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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Chlorine Dioxide Gas Generator**		
APPLICATION:	**BIOLOGICAL AGENT DECONTAMINATION**		
TECHNOLOGY NAME:	***Gas:Solid* Bench-Scale Unit**		
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The U.S. Environmental Protection Agency (EPA) has established 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. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations; with stakeholder groups that consist 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 Building Decontamination Technology (BDT) Center, under ETV, is operated by Battelle under contract with EPA's Office of Research and Development. During the period of March through May 2004, the BDT Center evaluated the performance of a chlorine dioxide technology for decontaminating indoor surfaces contaminated with biological agents. This verification statement provides a summary of the test results for the CDG chlorine dioxide (ClO_2) *Gas:Solid* bench-scale unit for decontaminating building materials after biological contamination.

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VERIFICATION TEST DESCRIPTION

The CDG bench-scale unit was verified in terms of its ability to achieve a reduction in the quantity of viable biological agent or surrogate spores on representative indoor surfaces. Qualitative factors were also evaluated, including ease of use and physical degradation of the indoor materials used as test materials.

The verification test consisted of using the CDG bench-scale unit to decontaminate seven types of surfaces contaminated with biological agent (or surrogate) spores. The surfaces included industrial-grade carpet, bare wood (pine), glass, decorative laminate, galvanized metal ductwork, painted wallboard paper, and painted concrete. The condition of test surfaces was determined by visual examination.

Test surfaces, 0.75 inch (in) x 3 in [1.9 centimeters (cm) x 7.6 cm], were wiped with 70% isopropanol and subsequently contaminated at challenge levels of approximately 1×10^8 viable biological spores per coupon. Spore suspensions were enumerated each day of use to confirm application density. Efficacy was evaluated using spores from *Bacillus anthracis* Ames strain, as well as the surrogates, *Bacillus subtilis* [American Type Culture Collection (ATCC) 19659] and *Geobacillus stearothermophilus* (ATCC 12980). In addition, surrogate biological indicators [*Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980)] and biological spore strips [*Bacillus atrophaeus* (ATCC 9372)] were used to further evaluate decontamination efficacy.

The CDG bench-scale unit was operated using cycle parameters specified by the vendor to introduce the ClO_2 into a test chamber. The cycle parameters were as follows:

- ClO_2 Concentration: 2,000 parts per million (ppm)
- Exposure Time: 6 hours
- Relative Humidity: 70% minimum (RH during testing ranged from 70% to 80%)
- Temperature: room temperature (temperature inside chamber during testing ranged from 23 to 27 ^{0}C)

The test chamber, containing the contaminated test samples, consisted of a Plas-Labs Compact Glove Box modified to CDG's specifications and equipped to enable generation of required humidity as well as promote circulation of gases. ClO_2 is light sensitive; therefore, the test chamber was also wrapped in brown paper. During testing, the lights in the laboratory were turned off and the only light source used was a flashlight. The humidity generator, not included with the CDG bench-scale unit, was incorporated into the test chamber to maintain 70% (minimum) relative humidity. At the end of each ClO_2 exposure, the chamber was evacuated overnight. The exhausted ClO_2 passed through a liquid scrubber containing 10% sodium hydroxide/10% sodium thiosulfate. Replacement air entered the chamber through high-efficiency particulate air filters.

Subsequent to using the CDG bench-scale unit, the samples were visually examined for surface damage. Spores were extracted from the surfaces and, after appropriate serial dilutions, plated onto tryptic soy agar and incubated at appropriate growth conditions. Colonies were enumerated the day following decontamination treatment. Efficacy of the decontamination procedure was evaluated by comparing the number of viable spores after decontamination to the number of viable spores from a control surface (of the same material, size, and challenge) that was not subjected to the decontamination. Efficacy was expressed in terms of a log reduction.

The extraction procedure did not remove 100% of the spores on the surface due to material-dependent characteristics, such as texture and porosity. To determine whether viable organisms remained on the test surface, the test coupon was placed in a liquid tryptic soy broth culture medium. The broth was checked after one and seven days for cloudiness, which indicated growth of residual viable organisms on the coupon. Growth may have resulted from the other microorganisms originally in or on the test coupon and not killed by the 70% isopropanol wipe or by the subsequent chlorine dioxide treatment.

QA oversight of verification testing was provided by both EPA and Battelle. Battelle performed a technical systems audit. Battelle QA staff conducted a data quality audit (minimum 10%) of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification are all available at www.epa.gov/etv/centers/center9.html.

TECHNOLOGY DESCRIPTION

The following description of the CDG bench-scale unit is based on information provided by the vendor. The technology description was not verified in this test.

The CDG bench-scale unit generates ClO_2 gas for decontaminating an enclosed space (e.g., a sealed room) by producing a blend of ClO_2 gas in nitrogen or air. A mixture of nitrogen (or air) and chlorine gas is passed through a reactor cartridge containing processed pellets of sodium chlorite. The chlorine reacts with the sodium chlorite to form ClO_2 gas and sodium chloride. As long as the chlorine concentration in the feed gas does not exceed 5%, the concentration of ClO_2 cannot enter the range (20% or greater) in which it can spontaneously undergo a self-propagating reaction. The production rate of ClO_2 is controlled either by adjusting the flow rate of the nitrogen/chlorine gas mixture or by using a compressed gas with a different chlorine:nitrogen ratio.

The CDG bench-scale unit consists of a cabinet about 20 in (51 cm) high by 16 in (41 cm) wide by 9 in (23 cm) deep, plus the required gas cylinders. Two compressed gas cylinders are required. One cylinder contains 4% chlorine in nitrogen. The other contains nitrogen for purging the system prior to shutdown. The other CDG bench-scale unit components include a sodium chlorite cartridge containing Saf-T-Chlor thermally stable sodium chlorite pellets, a supply of nitrogen for purging the system prior to shutdown, a flow meter and valve for controlling the flow rate of nitrogen/chlorine, a pressure gauge for controlling gas pressure in the generator, safety pressure relief valves, and on-off valves for the nitrogen/chlorine and nitrogen purge. The CDG bench-scale unit does not include equipment to monitor relative humidity or ClO_2 concentration; such additional equipment must be acquired and operated.

VERIFICATION RESULTS

For biological agents and surrogates, a quantitative analysis of efficacy was performed by comparing the number of spores extracted from control coupons to the number of spores from decontaminated test coupons. Because of the magnitudes of difference, efficacy is reported as the log of the ratio. Thus, a 1,000-fold reduction in spores after treatment is reported as 3 (the log of 1,000). Quantitative performance results for efficacy, based on extraction of spores in triplicate from the test materials, are summarized in Table 1. These results are the mean values that are significantly different from 0 ($P \leq 0.05$).

Table 1. Mean Efficacy (Log Reduction) for Spores

Material[a]		*B. anthracis*[b]	*B. subtilis*[b]	*G. stearothermophilus*[b]
Porous	Industrial-Grade Carpet	4.62 (4.11-5.50)	4.44 (4.28-4.62)	3.22 (3.17-3.28)[c]
	Painted Concrete	7.25 (6.24-7.76)	4.74 (4.44-4.93)[c]	5.79 (5.08-6.90)[c]
	Bare Wood	4.33 (4.10-4.48)	4.48 (4.14-4.79)	3.78 (3.70-3.87)
Non-porous	Glass	5.70 (5.35-6.06)	5.23 (4.89-5.49)	3.87 (3.64-4.20)[c]
	Decorative Laminate	4.57 (4.19-4.85)	5.14 (4.83-5.34)	4.44 (4.29-4.59)
	Painted Wallboard Paper	≥ 7.68 (7.68)	4.62 (3.24-5.47)[c]	5.62 (4.65-6.87)[c]
	Galvanized Metal Ductwork	≥ 7.79 (7.79)	5.57 (5.55-5.63)[c]	3.43 (3.33-3.56)[c]

[a] Three replicates were used for each test material for each organism.
[b] Log reduction in spores with range in parentheses.
[c] Surrogate significantly different from *B. anthracis* for specified material ($P \leq 0.05$).

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The results from the qualitative analysis of residual test spores or other surviving microorganisms following decontamination are summarized in Table 2.

Table 2. Growth (After Seven Days) of Residual Organisms on the Materials

Material[a]		*B. anthracis*[c]	*B. subtilis*[c]	*G. stearothermophilus*[c]
Porous	**Industrial-Grade Carpet**[b]	0	0	0
	Painted Concrete	0	+++	0
	Bare Wood	+++	+++	+++
Non-Porous	**Glass**	+	0	++
	Decorative Laminate	+++	+++	+++
	Painted Wallboard Paper	+	+	+
	Galvanized Metal Ductwork	0	++	+++

[a] Three replicates were used for each test material for each organism.
[b] The carpet, as manufactured, contains a broad-spectrum antimicrobial chemical. Although no bacterial growth was observed for these samples, no conclusions can be drawn as to residual organisms on the carpet.
[c] 0 indicates no growth in media for any of the samples after 7 days. + indicates growth in media for one of the samples. ++ indicates growth in media from two samples. +++ indicates growth in media in all three samples.

Surrogate biological indicators (*Bacillus subtilis* and *Geobacillus stearothermophilus*) and biological spore strips (*Bacillus atrophaeus*) showed no growth after decontamination (Table 3).

Table 3. Post-Decontamination Growth of Surrogate Indicators and Spore Strips

Biological Indicators/ Spore Strips[a]	Growth[b]
Biological Indicator (*B. subtilis*)	0
Biological Indicator (*G. stearothermophilus*)	0
Spore Strip (*B. atrophaeus*)	0

[a] For each testing day, 2 to 3 replicates were evaluated for each of the biological indicators and spore strips.
[b] 0 indicates no growth in media for samples after 7 days; all controls exhibited growth in media after 1 and 7 days.

Subsequent to decontamination, the test coupons were evaluated qualitatively (visual inspection) for visible surface damage. No damage (e.g., change in surface texture, color) to any of the test materials was observed, except for a bleaching effect observed on industrial-grade carpet.

The CDG bench-scale unit was set up for operation within minutes. The CDG bench-scale system was operated by manually regulating the introduction of ClO_2 into the exposure chamber. The ClO_2 concentration in the test chamber gradually decreased during testing. With the real-time measurement of ClO_2, using a spectrophotometer not supplied with the CDG bench-scale unit, the operator was able to maintain the desired concentration of ClO_2 by manually increasing or decreasing the flow rate of the nitrogen/chlorine gas mixture. No maintenance was required for the CDG bench-scale unit during this test. The effect of operator skill level on CDG bench-scale unit decontamination effectiveness, while not verified, should be minimal.

In summary, the CDG bench-scale unit did not change or damage any of the materials evaluated in the test, except for bleaching the carpet. Testing of the CDG bench-scale unit provided a range of results, from log reductions for *B. anthracis* of 4.33 to ≥ 7.79, depending on the material being decontaminated. Significant differences in efficacy between *B. anthracis* and both surrogate organisms were observed for painted concrete, painted wallboard, and galvanized metal ductwork. In addition, significant differences in efficacy between *B. anthracis* and *G. stearothermophilus* were observed for industrial grade carpet and glass. Surrogate biological indicators (*B. subtilis* and *G. stearothermophilus*) and biological spore strips (*B. atrophaeus*) showed results consistent with the high log reductions.

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Vice President	Director
Energy and Environment Division	National Homeland Security Research Center
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