl_2 residual before GAC, mg/l:
1.0 mg/l free, 1.25 mg/l total

	Before-*	After-*
pH value	6.4	6.4
TOC, mg/l	3.1	1.5
Endotoxins (LAL) Unfiltered, ng/ml	12.5	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	4.0	4.0
Temperature on arrival, °C	9.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	320
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	100
Percent of total plate count *Pseudomonas aeruginosa*	-	0
Percent of total plate count *P. fluorescens*	-	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 26 A-14

Treatment prior to GAC: Prechlorination, Precaustic (NaOH), Alum-coagulation, Sedimentation
GAC installation date: March 1978 (15 days-operation)
Last regeneration: None
Depth of GAC, in.: 33
Flow rate, gpm/ft^2: 2.15
Empty bed contact time, min: 9.35
Cl_2 residual before GAC, mg/l:
1.0 mg/l free, 1.25 mg/l total

	Before-*	After-*
pH value	6.4	6.4
TOC, mg/l	3.1	1.5
Endotoxins (LAL) Unfiltered, ng/ml	12.5	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	4.0	4.0
Temperature on arrival, °C	9.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	980
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	100
Percent of total plate count *Pseudomonas aeruginosa*	-	0
Percent of total plate count *P. fluorescens*	-	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 26 A-16

Treatment prior to GAC: Prechlorination, Precaustic (NaOH), Alum-coagulation, Sedimentation
GAC installation date: March 1978 (15-days operation)
Last regeneration: None
Depth of GAC, in.: 33
Flow rate, gpm/ft^2: 2.15
Empty bed contact time, min: 9.35
Cl_2 residual before GAC, mg/l:
1.0 mg/l free, 1.25 mg/l total

	Before-*	After-*
pH value	6.4	6.4
TOC, mg/l	3.1	1.4
Endotoxins (LAL) Unfiltered, ng/ml	12.5	12.5
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	4.0	4.0
Temperature on arrival, °C	9.0	9.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*	-	-

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 28

Treatment prior to GAC:
Prechlorination, Powered-carbon, Potassium Permanganate
GAC installation date: 1972
Last regeneration: Oct. 1977
Depth of GAC, in.: 24
Flow rate, gpm/ft^2: 1.9
Empty bed contact time, min: 9.18
Cl_2 residual before GAC, mg/l:
0.15 mg/l free, 0.4 mg/l total

	Before-*	After-*
pH value	7.6	7.6
TOC, mg/l	3.5	2.4
Endotoxins (LAL) Unfiltered, ng/ml	25.0	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	14.0	14.0
Temperature on arrival, °C	7.0	7.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	0	0
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	-	-
Percent of total plate count *P. fluorescens*	-	-

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 30 Primary GAC

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: Dec. 1977
Last regeneration: None
Depth of GAC, in.: 60
Flow rate, gpm/ft^2: 4.0
Empty bed contact time, min: 9.35
Cl_2 residual before GAC, mg/l:
1.5 mg/l free, 1.7 mg/l total (spent carbon)

	Before-*	After-*
pH value	6.8	6.8
TOC, mg/l	3.5	4.5
Endotoxins (LAL) Unfiltered, ng/ml	25.0	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	16.7	16.7
Temperature on arrival, °C	12.0	12.0
Dissolved oxygen, mg/l		
Total plate count organisms per ml	1	64
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # 30 Secondary GAC

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: Dec. 1977
Last regeneration: None
Depth of GAC, in.: 60
Flow rate, gpm/ft^2: 4.0
Empty bed contact time, min: 9.35
Cl_2 residual before GAC, mg/l:
<0.1 mg/l free, 0.1 mg/l total (virgin carbon)

	Before-*	After-*
pH value	6.8	6.8
TOC, mg/l	4.5	2.7
Endotoxins (LAL) Unfiltered, ng/ml	25.0	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	16.7	16.7
Temperature on arrival, °C	12.0	12.0
Dissolved oxygen, mg/l	64	241
Total plate count organisms per ml		
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	100	90
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T1

Treatment prior to GAC:
Prechlorination, Precaustic(NaOH), Alum-coagulation, Sedimentation
GAC installation date: 1973
Last regeneration: July 1976
Depth of GAC, in.: 48
Flow rate, gpm/ft^2: 0.75
Empty bed contact time, min: 39.89
Cl_2 residual before GAC, mg/l:
0.35 mg/l free, - total

	Before-*	After-*
pH value	5.8	5.8
TOC, mg/l	5.2	3.6
Endotoxins (LAL) Unfiltered, ng/ml	25.0	25.0
Endotoxins (LAL) Filtered, ng/ml	12.5	12.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	13.0	13.0
Temperature on arrival, °C	7.0	7.0
Dissolved oxygen, mg/l	9.4	9.3
Total plate count organisms per ml	3	3
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	50	50
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T2

Treatment prior to GAC: Prechlorination, Precaustic (NaOH), Diatomatious Earth, Alum-coagulation, Sedimentation
GAC installation date: Sept. 1974
Last regeneration: None
Depth of GAC, in.: 11
Flow rate, gpm/ft^2: 0.64
Empty bed contact time, min: 10.71
Cl_2 residual before GAC, mg/l:
- mg/l free, 0.0 mg/l total

	Before-*	After-*
pH value	5.9	5.9
TOC, mg/l	2.9	3.7
Endotoxins (LAL) Unfiltered, ng/ml	12.5	10.0
Endotoxins (LAL) Filtered, ng/ml	2.5	2.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	13.0	13.0
Temperature on arrival, °C	8.0	8.0
Dissolved oxygen, mg/l	10.8	10.7
Total plate count organisms per ml	8	11
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	100	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T3

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: March 1977
Last regeneration: None
Depth of GAC, in.: 48
Flow rate, gpm/ft^2: 1.6
Empty bed contact time, min: 18.70
Cl_2 residual before GAC, mg/l:
- mg/l free, 0.3 mg/l total

	Before-*	After-*
pH value	5.9	6.1
TOC, mg/l	3.7	3.5
Endotoxins (LAL) Unfiltered, ng/ml	12.5	10.0
Endotoxins (LAL) Filtered, ng/ml	7.5	7.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	10.0	10.0
Temperature on arrival, °C	8.0	8.0
Dissolved oxygen, mg/l	11.0	10.6
Total plate count organisms per ml	1	2
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	100	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T4

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: Sept. 1971
Last regeneration: May 1977
Depth of GAC, in.: 33
Flow rate, gpm/ft^2: 2.7
Empty bed contact time, min: 7.62
Cl_2 residual before GAC, mg/l:
0.5 mg/l free, 1.0 mg.l total

	Before-*	After-*
pH value	6.4	6.0
TOC, mg/l	3.5	2.0
Endotoxins (LAL) Unfiltered, ng/ml	25.0	10.0
Endotoxins (LAL) Filtered, ng/ml	12.5	7.5
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	12.0	12.0
Temperature on arrival, °C	6.0	6.0
Dissolved oxygen, mg/l	10.9	10.7
Total plate count organisms per ml	3	1
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	-
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T5

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation
GAC installation date: 1971
Last regeneration: June 1977
Depth of GAC, in.: 11
Flow rate, gpm/ft^2: 3.0
Empty bed contact time, min: 2.29
Cl_2 residual before GAC, mg/l:
0.1 mg/l free, 0.7 mg.l total

	Before-*	After-*
pH value	6.9	7.0
TOC, mg/l	3.0	3.7
Endotoxins (LAL) Unfiltered, ng/ml	12.5	10.0
Endotoxins (LAL) Filtered, ng/ml	10.0	5.0
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	10.0	10.0
Temperature on arrival, °C	7.0	7.0
Dissolved oxygen, mg/l	11.3	11.2
Total plate count organisms per ml	3	1
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	50	50
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

WATER TREATMENT PLANT SAMPLES

Sample # T6

Treatment prior to GAC:
Prechlorination, Alum-coagulation, Sedimentation, Sand Filtration
GAC installation date: July 1968
Last regeneration: March 1975
Depth of GAC, in.: 10
Flow rate, gpm/ft^2: 2.8
Empty bed contact time, min: 2.2
Cl_2 residual before GAC, mg/l:
0.15 mg/l free, 0.45 mg/l total

	Before-*	After-*
pH value	5.6	5.6
TOC, mg/l	3.3	6.7
Endotoxins (LAL) Unfiltered, ng/ml	25.0	25.0
Endotoxins (LAL) Filtered, ng/ml	25.0	25.0
Endotoxins (Rabbit Assay) Filtered, ng/ml**	<2	<2
Chlorine Residual, mg/l	<0.05	<0.05
Temperature at sampling, °C	11.0	11.0
Temperature on arrival, °C	6.0	6.0
Dissolved oxygen, mg/l	11.8	11.8
Total plate count organisms per ml	2	3
Coliforms per 100 ml, Millipore	0	0
Coliforms per 100 ml, MPN	<2	<2
Fecal coliforms per 100 ml, MPN	<2	<2
Coliphage, Pfu/ml	<2	<2
Percent of total plate count Gram negative	-	100
Percent of total plate count *Pseudomonas aeruginosa*	0	0
Percent of total plate count *P. fluorescens*	0	0

* GAC Filtration.
** Lower detection limit.

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(Please read Instructions on the reverse before completing)

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15. SUPPLEMENTARY NOTES

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16. ABSTRACT The endotoxin content and pyrogenic response of granular activated carbon (GAC) filtered waters were studied. GAC-filtered secondary effluent from an activated sludge pilot plant contained free endotoxins in the range 6-250 μg/l yielding positive pyrogenic responses in 18 of 20 trials. Samples obtained from 27 different water supplies in the U.S. that utilize GAC adsorption contained free endotoxin ranging from 1.2-25 μg/l but none gave a pyrogenic response. No relationship was discernible between endotoxin content and pyrogenic response.

Small removals of total organic carbon (TOC) by GAC beds which had been in operation in water treatment plants without regeneration for as long as 110 months were observed. However, 5 of 28 samples showed an increase in TOC through GAC and 8 of 28 samples showed an increase in standard plate count. One of 25 samples yielded pseudomonads, but none of the 28 samples contained coliforms.

Good correlations were observed on non-disinfected AWT effluent samples between standard plate count and total endotoxin ($r = 0.945$), standard plate count and free endotoxin ($r = 0.932$), and total coliforms and free endotoxin ($r = 0.939$). Lack of good correlations, however, were observed in assaying AWT samples that had been subject to the disinfecting procedures of chlorination, ozonation, pH or UV irradiation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Lipopolysaccharides, Pyrogens, Water Treatment, Carbon, Potable Water, Water Quality, Water Reclamation	Endotoxin Granular Activated Carbon Advanced Waste Treatment	57 U

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