ECOSENSE
Bio-Decontamination Services

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Pinworm Egg Decontamination Services

Overview: The Ecosense Company decontaminates rooms, buildings, equipment, HVAC systems and confined spaces where pinworm egg infestation is present. We routinely decontaminate research facilities on an emergency basis or as a precautionary measure.

We offer on-site decontamination services as well as convenient off-site contract sterilization services. Our decontamination process is performed using ClorDiSys chlorine dioxide generation equipment and their EPA approved sterilant. The decontamination treatment is conducted following ClorDiSys' proven operating protocol for the inactivation of pinworm eggs. After completion of each treatment, customers receive a comprehensive report validating the procedural, chemical and biological results.

Summary: ClorDiSys chlorine dioxide gas effectively eliminates pinworm eggs on equipment, or in facilities, so that there is no risk of cross-contamination. Post-treatment, the equipment or facilities can be used without the risk of compromising the integrity of future research results.

The ClorDiSys technology is ideally suited to address the unique challenges associated with eliminating pinworm egg contamination. Pinworm eggs can persist on surfaces for extended periods, multiplying and binding to subsequent research projects in the process. Complicated by the microscopic nature of many pinworm eggs, contamination can reside in cracks and crevices that traditional cleaning methods often fail to adequately address. Especially challenging, pinworm eggs are easily aerosolized, spreading to and residing in difficult to reach areas such as HVAC systems, leading to persistent facility recontamination and compromised research.

Chlorine dioxide gas inactivates pinworm eggs in a non-invasive and non-corrosive manner. As a true gas with a molecular size of approximately 0.124 nanometers, contamination residing in even the most remote area is eliminated. The ClorDiSys process uses a precise photospectrometer to insure that the proper concentration level is achieved and maintained for the duration of the decontamination treatment.

In the attached study, The University of Tennessee validated that the ClorDiSys decontamination method provided a more practical and effective method for inactivating pinworm eggs than either ethylene oxide or dry heat. Since then, numerous pinworm egg decontamination projects have been performed and all customer post-treatment analysis have been satisfactory.

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Pinworm Egg Inactivation Research Study Summary

Introduction:

The University of Tennessee at Knoxville performed a study on chlorine dioxide gas' effect on pinworm eggs to see if it was a viable option for treating contaminated spaces. Pinworm eggs are microscopic and have been found on equipment, shelving, in dust, and in ventilation air intake ducts. Prior to this study, only ethylene oxide gas, and dry heat had been proven to eliminate pinworm eggs. Ethylene oxide is not used for space fumigation due to its carcinogenic and explosive properties and it is very difficult to uniformly establish and maintain the high temperatures needed for dry heat (212° F held for 30 minutes) to be effective, especially in floor drains and HVAC grills. Such high temperatures can also affect various temperature sensitive components and materials within the space, necessitating that they be removed and treated separately. Chlorine dioxide gas is able to treat entire spaces at once, as it is able to reach deep into crevices and around objects within the space to leave no surface untreated, which is why it was used in the study.

Test Materials and Methods:

Thirty two mice of unknown health status were procured from two local vendors. The animals were group housed 3-4 mice per cage in micro-isolator cages on corncob bedding. Animals were housed in an AAALAC, International accredited dedicated animal facility under environmental conditions consistent with the Guide for the Care and Use of Laboratory Animals. Mice were provided rodent chow and tap water ad libitum. All manipulations were reviewed and approved by the University of Tennessee's Institutional Animal Care and Use Committee.

In a controlled study, *Syphacia* spp. ova were affixed to a slide and exposed to a set concentration of chlorine dioxide gas for varying amounts of time.

Ova were collected on the day of the experiment by anal cellophane tape impression. Double sided tape was used to affix the tape to a slide allowing the anal impression side up. Slides with affixed tape were scanned using a compound microscope under low power to confirm the presence of Syphacia spp. ova. Syphacia spp. ova were identified based upon distinguishing characteristics and size. Slides were randomly designated as either control or experimental for time points of 1, 2, 3 or 4 hours. After being exposed to chlorine dioxide gas, the ova were placed in petri dishes, covered with a hatching medium, and incubated at 37° C for six hours. Positive control ova not exposed to chlorine dioxide gas were also processed and incubated. After incubation they were observed microscopically. Duplicate runs were performed for all time points except 1 hour.

Conclusion:

The parameters to achieve a 6 log level kill of viruses, bacteria, fungi, and spores are normally as follows: 1 mg/L chlorine dioxide gas (360 parts per million or ppm) for 2 hours of exposure contact time. This equates to a 720 ppm-hours (360 ppm x 2 hours) chlorine dioxide gas dosage. It was found that a dosage twice as long (1440 ppm-hour) was needed in order to eliminate all viable ova from hatching. Below is a table showing the results of the study:

Exposure time	Chlorine Dioxide Gas Dosage	% of *Syphacia*, spp. ova hatched	
		Treated with CD Gas	Untreated (Positive Control)
1 hour	360 ppm-hour	14%	71%
2 hours	720 ppm-hour	12%	82.5%
3 hours	1080 ppm-hour	2%	80.5%
4 hours	1440 ppm-hour	0%	83%

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Exposure to Chlorine Dioxide Gas for 4 Hours Renders *Syphacia* Ova Nonviable

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The purpose of our study was to evaluate the efficacy of chlorine dioxide gas for environmental decontamination of *Syphacia* spp. ova. We collected *Syphacia* ova by perianal cellophane tape impression of pinworm-infected mice. Tapes with attached ova were exposed to chlorine dioxide gas for 1, 2, 3, or 4 h. After gas exposure, ova were incubated in hatching medium for 6 h to promote hatching. For controls, tapes with attached ova were maintained at room temperature for 1, 2, 3, and 4 h without exposure to chlorine dioxide gas and similarly incubated in hatch medium for 6 h. Ova viability after incubation was assessed by microscopic examination. Exposure to chlorine dioxide gas for 4 h rendered 100% of *Syphacia* spp. ova nonviable. Conversely, only 17% of ova on the 4-h control slide were nonviable. Other times of exposure to chlorine dioxide gas resulted in variable effectiveness. These data suggest that exposure to chlorine dioxide gas for at least 4 h is effective for surface decontamination of *Syphacia* spp. ova.

Pinworms of the genus *Syphacia* are common contaminants of contemporary laboratory animal facilities and can inhabit the cecum and colon of rodents.[7] Although usually nonpathogenic in immunocompetent rodents, pinworm infections can have adverse effects on behavior, growth, intestinal physiology, and immunology.[14,16,18,21] These effects, coupled with the demand for defined experimental rodents and possible hindrances to interinstitutional collaborations, make effective pinworm surveillance and eradication important for many laboratory animal facilities. Eradication of *Syphacia* spp. infections is complicated by the ability of ova to aerosolize and remain viable in the environment for lengthy, but unknown, periods.[6] Various agents and methods, including aqueous chlorine dioxide products, have been evaluated for destruction of *Syphacia* spp. ova.[5] Although chlorine dioxide gas has demonstrated efficacy as an environmental and surface biocide,[1,6,10,12,13,17] the agent's effectiveness in killing *Syphacia* spp. ova had not been evaluated previously. Here, we report on the efficacy of chlorine dioxide gas for environmental decontamination of *Syphacia* spp. ova.

Materials and Methods

Animals. A total of 32 mice of unknown health status were purchased from 2 local pet stores. The animals were group-housed at 3 or 4 mice per cage in microisolation cages (Zytem, Lab Products, Seaford, DE) on corncob bedding (Teklad 7087 Soft Cobs Enriched Bedding, Harlan Teklad, Madison, WI). Mice were housed in an AAALAC-accredited, dedicated animal facility under environmental conditions consistent with the *Guide for the Care and Use of Laboratory Animals*.[9] Mice were provided rodent chow (Teklad 8640, Harlan Teklad) and tap water ad libitum. All manipulations were reviewed and approved by the University of Tennessee's IACUC.

Collection of ova. Ova were collected by anal impressions using double-sided cellophane tape once daily, in the afternoon, from all 32 mice. All animals had at least one ova positive tape test. Double-sided tape was used to affix the test tape to a slide, with the anal impression side up. Slides with affixed tape were scanned by using a compound microscope under low power to confirm the presence of *Syphacia* spp. ova, which were identified according to distinguishing characteristics and size. The average number of ova per slide was 20.7. Slides were randomly designated as either control or experimental for time points of 1, 2, 3, or 4 h. Duplicate runs were performed for all time points except 1 h. Collection of ova and exposure to chlorine dioxide gas or room temperature took place on day 0.

Chlorine dioxide exposure. Chlorine dioxide gas (ClO_2) was generated by using the Minidox-M Decontamination System

Figure 1. Decontamination equipment, using a Minidox-M Decontamination System (ClorDiSys Solutions).

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Figure 2. (A) Nonviable *Syphacia* spp. Bar, 14.1 µm. (B) Viable *Syphacia* spp. ovum with open operculum. Bar, 13.4 µm. (C) Viable emerging *Syphacia* spp. larvae. Bar, 65.4 µm. (D) Viable emerging *Syphacia* spp. larvae. Bar, 8.84 µm.

(ClorDiSys Solutions, Lebanon, NJ; Figure 1) according to the manufacturer's instructions. To generate pure chlorine dioxide gas, 2% chlorine gas was passed through sodium chlorite catalyst cartridges. The resulting chemical reaction is 2 $NaClO_2$ + $Cl_2 \rightarrow 2ClO_2$ + 2 NaCl,[4,11] which generates ClO_2 gas. The system delivered ClO_2 gas through polyvinylidene fluoride tubing to an airtight, fabricated polypropylene chamber (ClorDiSys Solutions) measuring 0.028 m^3. The slides with attached pinworm ova were positioned 9 cm above the floor of the experimental chamber. A fan (Minebea, Mita, Tokyo, Japan) was placed on the floor of the chamber to continuously circulate the air. Temperature and relative humidity were monitored. Between treatment groups, relative humidity ranged from 52% to 67%, and temperatures ranged from 23 to 26 °C. Chlorine dioxide gas was delivered to the chamber until a concentration of 1 mg/L (360 ppm) was reached. The exposure periods were calculated by multiplying the desired ppm by the exposure period in hours. For all exposures, the concentration of chlorine dioxide gas was maintained at 1 mg/L and continuously monitored by the Minidox system. The exposure periods ended when 360, 720, 1080 and 1440 ppm×h (equivalent to 1-, 2-, 3-, and 4-h exposure periods, respectively) were achieved. At the end of each exposure period, the chamber was aerated by using a charcoal scrubber (ClorDiSys Solutions), and the slides with treated ova were removed from the chamber. To run control and treated samples simultaneously, control slides were placed in the same room for the same time, temperature, and humidity as the exposed ova slides.

Hatchability. The hatching medium used was adapted from and prepared according to a method provided previously.[5] We added 1.6 g sodium phosphate dibasic bio reagent (Na_2HPO_4, Sigma-Aldrich, St Louis, MO) to 95 mL sterile water, heated, and stirred to dissolve. Then, 0.07 g KH_2PO_4 (Sigma-Aldrich) was dissolved in 5 mL sterile water. These 2 solutions were combined

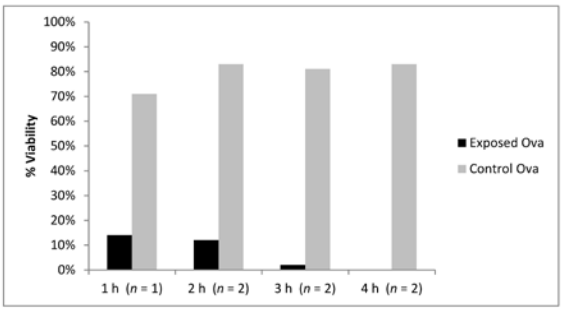

Figure 3. Comparison of hatch percentage at different time points for ClO_2-exposed (exposed) ova compared with unexposed (control) ova.

as the phosphate buffer. Next, 1.0 g trypsin (1000 to 2000 BAEE U/mg solid, Sigma-Aldrich), 0.26 g ox bile (dehydrated, purified for microbiology, Sigma-Aldrich) dissolved in 3 mL sterile water, and 0.2 g cysteine (Sigma-Aldrich) dissolved in 2.5 mL 1 N HCl were added to the phosphate buffer. Both control slides and slides exposed to chlorine dioxide gas were placed in covered culture dishes with a sufficient amount of hatching medium to cover the slide and the affixed tape. Slides were placed in an ambient air incubator at 37 °C for 6 h, then rinsed with sterile water, and observed microscopically.

Control and experimental slides were incubated concurrently in hatching medium on day 1. Slides were air dried and scanned at 40× magnification under a compound microscope to quantify hatched compared with not hatched *Syphacia* ova. Ova were considered nonviable and scored as not hatched if they contained larva (Figure 2 A). Ova without larva or those with an open operculum with larva emerging were considered viable and scored as a hatched (Figure 2 B through D). To reduce variability in the quantification of each condition, the same person (JKA) quantified all slides for hatching percentage. The person quantifying hatchability was not blinded to treatment groups.

Statistical analysis. All statistical analyses were performed by using SAS version 9.3 (SAS Institute, Cary, NC). An independent samples *t* test, corrected for unequal variances when necessary, was used to determine significant differences between experimental and control exposures. Some degrees of freedom are fractional because they had to be corrected for unequal variances between the experimental and control groups, between hatched and not hatched, or both. Duplicates were summed by exposure. Significance was set at $P < 0.001$.

Results

After 1 h of exposure, 14% of ClO_2-exposed ova were viable compared with 71% for the control group. The 2-h exposure group had a 12% viability rate compared with 82.5% for controls. The 3-h exposure group had a 2% viability rate compared with 80.5% for controls, and the 4-h exposure group had a 0% viability rate compared with 83% for the control group (Figure 3). Thus, viability rates decreased as exposure times increased. There were significant differences between the viability rates for exposed compared with control ova within each exposure period (Table 1).

Table 1. Hatch means (mean [95% CI]) for *Syphacia* ova exposed to chlorine dioxide gas

	Mean			
Exposure	Exposed	Control	*t*	df
1 h	0.14 ± 0.45 (0.62–0.81)	0.71 ± 0.35 (0.02–0.25)	6.96	1129
2 h	0.09 ± 0.29 (0.02–0.15)	0.84 ± 0.37 (0.81–0.88)	19.99	1134
3 h	0.04 ± 0.20 (0.02–0.15)	0.81 ± 0.39 (0.77–0.85)	32.69	1487
4 h	0.00	0.83 ± 0.38 (0.76–0.93)	17.18	1590

df, degrees of freedom.
Reported means are means of duplicate samples, except for the 1-h time point.
All treatment differences significant (one-sample *t* test; test value = 0) at $P < 0.001$.

Discussion

Chlorine dioxide destroys microorganisms by disrupting the transportation of nutrients across the cell wall.[20] In 1967, the Environmental Protection Agency first registered chlorine dioxide (in the form of a liquid) for use as a disinfectant and sanitizer at a variety of commercial sites, such as animal farms and food processing, handling, and storage plants. ClO_2 gas has been registered as a sterilizer since 1988,[20] and all microbial agents so far tested have been eradicated by ClO_2 gas.[2] For example, ClO_2 gas is effective at eradicating interior surfaces of *Bacillus anthracis* spores and *Salmonella*.[15,19]

To our knowledge, our study is the first that tests the effectiveness of ClO_2 gas against *Syphacia* spp. ova. All ClO_2 treatment times significantly decreased the hatching rates of the ova. The 1-h exposure decreased the hatching rate from 71% to 14%. Because some ova were still considered viable at 1 h, the exposure rate was increased. Chlorine dioxide gas fumigation effectively rendered 100% of the *Syphacia* ova nonviable at the 4-h exposure time. Hatching rates were significantly different between treated untreated eggs at each exposure, but a 4-h exposure was required to make 100% of the ova nonviable. Because the 4-h exposure time consistently rendered the *Syphacia* ova nonviable, longer exposure times were not evaluated. We shortened the incubation in hatching media from overnight to 6 h because the longer incubation led to digestion of hatched larvae. A previous study reported similarly altered egg walls after overnight incubation of eggs in hatching media.[5]

Syphacia spp. ova are known to be resilient in the environment,[8] and eradication of these eggs from the environment can be challenging. Using a gas would be ideal in eradicating *Syphacia* ova from a room because areas typically inaccessible to liquid decontaminants, such as light covers and vent covers, would be exposed to the gas. Our study addresses only a chamber method. For smaller items contaminated with *Syphacia* ova, the chamber method is ideal. In addition, items that could be damaged by high heat could be disinfected with the ClO_2 gas chamber system.

The ability of ClO_2 gas to permeate hard-to-reach areas makes it an attractive method for room-level eradication of *Syphacia* spp. Unlike liquid ClO_2 products, gaseous ClO_2 is not corrosive and thus is safe for many materials that could be damaged by other disinfectant methods or agents.[3] Although the 4-h exposure rendered 100% of *Syphacia* spp. ova nonviable in an enclosed environmental chamber, results may differ under other environmental conditions, such as those present in animal housing rooms. Additional large-scale studies should be performed to ensure the reproducibility of these data.

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