THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV

U.S. Environmental Protection Agency

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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**RAPID TOXICITY TESTING SYSTEM**		
APPLICATION:	**DETECTING TOXICITY IN DRINKING WATER**		
TECHNOLOGY NAME:	**POLYTOX™**		
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The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved 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 standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with 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 Advanced Monitoring Systems (AMS) Center, one of seven technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center has recently evaluated the performance of rapid toxicity testing systems used to detect toxicity in drinking water. This verification statement provides a summary of the test results for the POLYTOX™ testing system.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use bacteria, enzymes, or small crustaceans that will produce light or use oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in drinking water are indicated by a change in the color or intensity of light or by a decrease in the dissolved oxygen uptake rate (DOUR) in the presence of the

contaminants. As part of this verification test, which took place between July 14 and August 22, 2003, various contaminants were added to separate drinking water samples and analyzed by POLYTOX™. Response to interfering compounds in clean drinking water also was evaluated. Dechlorinated drinking water samples from Columbus, Ohio, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to concentrations several orders of magnitude less than the lethal dose and analyzed. Endpoint and precision, toxicity threshold for each contaminant, false positive/negative responses, ease of use, and sample throughput were evaluated.

Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the POLYTOX™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the POLYTOX™ results. The response of POLYTOX™ to compounds used during the water treatment process (interfering compounds) was evaluated by analyzing separate aliquots of DDW fortified with each potential interferent at approximately one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations guidance. For analysis of by-products of the chlorination process, 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.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials Type II deionized water; positive control samples fortified with cyanide; and negative control samples, which consisted of the unspiked DDW.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation audit, and a data quality audit of 10% of the test data. EPA QA staff also performed a technical systems audit while testing was being conducted.

TECHNOLOGY DESCRIPTION

The following description of POLYTOX™ was provided by the vendor and was not subjected to verification in this test.

POLYTOX™ 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.

The toxicity of a contaminant was detectable if its inhibition was significantly greater than the negative control. The average inhibition of the 50 negative controls analyzed using POLYTOX™ was 3 ± 15%; therefore, for any result to be detected, the inhibition had to be greater than 18%.

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, one- and two-liter containers for aerating the DI water (control), and pH adjusting solutions. 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 down-loaded 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.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents POLYTOX™ percent inhibition data and range of standard deviations for the contaminants and potential interferences that were tested. The toxicity thresholds also are shown for each contaminant tested.

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)
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	280	22	-3	-16	NA[b]	5–12	ND[c]
	Colchicine	240	-13	-6	-9	-13	6–23	ND
	Cyanide	250[a]	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	

(a) LD/10, LD/100, LD/1,000 concentrations for cyanide are 0.25, 0.0025, and 0.00025 mg/L respectively.
(b) NA = Not applicable.
(c) ND = Not detectable.
(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.

False Positive/Negative Responses: Average inhibitions for chloraminated and chlorinated water were 27% ± 4% and 10% ± 15%, respectively. The chloraminated water produced false positive responses because the inhibition was significantly greater than the negative control. However, both types of water had positive average inhibitions, 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. At the lethal concentration level, inhibitions produced by aldicarb, colchicine, dicrotophos, botulinum toxin, soman, and VX were not significantly different from the negative control or inhibition was generated by lower concentrations of the same contaminant, indicating false negative responses.

Field Portability: The performance of POLYTOX™ in the field was similar to its performance in the laboratory for the one contaminant (cyanide) that was tested in both locations. 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. The overflow upon inserting the 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 liters per day of waste were generated per oxygen probe. Oxygen probe membranes were changed once every 40 to 50 samples. Although the operators for this test had scientific backgrounds, based on the observations of the verification test coordinator, operators with little technical training would probably be able to use POLYTOX™ to successfully analyze samples.

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