Pinworms of the genus *Syphacia* are common contaminants of contemporary laboratory animal facilities and can inhabit the cecum and colon of rodents. Although usually nonpathogenic in immunocompetent rodents, pinworm infections can have adverse effects on behavior, growth, intestinal physiology, and immunology. 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. Various agents and methods, including aqueous chlorine dioxide products, have been evaluated for destruction of *Syphacia* spp. ova. Although chlorine dioxide gas has demonstrated efficacy as an environmental and surface biocide, 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 housed at 3 or 4 mice per cage in microisolation cages (Zytem, Lab Products, Seaford, DE) on corncob bedding (Teklad 7087 Soft Cob 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*. 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 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 (ClO₂) was generated by using the Minidox-M Decontamination System (ClorDiSys Solutions). Exposure to Chlorine Dioxide Gas for 4 Hours Renders *Syphacia* Ova Nonviable

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.
Using chlorine dioxide gas for *Syphacia* ova decontamination

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 x 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. We added 1.6 g sodium phosphate dibasic bio reagent (Na₂HPO₄, Sigma-Aldrich, St Louis, MO) to 95 mL sterile water, heated, and stirred to dissolve. Then, 0.07 g KH₂PO₄ (Sigma-Aldrich) was dissolved in 5 mL sterile water. These 2 solutions were combined

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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.
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 hatching ability 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**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Mean</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>0.14 ± 0.45</td>
<td>6.96</td>
<td>1129</td>
</tr>
<tr>
<td></td>
<td>(0.62–0.81)</td>
<td></td>
<td></td>
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<tr>
<td>2 h</td>
<td>0.09 ± 0.29</td>
<td>19.99</td>
<td>1134</td>
</tr>
<tr>
<td></td>
<td>(0.02–0.15)</td>
<td></td>
<td></td>
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<tr>
<td>3 h</td>
<td>0.04 ± 0.20</td>
<td>32.69</td>
<td>1487</td>
</tr>
<tr>
<td></td>
<td>(0.02–0.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>0.00</td>
<td>17.18</td>
<td>1590</td>
</tr>
<tr>
<td></td>
<td>0.83 ± 0.38</td>
<td></td>
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<tr>
<td></td>
<td>(0.76–0.93)</td>
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</table>

**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 $P < 0.001$.

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 viable 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.

*Syphacia* spp. ova are known to be resilient in the environment, 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 penetrate 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. Although the 4-h exposure rendered 100% of *Syphacia* 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.
Using chlorine dioxide gas for *Syphacia ova* decontamination

Acknowledgments

The Minidox-M Decontamination System was on loan from ClorDiSys Solutions. ClorDiSys was not part of the research team and put no conditions on our use of the Minidox system beyond that we return it when the study was completed. We acknowledge Ms Aly Chapman for her technical assistance and photography, Ms Ann Reed for the statistical analysis of the data, and Ms Misty Bailey for editorial comments.

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