Investigating Chlorine Dioxide as an Alternative for Inactivating *Cryptosporidium* in Aquatic Venues That Use Stabilized Chlorine

Vince Hill, Ph.D., PE

Dr. Hill is a research environmental engineer in the Waterborne Disease Prevention Branch at the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases. Dr. Hill's work focuses on the development and application of environmental sampling methods, microbial detection methods, and treatment technologies for water and wastewater systems. He is an author of over 50 peer-reviewed journal articles and scientific reports focused on environmental microbiology and engineering. In addition to directing the Waterborne Disease Prevention Branch's Environmental Microbiology Laboratory, Dr. Hill is also Team Lead for the Water, Sanitation, and Hygiene Laboratory Team.

Abstract

Cryptosporidium is an extremely chlorine-resistant pathogen. To effectively inactivate *Cryptosporidium*, the U.S. Centers for Disease Control and Prevention recommends that aquatic venue operators achieve free chlorine concentrations of 20 mg/L for 12.75 hours. Inactivation times might need to be at least 8 times longer when the chlorine stabilizer cyanuric acid (as a stand-alone additive or in the form of "dichlor" or "trichlor") is present in the water. These longer contact times are not feasible for most aquatics venues. Recent laboratory research indicates that chlorine dioxide (ClO_2) is highly effective against *C. parvum* and that the presence of free chlorine might shorten inactivation times. However, it is unclear what effect CYA has on ClO_2 inactivation rates. The aim of this study was to determine if ClO_2 can serve as an effective alternative to hyperchlorination of aquatic venues that utilize stabilized chlorine. The technical objective was to determine the time required to achieve a 3- $\log_{10}$ inactivation of *C. parvum* oocysts at 5 mg/L ClO_2 ; 2 mg/L free chlorine; and 50, 100, or 150 mg/L CYA.

Laboratory studies were conducted under ideal conditions (oxidant-demand- free water [ODF] at 2 mg/L free chlorine, pH 7.5, 25 °C) with CYA added to achieve a target concentration of 50, 100, or 150 mg/L. A concentrated solution of ClO_2 was prepared and added to triplicate experimental flasks to achieve a final ClO_2 concentration of 5 mg/L. A fourth flask was used to measure ClO_2 decay over experimental time periods. Control experiments included flasks containing: 1) 2 mg/L free chlorine and 5 mg/L ClO_2; 2) 20 mg/L free chlorine; and 3) ODF water to measure natural *C. parvum* decay.

Flasks were continuously stirred and samples were removed at select time points for *C. parvum* infectivity testing. Samples were quenched using sodium thiosulfate, concentrated by centrifugation, inoculated onto MDCK mammalian cells and incubated for 48–60 hours at 37 °C under 5% CO_2. A *Cryptosporidium*-specific monoclonal antibody was used to fluorescently label C. parvum living stages before microscopic counting. Images of fluorescing living stages were collected using a digital camera attached to a Zeiss Axiovert microscope at 100X magnification. Zeiss AxioVision and ImageJ software were used to quantify the number of living stages associated with sample and back-calculation provided an estimate of the log inactivation of oocysts over contact time.

A total of six individual experimental flasks were tested for each CYA concentration. At an average of 53 mg/L CYA and 2.4 mg/L free chlorine, dosing to 5 mg/L ClO_2 resulted in a 3- log_{10} inactivation of oocysts in < 3 hours. At an average of 119 mg/L CYA and 2.3 mg/L free chlorine, a 3-log_{10} reduction was achieved in 3 hours. At 186 mg/L CYA and 2.1 mg/L free chlorine, a 2.4-log_{10} reduction was achieved within 5 hours. All control assays indicated that oocysts did not exhibit differences in inactivation or die-off rates that were substantially different than those reported in previously published research reports.

These data indicate that ClO_2 might serve as an effective alternative to hyperchlorination in aquatic venues that utilize stabilized chlorine, even at CYA concentrations that exceed the maximum of 90 mg/L recommended by the U.S. Model Aquatic Health Code. Our previous work indicates that at CYA concentrations of 50–100 mg/L, utilizing hyperchlorination to achieve 3-log_{10} inactivation of *C. parvum* requires >102 hours at 20 mg/L free chlorine concentration. Alternatively, 3-log_{10} *C. parvum* inactivation in the presence of 50–100 mg/L CYA can be achieved within 3 hours at 5 mg/L ClO_2. Use of ClO_2 as an alternative to hyperchlorination, might allow aquatic venues to avoid or reduce the time for disruptive, potentially costly, closures. However, more research is needed before ClO_2 -based *C. parvum* remediation procedures that incorporate occupational and swimmer health and safety considerations are implemented.

National Center for Emerging and Zoonotic Infectious Diseases

Investigating Chlorine Dioxide as an Alternative for Inactivating *Cryptosporidium* in Aquatic Venues That Use Stabilized Chlorine

Vincent Hill, PhD, PE

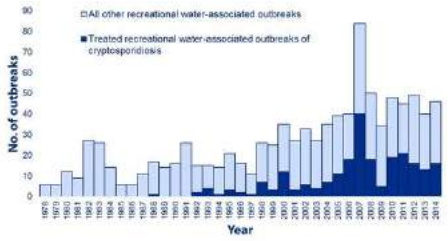
World Aquatic Health Conference
October 19, 2017

Battling Crypto: What's in the cards?

- Characterizing the creature, *Cryptosporidium*
- Establishing the epidemiologic landscape
- Chemical gear for battling pool pathogens
  - Chlorine: A good weapon most of the time
  - Cyanuric acid: Unwitting shield against chlorine attack
  - Chlorine dioxide to the rescue?
- Changing the landscape

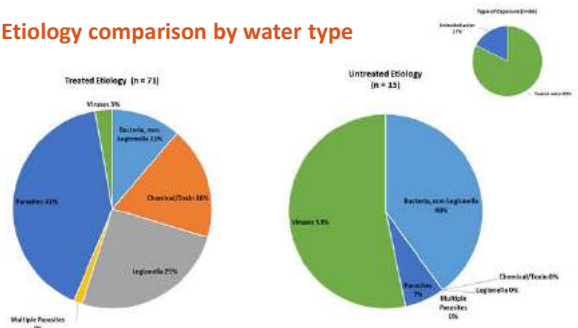
Current card we are dealt when battling *Cryptosporidium*

Outbreaks associated with recreational water, by year — United States, 1978–2014

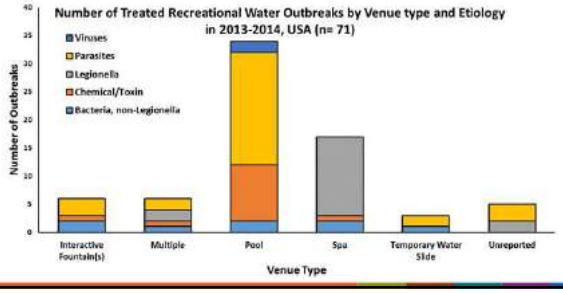


Source: Hlavsa et al (unpublished)

Etiology comparison by water type



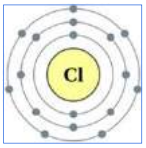
Etiology comparison by venue type



Chlorine: A good weapon most of the time

Chlorine's mode of action against microbes

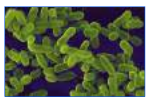
- Oxidation of nucleic acids and enzymes
- Chlorination of amino acids
- Loss of intracellular contents
- Decreased uptake of nutrients and/or oxygen
- Decreased energy production
- Breaks in DNA
- Combination of these factors



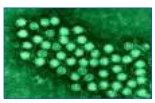
Rutala WA *et al.* 2008. Guideline for Disinfection and Sterilization in Healthcare Facilities, www.cdc.gov/infectioncontrol/guidelines/disinfection

Primary disinfection effective in properly maintained pools

- 1-3 mg/L free chlorine, pH 7.2-7.8
- Chlorine is effective against most enteric microbes



E.coli
99.9% reduction in seconds



Enteroviruses
99.9% reduction in minutes

But what about parasites?

Giardia duodenalis	*Cryptosporidium* (*parvum*)
3-log FC Ct = 40-50 mg/L *	3-log FC Ct = 10,400-15,300 mg/L ‡


CDC


CDC


CDC


http://www.hominis.mic.vcu.edu/

* USEPA. 1999. Disinfection Profiling and Benchmarking Guidance. 815-R-99-013.
‡ Shields, JM et al. 2008. *J Water and Health*, 6:513-520.

Cyanuric acid: Unwitting shield against chlorine attack

Cyanuric acid (CYA)

- Combines with chlorine to form a chlorinated isocyanurate
- Three forms available for pool use:
  1. CYA alone ("conditioner") can be added to a regularly chlorinated pool
  2. Dichloro-isocyanuric acid ("dichlor")
  3. Trichloro-isocyanuric acid ("trichlor")

CYA and chlorine combined into one molecule

CYA chemistry

- Up to 3 chlorine atoms can combine with CYA molecule
  - Form temporary weak bonds
  - Chlorine protected from UV degradation
- CYA/chlorine + H_2O ⇔ HOCl + CYA ion
  - CYA/chlorine: Minimal disinfection power
  - HOCl: Available for disinfection
- HOCl eventually depleted
  - More chlorine must be added
- CYA remains and builds up over time if dichlor or trichlor are used

Effect of CYA on bacteria and viruses

- 3-log_{10} CT inactivation values (0.5 mg/L FC, pH 7.5, 25°C)

S. aureus

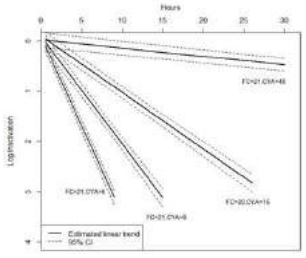
CYA conc (mg/L)	CT (mg-min/L) (SD)	Remediation time (mins)
0	0.5 (0.1)	1.0
10	2.6 (0.1)	5.2
25	3.8 (0.5)	7.6
50	5.2 (1.3)	10.4
100	6.3 (0.7)	12.6

MS2 (virus)

CYA conc (mg/L)	CT (mg-min/L) (SD)	Remediation time (mins)
0	1.3 (0.2)	2.6
10	8.5 (1.4)	17.0
25	13.2 (1.8)	26.4
50	Coming	Coming
100	Coming	Coming

CYA impact on chlorine disinfection

- Log_{10} inactivation (% kill) vs. time for 20 mg/L FAC @ 0-50 mg/L CYA levels
- Significant differences in disinfection rates after 2 hrs
- Source: Murphy JL et al. 2015. *Environ Sci & Technol*, 49:7348-7355.



Cl-CYA chemistry 101 (school of Richard Falk)

- Additional free chlorine (FC) needed at higher CYA levels to maintain equivalent with no CYA
- pH increases with added amounts of hypochlorite source, requiring acid addition to maintain pH 7.5—and target level of 5 mg/L HOCl (in this example for 10,000-gal pool)

CYA (ppm)	Added FC (ppm)	Leads to pH	HOCl (ppm)	Req'd Acid (oz)	Leads to pH	HOCl (ppm)
0	10	7.76	3.51	9.1	7.50	4.94
10	20	8.24	1.87	24.7	7.50	5.30
20	29	8.56	1.20	39.5	7.50	5.19
30	38	8.78	0.94	54.1	7.50	5.11
40	47	8.94	0.79	68.7	7.50	5.02

Impact for hyperchlorination

- CYA is an HOCl buffer and resists changes in HOCl
- When adding hypochlorite sources of chlorine, the pH rises more than it would if CYA is not present
- When CYA level is elevated, it is impractical to try and hyperchlorinate
  - Large amounts of FC needed
  - Also requires large amounts of acid to be added to lower the pH and maintain an effective level of HOCl

Current pool remediation guidance

Standard hyperchlorination applies (e.g., 20 mg/L FC for 12.75 hrs)

If CYA > 15 mg/L, hyperchlorination not feasible. Dump water to get ≤ 15 mg/L, then treat @ 20 mg/L for 28 hrs

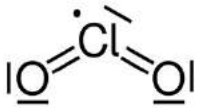
Chlorine dioxide to the rescue?

Chlorine dioxide chemistry

- ClO_2 is a neutral chlorine compound in the +IV oxidation state
- Highly selective oxidant, transfers only one electron
- $ClO_{2(aq)} + e^- \leftrightarrow ClO_2^-$ (chlorite)
  - Chlorite can also react to form chlorate and chloride
  - pKa of chlorite is very low (pH 1.8), so chlorite will exist as the dominant species in treated aquatics water, ~70% chlorite and 30% chlorate and chloride
  - ClO_2 does not react with organics to form trihalomethanes (THMs) or haloacetic acids (HAAs), common DBPs for chlorine treatment
- ClO_2 highly soluble in water, but remains primarily as a dissolved gas
- ClO_2 must be made onsite (using onsite generator or oxidizing tablets)

Chlorine Dioxide's mode of action against microbes

- Modes of action unclear
- Oxidize thiol groups, denature proteins (cell wall & intracellular)
- Disrupts protein synthesis



Chlorine dioxide disinfection efficacy research

- 1940s - 1960s
  - Multiple studies in 1940s and 1950s found ClO_2 more effective than chlorine for killing bacteria at 1-5 mg/L dosages
  - Benarde MA et al (1965, 1967) found that *E. coli* killed at rate of ~99.99% within 30 seconds at 20 °C using 0.75 mg/L ClO_2
  - Disinfection rate slowed by lower temperatures
- ClO_2 comparison with chlorine
  - Not as reactive with organic compounds, ammonia
  - pH has less impact on ClO_2 disinfection, with increased kill of poliovirus (Scarpino et al., 1979) and *N. gruberi* (Chen et al., 1984) as pH increases
  - Hoffman et al (1997): ClO_2 effective against *Giardia* and Crypto

Crypto inactivation times and CT Values for 5 mg/L ClO_2 when no CYA present

ClO_2 Conc. (mg/L)	Avg FC Conc. (mg/L)	1-log Inact. Time (hr)	2-log Inact. Time (hr)	3-log Inact. Time (hr)	3-log CT Value (mg·min/L)
0	21	2.5	5.1	7.6	9555
5	0	1.2	1.7	2.1	640
5	2.6	0.88	1.4	1.8	525

Source: Murphy JL et al. 2014. *Environ Sci & Technol*, 48:5849-5856.

3-log_{10} Crypto inactivation possible within 3-5 hours

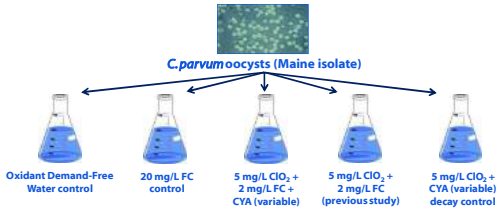
ClO_2 Conc. (mg/L)	Avg FC Conc. (mg/L)	1-log Inact. Time (hr)	2-log Inact. Time (hr)	3-log Inact. Time (hr)	
0	21	2.5	5.1	7.6	←Hyperchlorination
1.4	0	4.8	9.5	14.3	
1.4	3.6	1.6	3.3	4.9	
5	0	1.2	1.7	2.1	
5	2.6	0.9	1.4	1.8	

- Chlorine dioxide treatment was as effective, or more effective, than hyperchlorination

Source: Murphy JL et al. 2014. *Environ Sci & Technol*, 48:5849-5856.

Studying ClO_2 disinfection with CYA & Cl present

- ClO_2 concentrations: 5 mg/L using Aseptrol S-Tab10 tablets (BASF)



Flasks were covered, continuously stirred, and maintained at 25 °C

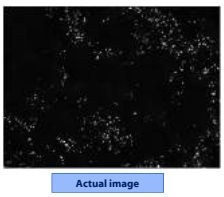
ClO_2-CYA-Cl study procedure and methods

- Measure initial pH (target = 7.5), free chlorine (DPD method), ClO_2 (glycine-DPD method) (Hach), and oxidation-reduction potential (ORP)
- Take samples over time
  1. Measure free chlorine, ClO_2 and ORP
  2. Perform infectivity assay
- Infectivity assay
  1. Neutralize chlorine & ClO_2 and concentrate oocysts
  2. Induce excystation
  3. Apply oocysts to MDCK cells
  4. Incubate at 37°C with CO_2 for 48 - 60 hours

Crypto infectivity data collection

- Process slides
  - Affix monolayers to slides
  - Wash monolayers
  - Fluorescent antibody labeling
- Microscopy and analysis
  - 100X immunofluorescence microscopy and image capture
  - Life stages defined by size, shape, and fluorescence
  - Back-calculate to determine Crypto concentrations and log_{10} inactivation estimates

Automated detection & counting of Crypto life stages

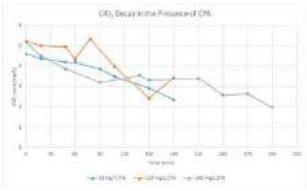


Actual image

Counted image

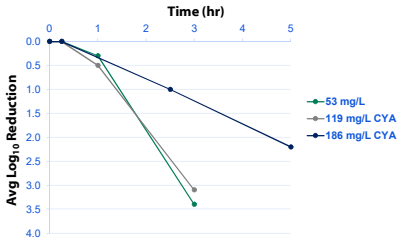
Accounting for disinfectant decay

- ClO_2 decayed with time in each experiment
  - Accounted for in Hom model to evaluate Crypto inactivation kinetics and estimate CT values (disinfectant concentration [C, in mg/L] x time [T, in minutes]



- Free chlorine decay was less substantial (concentrations did not go below 1 mg/L)

Average log_{10} reduction of *C. parvum* oocysts with CYA and ~2 mg/L free chlorine and ~5 mg/L ClO_2



Preliminary CDC ClO_2-CYA-Cl disinfection results *

CYA (mg/L)	ClO_2 (mg/L)	Free Chlorine (mg/L)	1-log Kill Time (hr)	2-log Kill Time (hr)	3-log Kill Time (hr)
53	4.6	2.4	1.0	1.9	2.8
119	5.2	2.3	1.1	2.2	3.2
186	5.2	2.1	2.2	4.4	~6-7

- * Study performed using same experimental set-up as described by Murphy et al (2014), except used *C. parvum* Maine isolate (not Iowa)
- N = 6 for each condition; pH = 7.3-7.5; ClO_2 decay varied (roughly 0.4-0.6 mg/L per hour), requiring statistical modeling

Changing the landscape for remediation of CYA-stabilized pools

Conclusions

- CYA generally precludes effective/feasible use of hyperchlorination for Crypto control
  - Pools using stabilized chlorine are in difficult position to remediate suspected *Cryptosporidium* contamination
- Chlorine dioxide was effective in presence of CYA
  - ~5 mg/L achieved 3-log kill of *C. parvum* oocysts when free chlorine present at ~ 2 mg/L and CYA present at 53-190 mg/L

Possible future state (if ClO_2 is approved for the battle)

Next steps

- Complete modeling and estimate CT values, publish study results
- CMAHC: incorporate results into Model Aquatic Health Code Annex
  - Potential for MAHC change request for pool remediation
- Consider safety issues
  - Chemical handling, storage, chemical exposure
  - Drinking water MRDL for ClO_2 is 0.8 mg/L; MCL for chlorite is 1.0 mg/L, but pool chemistry and exposure conditions might make chlorite and chlorate exposure less of an issue than for drinking water systems
- Partner engagement re: potential use conditions, additional data needs
  - USEPA, industry partners, academic partners

Acknowledgments

- Research supported through a grant by National Swimming Pool Foundation to CDC Foundation
- CDC colleagues: Jennifer Murphy, Mike Arrowood, Michele Hlavsa, Marissa Vigar, Bryanna Cikesh, Jon Kennon, Michael Beach
- Richard Falk
- Ellen Meyer, Lonza

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

To infinity and beyond . . . .