November 2003

Environmental Technology Verification Report

INTERLAB SUPPLY, LTD.
POLYTOX™
RAPID TOXICITY TESTING SYSTEM

Prepared by
Battelle

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EPA U.S. Environmental Protection Agency

ETV ETV ETV

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

InterLab Supply, Ltd.
POLYTOX™
Rapid Toxicity Testing System

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Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

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Contents

Page

Figures

Tables

List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
BOD	biological oxygen demand
DI	deionized water
DOUR	dissolved oxygen uptake rate
DDW	dechlorinated drinking water from Columbus, Ohio
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
ID	identification
LD	lethal dose
mg	milligram
mL	milliliter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
QA	quality assurance
QC	quality control
QMP	quality management plan
SOP	standard operating procedure
TSA	technical systems audit

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the InterLab Supply, Ltd. POLYTOX™ rapid toxicity testing system. Rapid toxicity testing systems were identified as a priority technology verification category through the AMS Center stakeholder process.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of POLYTOX™. Following is a description of POLYTOX™, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

POLYTOX™ (Figure 2-1) uses the respiration of microorganisms to indicate the toxicity of a water or wastewater stream. When activated in water, the mixture of bacterial cultures in POLYTOX™ begins to "breathe" like all other living organisms. They breathe in oxygen and respire carbon dioxide. The inhibitory effect of toxicants in potable tap water (or any water-based medium) to the bacterial cultures in POLYTOX™ is measured by evaluating the culture's respiration rate in the presence of different concentrations of toxicants. The respiration rate is the oxygen consumed by aerobic and facultative cultures (the dissolved oxygen update rate—DOUR) and is expressed as milligrams (mg) of oxygen consumed per liter per minute.

The DOUR is determined by measuring the dissolved oxygen concentration at 19 and 21 minutes after adding the POLYTOX™ microbial mixture to 300 milliliters (mL) of a drinking water sample. The DOUR of each drinking water sample is compared to a baseline DOUR measured at the beginning of each day by adding POLYTOX™ to a clean water matrix and measuring the oxygen concentrations in a manner similar to the test samples. For this verification test, the vendor provided YSI 5000 and 5100 dissolved oxygen probes.

Figure 2-1. POLYTOX™ Bacterial Cultures

The POLYTOX™ test components include standard 300-mL biological oxygen demand (BOD) bottle(s) and a dissolved oxygen probe (with stirrer) and meter. The probe must fit snugly into the neck of the BOD bottle, eliminating all headspace. Also required, but not included in the test kit, are an aeration device and

one- and two-liter containers for aerating the deionized (DI) water (control). A thermometer and a stopwatch are also provided.

The dimensions of the POLYTOX™ test kit are 8 inches x 8 inches x 4 inches**.** With all necessary components, the kit size is approximately 18 inches x 18 inches x 16 inches. The dissolved oxygen probe and meter are 9-½ inches x 8-½ inches x 6 inches. When a large number of tests are performed, data can be downloaded directly from the dissolved oxygen meter to a laptop or desktop computer for manipulation into a usable form. The suggested price of the POLYTOX™ culture is $147 for 20 tests. The dissolved oxygen probe and meter provided by the vendor for use during testing cost approximately $1,600 for the complete unit.

Chapter 3
Test Design and Procedures

3.1 Introduction

The objective of this verification test of rapid toxicity technologies was to evaluate their ability to detect certain toxins and to determine their susceptibility to interfering chemicals in a controlled experimental matrix. Rapid toxicity technologies do not identify or determine the concentration of specific contaminants, but serve as a screening tool to quickly determine whether water is potentially toxic. Rapid toxicity technologies use bacteria (e.g., *Vibrio fischeri*), enzymes (e.g., luciferase), or small crustaceans (e.g., *Daphnia magna*) that either directly, or in combination with reagents, produce a background level of light or use dissolved oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in water are indicated by a change in the color or intensity of light produced or by a decrease in the DOUR in the presence of the contaminants.

As part of this verification test, POLYTOX™ was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether POLYTOX™ can detect the toxicity caused by each contaminant, its response to interfering compounds in clean drinking water, such as water treatment chemicals and by-products, was evaluated. Table 3-1 shows the contaminants and potential interferences that were evaluated during this verification test.

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies*.[1] POLYTOX™ was verified by analyzing a dechlorinated drinking water (DDW) sample from Columbus, Ohio, fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Hereafter in this report, DDW will refer to dechlorinated drinking water from Columbus, Ohio. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

Table 3-1. Contaminants and Potential Interferences

Category	Contaminant
Carbamate pesticide	aldicarb
Pharmaceutical	colchicine
Industrial chemical	cyanide
Organophosphate pesticide	dicrotophos
Rodenticide	thallium sulfate
Biological toxins	botulinum toxin, ricin
Nerve agents	soman, VX
Potential interferences	aluminum, copper, iron, manganese, zinc, chloramination by-products, and chlorination by-products

POLYTOX™ was evaluated by

- Endpoint and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant
- False negative responses—contaminants that were reported as producing inhibition results similar to the negative control when the contaminant was present at lethal concentrations
- False positive responses—occurrence of inhibition significantly greater than the inhibition reported for unspiked American Society for Testing and Materials (ASTM) Type II DI water samples (zero inhibition)
- Field portability
- Ease of use
- Throughput.

3.2 Test Design

POLYTOX™ was used to analyze the DDW sample fortified with contaminants at concentrations ranging from lethal levels to concentrations several orders of magnitude times less than the lethal dose. The lethal dose concentration was determined by calculating the concentration of each contaminant at which 250 mL of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant. For soman and VX the stock solution confirmation showed degradation in the water; therefore, the

concentrations analyzed were less than anticipated. Whether the concentration is still a lethal dose, as is the case for all contaminants, depends on the characteristics of the individual person and the amount of contaminant ingested. Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of POLYTOX™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of POLYTOX™ results.

The response of POLYTOX™ to compounds used during the water treatment process (identified as potential interferences in Table 3-1) was evaluated by analyzing separate aliquots of DDW fortified with each potential interference at approximately one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations (NSDWR)[(2)] guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample from St. Petersburg, Florida, which uses chloramination as its disinfection process, was obtained. The samples were analyzed after residual chlorine was removed using sodium thiosulfate.

Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators and the verification test coordinator. In addition to comprehensive testing in Battelle laboratories, POLYTOX™ was operated in the basement of a Columbus, Ohio, home to test its ability to be transported and operated in a non-laboratory setting.

3.3 Test Samples

Test samples used in the verification test included drinking water and quality control (QC) samples. Table 3-2 shows the number and type of samples analyzed. QC samples included method blanks and positive and negative control samples. The fortified drinking water samples were prepared from a single drinking water sample collected from the Columbus, Ohio, system. The water was dechlorinated using sodium thiosulfate and then fortified with various concentrations of contaminants and interferences. Using this DDW (Columbus, Ohio, dechlorinated drinking water), individual solutions containing each contaminant and potential interference were prepared and analyzed. The DDW containing the potential interferences was analyzed at a single concentration level, while four concentration levels (made using the DDW) were analyzed for each contaminant using POLYTOX™. Mixtures of contaminants and interfering compounds were not analyzed. One concentration level of cyanide was analyzed in the field setting.

3.3.1 Quality Control Samples

QC samples included method blank samples, which consisted of ASTM Type II DI water; positive control samples, which consisted of ASTM Type II DI water or DDW (depending on vendor preference) fortified with a contaminant and concentration selected by the vendor; and negative control samples, which consisted of the unspiked DDW. The method blank samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. Cyanide was suggested by the vendor for use as the positive

Table 3-2. Summary of Quality Control and Contaminant Test Samples

Type of Sample	Sample Characteristics	Concentration Levels (mg/L)	No. of Sample Analyses
Quality control	Method blank	NS[a]	7
	Positive control (cyanide)	8	15
	Negative control (unspiked DDW)	NS	50
DDW fortified with contaminants	Aldicarb	280; 28; 2.8	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24	4 per concentration level
	Cyanide	250; 0.25; 0.0025; 0.00025	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4	4 per concentration level
	Thallium sulfate	2,400; 240; 24; 2.4	4 per concentration level
	Botulinum toxin[b]	0.30; 0.030; 0.0030; 0.00030	4 per concentration level
	Ricin[c]	15; 1.5; 0.15; 0.015	4 per concentration level
	Soman	0.18[d]; 0.018; 0.0018; 0.00018	4 per concentration level
	VX	0.088[d]; 0.0088; 0.00088; 0.000088	4 per concentration level
Field location	Cyanide	0.25	4
DDW fortified with potential interferences	Aluminum	0.36	4
	Copper	0.65	4
	Iron	0.069	4
	Manganese	0.26	4
	Zinc	3.5	4
Disinfectant by-products	Chloramination by-products	NS	4
	Chlorination by-products	NS	4

[a] NS = Samples not fortified with any contaminant or potential interference.
[b] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[c] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[d] Due to the degradation of soman and VX in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 61% of the expected concentration of 0.30 mg/L for soman, and 44% of the expected concentration of 0.20 mg/L for VX.

control sample. While performance limits were not placed on the results, inhibition of at least 50% indicated to the operator that POLYTOX™ was functioning properly. The negative control sample was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared.

3.3.2 Drinking Water Fortified with Contaminants

Approximately 150 liters (L) of Columbus, Ohio, tap water were collected in a high-density polyethylene (HDPE) container. The sample was dechlorinated with 0.5 mL of 0.4 M sodium thiosulfate for every liter of water. All subsequent test samples were prepared from this DDW and stored in glass containers to avoid chlorine leaching from HDPE containers.

A stock solution of each contaminant was prepared in ASTM Type II DI water at concentrations above the lethal dose level. The stock solution was diluted in DDW to obtain one sample containing the lethal dose concentration for each contaminant and three additional samples with concentrations 10, 100, and 1,000 times less than the lethal dose. Exceptions to this include aldicarb, which was non-inhibitory at the 10- and 100-fold dilution levels, so no additional dilutions were analyzed, and cyanide, which was diluted by factors of 10^5 and 10^6 from the lethal concentration to reach non-inhibitory levels. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

3.3.3 Drinking Water Fortified with Potential Interferences

Individual aliquots of the DDW were fortified with one-half the concentration specified by the EPA's NSDWR for each potential interference. Table 3-2 lists the interferences, along with the concentrations at which they were tested. Four replicates of each of these samples were analyzed. To test the sensitivity of POLYTOX™ to by-products of the chlorination process as potential interferences, the unspiked DDW (same as the negative control) was used since the water sample originated from a utility that uses chlorination as its disinfectant procedure. In a similar test involving the by-products of the chloramination process, an additional water sample was obtained from St. Petersburg, Florida, a city that uses chloramination as its disinfectant procedure. The residual chlorine in both of these samples was removed using sodium thiosulfate, and then the samples were analyzed in replicate with no additional fortification of contaminants.

3.4 Test Procedure

3.4.1 Test Sample Preparation and Storage

A drinking water sample was collected as described in Section 3.3.2 and, because free chlorine kills the microorganisms within the POLYTOX™ and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Prior to preparing each stock solution, dechlorination of the water sample was qualitatively confirmed by adding an n,n-diethyl-p-phenylenediamine tablet to a 25-mL aliquot of the DDW. Once dechlorination was confirmed, all the contaminant samples, potential interference samples, and negative control QC samples were made from this DDW, while the method blank sample was prepared from ASTM Type II DI water. The positive control samples were made using ASTM Type II DI water in Class A volumetric glassware. All QC samples were prepared prior to the start of the testing and stored at room temperature for a maximum of 60 days. The aliquots of DDW containing the contaminants were prepared within seven days of testing and stored in the dark at room temperature without chemical preservation. Aliquots to be analyzed by each technology were

placed in uniquely labeled sample containers. The sample containers were assigned an identification (ID) number. A master log of the samples and sample ID numbers for each technology was kept by Battelle.

3.4.2 Test Sample Analysis Procedure

Each day, prior to analyzing the test samples, the oxygen probes used in conjunction POLYTOX™ were calibrated by adjusting the probe to 100% dissolved oxygen when it was placed in BOD bottles containing ASTM Type II DI water that had been aerated for at least 30 minutes. Then a background sample was analyzed by inserting the oxygen probes into BOD bottles containing only DDW and recording oxygen concentrations at 19 and 21 minutes after inserting the probes. The last step prior to sample analysis was to analyze a baseline sample to determine the dissolved oxygen uptake rate of unspiked DDW, the matrix in which each test sample was prepared. This was done by starting a stopwatch and adding the contents of one POLYTOX™ container to a BOD bottle and approximately 300 mL of DDW to fill the BOD bottle. The oxygen probes were inserted, and, according to the vendor protocol, oxygen concentrations were recorded every two minutes until Minute 18 and every minute until Minute 24. Test samples were analyzed in a manner identical to the baseline except that only oxygen concentrations at Minutes 19 and 21 were recorded. For both the baseline and test samples, only the data points at Minutes 19 and 21 were used for the calculation of DOUR. The additional data acquired during the analysis of the baseline sample were not used for any additional calculations.

For each contaminant, POLYTOX™ analyzed the lethal dose concentration and three additional concentration levels four times. Only one concentration of potential interference was analyzed. To test the field portability of POLYTOX™, a single concentration level of cyanide, prepared in the same way as the other DDW samples, was analyzed in replicate by POLYTOX™ in the basement of a Columbus, Ohio, home. Sample analysis procedures were performed in the same way as during testing in the laboratory. Two operators performed all the analyses using POLYTOX™. Both held bachelor's degrees in the sciences and spent approximately eight hours with the vendor to become accustomed to performing tests using POLYTOX™.

3.4.3 Stock Solution Confirmation Analysis

The concentrations of the contaminant and interfering compound stock solutions were verified with standard analytical methods, with the exception of colchicine, ricin, and botulinum toxin—contaminants without standard analytical methods. Aliquots to be analyzed by standard methods were preserved as prescribed by the method. In addition, the same standard methods were used to measure the concentrations of each contaminant/potential interference in the unspiked DDW so that background concentrations of contaminants or potential interferences were accounted for within the displayed concentration of each contaminant/potential interference sample. Table 3-3 lists the standard methods used to measure each analyte; the results from the stock solution confirmation analyses (obtained by reporting the lethal dose concentration for the contaminants and the single concentration that was analyzed for the potential interferences); and the background levels of the contaminants and potential interferences measured in the DDW sample, which were all non-detect or negligible.

Table 3-3. Dose Confirmation Results

	Method	Average Concentration ± Standard Deviation N = 4 (mg/L)	Background in DDW Sample (mg/L)
Contaminant			
Aldicarb	EPA 531.1[3]	280 ± 28	<0.0007
Colchicine	[a]	NA[b]	NA
Cyanide	EPA 335.1[4]	250 ± 15	0.008
Dicrotophos	EPA SW846 (8141A)[5]	1,400 ± 140	<0.002
Thallium sulfate	EPA 200.8[6]	2,400 ± 24	<0.001
Botulinum toxin	[a]	NA	NA
Ricin	[a]	NA	NA
Soman	[c]	0.184[d] ± 0.001	<0.05
VX	[c]	0.088 ± 0.001	<0.05
Potential Interference			
Aluminum	EPA 200.8	0.36 ± 0.01	<0.10
Copper	EPA 200.8	0.65 ± 0.01	0.011
Iron	EPA 200.8	0.069 ± 0.008	<0.04
Manganese	EPA 200.8	0.26 ± 0.01	<0.01
Zinc	EPA 200.8	3.5 ± 0.35	0.30

[a] No standard method available. QA audits and balance calibration assured accurately prepared solutions.
[b] NA = Not applicable.
[c] Purity analyses performed on chemical and biological agent materials using Battelle standard operating procedures.
[d] The result of the dose confirmation analysis for soman was 61% of the expected concentration of 0.30 mg/L and for VX was 44% of the expected concentration of 0.20 mg/L.

Standard methods were also used to characterize several water quality parameters such as the concentration of trihalomethanes, haloacetic acids, and total organic halides; turbidity; dissolved organic carbon content; pH; alkalinity; specific conductivity; and hardness. Table 3-4 lists these measured water quality parameters for both the water sample collected in Columbus, Ohio, representing a water system using chlorination as the disinfecting process, and the water sample collected in St. Petersburg, Florida, representing a water system using chloramination as the disinfecting process.

Table 3-4. Water Quality Parameters

Parameter	Method	Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)	Dechlorinated St. Petersburg, Florida, Tap Water (disinfected by chloramination)
Turbidity	EPA 180.1[7]	0.1 NTU[a]	0.3 NTU
Organic carbon	SM 5310[8]	2.5 mg/L	2.9 mg/L
Specific conductivity	SM 2510[8]	364 µmho	460 µmho
Alkalinity	SM 2320[8]	42 mg/L	97 mg/L
pH	EPA 150.1[9]	7.65	7.95
Hardness	EPA 130.2[9]	112 mg/L	160 mg/L
Total organic halides	SM 5320B[8]	190 µg/L	83 µg/L
Total trihalomethanes	EPA 524.2[10]	52.8 µg/L	2.4 µg/L
Total haloacetic acids	EPA 552.2[11]	75.7 µg/L	13.5 µg/L

[a] NTU = nephelometric turbidity unit.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[12] and the test/QA plan for this verification test.[1]

4.1 Quality Control of Stock Solution Confirmation Methods

The stock solutions for aldicarb, cyanide, dicrotophos, and thallium sulfate were analyzed using a standard reference method at ATEL. As part of ATEL's standard operating procedures (SOPs) various QC samples were analyzed with each sample set. These included matrix spike, laboratory control spike, and method blank samples. According to the standard methods used for the analyses, recoveries of the QC spike samples analyzed with samples from this verification test were within acceptable limits of 75% to 125%, and the method blank samples were below the detectable levels for each analyte. For VX and soman, the confirmation analyses were performed at Battelle using a Battelle SOP. Calibration standard recoveries of VX and soman were always between 69% and 130%, and most of the time were between 90% and 100%. Standard analytical methods for colchicine, ricin, and botulinum toxin were not available and, therefore, were not performed. QA audits and balance calibrations assured that solutions for these compounds were accurately prepared.

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of ASTM Type II DI water was analyzed once by POLYTOX™ for approximately every 20 drinking water samples that were analyzed. The method blank samples were used as baseline samples to compare the inhibition produced by the DDW with that produced by the method blank. A baseline sample of unspiked DDW was analyzed to obtain a DOUR of unspiked DDW to compare the results with test samples prepared in DDW. A negative control sample (unspiked DDW) was analyzed with approximately every four samples and compared to the DDW baseline analyzed on the same day to determine its inhibition. Since the negative controls were compared with a baseline sample of unspiked DDW (same as the negative control), its inhibition should be near zero. Therefore, the scatter of the negative control results around zero show the precision of POLYTOX™ near its detection limit. A positive control sample of 8 mg/L cyanide also was analyzed once for approximately every 20 drinking water samples. While performance limits were not placed on the results of the positive control sample, the vendor informed Battelle that if the positive control samples did not

cause greater than approximately 50% inhibition, it would indicate to the operator that POLYTOX™ was operating incorrectly. For 15 positive control samples, the average inhibition was 85% ± 7%, indicating the proper functioning of POLYTOX™.

4.3 Audits

4.3.1 Performance Evaluation Audit

The concentration of the standards used to prepare the contaminant and potential interferences was confirmed by analyzing solutions of each analyte prepared in ASTM Type II DI water from two separate commercial vendors using the confirmation methods. The standards from one source were used to prepare the stock solutions during the verification test, while the standards from a second source were used exclusively to confirm the accuracy of the measured concentration of the first source. The percent difference (%D) between the measured concentration of the performance evaluation (PE) sample and the prepared concentration of that sample was calculated using the following equation:

$$\%D = \frac{M}{A} \times 100\% \quad (1)$$

where M is the absolute value of the difference between the measured and the prepared concentration and A is the prepared concentration. The %D between the measured concentration of the PE standard and the prepared concentration had to be less than 25 for the measurements to be considered acceptable. Table 4-1 shows the results of the PE audit for each compound. All %D values were less than 25.

Given the lack of confirmation methodology for some of the contaminants in this verification test, PE audits were not performed for all of the contaminants. PE audits were performed when more than one source of the contaminant or potential interference was commercially available and when methods were available to perform the confirmation. To assure the purity of the other standards, documentation, such as certificates of analysis, was obtained for colchicine, botulinum toxin, and ricin. In the case of VX and soman, which were obtained from the U.S. Army, the reputation of the source, combined with the confirmation analysis data, provided assurance of the concentration analyzed.

4.3.2 Technical Systems Audit

The Battelle Quality Manager conducted a technical systems audit (TSA) to ensure that the verification test was performed in accordance with the test/QA plan[1] and the AMS Center QMP.[12] As part of the audit, the Battelle Quality Manager reviewed the contaminant standard and stock solution confirmation methods, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle verification test coordinator for response. No findings were documented that required any significant action. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

Table 4-1. Summary of Performance Evaluation Audit

		Average Measured Concentration ± Standard Deviation (mg/L)	Actual Concentration (mg/L)	Percent Difference
Contaminant	Aldicarb	0.00448 ± 0.000320	0.00500	11
	Cyanide	0.207 ± 0.026	0.200	4
	Dicrotophos	0.00728 ± 0.000699	0.00748	3
	Thallium sultate	0.090 ± 0.004	0.100	10
Potential interference	Aluminum	0.512 ± 0.013	0.500	2
	Copper	0.106 ± 0.002	0.100	6
	Iron	0.399 ± 0.004	0.400	0.30
	Manganese	0.079 ± 0.003	0.100	21
	Zinc	0.106 ± 0.016	0.100	6

The EPA Quality Manager also conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan[1] and the AMS Center QMP.[12] As part of the audit, the EPA Quality Manager compared actual test procedures with those specified in the test/QA plan and reviewed data acquisition and sample preparation records and procedures. No significant findings were observed during the EPA TSA. The records concerning the TSA are permanently stored with the EPA Quality Manager.

4.3.3 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 QA/QC Reporting

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.[12] Once the assessment report was prepared, the Battelle verification test coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.5 Data Review

Records generated in the verification test were reviewed before these records were used to calculate, evaluate, or report verification results. Table 4-2 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-2. Summary of Data Recording Process

Data to be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data[(a)]
Dates, times of test events	Battelle	Laboratory record books	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample preparation (dates, procedures, concentrations)	Battelle	Laboratory record books	When each sample was prepared	Used to confirm the concentration and integrity of the samples analyzed, procedures entered into laboratory record books
Test parameters (contaminant concentrations, location, etc.)	Battelle	Laboratory record books	When set or changed	Used to organize/check test results, manually incorporated in data spreadsheets as necessary
Stock solution confirmation analysis, sample analysis, chain of custody, and results	Battelle or contracted laboratory	Laboratory record books, data sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report

[(a)] All activities subsequent to data recording were carried out by Battelle.

Chapter 5
Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.1.

5.1 Endpoints and Precision

For both baseline and sample analysis using POLYTOX™, dissolved oxygen concentrations were measured at 19 and 21 minutes after the water was added to POLYTOX™. DOURs were calculated from these results using the following equation

$$DOUR = \frac{C_{19\,min} - C_{21\,min}}{2\,min} \tag{2}$$

where C_{19min} and C_{21min} are the dissolved oxygen concentrations measured at 19 and 21 minutes. Percent inhibitions (*%I*) were calculated by comparing the baseline DOUR to the sample DOUR as follows:

$$\%I = 1 - \left(\frac{DOUR_{sample} - DOUR_{background}}{DOUR_{baseline}} \right) \times 100\% \tag{3}$$

During this testing, $DOUR_{background}$ was always zero. For all of the test samples, $DOUR_{baseline}$ is the baseline DOUR measurement using unspiked DDW analyzed at the start of each testing day. The only exceptions to this were the disinfectant by-product samples, which were compared to an ASTM Type II DI water baseline.

The standard deviation (*S*) of the results for the replicate samples was calculated, as follows, and used as a measure of technology precision at each concentration.

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(I_k - \bar{I} \right)^2 \right]^{1/2} \tag{4}$$

where n is the number of replicate samples, I_k is the percent inhibition measured for the k^{th} sample, and $\bar{I}$ is the average percent inhibition of the replicate samples. Because the average inhibitions were frequently near zero for this data set, relative standard deviations often would have greatly exceeded 100%, making the results difficult to interpret. Therefore, the precision results were left in the form of standard deviations so the reader could easily view the uncertainty around the average for results that were both near zero and significantly larger than zero.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly different from the negative control. The average inhibition of the 50 negative control samples analyzed using POLYTOX™ was 3% ± 15%; therefore, for any result to be significantly different from that of the negative control, the inhibition would have to be greater than 18%.

5.3 False Positive/Negative Responses

A response would be considered false positive if an unspiked drinking water sample produced an inhibition significantly greater than zero when determined with respect to ASTM Type II DI water. Depending on the degree of inhibition in the sample, toxicity due to subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. To test for this possibility, the percent inhibition of the unspiked drinking water was determined with respect to ASTM Type II DI water. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner. Since the inhibition of ASTM Type II DI water is defined as zero, for the same reason as described in Section 5.2, samples significantly different than zero would have to have an inhibition greater than 18%.

A response was considered false negative when POLYTOX™ was subjected to a lethal concentration of some contaminant in the DDW and did not indicate inhibition significantly greater than the negative control and the other concentration levels analyzed. Requiring the inhibition of the lethal dose sample to be significantly greater than the negative control (3% ± 15%) and the other concentration levels more thoroughly incorporated the uncertainty of all the measurements made by POLYTOX™ in determining a false negative response.

5.4 Field Portability

The results obtained from the measurements made on DDW samples in the laboratory and field setting were compiled independently and compared to assess the performance of the POLYTOX™ under different analysis conditions. Means and standard deviations of the endpoints generated in both locations were used to make the comparison. Also, qualitative observations of POLYTOX™ in a non-laboratory setting were made by the verification test

coordinator and operators. Factors such as ease of transport and set-up, demand for electrical power, and space requirement were documented.

5.5 Other Performance Factors

Ease of use (including clarity of the instruction manual, user-friendliness of software, and overall convenience) was qualitatively assessed throughout the verification test through observations of the operators and verification test coordinator. Sample throughput was evaluated quantitatively based on the number of samples that could be analyzed per hour.

Chapter 6
Test Results

6.1 Endpoints and Precision

Tables 6-1a-i present the percent inhibition data for nine contaminants, and Table 6-2 presents data for five potential interferences and the drinking water samples disinfected by both chlorination and chloramination. Given in each table are the concentrations analyzed, the percent inhibition results for each replicate at each concentration, and the average and standard deviation of the inhibition of the four replicates at each concentration. Samples that produced negative percent inhibition values indicated that the sample caused an increase in the respiration of the bacteria within POLYTOX™ relative to the negative control and was considered not toxic to the bacteria.

6.1.1 Contaminants

The contaminants that were analyzed by POLYTOX™ during this verification test resulted in percent inhibition data that varied considerably among the contaminants. The percent inhibitions for thallium sulfate and ricin were significantly different from the negative control (3 ± 15%) for only the highest concentration level (lethal dose). POLYTOX™ was especially sensitive to cyanide at concentrations near the lethal dose. Cyanide concentrations as low as 0.25 mg/L (one thousand times less concentrated than the lethal dose) produced an average percent inhibition of 61%. No inhibition significantly greater than the negative control was produced by aldicarb, colchicine, dicrotophos, botulinum toxin, soman, and VX. The lethal concentration of ricin was significantly different from the negative control, but not significantly different from the lower concentrations analyzed. However, one low result (13%) of the four replicates heavily influenced the average inhibition. If that outlier was removed, the lethal concentration was significantly different from results from the lower concentration levels. The three lowest concentrations of ricin produced average inhibitions ranging from 16 to 26%, but the uncertainty (7 to 12%) suggests that these results are not significantly different from the negative control.

6.1.2 Potential Interferences

Table 6-2 presents the results from the samples that were analyzed to test the effect of potential interferences on POLYTOX™. All five potential interferences exhibited percent inhibitions that were not significantly different from the negative control samples.

Table 6-1a. Aldicarb Percent Inhibition Results[a]

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
2.8	-9 -6 -32 -16	-16	12
28	6 0 -3 -13	-3	8
280 (Lethal Dose)	27 26 19 16	22	5

[a] Only three concentration levels of aldicarb were analyzed.

Table 6-1b. Colchicine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.24	-18 -15 -15 -4	-13	6
2.4	-18 -7 -33 22	-9	23
24	-9 -21 6 1	-6	12
240 (Lethal Dose)	-24 -13 -7 -7	-13	8

Table 6-1c. Cyanide Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00025	11 15 7 10	11	3
0.0025	9 0 -3 7	3	6
0.25	57 62 62 64	61	3
250 (Lethal Dose)	84 84 87 87	86	2
0.25 (Field Location)	61 67 61 67	64	3

Table 6-1d. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
1.4	11 30 13 10	16	10
14	8 16 19 30	18	9
140	-38 15 23 33	8	31
1,400 (Lethal Dose)	-8 -3 0 -10	-5	4

Table 6-1e. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
2.4	-13 0 25 0 -3	2	14
24	-6 6 9 3	3	7
240	22 19 25 16	20	4
2,400 (Lethal Dose)	25 34 34 41	34	6

Table 6-1f. Botulinum Toxin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00030	16 0 0 18	9	10
0.0030	8 20 16 14	14	5
0.030	12 20 -5 -5	6	12
0.30 (Lethal Dose)	-4 24 -9 10	3	15

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.015	16 17 18 5	16	8
0.15	30 32 20 18	25	7
1.5	37 36 14 18	26	12
15 (Lethal Dose)	48 56 13 59	44	21

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00018	-32 -11 14 5	-6	20
0.0018	-5 -5 14 14	5	11
0.018	-16 37 10 -5	6	23
0.18[(a)] (Lethal Dose)	5 5 -5 0	1	5

[(a)] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 61% of the expected concentration of 0.30 mg/L.

Table 6-1i. VX Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.000088	-4 9 -10 5	0	9
0.00088	17 4 -5 -15	0	14
0.0088	26 39 -5 15	19	19
0.088[(a)] (Lethal Dose)	17 22 -10 -15	4	19

[(a)] Due to the degradation of VX in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 44% of the expected concentration of 0.20 mg/L.

All of the contaminant and potential interference samples were prepared in the DDW and compared with the baseline sample of unspiked DDW. Therefore, any background inhibition in the DDW should be accounted for in the percent inhibition calculation. To investigate whether POLYTOX™ is sensitive to by-products of the disinfecting processes, dechlorinated drinking water samples from water systems that use chlorination and chloramination were analyzed and compared with ASTM Type II DI water as the baseline sample. This determination is crucial because the ability of POLYTOX™ to detect toxicity is dependent on its baseline DOUR in a clean drinking water matrix. If clean drinking water produces 100% inhibition of DOUR, the detection of subsequently added contaminants would not be possible. On average, the chlorinated sample exhibited inhibitions of 10 ± 15%, while the chloraminated sample exhibited inhibitions of 27% ± 4%. This suggests that by-products of either disinfection process that may be present in drinking water could interfere with POLYTOX™ results if they are compared with baseline measurements in ASTM Type II DI water. If a matrix similar to the drinking water sample being analyzed is used as the baseline sample, there would probably be no interference.

Table 6-2. Potential Interferences Results

Compound	Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Aluminum	0.36	3 0 -17 -17	-8	11
Copper	0.65	3 5 0 10	5	4
Iron	0.069	0 3 5 20	7	9
Manganese	0.26	3 3 13 5	6	5
Zinc	3.5	15 10 13 8	11	3
Chlorination by-products	NA[(a)]	[(b)]	10	15
Chloramination by-products	NA	31 21 28 28	27	4

[(a)] Sample not fortified with any contaminant or potential interferent.
[(b)] Chlorination by-product data averaged over the negative control results with respect to the inhibition of ASTM Type II DI water (N = 11).

6.1.3 Precision

Across all the contaminants, the standard deviation was measured and reported for each set of four replicates to evaluate POLYTOX™ precision. For the test samples, the standard deviations ranged from 2% to 31%. Out of 43 sample sets, 22 had standard deviations less than 10%, 17 had standard deviations of between 10% and 20%, and four were greater than 20%. However, when the average inhibition of the sample was greater than 20% (with the exception of ricin), the standard deviations were always below 10%. These precision results were consistent with those of the negative and positive control samples, where the average inhibition for the negative control samples was 3 ± 15% and 85% ± 7% for the positive control sample. When the inhibition was high, the uncertainty was low; and, near the detectable limit, the uncertainty was high.

6.2 Toxicity Threshold

Table 6-3 gives the toxicity thresholds (i.e., lowest concentration of contaminant with percent inhibition significantly greater than the negative control) for each contaminant. The lowest toxicity threshold concentration was for cyanide at 0.25 mg/L, indicating that POLYTOX™ was most sensitive to cyanide. For aldicarb, colchicine, dichrotophos, botulinum toxin, soman, and VX, inhibition significantly greater than the negative control was not detected, regardless of the concentration level, indicating that the technology was not highly responsive to these contaminants.

Table 6-3. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	ND[(a)]
Colchicine	ND
Cyanide	0.25
Dicrotophos	ND
Thallium sulfate	2,400
Botulinum toxin	ND
Ricin	15
Soman	ND
VX	ND

[(a)] ND = Significant inhibition was not detected.

6.3 False Positive/Negative Responses

The drinking water sample from the water system using chloramination as its disinfection process caused a false positive response. When compared to ASTM Type II DI water, four replicate samples of the chloraminated water produced an average inhibition of 27% ± 4% (N=4). In the absence of a baseline sample of a matrix similar to the sample, there is considerable risk that an analysis of POLYTOX™ in clean chloraminated water would produce inhibition greater than the negative control. However, since the inhibition is not complete, it is possible that the addition of toxic contamination could be detected, but the inhibition may be exaggerated.

The drinking water from a system that uses chlorination caused an average inhibition of 10% ± 15% (N=11). This result is not significantly different from zero inhibition, and therefore not a false positive response. However, the mean inhibition was greater than zero, so care should be taken to use a baseline sample as similar to the test sample matrix as possible to avoid exaggerated inhibitions.

A false negative response is when a lethal dose of contaminant is present in the drinking water sample and the inhibition is not significantly different from the negative control. Table 6-4 gives these results. The inhibition induced by lethal doses of cyanide, thallium sulfate, and ricin was detectable by POLYTOX™, while aldicarb, colchicine, dicrotophos, botulinum toxin, soman, and VX did not indicate inhibition significantly greater than the negative control, indicating false negative results.

Table 6-4. False Negative Responses

Contaminant	Lethal Dose Concentration (mg/L)	False Negative Response
Aldicarb	280	yes
Colchicine	240	yes
Cyanide	250	no
Dicrotophos	1,400	yes
Thallium sulfate	2,400	no
Botulinum toxin	0.30	yes
Ricin	15	no
Soman	0.18[(a)]	yes
VX	0.088[(a)]	yes

[(a)] Due to the degradation of soman and VX in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose of soman was 61% of the expected concentration of 0.30 mg/L and of VX was 44% of the expected concentration of 0.20 mg/L.

6.4 Field Portability

POLYTOX™ was used to prepare and analyze a single concentration of cyanide in replicate at a field location to examine its use in a non-laboratory setting. POLYTOX™, the oxygen probe and meter, and necessary accessories were transported to the field location in a medium-sized cardboard box because a single carrying case was not provided by the vendor. At the field location, the oxygen probe was operated, using batteries as the power source, on a small table in the basement of a house. A flat, sturdy surface is necessary to operate POLYTOX™ because of the requirement for using BOD bottles and oxygen probes. Also, because some overflow of the water sample occurs when the oxygen probe is inserted, a secondary container was used to contain the planned spill. Since 300 mL of sample are required for each sample, a sizable waste container was necessary for field work. Table 6-1c shows the results for the cyanide samples analyzed at the field location, along with the results of the cyanide samples analyzed in the laboratory. The concentration of the solution analyzed in the field was 0.25 mg/L. The inhibition produced in the field was 64% ± 3%, and the inhibition produced in the laboratory at the same concentration was 61% ± 3%. These inhibitions are not significantly different from one another. The agreement of the field results with those in the laboratory indicate that POLYTOX™

functioned similarly at both locations. POLYTOX™ can be kept at room temperature prior to use, making it convenient for long-term field deployment.

6.5 Other Performance Factors

The step-by-step pictorial instruction manual for POLYTOX™, combined with a half-day training session with the vendor, enabled operators to become quickly adept at analyzing samples. POLYTOX™ was very straightforward to operate. Baseline and background measurements took approximately one hour to complete. After those were complete, the ETV operators analyzed three samples per hour. Each sample required approximately 300 mL of water; therefore, 8 L of waste were generated each day when analyzing large sample sets. The membranes on the oxygen probes were changed every 40 to 50 samples. Although the operators had scientific backgrounds, based on observations of the verification test coordinator, an operator with little technical training would probably be able to successfully analyze drinking water samples using POLYTOX™.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibitions at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)[a]
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	280	22	-3	-16	NA[b]	5–12	ND
	Colchicine	240	-13	-6	-9	-13	6–23	ND
	Cyanide	250[c]	86	61	3	11	2–6	0.25
	Dicrotophos	1,400	-5	8	18	16	4–31	ND
	Thallium sulfate	2,400	34	20	3	2	4–14	2,400
	Botulinum toxin[d]	0.30	3	6	14	9	5–15	ND
	Ricin[e]	15	44	26	25	16	7–21	15
	Soman	0.18[f]	1	6	5	-6	5–23	ND
	VX	0.088[f]	4	19	0	0	9–19	ND
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**	
	Aluminum	0.36	-8				11	
	Copper	0.65	5				4	
	Iron	0.069	7				9	
	Manganese	0.26	6				5	
	Zinc	3.5	11				3	
False positive response	Average inhibitions for chloraminated and chlorinated drinking water were 27% ± 4% and 10% ± 15%, respectively. The chloraminated water result was false positive because the inhibition was significantly greater than the negative control. However, both types of water had positive average inhibition, so a water matrix similar to the negative control should be used to compare background inhibition due to disinfectant by-products and inhibition due to toxic contaminants.							
False negative response	At the lethal concentration level, aldicarb, colchicine, dicrotophos, botulinum toxin, soman, and VX inhibitions were not significantly different from the negative control or inhibition was generated by lower concentrations of the same contaminant, indicating false negatives.							
Field portability	Performance in the field was similar to performance in laboratory. A flat, sturdy surface is needed for BOD bottles and oxygen probes. Not including reference and background samples, 300 mL of waste were generated for every sample. A carrying case was not provided. Overflow upon inserting oxygen probe required a secondary container.							
Other performance factors	The pictorial manual was useful, and sample throughput was three samples per hour. Each sample required 300 mL of water; 8 L/day of waste were generated per oxygen probe. Oxygen probe membranes changed once per 40 to 50 samples. Although the operators had scientific backgrounds, operators with little technical training would probably be able to successfully analyze samples.							

[a] See Tables 6-1a-I in the report for the precision around each individual inhibition result.
[b] ND = Not detectable.
[c] LD/10, LD/100, LD/1,000 concentrations for cyanide are 0.25, 0.0025, and 0.00025 mg/L respectively.
[d] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[e] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[f] Due to the degradation of soman and VX in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose of soman was 61% of the expected concentration of 0.30 mg/L and of VX was 44% of the expected concentration of 0.20 mg/L.

Chapter 8
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