Efficacy of chlorine dioxide gas for the destruction of *Syphacia muris* eggs

LORNA CLEVERLEY, CALLUM LOGAN and REBECCA LAWSON

Fera Science Ltd, York, UK

Correspondence: Lorna.Cleverley@fera.co.uk

Introduction

Chlorine dioxide gas is a powerful oxidant with excellent anti-microbial activity against a variety of micro-organisms including viruses, bacteria and parasites.[1] It is considered an environmentally friendly safe disinfectant that could be used to kill pinworms eggs. The pinworm nematodes, *Syphacia muris*, (Figure 1) are commonly encountered in bioresearch facilities infecting laboratory rodents.

The availability of disinfectants to successfully eradicate pinworm eggs from the environment is limited to potent phenolic compounds such as *Neopredisan* and traditional disinfectants such as *Formaldehyde* and *Ethylene oxide*.

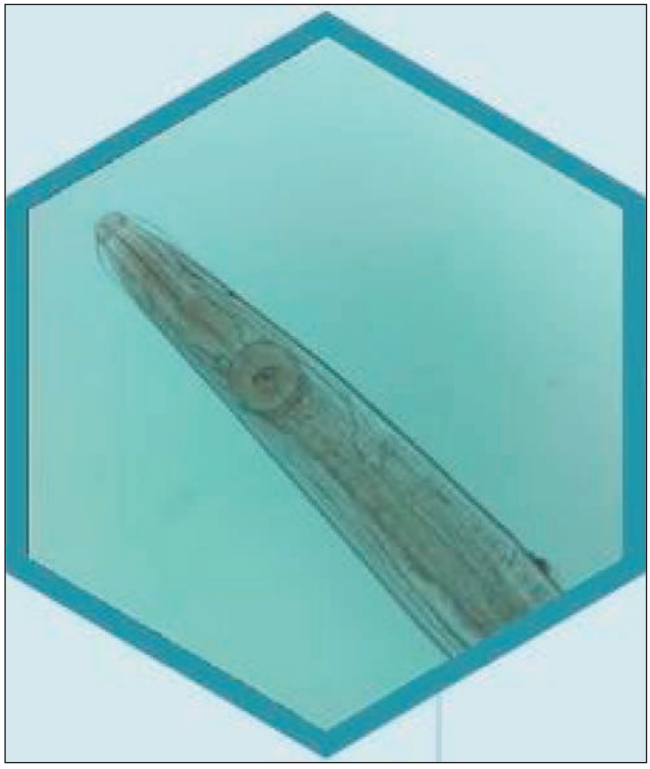

Figure 1. Head region of female *Syphacia muris*.

These disinfectants are limited in their use due to flammability of *Neopredisan* and carcinogenic properties of formaldehyde and ethylene oxide. A publication by Czara, Adams and Carter[2] *et al*. (2014) demonstrated that chlorine dioxide exposure at a concentration of 1mg/L for 4 hours renders pinworm eggs non-viable. We sought to confirm this finding and determine if the exposure time could be reduced by increasing the concentration of chlorine dioxide gas.

Method

Animals

A total of 33 Cax mice (Cambridge cream) of known health status with an established *Syphacia muris* infection were sampled. The animals were group housed as 3 to 4 mice with husbandry procedures carried out in accordance with the Animals (Scientific Procedures) Act 1986 (ASPA).

Collection of eggs

Eggs were collected by sellotape impressions of the anal area. Samples were taken in the afternoon to optimise collection. Each slide was scanned to identify the number of eggs present. The sellotape impressions were dissected at 40x magnification into sections containing 10 viable eggs and placed sticky side up and fixed to slides in the centre of 55mm petri dishes using 10mm acid free craft dots (Figure 2).

Non-viable eggs were identified by the presence of degradation of the lipids inside infective juveniles, this indicated that the nematodes within the eggs were unable to hatch. These eggs were omitted from the tapes. The tapes were allocated as treatment or control replicates.

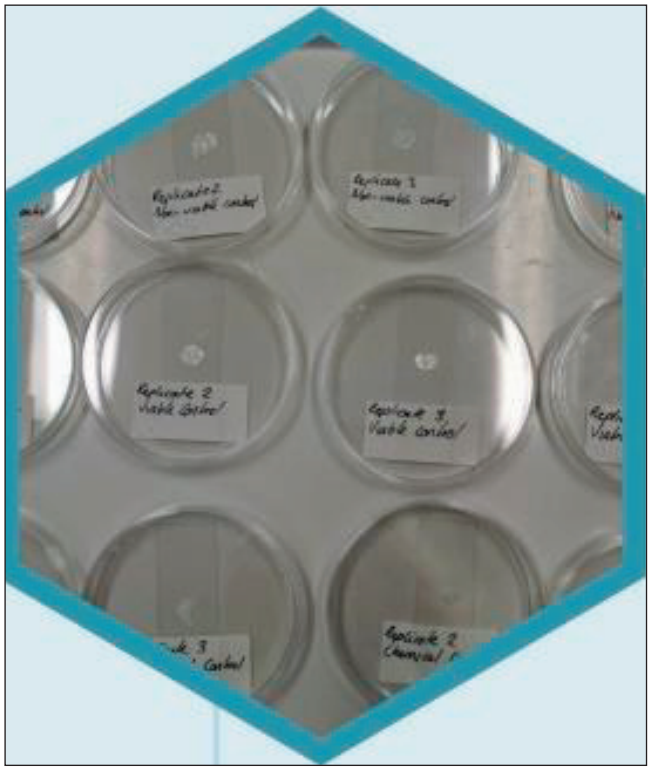

Figure 2. 55mm petri dishes containing *Syphacia muris* eggs.

Chlorine dioxide exposure

Chlorine dioxide gas was generated using a Serosep minidox-M decontamination system into a sealed 62.5 L chamber (Figures 3 and 4).

Figure 3. Minidox-M Chlorine Dioxide management system.

Figure 4. Minidox-M system (courtesy of Castium Ltd).

Two exposure concentrations and times were investigated. Replicates (Figure 2) 1 to 5 were exposed for 4 hours at 1mg/L. Humidity was maintained at 58.5 to 60.1% rH. Temperature was maintained at 20.8 to 23.3°C. Replicates 6 to10 were exposed for 1 hour at 4mg/L, humidity was maintained at 51.8 to 55.8 % rH, temperature was maintained at 24.6 to 25.9°C. The treatment replicate petri-dishes were placed in the following conformation in (Figure 5).

The chamber was monitored throughout the process to maintain the concentration of chlorine dioxide gas. At the end of each exposure period the chamber was aerated with a charcoal scrubber before removing the petri dishes from the chamber.

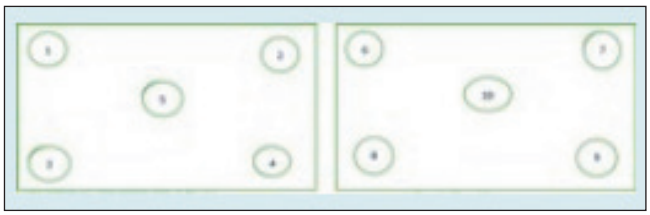

Figure 5. Replicate positions in chlorine dioxide chamber.

Staining

A 0.05% solution of Medola's blue stain in an 0.85% saline solution was added to the treatment and control petri dishes and incubated for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature.

Treatment and control tapes were scanned at x60 magnification and the number of viable and non-viable eggs was recorded. Eggs were considered non-viable if they appeared blue (Figure 6) and viable if they did not take up the Medola's blue stain.

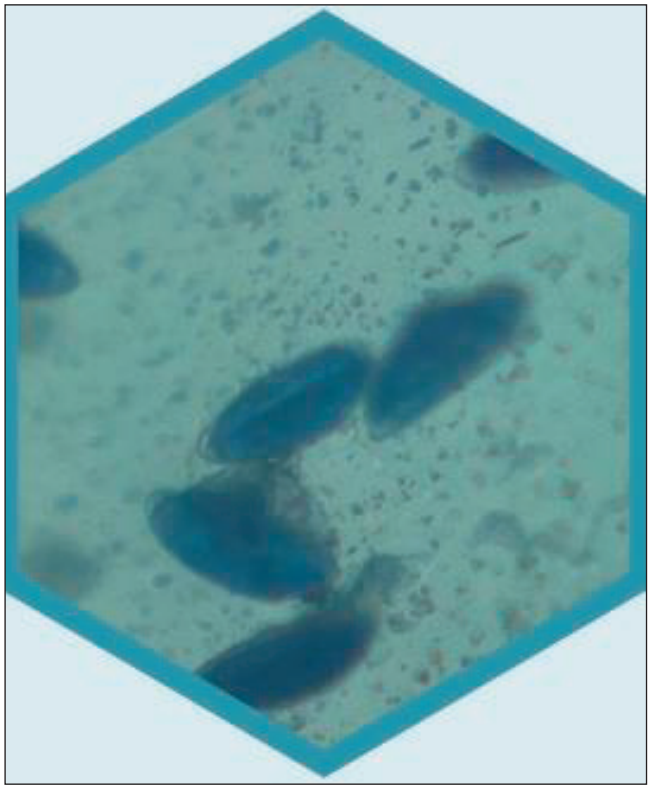

Figure 6. Non-viable eggs stained with Medola's blue (x60 mag).

Hatching

The hatching medium was prepared as described previously by Dix, Astill and Whelan.[3]

Treatment and control eggs were covered in hatching medium and incubated in ambient air at 37°C overnight. After incubation tapes were scanned x60 magnification and the number of hatched and non-hatched eggs recorded. Eggs were considered non-viable if the operculum was intact or the eggs contained larva. Any eggs without larva or those with an open operculum were considered viable (Figures 7 and 8). The data was recorded for each replicate.

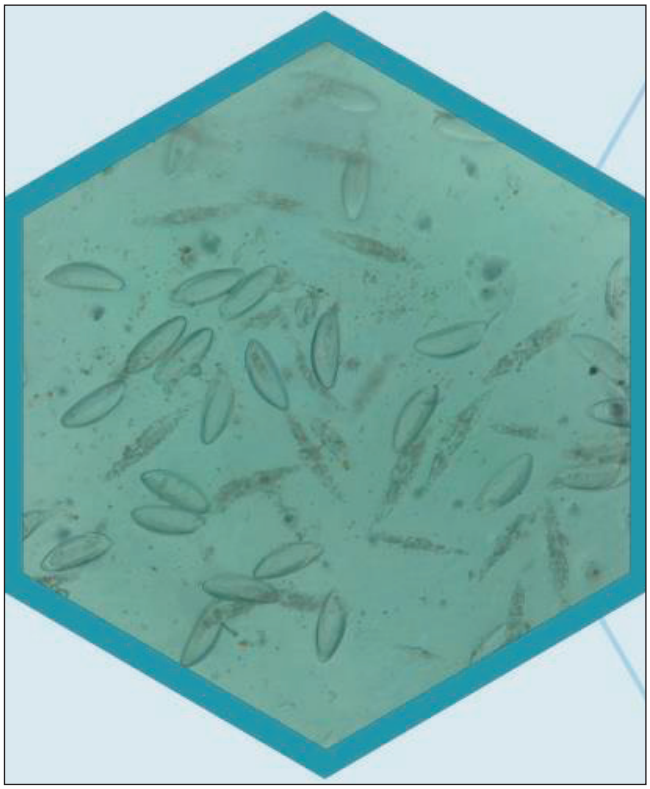

Figure 7. Non-viable eggs showing an open operculum (x60 mag).

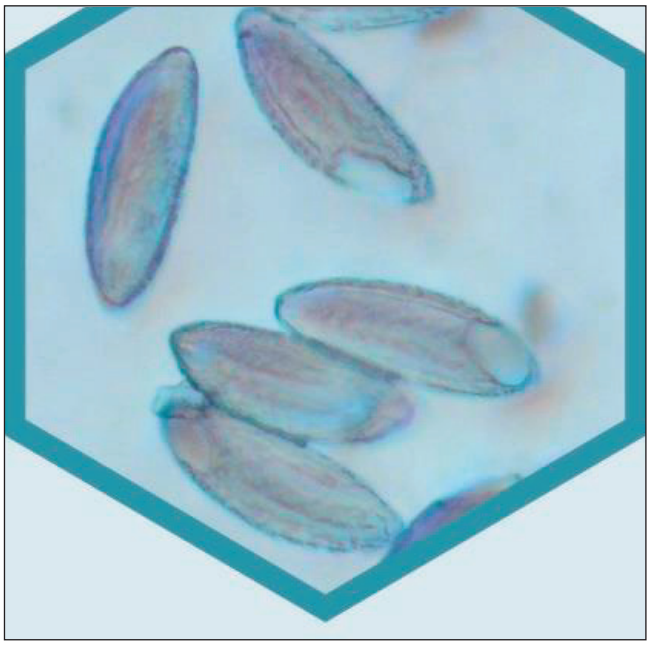

Figure 8. Hatched larvae after 6 hours incubation at 37°C.

Results

Medola's blue viability stain – the number of non-viable eggs

Replicate	1	2	3	4	5	6	7	8	9	10
Test Tapes	10	10	10	10	10	10	10	10	10	10
Control Tapes	0	0	0	0	0	0	0	0	0	0

Number of eggs that hatched

Replicate	1	2	3	4	5	6	7	8	9	10
Test Tapes	0	0	0	0	0	0	0	0	0	0
Control Tapes	10	10	10	10	10	10	10	10	10	10

Summary

Chlorine dioxide gas resulted in 100% kill of *Syphacia muris* eggs at a concentration of 1mg per 4 hours and 4mg per 1 hour under the temperature and humidity conditions described. The current disinfectants used in animal facilities include Neopredisan which is flammable and cannot be used for electrical items. Ethylene oxide is carcinogenic.

The use of Chlorine dioxide could provide a useful tool in our arsenal against highly successful pinworm species particularly as this chemical is considered safe for use.

Acknowledgements

The Chlorine dioxide gas generation in the study and photographs of the Minidox-M system were supplied by Castium Ltd.

References

1 **Jefri, U.H.N.M., Khan, A., Lim Y.C., Lee, K.S., Liew, K.B., Kassab, Y.W., Choo, C.-Y., Al-Worafi, Y.M., Ming, L.C., Kalusalingam, A.** (2022). A systemic review on chlorine dioxide as a disinfectant. Journal of Medicine and Life 15:3; 313-318.

2 **Czarra, J.A., Adams, J.K., Carter, C.L., Hill, W.A., Coan**, **P.N.** (2014). Exposure to Chlorine Dioxide Gas for 4 Hours Renders Syphacia eggs Nonviable. Journal of the American Association for Laboratory Animal Science. 53: 4; 364–367.

3 **Dix, J., Astill, J., Whelan, J.** (2003). Assessment of methods for the destruction of *Syphacia muris* eggs. Laboratory Animals (2004) 38, 11–16.