ORIGINAL ARTICLE

Gaseous Chlorine Dioxide as an Alternative for Bedbug Control

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OBJECTIVE. This study evaluated the efficacy of gaseous chlorine dioxide (ClO_2) for extermination of bedbugs (*Cimex lectularius* and *Cimex hemipterus*).

BACKGROUND. Bedbugs have received attention because of recent outbreaks. Bedbug eradication is difficult and often requires a time-consuming multifaceted approach.

SETTING. Laboratory and hospital room.

METHODS. Bedbugs were exposed to concentrations of ClO_2 of 362, 724, and 1,086 parts per million (ppm) in an exposure chamber. Bedbug mortality was then evaluated. The ability of ClO_2 to penetrate various spaces in a hospital room was evaluated using *Bacillus atropheus* as a surrogate organism.

RESULTS. Concentrations of 1,086 and 724 ppm of ClO_2 yielded 100% bedbug mortality assessed immediately after exposure. Live young were not observed for any eggs exposed to ClO_2 gas. ClO_2 at a concentration of 362 ppm for 1,029 parts per million hours (ppm-hours) achieved 100% mortality 6 hours after exposure. A ClO_2 concentration of 362 ppm for 519 ppm-hours had 100% mortality 18 hours after exposure. Up to a 6-log reduction in *B. atropheus* spores was achieved using similar concentrations of ClO_2 in a hospital room, indicating that the concentrations needed to kill bedbugs can be achieved throughout a hospital room.

CONCLUSIONS. ClO_2 is effective at killing bedbugs in the laboratory, and similar concentrations of ClO_2 gas can be achieved in a hospital room. ClO_2 can be removed from the room without residuals.

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Bedbugs (*Cimex lectularius* and *Cimex hemipterus*) have received significant attention because of recent outbreaks. Bedbugs are capable of surviving for several months without an available food source. Infestations have been reported in residential areas, retail stores, and hotels as well as within the healthcare system.[1-3] Infestations have lead to operational closure of facilities, including healthcare facilities.

Human immunodeficiency virus, hepatitis B virus, methicillin-resistant *Staphylococcus aureus*, and other pathogens have been detected in bedbugs. Studies have not demonstrated transmission to humans, but concern remains that bedbugs may serve as vectors for the transmission of infectious diseases.[1,4-6] They are classified by the US Environmental Protection Agency (EPA) as "a pest of significant public health importance."[7]

Bedbug eradication is multifaceted and may consist of environmental measures, such as superheating and freezing, as well as the use of chemical measures.[8] Bedbugs are resistant to multiple commercially available pesticides, and the use of conventional pesticides for bedbug control resulted in 39 cases of pesticide poisoning in 2010.[9,10]

Chlorine dioxide (ClO_2) is an effective gaseous decontaminant and was used in US governmental facilities after the 2001 anthrax attacks.[11-14] ClO_2 has the potential to reach small cracks and crevices where bedbugs reside, but its effect on bedbugs has not been investigated. ClO_2 can be removed from the environment after treatment to reduce concerns associated with chemical residuals. This study evaluated the efficacy of gaseous ClO_2 in extermination of bedbugs.

METHODS

A laboratory study was conducted to determine the ability of ClO_2 to kill bedbugs at various concentrations within an experimental chamber. Because of concerns over the accidental release of bedbugs into the hospital environment, the bedbugs were not tested in a hospital room. However, a field evaluation of the decontamination of a hospital room with the concentration of ClO_2 used in the laboratory was performed.

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TABLE 1. *Bacillus atropheus* Spore Strip Placement Sites Used for Hospital Room Evaluation

Site number	Description	Meters from Injection site	Meters from Floor	Meters from Ceiling
1	Floor next to injection tubing	0.3	0.0	3.3
2	Small wall-mounted countertop	1.3	1.0	2.3
3	Floor	1.6	1.3	2.0
4	On top of bed mattress	3.0	2.3	1.0
5	Top of wall-mounted light fixture	3.7	1.0	2.3
6	Top of wall-mounted light fixture	4.0	1.3	2.0
7	On window countertop	4.3	2.7	0.7
8	Between wall-mounted television and VCR	5.3	2.7	0.7
9	Top of sink in bathroom	3.3	1.3	2.0
10	Inside metal cabinet	2.7	0.0	3.3
11	Inside pulse oximeter	2.3	1.3	2.0
12	Inside ventilator	2.3	1.3	2.0
13	Inside specimen incubator	2.3	1.3	2.0
14	Inside computer keyboard	2.3	1.3	2.0
15	Inside linen bag	2.3	1.3	2.0

Chlorine Dioxide Generation

ClO_2 was generated using the Minidox-M Decontamination System (Clordisys Solutions, Lebanon, NJ). Minidox is an EPA-registered antimicrobial sterilizer (EPA registration 80802-1). ClO_2 gaseous decontamination of microorganisms has 3 critical elements: ClO_2 concentration, relative humidity, and exposure time. Minidox continuously monitors these critical elements to ensure target levels during exposure time. ClO_2 gas was removed from both the chamber and the hospital patient care room by means of an activated charcoal scrubber.

Bedbug Chamber Evaluations

Bedbugs were collected and identified by an entomologist from local residences. *C. lectularius* and *C. hemipterus* were the 2 species recovered in approximately the same numbers. The insects included a mixture of adults, juveniles, and eggs. Bedbugs were sorted and placed in groups of 30 (adults and juveniles) into glass flasks. Three flasks of bedbugs were placed in an exposure chamber within a chemical fume hood for each ClO_2 exposure. A control flask of organisms was also maintained for each exposure at 25°C and 70% relative humidity. This facilitated evaluation of 90 bedbugs with similar numbers of each species in each group—30 in 3 groups for each exposure and a group of 30 control bedbugs for each exposure scenario. All insects were tested within 96 hours after collection. Eggs that were laid by the bedbugs we had collected were exposed in triplicate for groups of 5–10 egg sacks at the 724 parts per million (ppm) exposures. No attempt was made to determine which species of bedbug had laid each egg.

After placement of the insect groups, the decontamination area was sealed to form a gas-tight chamber. Once the area was sealed, relative humidity was increased by the Minidox system. ClO_2 gas was generated in the chamber until the target concentrations (362, 724, and 1,086 ppm) were reached and the desired exposures in parts per million hours (ppm-hours) were achieved. Ambient ClO_2 gas concentrations were monitored in adjacent areas to ensure minimal leakage. After ClO_2 gas exposure was complete, the bedbugs were immediately moved to the ambient air environment and evaluated. They were maintained under the same conditions as the control organisms during the evaluation periods.

The Minidox system allows variation of gas concentration and exposure time, which allowed for adjustment of ppm-hours, the measure of ClO_2 exposure. Temperature ranged from 20° to 21°C throughout the exposure periods. Following exposure, all bedbugs (controls and exposed) were evaluated every 6 hours to determine whether they were dead. Bedbugs were determined to be dead during evaluation by a lack of movement when agitated using forceps for 30 seconds under a dissecting scope, the standard practice for determination of bedbug mortality.[15] After insects were labeled as dead, they were evaluated every 6 hours for an additional 96 hours and then evaluated daily over 2 weeks for confirmation. All bedbug eggs were maintained at an optimal temperature of 25°C in ambient air and relative humidity of 70% for 4 weeks to determine whether they remained viable, which is twice as long as the eggs normally take to hatch.[10]

Hospital Room Evaluation for Chlorine Dioxide Penetration and Material Compatibility

The ClO_2 generator was set up outside a patient care hospital room in the Nebraska Medical Center. Tubing ran from the generator under the door and into the room to provide gas flow, ClO_2 sampling, and in-room monitoring of temperature and relative humidity. A 10-inch portable fan was placed in the room 2 feet from the gas inflow to help distribute the gas throughout the room. A portable steamer (not included with the Minidox system) was added to the room and con-

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TABLE 2. Scenarios for Bedbug Exposure to Chlorine Dioxide (ClO_2) Gas

ClO_2 concentration	Exposure time, minutes	Exposure, ppm-hours	Relative humidity, %	Mortality after exposure to ClO_2, % (SD)			
				0 hours	6 hours	12 hours	18 hours
1,086 ppm	167	3,024	82	100	...	...	...
724 ppm	94	1,132	64	100	...	...	...
362 ppm	176	1,029	43	84.3 (4.8)	100	...	...
362 ppm	89	519	43	59.8 (14.5)	86.9 (6.3)	98 (1.8)	100

NOTE. All trials were performed at temperatures of 20°–21°C. ppm-hours, parts per million hours; SD, standard deviation.

trolled from outside the room to maintain relative humidity. Biological indicators were placed in the room. Duct tape and plastic were used to seal the room. This process took less than 1 hour. ClO_2 was generated as described above, and a total of 6 runs were completed, with each run taking less than 3 hours. A PortSens (Analytical Technology, Oaks, PA) portable gas detector was used to monitor for ClO_2 outside the room during exposure periods. ClO_2 gas was removed from the hospital patient care room by means of an activated charcoal scrubber, a piece of equipment separate from the Minidox system. The gas was absorbed by the activated charcoal, and no liquid or other material was generated. The gas was cleared from the room during the aeration process in approximately 30 minutes to the safe concentration of 0.1 ppm; however, the smell of the gas was present in the room for 2–3 hours following completion of the protocol.

B. atropheus spore strip (Raven Labs; catalog 1-6100) biological indicators were used to validate ClO_2 penetration. Each of these biological indicator strips are impregnated with a median value of 10^6 spores contained in sterile Tyvek envelopes for aseptic processing (http://www.mesalabs.com/products-services/raven-labs/RCTkit-ClO2.html). These biological indicators are the standard for determining ClO_2 decontamination and require a relative humidity of approximately 65%. Biological indicators were placed at 15 sites throughout the hospital room in duplicate pairs, including several inaccessible sites (Table 1). Indicator placement sites 1–10 represent locations throughout the room selected to ensure complete room coverage, including places bedbugs were likely to inhabit. Biological indicator placement sites 11–15 were located inside medical devices. Following exposure to ClO_2 gas, indicators were processed according to manufacturer recommendations and incubated in tryptic soy broth at 35°C for 7 days. Positive and negative controls of biological indicators were performed for each run.

In addition to the biological indicators, several pieces of electronic equipment (Table 1), furniture, bedding, and various fabric and material samples were placed in the room for qualitative evaluation of the impact of ClO_2. These items were present during all 6 runs and were evaluated at the completion of the room study to determine whether they still were operational or underwent any material change in either texture or coloration.

RESULTS

The ability of ClO_2 to exterminate bedbugs in the laboratory setting is presented in Table 2. Given the information regarding the hardiness of bedbugs and their resistance to pesticides, higher concentrations of ClO_2 were initially used. As shown in Table 2, 100% mortality was achieved under each scenario by the 18-hour postexposure time point even when a lower ClO_2 concentration (362 ppm) was used. There was no mortality in the control groups throughout the study, and no difference in susceptibility was identified between *C. lectularius* and *C. hemipterus*.

The ability of ClO_2 to penetrate throughout a hospital patient care room and into various medical devices was evaluated under the conditions described in Table 3. Six room decontaminations were completed to expose *B. atropheus* indicators to ClO_2. The target ClO_2 concentration used for the hospital room evaluation was 360 ppm, which was selected because of the substantial bedbug mortality achieved at 360 ppm in the experimental chamber. Additionally, *B. atropheus* has been shown (J. J. Lowe et al., unpublished data) to require approximately 720 ppm-hours of exposure to achieve a 6-log reduction.

Decontamination trials 1–4 used open doors on a metal cabinet and bathroom, resulting in 6-log reductions at all 10 room sites. Trials 5 and 6 were carried out with cabinet and bathroom doors closed to evaluate the gas's ability to penetrate closed compartments. Trials 5 and 6 resulted in a mean 3-log reduction of *B. atropheus* spores at site 9 (bathroom sink). All other room placement sites resulted in a 6-log reduction or complete inactivation, including site 8 in the closed cabinet.

B. atropheus indicators placed inside medical devices (sites 11–15) were evaluated during 6 hospital room decontaminations (Table 3). Complete inactivation (6-log reduction) of *B. atropheus* indicators was observed at placement sites inside the ventilator (site 12), computer keyboard (site 14), and linen bag (site 15). A 6-log reduction was not achieved within a pulse oximeter unit (site 11) or the specimen incubator (site 13).

Qualitative evaluation of the 50 fabric and material samples placed in the room showed no visible color damage or changes in material texture, with one exception. A clear plastic

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TABLE 3. Chlorine Dioxide (ClO_2) Reduction of *Bacillus atropheus* Spores Inside Medical Equipment by Site Number

Trial number	ClO_2 concentration, ppm	Exposure time, minutes	Exposure, ppm-hours	Relative humidity, %	*B. atropheus* spore log reduction by site[a]				
					11	12	13	14	15
1	352	116	677	50	3.8	6.0	5.0	6.0	6.0
2	377	142	890	65	5.5	6.0	5.4	6.0	6.0
3	379	121	767	65	3.5	6.0	5.5	6.0	6.0
4	385	120	770	65	6.0	6.0	6.0	6.0	6.0
5	376	126	788	64	2.4	6.0	5.4	6.0	6.0
6	383	122	781	66	4.0	6.0	5.0	6.0	6.0

NOTE. All trials were performed at temperatures of 21°–24°C. ppm-hours, parts per million hours.
[a] See Table 1 for site numbers.

sample did become yellow, similar to the ClO_2 gas. All electronic devices remained fully functional.

DISCUSSION

Conventional chemical treatment of bedbugs requires that the bedbug move through the applied pesticide, and a residual must be maintained for several weeks after treatment. This does not guarantee complete bedbug removal, as some bedbug populations may not move to feed or hatch until after the pesticide has dissipated. Pesticides then have to be reapplied while humans are occupying the space, which can result in adverse effects in humans.[9] These human health effects can be reduced or avoided by following proper procedures and regulations; however, this does not always occur. Even when pesticides are applied properly, there are a number of factors associated with their persistence in the environment that can contribute to human illness.[9]

In our evaluations of the hospital room in our study, we did detect levels of ClO_2 outside the hospital room. Tape reinforcement eliminated the detection. No gas was detected on the floor below the exposure room, in the adjacent room, or more than several feet beyond the taped doors, all of which were off limits to all but study personnel to maintain safety. Study personnel wore proper personal protective equipment as they conducted evaluations. There were no adverse events reported during the study.

Bedbugs are widely known to live in cracks and crevices at close proximity to their human food source.[16] Healthcare facilities face numerous challenges associated with bedbugs, which can enter through a number of routes, mainly with patients and visitors.[16] Healthcare facilities and other public facilities must be able to remediate any potential infestation with minimal interruption to operations. Any remediation must be done quickly to allow for room or space occupancy and to confine spread. Avoidance of harmful chemical residuals that may adversely impact a vulnerable patient population is optimal. Standard methods for bedbug remediation often make it difficult to meet these goals. Temperature-related controls are time and labor intensive, and bedbugs have been found to be resistant to a number of conventional pesticides that maintain residuals.[17]

The resistance of bedbugs to pesticides, such as pyrethroids, coupled with concerns related to human exposure to persistent chemical control measures led to the evaluation of other options for bedbug control.[15] In our study, it would take about 2 hours of exposure time with 362 ppm of ClO_2 to achieve 100% mortality at 18 hours. After exposure time has been achieved, the ClO_2 can be removed with a scrubber, allowing humans to safely enter the room in under 30 minutes.

It is important that the agent used for bedbug eradication kills adult bedbugs and bedbug eggs. In our study, 100% mortality of all stages of the bedbug life cycle 0 hours after exposure was achieved with 724 ppm for 1,132 ppm-hours, which could be useful in multiple environments. This concentration has been maintained safely in hospital and university rooms for longer periods of time than necessary to achieve the 1,132 ppm-hours of exposure (J. J. Lowe et al., unpublished data). ClO_2 was found to achieve 100% mortality 18 hours after exposure for all concentrations evaluated, down to a concentration of 362 ppm for 89 minutes. ClO_2 is a toxic compound that requires training and personal protective equipment to work with, as are the other compounds currently used for bedbug control. ClO_2 detectors can detect any leak coming from a sealed room, which can be quickly stopped with duct tape. Additionally, the odor threshold for ClO_2 is approximately 0.1 ppm; hence, one can smell the gas at concentrations much lower than those that have adverse human health effects.[18]

Limitations of our study include that it was a small pilot study and that bedbugs were not placed in a hospital room. Spores were used in the hospital room as a surrogate for bedbugs, and ClO_2 demonstrated excellent killing activity and penetration in the laboratory and against the bedbug surrogates in the hospital room. The spores used in the room are well-known ClO_2 biological indicators because of their difficulty to kill. We did not conduct formal structural testing of synthetic materials and electronic equipment used within hospital rooms. Additionally, further evaluation may be

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needed to determine the proper aeration time for areas with extensive porous surfaces.

The results of this study are encouraging and demonstrate that ClO_2 may be useful for eradication of bedbugs. Further study is necessary to determine the effects of gaseous ClO_2 on bedbug infestations in the healthcare environment and in other settings (e.g., hotels and businesses) where they have been found. Further studies involving the application of ClO_2 to a bedbug-infested area and evaluation of its practical use are necessary.

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