

EXPOSURES AND RISKS ASSOCIATED WITH ACTIVITIES AND BEHAVIORS  
IN SWIMMING POOL ENVIRONMENTS

by

Laura Michele Suppes

---

A Dissertation Submitted to Faculty of the

MEL AND ENID ZUCKERMAN COLLEGE OF PUBLIC HEALTH

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

With a Major in Environmental Health Sciences

In the Graduate College

THE UNIVERSITY OF ARIZONA

2013

THE UNIVERSITY OF ARIZONA  
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Laura M. Suppes entitled Exposures and Risks Associated with Activities and Behaviors in Swimming Pool Environments and recommend that it be accepted as fulfilling the dissertation requirement for the degree of Doctor of Philosophy.

\_\_\_\_\_  
Date: 4/1/2013  
Kelly A. Reynolds

\_\_\_\_\_  
Date: 4/1/2013  
Mary Kay O'Rourke

\_\_\_\_\_  
Date: 4/1/2013  
Charles P. Gerba

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College. I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

\_\_\_\_\_  
Date: 4/1/2013  
Dissertation Director: Kelly A. Reynolds

### STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library. Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department of the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Laura M. Suppes

## ACKNOWLEDGEMENTS

Over the past three years I have received support and encouragement from many individuals. Dr. Kelly A. Reynolds has been a mentor, colleague, and friend. Her guidance has made this a thoughtful and rewarding journey. I would like to thank my dissertation and comprehensive exam committee members Mary Kay O'Rourke, Charles Gerba, Sharon Megdal, and Kelly Reynolds for their support over the past two years as I moved from doctoral student to candidate. In addition, Leif Abrell and Robert Canales provided valuable analytical and statistical advice.

I would also like to thank my family and friends for supporting my decision to pursue a PhD. Thank you Kent for your love, patience, and support; Mom and Dad for always encouraging my pursuit of happiness; and Gabe and Britta for always setting the bar high. Thank you Kim, Ellie, Grace, Martina, and Anne as well for your support throughout this process.

## DEDICATION

In loving memory of my grandfather, Dr. Curtis Larson,  
for his inspiration and unwavering love and support.

## TABLE OF CONTENTS

|  |    |
|--|----|
| LIST OF TABLES.....                            | 8  |
| LIST OF FIGURES.....                           | 9  |
| ABSTRACT.....                                  | 10 |
| INTRODUCTION.....                              | 12 |
| 1. Explanation of the Problem.....             | 12 |
| 2. Specific Aims and Hypotheses.....           | 14 |
| 2.1 Specific Aim 1.....                        | 14 |
| 2.2 Specific Aim 2.....                        | 14 |
| 2.3 Specific Aim 3.....                        | 15 |
| 3. Dissertation Format.....                    | 15 |
| 4. Review of the Literature.....               | 16 |
| 4.1 Acute Gastrointestinal Illness.....        | 16 |
| 4.2 Acute Respiratory Illness.....             | 23 |
| 4.3 Skin and Ear-related Infections.....       | 27 |
| 4.4 Pool Operation and Maintenance.....        | 28 |
| 4.5 Pool Water Ingestion and Swimmer Risk..... | 29 |
| PRESENT STUDY.....                             | 34 |
| Methods.....                                   | 35 |
| Results.....                                   | 39 |
| Discussion.....                                | 42 |
| Limitations.....                               | 44 |

TABLE OF CONTENTS - *Continued*

|   |     |
|---|-----|
| Conclusions and Recommendations.....  | 46  |
| REFERENCES.....   | 49  |
| APPENDIX A: VALIDATION AND STANDARDIZATION OF SWIMMING<br>EXPOSURE AND POOL OPERATIONS QUESTIONNAIRES ..... | 55  |
| APPENDIX B: ASSESSMENT OF SWIMMER HEAD SUBMERSION INFLUENCE<br>ON POOL WATER INGESTION .....                | 89  |
| APPENDIX C: <i>CRYPTOSPORIDIUM</i> INFECTION RISK IN SWIMMING POOL<br>RECREATIONAL SWIMMERS.....            | 118 |

## LIST OF TABLES

|  |    |
|--|----|
| Table 1: Reported outbreaks in treated recreational water associated with Accidental Fecal Releases, 1995–2009.....        | 20 |
| Table 2: Trichloramine range and average concentrations measured in indoor swimming pool air.....                          | 25 |
| Table 3: Recreational Waterborne Illness outbreaks associated with improper pool operation and maintenance, 2000-2006..... | 29 |

LIST OF FIGURES

Figure 1: Questionnaire, urinalysis, videography, and environmental sensor participation  
flowchart.....38

## ABSTRACT

Enteric pathogens in pool water can be unintentionally ingested during swimming, increasing the risk of Acute Gastrointestinal Illness. Swimmer activities and behaviors influence pool water ingestion rates, and can be quantified for use in risk assessment. Enteric infection risk estimates help identify data gaps, areas to focus resources, and research needs. Primary objectives of this study were to develop electronic, self-administered “exposure” and “pool operations” questionnaires; to gather swimmer behavior and activity data for use in risk assessment; and to estimate *Cryptosporidium parvum* infection risk in swimmers. Results were used to identify data gaps and future research needs relative to treated recreational water. To achieve these objectives, 126 swimmers were recruited at four pool sites in Tucson, Arizona, video-taped, and asked to complete a post-swim questionnaire. Forty-six of the 126 swimmers submitted a 24 hr post-swim urine sample for quantifying pool water ingestion. Head submersion frequency and duration and splashes to the face were observed and quantified in video analysis, and activities and behaviors were reported on the exposure questionnaire. Variable data were analyzed for associations with pool water ingestion estimated by urinalysis. Results indicate questionnaires can be self-administered electronically; the exposure questionnaire can be used to estimate ingestion magnitude in place of urinalysis; leisure swimming activities (diving, playing, splashing, wading, sitting) and frequency of face splashes are ingestion exposure factors; and that *Cryptosporidium* infection risk is greatest among leisure swimmers. Other activities observed and suspected of having associations with ingestion were short submersion durations (<1 sec), and spitting and

spouting water. More research and resources focused on improving treated recreational water environments and reducing risks among swimmers are needed. Developing an indicator organism test representative of *Cryptosporidium*, a monitoring program for treated recreational water, education aimed at leisure swimmer, and routine engineering and administrative controls are recommended. Swimming is a unique activity that can be enjoyed by people of all ages and abilities. Controlling hazards in pool environments reduces Recreational Waterborne Illness risks associated with pool water ingestion and improves the health and safety of swimmers.

## INTRODUCTION

### 1. Explanation of the Problem

Exposures to microbial and chemical contaminants in swimming pool water can cause Recreational Waterborne Illness (RWI). Recreational Waterborne Illness in the United States was at an all-time high between 2007 and 2008 (the latest published data) since reporting began in 1978, with 38 states reporting a total of 134 outbreaks (Hlavsa et al., 2011; Yoder et al., 2008). Ingestion was the most common source of exposure (60.4%), but direct skin contact (18.7%), inhalation (13.4%), mixed exposure routes (4.5%), and “other” exposures (3.0%) were also identified. Health outcomes ranged from Acute Gastrointestinal Illness (AGI), respiratory ailments, skin infections and irritations, and hospitalization. Microbial agents included *Cryptosporidium* spp., Norovirus, *Giardia lamblia*, *Leptospira* spp., *Pseudomonas* spp., *Vibrio* spp., *Campylobacter jejuni*, *Legionella* spp., and *Shigella sonnei*, and chemical agents from chlorine gas to disinfection by-products (DBP).

Several trends in RWI outbreaks between 2007 and 2008 were notable: 1) outbreaks (60.4%; 81/134) resulted in AGI; 2) the majority of outbreaks and illnesses (86.6%; 13,480/13,966) occurred in treated water venues; and 3) most were caused by the protozoa, *Cryptosporidium parvum* or *C. hominis* (44.8%; 60/134) (Hlavsa et al., 2011). *Cryptosporidium parvum* is the species associated most often with swimming pool AGI outbreaks. Although inhalation was not the primary exposure associated with RWIs between 2007 and 2008, the number of Acute Respiratory Illness (ARI) outbreaks related to DBPs in air from 2005-2006 increased when chemical-associated outbreaks were first

reported (3 suspected in 2005-2006, and 9 suspected, 3 confirmed in 2007-2008) (Hlavsa et al., 2011; Yoder et al., 2008).

AGI is the most common RWI, and affects swimmers when exposures occur via the fecal oral route. Investigations following AGI outbreaks in pools suggest head submersion and grater water ingestion during swimming are risk factors of AGI (Boehmer et al., 2009; Causer et al., 2006). However, no quantitative, observational information exists to support this hypothesis.

One study successfully assessed the volume of pool water ingested by swimmers during a 45 min observation period applying urinalysis (Dufour et al., 2006). Dufour et al. compared child ( $\leq 18$ ) and adult ( $> 18$ ) ingestion rates and found children are more likely to swallow pool water. Schets et al. (2011) through a questionnaire found children ( $\leq 15$ ) ingest more than adults ( $> 15$ ). Although both research groups found children ingest more water than adults while swimming, neither group observed behaviors and activities of children or adults leading to ingestion.

Quantitative exposure data on specific swimming behaviors and activities associated with ingestion are useful for risk assessment and risk comparison. Evaluating *Cryptosporidium parvum* infection risk from swimming in pool water provides a useful reference value for identifying vulnerable populations and activities, and research needs. Further, identifying sub-populations with elevated risks can focus healthy swimming education campaigns by targeting specific activities and behaviors. Currently, state and local health departments lack standardized swimming pool safety regulations since there is no federal pool code in the U.S. No infection risk limits exist for pool water, but fresh

and marine recreational water have limits (8 and 19/1,000 swimmers/year, respectively). Risk estimates can be useful for identifying future research needs related to treated recreational water quality.

## **2. Specific Aims and Hypotheses**

Three aims and hypotheses are the focus of this dissertation and represent three separate manuscripts appended to this document:

**2.1 Specific Aim 1:** Create comprehensive, electronic, self-administered exposure and pool operation questionnaires and test for reliability of self-reported pool water ingestion assessment (Appendix A). To achieve this aim, “exposure” and “operations” questionnaires were developed electronically and administered to 126 swimmers and four pool sites. Forty-six of 126 swimmers submitted a urine sample used to measure pool water ingestion. Responses from the exposure questionnaire were analyzed for associations with measured ingestion. It is hypothesized the magnitude of pool water ingestion will not differ between measured and reported amounts, and that young age and boisterous activities, like diving, will be considered risk factors of ingestion.

**2.2 Specific Aim 2:** The second aim was to assess demographic, activity, and behavioral factors associated with increased pool water ingestion through questionnaires and observation and gather exposure factor data useful for risk assessment (Appendices A and B). To achieve this aim, activities, behavioral factors, and demographics suspected of

increasing pool water ingestion were quantified by videography and questionnaire and analyzed for associations with measured ingestion rates. It is hypothesized that pool water ingestion is positively associated with head submersion frequency and duration, as well as frequency of receiving a splash to the face.

**2.3 Specific Aim 3:** Assess the risk of developing *Cryptosporidium parvum* infection in different age and activity groups from swimming in treated recreational water (Appendix C). To achieve this aim, demographic and activity data collected by the questionnaire described in Appendix A, measured ingestion rates described in Appendix B, and *Cryptosporidium* prevalence and concentrations in swimming pool water described in a separate study were applied in a Quantitative Microbial Risk Assessment model. It is hypothesized that children and swimmers reporting leisure activities will have higher annual risks of infection than the adult and lap swimming comparison groups.

### **3. Dissertation Format**

This dissertation contains three manuscripts ready for publication submission. Appendix A contains the manuscript “Validation and Standardization of Swimming Exposure and Pool Operations Questionnaires,” which evaluates the validity of a recreational swimming exposure questionnaire. This manuscript will be submitted to Water Research. Appendix B contains “Assessment of Swimmer Head Submersion Influence on Pool Water Ingestion,” which will be submitted to the Journal of Water and

Health. This manuscript assesses swimmer behaviors and activities associated with pool water ingestion. The manuscript in Appendix C will be submitted to Water Research, and is titled “*Cryptosporidium* Infection Risk in Swimming Pool Recreational Swimmers.” This manuscript evaluates the risk of *Cryptosporidium parvum* infection from swimming in treated recreational water venues.

I am responsible for data collection, analysis, and writing of the manuscripts in all appendices.

#### **4. Review of the Literature**

##### **4.1 Acute Gastrointestinal Illness**

In 2007 and 2008, 116 waterborne outbreaks were linked with treated water venues. Eighty-one of the 116 outbreaks were associated with AGI, accounting for 12,411 illnesses (Hlavsa et al., 2011). Acute Gastrointestinal Illness is spread by the fecal-oral route, and symptoms include: diarrhea, abdominal cramps, vomiting and nausea (Dupont et al., 1995). Acute Gastrointestinal Illness can be life threatening in sensitive populations, which represent 20-25% of the U.S. population (HIV/AIDS patients, people greater than 65 and less than 5 years old, people with diabetes, cancer and organ transplants, and pregnant women especially) (Chen et al., 2002; Dupont et al., 1995; Okhuysen et al., 1998; Reynolds et al., 2008). Bacteria were associated with 4 of the 81 AGI outbreaks, predominantly from *Shigella sonnei* and *Escherichia coli* O157:H7 (Hlavsa et al., 2011). The majority of AGI outbreaks (58/81, 71.6%) were associated with *Cryptosporidium parvum* and *C. hominis*. *Cryptosporidium spp.* are waterborne, oocyst-

forming protozoa spread by the fecal oral route that is highly resistant to chlorine, and can cause gastroenteritis in healthy populations and more severe health outcomes, including cholera-like diarrhea, cholangitis, pancreatitis, and death, in immunocompromised populations (Dupont et al., 1995; Okhuysen et al., 1998). The infectious dose of *Cryptosporidium parvum* (referred to as *Cryptosporidium* throughout the remaining text) in healthy, immunocompetent adults is 10-30 oocysts (Okhuysen et al., 1998) and as low as one oocyst in the immunocompromised (Eisenberg et al., 1998). The incubation period of *Cryptosporidium* is 7-10 days (Chen et al., 2002; Okhuysen et al., 1998). Symptoms of watery diarrhea (an average of eight per day) may occur and last 2-4 days in healthy people (Chen et al., 2002; Okhuysen et al., 1998). Water ingestion is the most common route of *Cryptosporidium* exposure (Yoder & Beach, 2010). Oocysts cause disease by attaching to intestinal walls, which prevents nutrient absorption (Chen et al., 2002). One hundred forty-three waterborne outbreaks of cryptosporidiosis occurred between 1984 and 2007 (Yoder & Beach, 2010). Of the 143 outbreaks, 128 are associated with treated recreational water venues. Fifteen were associated with drinking water, and 14 with untreated recreational water. Treated water venues are ideal for cryptosporidiosis outbreaks because swimming is essentially community bathing (Shields et al., 2009; Yoder & Beach, 2010). Bathers can excrete *Cryptosporidium* when ill in amounts between  $10^8$  and  $10^9$  oocysts per fecal release, and low amounts (10-30 oocysts) of oocysts can cause infection in a healthy adult (bathers also shed on average 0.14 grams/swim of fecal matter from the body, which may be a contamination source when swimmers are ill) (Gerba, 2000). Treated water venues are ideal for cryptosporidiosis

outbreaks because oocysts can be released up to 50 days post-diarrhea cessation, and are highly resistant to chlorine disinfection, especially in pools that use cyanuric acid.

Cyanuric acid and elevated pH in pool water (7.2–7.8 compared with 7.0 in tap water) decrease the disinfection efficacy of chlorine, and thus increase the chlorine inactivation time for *Cryptosporidium* (Shields et al., 2008; 2009). Cyanuric acid is a stable chemical reduced in concentration primarily by dilution that is used in outdoor pools to reduce photodegradation rates of chlorine (Andersen, 1965). Cyanuric acid can be found in most pools in concentrations between 50-100 mg/L (Johnston et al., 1999). Currently, the Centers for Disease Control and Prevention (CDC) recommend a 15,300 min contact time (*Ct*) following Accidental Fecal Releases (AFR) as a precautionary measure against *Cryptosporidium*, compared to a 9,600 min *Ct* required for drinking water treatment (Shields et al., 2008) because of cyanuric acid presence and pH differences. Contact time values describe the length of time a disinfectant must contact a pathogen for inactivation to occur. The 15,300 min contact time for *Cryptosporidium* in pool water can thus be described as 20 mg/L chlorine contact for 12.75 hrs. Laboratory experiments that control pH and temperature are used to determine pathogen *Ct* values. The 15,300 and 9,600 *Ct* values are based on studies of *Cryptosporidium* in pH conditions similar to pool water (7.5) and drinking water (7.0), respectively. Treatment of pool water after a loose AFR requires 12.75 hours of a 20 mg/L free chlorine to reduce oocysts by 3 logs (Chappell et al., 1996; Shields et al., 2008), and most state pool codes require a chlorine residual between 1 and 5 mg/L (Johnston et al., 1999). Five mg/L would not inactivate *Cryptosporidium* for over two days (51 hours) in the presence of 50

mg/L cyanuric acid. Recent research shows that a 67,200 *Ct* at a lower pH (6.5) and in the presence of 50 mg/L cyanuric acid is necessary to achieve a 3 log reduction of oocysts (Shields et al., 2009).

To reduce fecal contamination, pool users experiencing diarrhea are restricted from swimming at facilities that enforce rules; however, these rules are often unknown or ignored by users. Swimmers with asymptomatic infection may also unknowingly contaminate pools. Between 1995 and 2009, 12 documented outbreaks occurred in swimming pools that originated from AFRs (Table 1). One child contaminated three different Wisconsin pools in 1998 after swimming with a parasitic infection (Barwick et al., 2000). The child had an AFR on three consecutive days in different pools and was later diagnosed with cryptosporidiosis. Pool operators were unaware of the AFRs until the mother reported the incidences two weeks later. Other cryptosporidiosis outbreaks occurred in Florida (three in one year), Ohio, and Nebraska in 2000 after AFRs occurred in swimming pools (Lee et al., 2002). Nine hundred twenty-five combined illnesses resulted from the Nebraska and Ohio outbreaks. In 1995, 5,449 people contracted cryptosporidiosis following an AFR in a Georgia community pool (Lee et al., 2002). An outbreak of *E. coli* 0157:H7 in a different Georgia swimming pool caused 26 illnesses and one death after an AFR in 1998 (Lee et al., 2002). A state-wide outbreak of cryptosporidiosis suspected to have originated from a swimming pool caused 1,902 illnesses in Utah in 2007 (Rolfs et al., 2008). *Cryptosporidium* contamination from an AFR caused 12 illnesses of 17 exposures in a 2009 Colorado outbreak (Boehmer et al., 2009). *Cryptosporidium* ingestion exposures occurred despite adequate chlorine

concentrations and Ultraviolet light (UV) disinfection, a secondary prevention measure against *Cryptosporidium* and other chlorine resistant parasites. UV treatment likely failed to prevent the outbreak because swimmers were exposed to oocysts before pool water was recirculated through the UV system, or because suspended solids interfered with the UV light source. Outbreaks of cryptosporidiosis in pool water associated with suspected AFRs also occurred internationally in Sweden (Insulander et al., 2005) and Australia (Hellard et al., 2000).

Table 1: Reported outbreaks in treated recreational water associated with AFR, 1995-2009.

| Factor  | Agent                  | Location  | Year | Source                  |
|---|------------------------|-----------|------|-------------------------|
| Reported and Suspected Accidental Fecal Release | <i>Cryptosporidium</i> | GA        | 1995 | Lee et al., 2002        |
|   | <i>E. coli</i> 0157:H7 | GA        | 1998 | Lee et al., 2002        |
|   | <i>Cryptosporidium</i> | WI        | 1998 | Barwick et al., 2000    |
|   | <i>Cryptosporidium</i> | FL*       | 2000 | Lee et al., 2002        |
|   | <i>Cryptosporidium</i> | OH        | 2000 | Lee et al., 2002        |
|   | <i>Cryptosporidium</i> | NE        | 2000 | Lee et al., 2002        |
|   | <i>Cryptosporidium</i> | CO        | 2009 | Boehmer et al., 2009    |
|   | <i>Cryptosporidium</i> | Sweden    | 2002 | Insulander et al., 2005 |
|   | <i>Cryptosporidium</i> | Australia | 1998 | Hellard et al., 2000    |
|   | <i>Cryptosporidium</i> | UT        | 2007 | Rolfs et al., 2008      |

\*Three *Cryptosporidium* outbreaks related to AFRs occurred in Florida in 2000 (Lee et al., 2002).

There is a need for alternative or secondary disinfection in pool facilities to control *Cryptosporidium* because chlorination does not inactivate oocysts at recommended levels. Protozoa can be eliminated or reduced in water by applying heat, filtration, radiation, or chemical treatments (Erickson & Ortega, 2006). Most states require secondary disinfection systems for swimming pools to be certified by a third

party company approved by the American National Standards Institute to reduce *Cryptosporidium* oocysts by 3 logs (National Sanitation Foundation, 2009).

Currently, there is no peer-reviewed research that assesses heat-treating pool water to inactivate *Cryptosporidium*, but applying heat to water can effectively inactivate oocysts (Fayer, 1994). The Thermal Death Time of *Cryptosporidium* is 72.4°C/1 minute (Fayer, 1994). Pool water temperatures would need to be raised uniformly to 72.4°C for 1 minute to inactivate *Cryptosporidium* (typical pool water temperatures are maintained below 40°C) (Johnston et al., 1999), which may be possible in smaller volume spas or wading pools (~8,000 gal), but would be difficult in larger lap pools or waterparks (~200,000 gal) because of time limitations and heater capacities.

*Cryptosporidium* can be physically removed from water by filtration. Common pool filters use sand, diatomaceous earth, perlite, and cartridges (Amburgey et al., 2012). When tested in simulated spa water, researchers found sand and cartridge filters had average *Cryptosporidium* removal efficiencies of 0.19 log; diatomaceous earth 2.3 log; and perlite 4.4 log (Amburgey et al., 2012). In drinking water treatment, Granular Activated Carbon filters achieve a 2.7 log reduction, and reverse osmosis achieves a 3 log reduction of oocysts (Gerba et al., 1997; Hijnen et al., 2010). Although drinking and pool water do not have the same chemical properties (different pH, chlorine content, salt levels, etc.), filtration treatment studies for *Cryptosporidium* removal in drinking water may inform the pool health and safety industry. Other treatment mechanisms are needed in addition to filtration, as most states require pool water to recirculate every six hours

(Johnston et al., 1999). This leaves a six hr exposure window for swimmers following a contamination event.

UV is also used to inactivate *Cryptosporidium* and other waterborne pathogens (Craik et al., 2001). Low and medium pressure lamps inactivate oocysts by 3 logs with 25 mJ/cm<sup>2</sup> and 1 mJ/cm<sup>2</sup> doses, respectively (Craik et al., 2001; Zimmer et al., 2003). Disinfection efficiencies of UV systems are limited since the lamps must be installed on pool piping lines. UV systems thus share the same dependence in water recirculation as filtration. UV is limited by high suspended particle loads in water that interfere with the light source (Loge et al., 1999).

Heat, filtration, and radiation are considered secondary treatment mechanisms because none leave disinfectant residuals in water. Chlorine leaves a residue, and thus is commonly used for primary treatment of pool water. The resistance of *Cryptosporidium* to chlorine has initiated exploration of alternative chemical treatments, such as chlorine dioxide, aluminum sulfate, and ozone, for pools. Chlorine dioxide achieves a 3 log reduction in *Cryptosporidium* oocysts with 54.2 mg/L for 1 min at 30°C (Ruffell et al., 2000). Aluminum sulfate acting as a coagulant can achieve 1.6 log removal of *Cryptosporidium* surrogates (1-7 µm fluorescent polystyrene particles) after sand filtration, compared to a 0.3 log reduction without aluminum sulfate (Croll et al., 2007). Ozone oxidizes and inactivates pathogens quickly when dissolved in water that passes through pool piping in relatively low concentrations (Loeb, 2009). Ozone is considered the most powerful oxidizing and disinfecting agent for water treatment (World Health Organization, 2003), and is not limited by suspended solids (Rennecker et al., 1999). The

*Ct* value to achieve a 3 log reduction in *Cryptosporidium* for ozone is 2.57 min at 30°C (Rennecker et al., 1999), much lower than the *Ct* value of chlorine in pool water (15,300 min). Like filtration and UV, however, water must pass through an ozone treatment system to be disinfected, which limits the use of ozone in pools. The most effective mechanism to control *Cryptosporidium* in pool water may therefore be a multi-barrier approach that applies a combination of primary and secondary treatment techniques.

#### **4.2 Acute Respiratory Illness**

Inhalation exposures in swimming pool environments can result in Acute Respiratory Illness (ARI). Seventeen of the 116 pool-related outbreaks in 2007 and 2008 were associated with ARI (Hlavsa et al., 2010). Pool-related outbreaks occur when common symptoms are reported by >2 people in separate parties who also report visiting the same pool facility within a given timeframe relative to symptom manifestation. Both bacteria and chemicals can cause ARI. Ten of the 17 ARI outbreaks were caused by *Legionella* spp., waterborne pathogens that proliferate in man-made and natural waters maintained between 25 and 43°C (Craun et al., 2010; Leoni et al., 2001). *Legionella* spp. are common in spa and shower water because they survive temperatures up to 60°C (Leoni et al., 2001). *Legionella* spp. can grow in pool-facility piping, and can be aerosolized by spa jets and showers used for pre- and post-swim rinsing. In a study that surveyed *Legionella* spp. in spa and shower water from the same source, 2 of 48 (4.2%) pool water samples, and 27 of 48 (56.3%) shower samples were positive for *Legionella*

spp. (Leoni et al., 2001). *Legionella* spp. exposures in pool facilities may therefore occur during pre- or post-swim showering more often than swimming.

Seven additional ARI outbreaks were caused or suspected to be caused by chemicals between 2007 and 2008 (Hlavsa et al., 2011). Tri-chloramine ( $\text{NCl}_3$ ) is a disinfection by-product (DBP) suspected in 3 of the 17 ARI outbreaks (drinking water treatment applies mono-chloramine as a disinfectant, which has not been associated with ARI). Formation of tri-chloramine and other DBPs occurs when organic matter, naturally or artificially present in water, reacts with chlorine (Bull et al., 1995; Weng et al., 2011). Tri-chloramine is a mucous membrane irritants associated with ARI symptoms, including: cough, wheeze, shortness-of-breath, chest tightness, sore throat, and nose irritation (Hery et al. 1995; Dang et al., 2010). More researchers are focusing on acute health effects from DBPs due to recent ARI outbreaks where tri-chloramine was suspected as the causative agent.

A Lowest Observed Adverse Effect Level (LOAEL) for tri-chloramine in air of  $0.355 \text{ mg/m}^3$  has been proposed, but concentrations as low as  $0.1 \text{ mg/m}^3$  have been associated with acute respiratory and ocular symptoms (Bonvallet et al., 2010). The LOAEL was determined by Bonvallet et al. using existing literature and data from animal and human research studies that assessed frequent exposures over a subject's lifetime that lasted several hours. Tri-chloramine measured in air at indoor swimming pools range from  $0.1\text{-}1.34 \text{ mg/m}^3$  (Table 2) (Bessonneau et al., 2011; Carbonnelle et al., 2008; Carbonnelle et al., 2002; Hery et al., 1995; Jacobs et al., 2007; Massin et al., 1998; Thickett et al., 2002; Weng et al., 2011). Manifestation of ARI symptoms were observed in study

subjects following exposure to 0.1 mg/m<sup>3</sup> tri-chloramine for 2,880 min and 1.34 mg/m<sup>3</sup> for 120 min. All four studies found a statistically significant relationship between reported ARI symptoms in adult volunteers following at least 30 min of exposure.

Table 2: Tri-chloramine range and average concentrations measured in indoor swimming pool air.

| <b>NCL<sub>3</sub> in Air (mg/m<sup>3</sup>)</b> | <b>Observed Exposure Period</b> | <b>Acute Respiratory Symptoms</b> | <b>Subject Description</b> | <b>Reference</b>         |
|--|---------------------------------|-----------------------------------|----------------------------|--------------------------|
| 0.9*   | -                               | Not assessed                      | -                          | Bessoneau et al., 2011   |
| 0.48*  | -                               | Not assessed                      | -                          | Carbonnelle et al., 2002 |
| 0.16-0.28  | -                               | Not assessed                      | -                          | Carbonnelle et al., 2008 |
| 0.15-0.87  | 30–180 min                      | Yes                               | Pool Employees             | Hery et al., 1995        |
| 0.56-1.34  | 120 min                         | Yes                               | Pool Employees             | Jacobs et al., 2007      |
| 0.14-0.67  | 180-240 min                     | Yes                               | Lifeguards                 | Massin et al., 1998      |
| 0.1-0.57   | 2,880 min                       | Yes                               | Pool Employees             | Thickett et al., 2002    |
| 0.2-0.7  | -                               | Not assessed                      | -                          | Weng et al., 2011        |

\*Average trichloramine concentration in air

Reports of swimming-associated respiratory symptoms have identified the need for improved exposure assessment to determine associations (Weisel et al., 2009). Weisel et al. recommend developing an exposure characterization for aerosolized DBP to estimate inhalation rates of swimmers, and collecting data on maintenance to determine how poor conditions impact DBP in pool water. Pool-user behaviors that impact water quality before and during swimming should also be studied. It is estimated that swimmers excrete 50 mL urine and 200 mL of sweat during a swim event, which are composed of organic constituents associated with DBP formation (Judd & Bullock, 2003). Pollutants released from bathers (lotions, detergents, skin cells, etc.) can also form DBPs, but are removed within 60 seconds of pre-swim showering (Keuten et al., 2012). Requiring a

pre-swim shower may therefore reduce DBP formation and improve pool facility air and water quality. Future efforts to reduce DBPs in pool water should focus on effective interventions like pre-swim showering.

Exposures to other DBPs associated with chronic illnesses, like Trihalomethanes (THM), are also a research focal point. Less information is known about chronic illness and DBP exposures in pools because time and resource intensive study designs are required to infer relationships. The difficulty of identifying pool-only DBP exposures associated with chronic disease is also a barrier.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, dibromochloromethane) are present in swimming pools ranging from 0.2-250  $\mu\text{g/L}$  (Cammann & Hubner, 1995; Dyck et al., 2011). Ingestion has been the primary exposure route of concern for THMs, as both controlled toxicity tests in rodents and epidemiological studies in humans have shown associations between chloroform exposures and bladder, rectal, and colon cancers (Bull et al., 1995). Researchers in 1981 were among the first to conduct a longitudinal study that showed increased relative risk of bladder cancer in participants exposed to THMs in drinking water (Wilkins & Comstock, 1981). In a later study, populations exposed to 250  $\mu\text{g/L}$  compared with 0.1  $\mu\text{g/L}$  chloroform in drinking water had increased risks of bladder cancer (International Agency for Research on Cancer, 1991). A more recent, retrospective study assessed associations between bladder cancer and shower, drinking water, and swimming pool exposures (Villanueva et al., 2007). Pool-users were 1.5 times more likely to have bladder cancer than non-users. Although the frequency of pool water ingestion is less

than drinking water for most people, those who swim often may be at increased risk of disease because THMs in pools are present at levels associated with cancer development.

#### **4.3 Skin and Ear-related Infections**

Dermal exposures in swimming pool environments can result in skin- and ear-related infections. Twenty-one of the 24 skin-related outbreaks (83.3%) between 2007 and 2008 occurred in treated recreational water (Hlavsa et al., 2011). Four of 21 were confirmed to be caused by *Pseudomonas* spp. resulting in folliculitis. In addition to folliculitis, *Pseudomonas* causes otitis externa (swimmers ear), and is responsible for 79% of ear-related symptoms in swimmers (Hajjartabar, 2004). *Pseudomonas* is one of the most commonly isolated pathogens in pool and spa water because it is naturally present in bathers and source water, and can form biofilms on pool surfaces for protection (Hogan et al., 2004). Human contamination also occurs when *Pseudomonas* bacteria enter pool water through AFRs or fecal shedding (Hogan et al., 2004). *P. aeruginosa* is the *Pseudomonas* species often responsible for ear and skin-related infections in swimmers (Hogan et al., 2004). Recently, 96% of *P. aeruginosa* assayed from pool water samples were found to be multi-drug resistant (Lutz & Lee, 2011). Pool water was positive for *P. aeruginosa* in 21% (23/108) of pools tested by Lutz and Lee, even when chlorine residuals were within acceptable concentration ranges. Swimmers should shower before entering pool or spa water, and biofilms should be routinely removed from pool wall, floor, and skimmer basket surfaces to prevent *Pseudomonas* spp. infections.

#### 4.4 Pool Operation and Maintenance

Improper maintenance, operation, disinfectant concentrations and chemistry contributed to 52% of all outbreaks in treated water venues reported between 1971 and 2000 (Craun et al., 2010). Between 2000 and 2006, eight reported outbreaks have been associated with improper pool operation and maintenance (Table 3). Cloudy water, “poor water quality,” and low chlorine levels were observed in two Florida outbreaks in 2000 (Lee et al., 2002). Low chlorine at a Georgia swimming pool was also recorded prior to an *E. coli* 0157:H7 outbreak in 2000, as well as a *Legionella* sp. outbreak in Illinois in 2005 (Lee et al. 2002; Yoder et al. 2008). The *E. coli* 0157:H7 and *Legionella* outbreaks caused 26 and 43 illnesses, respectively. Improper ventilation, maintenance staff training, bather over-loading (more bathers per square ft. pool water than allowed), and disinfectant concentrations were associated with three outbreaks of ocular and respiratory symptoms in 2006 (Bowen et al., 2007; Dang et al., 2010). Collecting data on maintenance to determine how poor conditions impact microbial proliferation and DBP formation in pool water has been recommended to reduce operation-related outbreaks (Weisel et al., 2009). Active surveillance and a standardized reporting system for both public and residential pools would also improve outbreak and causative agent identification.

Table 3: Recreational Waterborne Illness outbreaks associated with improper pool operation and maintenance, 2000-2006.

| Factor   | Agent                    | Location | Year | Source             |
|--|--------------------------|----------|------|--------------------|
| Disinfectant concentrations and imbalanced chemistry | <i>Cryptosporidium</i>   | FL*      | 2000 | Lee et al., 2002   |
|  | <i>E. coli</i> 0157:H7   | GA       | 2000 | Lee et al., 2002   |
|  | <i>Legionella</i>        | IL       | 2005 | Yoder et al., 2008 |
| Ventilation  | Disinfection by-products | IL       | 2006 | Bowen et al., 2007 |
|  |                          | OH       | 2006 | Dang et al., 2010  |
| Staff training                                       | Disinfection by-products | IL       | 2006 | Bowen et al., 2007 |
| Bather over-loading                                  | Disinfection by-products | IL       | 2006 | Bowen et al., 2007 |

\*Two *Cryptosporidium* outbreaks associated with imbalanced chemistry occurred in Florida in 2000 (Lee et al., 2002)

#### 4.5 Pool Water Ingestion and Swimmer Risk

Pool water ingestion was the primary pathway of exposure associated with RWI between 2007 and 2008 (Hlavsa et al., 2011). Ingestion by swimmers has been estimated with both qualitative and quantitative tools. Using questionnaires to estimate swimming ingestion rates, the World Health Organization (2003) found swimmers swallow 20–50 mL/hr. Reported volumes, however, underestimate ingestion rates measured quantitatively. Pool water ingestion quantified by Dufour et al. (2006) using a chemical tracer method that compared cyanuric acid in urine and pool water showed swimmers ingest between 0 and 154 mL/hr. Cyanuric acid is a chlorine stabilizer added to outdoor pool water, and when ingested, passes through the human body unmetabolized (Allen, et al., 1982; Briggie et al., 1981). Controlled studies show that 98% of cyanuric acid ingested could be recovered in a 24 hr period in urine (Allen et al., 1982). On average, Dufour et al. found child and adult swimmers ingest 49 and 21 mL/hr of pool water, respectively, suggesting younger swimmers have a higher risk of developing AGI. Child

activities and behaviors suspected to increase pool water ingestion, however, were not observed or quantified by Dufour et al.

The majority of AGI outbreaks in swimming pools (58 of 81) between 2007 and 2008 were associated with *Cryptosporidium* (Hlavsa et al., 2011). In 2009, cryptosporidiosis incidence was higher in children 1-4 (5.6/100,000) than in adults (2.5/100,000) (Centers for Disease Control and Prevention [CDC], 2011; Yoder & Beach, 2010). Young children may have higher incidence rates because their immune systems are underdeveloped and they ingest more pool water than adults (Dufour et al., 2006; Teunis et al., 2002). Infection incidence may also be higher because children participate in swimming activities that lead to pool water ingestion; however, relationships between these variables have not been evaluated previously.

*Cryptosporidium* is the primary cause of AGI outbreaks in pools because oocysts resist chlorine disinfection, are transmitted fecal-orally, are excreted by ill swimmers in high amounts ( $10^8$  to  $10^9$  oocysts/fecal release), and have a low infectious dose (Dufour et al., 2006; Teunis et al., 2002; Yoder & Beach, 2010). The resistance of oocysts to chlorine makes *Cryptosporidium* the ideal reference pathogen for assessing AGI risk from swimming in treated recreational water venues. Currently, there is no federal pool code in the U.S. The CDC are developing a Model Aquatic Health Code (MAHC) in an effort to standardize pool codes nationwide, but the MAHC is not enforceable. The MAHC serves as a model for state or local health departments needing to update pool codes, and for departments interested in data-driven, risk-reducing pool inspection programs (CDC, 2013). Residential pools are not associated with the MAHC, as pool

codes are enforceable in public venues only. A lack of federal oversight compromises the safety of swimmers because water treatment and monitoring regulations are not standard across jurisdictions. The United States Environmental Protection Agency (USEPA) is the federal agency that oversees the enforcement of policies related to water quality in the U.S. for untreated recreational water (Dufour, 1984). The acceptable risk of enteric pathogen infection from swimming in untreated fresh water is 8/1,000 swimmers/year and 19/1,000 swimmers/year in marine water. (United States Environmental Protection Agency [USEPA], 1986). No acceptable risk has been established by the U.S. government for swimming in treated recreational water venues. Risk estimates can be useful for identifying future research needs related to pool water treatment and monitoring.

Quantifying the risk of RWI caused by biological and chemical hazards in pools requires identification of exposures associated with disease, like water ingestion. While epidemiological studies have been employed to examine how pool activities relate to ingestion and thus illness, these studies are often criticized for not comprehensively assessing the dynamic factors related to pool water ingestion. Proper activity, behavior, and pool water ingestion collection tools are essential to accurately estimate the health effects of swimming, and assure the development of appropriate controls, policies, and regulations. Tools that quantify behaviors and activities suspected to cause water ingestion are needed. Quantitative data on activities and behaviors associated with pool water ingestion are useful for estimating and comparing infection risks between swimmer age and activity groups.

Risk assessment involves four steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization (Baram, 1983). Risk assessment is useful for estimating illness probabilities associated with low-dose exposures, which is difficult in epidemiological studies. Hazard identification recognizes agents associated with adverse health effects, and assumptions about causality are based on toxicological and epidemiological studies. A stronger correlation between exposure and illness increases the likelihood of a causal relationship.

The magnitude and duration of exposures to hazardous agents are measured in exposure assessment. Information about exposure route and magnitude (duration and volume), personal characteristics, agent concentration, and internal dose are necessary for exposure assessment. Human exposure factors, like pool water ingestion per hour, are necessary for assessing risk in a large population when not all individuals can be observed. Quantitative uncertainty analysis using exposure factor distributions improves the reliability of risk estimates by accounting for human variability.

Dose-response assessments describe the relationship between exposure and disease. Pathogens that are non-life threatening in healthy people, like *Cryptosporidium*, can be administered in controlled studies to determine dose-response relationships. Data from *Cryptosporidium* feeding studies show exposures to 10-30 oocysts (n=3) cause gastrointestinal symptoms in adult populations (Dupont et al., 1995; Messner et al., 2001; Okhuysen et al., 1998; Teunis et al., 2002). Feeding studies have not been conducted in children, but outbreak data suggest one oocyst can cause infection in

immunocompromised populations, including children <5 (Chen et al., 2002; Eisenberg et al., 1998).

Risk characterization combines information from the three risk assessment steps to estimate risk severity (Baram, 1983). Risks can be estimated for different exposure scenarios, sub-populations, and activities and compared to determine where public health interventions are needed.

## PRESENT STUDY

The overarching goal of this study was to reduce pool water ingestion and thus Recreational Waterborne Illness (RWI) in swimmers by formulating recommendations for future research and controls that reduce pathogenic contaminants in pool water. This chapter provides an overview of the study design and highlights key findings related to each aim and the dissertation as a whole. Detailed analyses of study aims are presented in appended papers.

The overarching goal was achieved by recruiting a population of swimmers, administering a questionnaire to the study population, video-taping each swimmer, and collecting a biomarker measuring pool water ingestion in a sub-set of participants. Using this broad approach, risk factors of pool water ingestion were identified, along with exposure factors useful for risk assessment (Appendices A and B). Data generated from these papers provided the bases of *Cryptosporidium* infection risk assessment in swimming sub-populations (Appendix C).

Three specific aims were developed to formulate recommendations for future research and controls that reduce pathogenic contaminants in pool water. The first aim was to create a comprehensive, electronic, self-administered questionnaire and test for reliability of self-reported pool water ingestion assessment (Appendix A). The second aim was to assess demographic, activity, and behavioral factors associated with increased pool water ingestion through questionnaires and observation and gather exposure factor data useful for risk assessment (Appendices A and B). The third aim was to calculate the

risk of developing *Cryptosporidium* infection in different demographic and activity groups from swimming in treated recreational water (Appendix C).

## **Methods**

To address the specific aims, a study population of 126 swimmers at four pool sites was recruited and consented to participate using incentives and forms. All swimmers (n=126) were administered a post-swim activity and behavior questionnaire, video-taped (videography), and instructed to wear an environmental sensor headband prior to swimming. Swimmers were video-taped and instructed to wear environmental sensors for quantifying head submersion frequency and duration. Forty-six of 126 swimmers submitted a urine sample to evaluate pool water ingestion (urinalysis). Figure 1 illustrates the study population's participation in each data gathering step, when exclusions occurred, and the papers (appendices) developed to achieve the specific aims.

### *Questionnaire*

One hundred twenty-six swimmers were recruited and consented to participate at four pool sites. Participants were given the option of completing an electronic questionnaire immediately after swimming by tablet, or after returning home by accessing the questionnaire through email. Questionnaire responses were stored with restricted access in the DatStat Illume secure online database. Responses on age, gender, deep-end swimming, diving, playing, splashing, standing, walking, wading, lap swimming, length of swim, number of pool visits/yr, and time spent at any pool visit (independent

variables) were analyzed for associations with measured pool water ingestion to determine risk factors of ingestion. Independent variables were considered ingestion risk factors if positively associated with measured pool water ingestion.

Data gathered on length of swim, number of pool visits/yr, and time spent swimming at any pool visit were separated by age (child:  $\leq 18$ , and adult:  $>18$ ) and activity group (lap and leisure swimmers) for use in risk factor identification by age and activity, and for use in risk assessment. Leisure swimmers were defined as anyone reporting diving, playing, splashing, standing, walking, and wading. Lap swimmers reported lap swimming for exercise.

Reported pool water swallowing was also analyzed for a relationship with measured ingestion. Average mouthful volume measured in previous research was used to categorize measured ingestion data into “mouthfuls/swim.” The question asking swimmers how much pool water was swallowed was considered reliable if responses from swimmers did not differ from measured ingestion values categorized to match reported volumes ( $n=38$ ).

#### *Urinalysis and Pool Water Ingestion*

The 46 swimmers submitting urine were provided a sterile 3 L urine collection container for a 24 hr post-swimming composite sample. Pool water samples were also collected. Both urine and pool water were preserved within 24 hrs of collection and analyzed within 31 days following methods by Cantu et al. (2001a; 2000) and Smoker and Krynitsky (2008). Pool water ingestion measured by urinalysis was determined using a model developed by Briggles et al. (1981) and Allen et al. (1982) (Eq. 1). Ingestion data

were separated by age (child and adult) and activity group (lap and leisure swimmers) for use in risk factor identification by age and activity, and for use in risk assessment.

$$\text{Eq. 1: Water Ingestion (L)} = \left( [\text{Cyanuric Acid}]_{\text{urine}} \left( \frac{\mu\text{g}}{\text{L}} \right) \div [\text{Cyanuric Acid}]_{\text{pool water}} \left( \frac{\mu\text{g}}{\text{L}} \right) \right) \times \text{Urine Volume (L)}$$

### *Videography and Environmental Sensors*

Head submersion durations and frequencies, and frequencies of splashes to the face were quantified using videography. Two digital cameras with tripods were placed in different locations on pool decks to ensure each swimmer was filmed. Water entry times of participants on the two videos were recorded. Each swimmer was observed for the entire length of the swim on one video-tape in 10 min consecutive segments. Reviewing in 10 min segments was applied to reduce viewer fatigue (Ferguson et al., 2006).

Head submersion durations and frequencies were quantified using environmental sensors. The sensors were developed by attaching temperature-logging i-Button thermometers to the light mounting bracket on modified elastic headbands. Temperatures recorded by environmental sensors were uploaded using One-wire Viewer Software for analysis. Measurements lower than the air temperature by 0.5°C were considered a head submersion event.

Head submersion durations and frequencies (videography and environmental sensors), and frequencies of splashes to the face (videography) were analyzed for associations with ingestion in different age (child and adult) and activity groups (lap and leisure).

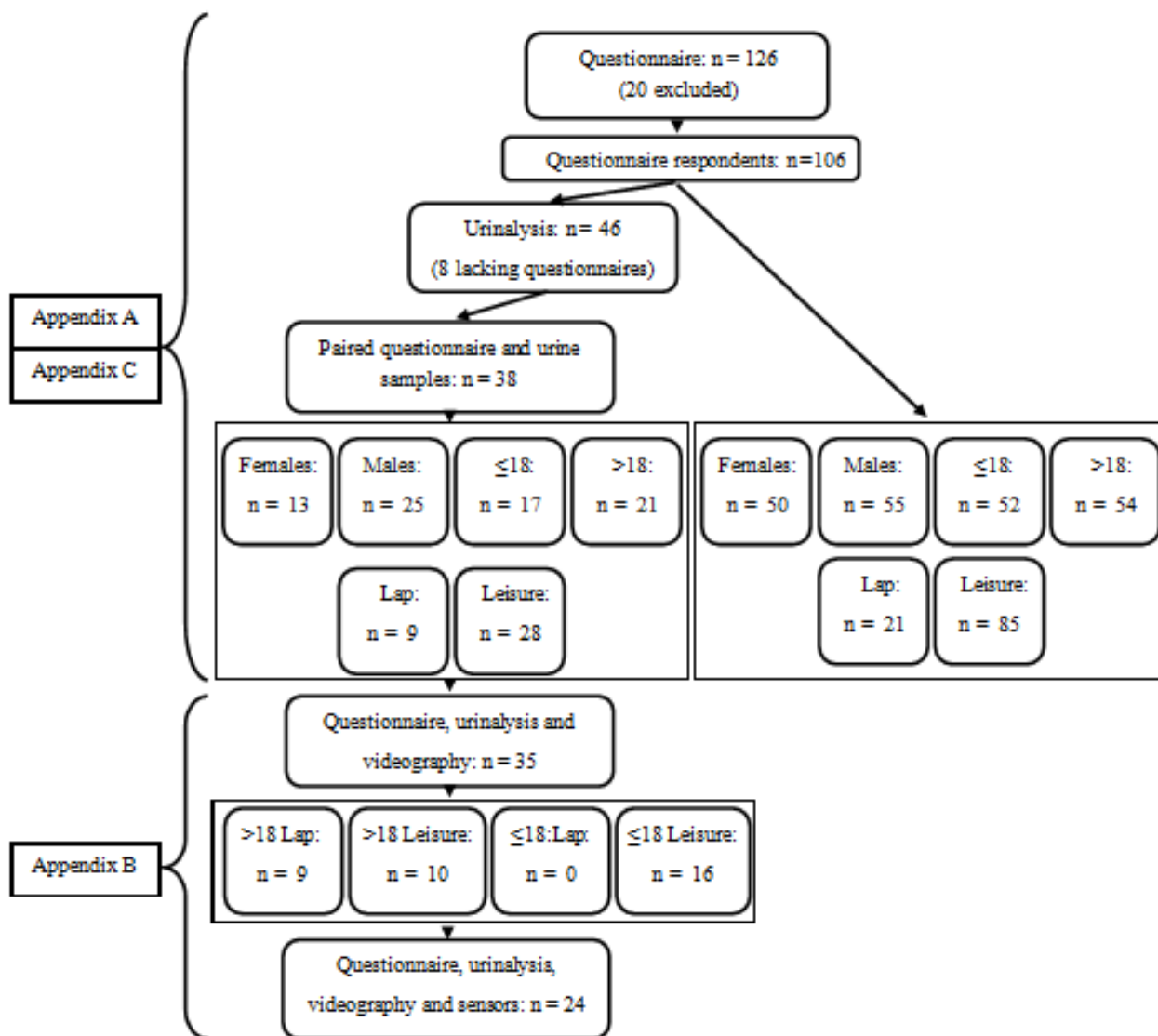


Figure 1: Questionnaire, urinalysis, videography, and environmental sensor participation flowchart.

### *Risk Assessment*

Measured pool water ingestion, pool visit frequency throughout the year, and visit duration data were used as beta poisson model variables to estimate annual and per-swim risks of *Cryptosporidium* infection in child, adult, lap, and leisure swimmers with R statistical analysis software. The literature was searched to obtain information needed in the model. Data on *Cryptosporidium* concentrations from 152 backwash samples collected in the Netherlands at seven pool sites were used as *Cryptosporidium* concentrations in pool water (Schets et al., 2004). Monte Carlo data simulation (10,000 iterations) results for each variable were multiplied to create a distribution of oocysts ingested per-swim, used to estimate per-swim risks (Haas et al., 1983; Teunis et al., 2002). The distribution of annual pool visit frequency was incorporated to estimate annual risks (Regli et al., 1991). Spearman's correlation coefficient was used to assess which model variables contributed most to risk of infection per-swim.

### **Results**

One hundred and six participants of 126 completed the questionnaire, and 38 of 46 collected urine samples could be matched with questionnaire responses; eight urine samples were submitted by swimmers who did not complete a questionnaire (Figure 1). Sixty-four swimmers were recorded on video-tapes, and 35 of 64 had measured pool water ingestion values from urinalysis. Twenty-four participants with measured ingestion had analyzable environmental sensor data. Fifty-two children ( $\leq 18$ ), 54 adults ( $>18$ ), 31

lap swimmers, and 85 leisure swimmers completed the questionnaire. Seventeen children ( $\leq 18$ ), 54 adults ( $> 18$ ), 9 lap swimmers, and 28 leisure swimmers submitted urine samples and questionnaires.

#### *Questionnaire Addressing Pool Water Ingestion and Swimmer Activity (Specific Aim 1)*

Specific aim 1 facilitated achievement of the overarching goal by developing a tool that assesses pool water ingestion magnitude. The questionnaire included 104 questions related to participant gender, age, perceived pool water ingestion, pool-use history, respiratory and gastrointestinal symptoms, and activity. The perceived ingestion question used to validate the questionnaire asks if no water or only a few drops; 1 to 2 mouthfuls (amount in a shot glass); 3 to 5 mouthfuls (amount in a coffee cup); or 6 to 8 mouthfuls (amount in a soda glass) of pool water were swallowed. Magnitude of reported and measured pool water ingestion did not differ among the 38 swimmers who submitted a urine sample and completed a questionnaire (Chi-2:  $p > 0.878$ ) when compared as mouthful categories.

#### *Identification of Pool Water Ingestion Risk Factors (Specific Aim 2)*

Specific aim 2 facilitated achievement of the overarching goal by identifying risk factors of pool water ingestion in all swimmers, and among age and activity groups, and exposure factor data useful for risk assessment. Of the 38 evaluated, risk factors of ingestion among all swimmers were age  $\leq 18$  and diving. Risk factors among children were leisure swimming activities (diving, playing, splashing, standing, wading) and

receiving a splash to the face. No associations were found using Pearson's correlation test between pool water ingestion volumes and head submersion frequency or duration measured by videography, but, swimmers ingesting more water tended to have fewer head submersions. These participants were primarily leisure swimmers. During videography, observers noted leisure swimmers having short and inconsistent submersions, and lap swimmers consistently and frequently submerging heads during activities like the front crawl. Lap swimmers appeared to hold their breath under water more frequently than leisure swimmers. These behavior differences relate to lap swimmers submerging heads more frequently and for longer durations, but ingesting less pool water. Head submersion and duration measured by environmental sensors showed poor accuracy with videography measurements when compared using a paired t-test. Submersion frequency was under-estimated by the sensors ( $p < 0.000$ ), and duration was over-estimated ( $p < 0.001$ ). Environmental sensor data was therefore not used for identifying ingestion exposure activity factors.

Analysis of exposure factors useful for risk assessment showed average reported frequency of pool visits/year and time spent at the pool facility/visit were 72.7 times/year ( $\pm 66.7$ ) and 1.57 hrs ( $\pm 1.0$ ) respectively. Urinalysis participants ( $n=38$ ) ingested on average 14.3 mL/hr of pool water ( $\pm 22.8$ ); 24.2 mL/hr ( $\pm 29$ ) if 18 or younger; and 6.3 mL/hr ( $\pm 16.6$ ) if older than 18. Unpaired t-tests identified children were more likely to spend more time at pools per visit ( $p < 0.0468$ ), and that frequency of swimming did not differ between children and adults ( $p > 0.6992$ ). Swimmers reporting leisure activities

spent more time swimming per-visit ( $p < 0.0302$ ) and visited pools less frequently than lap swimmers ( $p < 0.0314$ ).

### *Annual and Per-swim Risk Estimates for Cryptosporidium Infection (Specific Aim 3)*

Specific aim 3 facilitated achievement of the overarching goal by determining per-swim and annual risks of *Cryptosporidium* infection in child, adult, lap and leisure swimmers. Average estimated risk of infection per-swim of all swimmers is  $3.7 \times 10^{-4}$  swimmers/swim event. Leisure swimmers have the highest estimated average per-swim risk ( $5.2 \times 10^{-4}$  swimmers/swim event), followed by child and lap swimmers (same risk of  $3.5 \times 10^{-4}$ ), and adults (pg. 132). The most influential variable among all swimmers and groups comparing Spearman's rank correlation coefficients is *Cryptosporidium* oocyst concentration, followed by pool water ingestion. Average annual risk of *Cryptosporidium* infection in all swimmers is  $3.3 \times 10^{-2}$  swimmers/year (33/1,000 swimmers/year), and is highest among leisure swimmers ( $2.6 \times 10^{-1}$  swimmers/year), followed by lap, child, and adult swimmers (pg. 132).

## **Discussion**

This study produced a swimmer activity and behavior assessment questionnaire available electronically for use by outbreak investigators, and validated the questionnaire for assessing pool water ingestion magnitude. The self-administered, electronic questionnaire can reduce costs associated with survey administration, data organization, and statistical analysis that health department incur during outbreaks. A repository for

ingestion risk factor data provides a centralized and electronically accessible location to begin a database. Increasing swimmer sample sizes and expanding the geographic region where data is collected improves the likelihood risk factor differences across broader demographic groups and venues will be recognized. The questionnaire can be used in place of urinalysis methods when ingestion magnitude is a desired outcome, as opposed to measured volumes. Assessing ingestion magnitude by questionnaire facilitates interventions aimed at reducing pool water ingestion, assessing swimmer ingestion where cyanuric acid is not present, and evaluating outbreak investigations when ingestion exposures have already occurred.

This study identified risk factors of ingestion and quantified exposure data needed for risk assessment. Children are engaging in activities other than greater head submersion frequency and duration that lead to pool water ingestion, including leisure swimming (diving, playing, splashing, standing, and wading) activities and receiving a splash to the face. Three unquantified activities suspected to lead to ingestion were “bobbing” at the water surface, spouting water for fun, and intentionally allowing pool water to enter the mouth. “Bobbing” at the water surface is an activity within leisure swimming that leads to pool water ingestion, and should be studied in future exposure assessments, particularly among children.

Last, this study determined per-swim and annual risks of *Cryptosporidium* infection in age and activity groups. Currently, no infection risk guidelines exist for treated recreational water in the U.S. The most appropriate risk limits available for comparing estimates are the USEPA untreated fresh and marine water risks (8/1,000 and 19/1,000

swimmers/year, respectively). Annual risk estimates in the present study in all sub-populations exceeded these values. These findings demonstrate a need for routine, standardized monitoring of *Cryptosporidium* oocysts in pool water across the U.S. and internationally. The sensitivity analysis indicated *Cryptosporidium* concentrations in pool water contributed most to infection risk, suggesting a need for more robust and routine engineering and administrative controls in swimming pools. Pool water ingestion also influences infection risk, which was highest among leisure swimmers, suggesting leisure swimmers should be targeted in healthy swimming education.

### **Limitations**

Six limitations were evident in this study, and are summarized with recommendations to future work.

- Ingestion volumes recorded in the questionnaire are too broad. Estimates limited the accuracy of comparing measured and reported ingestion (1 to 2 mouthfuls = 27 to 54 mL). Improvement of the ingestion question by decreasing volume ranges inquired on the questionnaire is essential to estimate volumes with greater accuracy.
- The study location limits the generalizability of results. Participants in this study swam on average 72.7 times/year compared to 13 times/year in the Netherlands (Schets et al., 2011). Results can be used most accurately for assessing swimming risks in climates similar to Southern Arizona.

- i-Button thermometers were pre-set to read a temperature every 3 seconds, yet submersions lasting < 3 seconds were observed during videography. i-Buttons can be pre-set to collect a temperature every 1 second instead of every 3 to improve accuracy of the environmental sensors.
- No lap swimmers were children in this study. The child age group is most likely to ingest pool water. The observed difference in ingestion volumes between leisure and lap swimmers in this study may therefore be attributable to the lack of child, lap swimming participants. Observing child lap swimmers and measuring their ingestion is recommended for future research to determine associations between skill, age, lap swimming, and water ingestion.
- The assay for cyanuric acid in urine can be improved. Recovery after SPE was low (6%), and could be increased by improving urine cleaning techniques. An inexpensive and rapid SPE method removing less cyanuric acid from urine is needed for UHPLC-MS/MS analysis to improve sensitivity and quantification of low levels of cyanuric acid in urine samples.
- *Cryptosporidium* concentration data were collected in the Netherlands, which may not be representative of U.S. pool water quality. Data on *Cryptosporidium* concentrations in U.S. swimming pools beyond presence/absence, however, are not currently available. Future research should survey *Cryptosporidium* concentrations in U.S. swimming pools.

## **Conclusions and Recommendations**

The overarching goal of this study was to reduce pool water ingestion and thus RWI in swimmers by formulating recommendations for future research and controls that reduce pathogenic contaminants in pool water. Swimming is a unique activity that can be enjoyed by people of all ages and abilities. Maintaining safe swimming pool environments by implementing operational controls and educating swimmers about risk is a critical role of public health professionals. More research and resources focused on improving treated recreational water environments and reducing risks among swimmers are needed. Results of this study demonstrate five important implications for exposure scientists, risk assessors, and government officials. The following is an executive summary of the most important and relevant recommendations based on these implications.

Greater head submersion is not an activity driving ingestion. Frequency of receiving a splash to the face appears to increase the likelihood of ingestion of pool water. Including questions on outbreak surveys about participation in leisure swimming activities and frequency of splashes to the face is recommended. Developing public awareness campaigns that communicate activities to avoid, like splashing other in the face, is recommended to reduce pool water ingestion.

Water ingestion, swim duration, and swim frequency data collected with the questionnaire and urinalysis are useful for assessing infection risk from swimming in different venue types and hazard scenarios. Risk assessment applying dose-response data

on other waterborne pathogens is needed to identify additional research needs related to treated recreational water.

Swimmers reporting leisure activities are the sub-population most at-risk of developing *Cryptosporidium* infection. Development and distribution of healthy swimming education materials by health departments at the beginning of every pool-season (May-September) is recommended. Materials focusing on avoiding swimming for two weeks after experiencing diarrhea, intentional pool water ingestion, and splashing others in the face are recommended. Health departments can develop and provide literature to swim instructors and pool or facility managers for distribution among pool-users and parents of pool-users. Leisure swimmers (children) have the highest risk of infection among all sub-populations, and thus should be targeted by public awareness messages and education campaigns.

Last, Quantitative Microbial Risk Assessment indicates infection risk from swimming in treated recreation water is greater than infection risk in untreated water, and demonstrates a need for improving treated recreational water environments to reduce risks among swimmers. A monitoring program similar to the USEPA Beaches Environmental Assessment and Coastal Health Program for treated recreational water is recommended. To accomplish this, an indicator organism with chlorine resistant-properties similar to *Cryptosporidium* should be identified, monitored in swimming pools, and maintained at or below an accepted level that is established. The risk assessment identified *Cryptosporidium* concentration in pool water as the most influential parameter on infection probability. Applying more robust and routine engineering and

administrative controls, like more frequent shock chlorination of high-use leisure pools, ensuring proper water circulation, and training staff to recognize AFRs, are recommended to health departments. More research on swimming pool fluid dynamics is recommended to improve recirculation mechanisms in newly constructed and existing pools.

Preventing exposures to contaminants in pool water improves swimmer health and safety by reducing RWI incidence. This research identified operational and behavioral recommendations for improving treated recreational water environments and communicating risk to reduce RWI incidence among swimming populations. Future research exploring data gaps related to swimming exposures in treated recreational water venues is needed to improve treated recreational water venue safety and swimmer health.

## REFERENCES

- Allen, L. M., Briggles, T. V., & Pfaffenberger, C. D., 1982. Absorption and excretion of cyanuric acid in long distance swimmers. *Drug Metabolism Reviews*. 13 (3), 499-516.
- Amburgey, J. E., Walsh, K. J., Fielding, R. R., & Arrowood, M. J., 2012. Removal of *Cryptosporidium* and polystyrene microspheres from swimming pool water with sand, cartridge, and precoat filters. *Journal of Water and Health*. 10 (1), 31-42.
- Andersen, J. R., 1965. A study of the influence of cyanuric acid on the bactericidal effectiveness of chlorine. *American Journal of Public Health and the Nations Health*. 55 (10), 1629-1637.
- Baram, M., 1983. Risk assessment in the federal government - managing the process - National Academy of Science and National Resource Council. *Environment*. 25 (7), 25-27.
- Barwick, R. S., Levy, D. A., Craun, G. F., Beach, M. J., & Calderon, R. L., 2000. Surveillance for waterborne-disease outbreaks--United States, 1997-1998. *MMWR CDC Surveillance Summary*. 49 (4), 1-21.
- Bessonneau, V., Derbez, M., Clément, M., & Thomas, O., 2011. Determinants of chlorination by-products in indoor swimming pools. *International Journal of Hygiene and Environmental Health*. 215 (1), 76-85.
- Boehmer, T. K., Alden, N. B., Ghosh, T. S., & Vogt, R. L., 2009. Cryptosporidiosis from a community swimming pool: outbreak investigation and follow-up study. *Epidemiology and Infection*. 137 (11), 1651-1654.
- Bonvallot, N., Glorennec, P., & Zmirou, D., 2010. Derivation of a toxicity reference value for nitrogen trichloride as a disinfection by-product. *Regulatory Toxicology and Pharmacology*. 56 (3), 357-364.
- Bowen, A. B., Kile, J. C., Otto, C., Kazerouni, N., Austin, C., Blount, B. C., Wong, H.N., Beach, M.J., Fry, A. M., 2007. Outbreaks of short-incubation ocular and respiratory illness following exposure to indoor swimming pools. *Environmental Health Perspectives*. 115 (2), 267-271.
- Briggles, T. V., Allen, L. M., Duncan, R. C. & Pfaffenberger, C. D. 1981. High performance liquid chromatography determination of cyanuric acid in human urine and pool water. *Journal of the Association of Official Analytical Chemists*. 64 (5), 1222-1226.
- Bull, R. J., Birnbaum, L. S., Cantor, K. P., Rose, J. B., Butterworth, B. E., Pegram, R., & Tuomisto, J., 1995. Water chlorination: Essential process or cancer hazard? *Fundamental and Applied Toxicology*. 28 (2), 155-166.
- Cammann, K., & Hubner, K., 1995. Trihalomethane concentrations in swimmers and bath attendants blood and urine after swimming or working in indoor swimming pools. *Archives of Environmental Health*. 50 (1), 61-65.
- Carbonnelle, S., Bernard, A., Doyle, I. R., Grutters, J., & Francaux, M., 2008. Fractional exhaled NO and serum pneumoproteins after swimming in a chlorinated pool. *Medicine and Science in Sports and Exercise*. 40 (8), 1472-1476.

- Carbonnelle, S., Francaux, M., Doyle, I., Dumont, X., De Burbure, C., Morel, G., Michel, O., Bernard, A., 2002. Changes in serum pneumoproteins caused by short-term exposures to nitrogen trichloride in indoor chlorinated swimming pools. *Biomarkers*. 7( 6), 464-478.
- Causer, L. M., Handzel, T., Welch, P., Carr, M., Culp, D., Lucht, R., Mudahar, K., Robinson, D., Neavear, E., Fenton, S., Rose, C., Craig, L., Arrowood, M., Wahlquist, S., Xiao, L., Lee, Y.M., Mirel, L., Levy, D., Beach, M.J., Poquette, G., Dworkin, M. S., 2006. An outbreak of *Cryptosporidium* hominis infection at an Illinois recreational waterpark. *Epidemiology and Infection*. 134 (1), 147-156.
- Centers for Disease Control and Prevention, 2013. Healthy swimming and recreational water, Model Aquatic Health Code. Accessed March, 2013 from: <http://www.cdc.gov/healthywater/swimming/pools/mahc/about.html>
- Centers for Disease Control and Prevention, 2011. Morbidity and Mortality Weekly Report, Summary of Notifiable Diseases - United States, 2009. May 13, 2011. 58 (53), 1-100.
- Chappell, C. L., Okhuysen, P. C., Sterling, C. R., & DuPont, H. L., 1996. *Cryptosporidium parvum*: Intensity of infection and oocyst excretion patterns in healthy volunteers. *Journal of Infectious Diseases*. 173 (1), 232-236.
- Chen, X. M., Keithly, J. S., Paya, C. V., & LaRusso, N. F., 2002. Current concepts: Cryptosporidiosis. *New England Journal of Medicine*. 346 (22), 1723-1731.
- Craik, S. A., Weldon, D., Finch, G. R., Bolton, J. R., & Belosevic, M., 2001. Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Research*. 35 (6), 1387-1398.
- Craun, G. F., Brunkard, J. M., Yoder, J. S., Roberts, V. A., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J.M., Beach, M.J., Roy, S. L., 2010. Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews*. 23 (3), 507.
- Croll, B. T., Hayes, C. R., & Moss, S., 2007. Simulated *Cryptosporidium* removal under swimming pool filtration conditions. *Water and Environment Journal*. 21 (2), 149-156.
- Dang, B., Chen, L. L., Mueller, C., Dunn, K. H., Almaguer, D., Roberts, J. L., & Otto, C. S., 2010. Ocular and Respiratory Symptoms Among Lifeguards at a Hotel Indoor Waterpark Resort. *Journal of Occupational and Environmental Medicine*. 52 (2), 207-213.
- Dufour, A. P., 1984. Health effects criteria for fresh recreational waters. Cincinnati, OH: U.S. United States Environmental Protection Agency Research and Development. EPA-600/1-84-004.
- Dufour, A. P., Evans, O., Behymer, T. D., & Cantu, R., 2006. Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water Health*. 4 (4), 425-430.
- DuPont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B., & Jakubowski, W., 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* 332 (13), 855-859.

- Dyck, R., Sadiq, R., Rodriguez, M. J., Simard, S., & Tardif, R., 2011. Trihalomethane exposures in indoor swimming pools: A level III fugacity model. *Water Research*. 45 (16), 5084-5098.
- Eisenberg, J. N. S., Seto, E. Y. W., Colford, J. M., Olivieri, A., & Spear, R. C., 1998. An analysis of the Milwaukee cryptosporidiosis outbreak based on a dynamic model of the infection process. *Epidemiology*. 9 (3), 255-263.
- Erickson, M. C., & Ortega, Y. R., 2006. Inactivation of protozoan parasites in food, water, and environmental systems. *Journal of Food Protection*. 69 (11), 2786-2808.
- Fayer, R., 1994. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. *Applied and Environmental Microbiology*. 60 (8), 2732-2735.
- Ferguson, A. C., Canales, R. A., Beamer, P., Ayeung, W., Key, M., Munninghoff, A., Tse-Wing Lee, K., Robertson, A., Leckie, J. O., 2006. Video methods in the quantification of children's exposures. *Journal of Exposure Science and Environmental Epidemiology*. 16, 287-298.
- Gerba, C. P., Naranjo, J. E., & Hansan, M. N., 1997. Evaluation of a combined portable reverse osmosis and iodine resin drinking water treatment system for control of enteric waterborne pathogens. *Journal of Environmental Science and Health Part a-Environmental Science and Engineering & Toxic and Hazardous Substance Control*. 32 (8), 2337-2354.
- Gerba, C., 2000. Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality. *Quantitative Microbiology*. 2 (1), 55-68.
- Haas, C. N., 1983. Estimation of risk due to low-doses of microorganisms. *American Journal of Epidemiology*. 118 (4), 573-582.
- Hajjartabar, M., 2004. Poor-quality water in swimming pools associated with a substantial risk of otitis externa due to *Pseudomonas aeruginosa*. *Water Science and Technology*. 50 (1), 63-67.
- Hellard, M. E., Sinclair, M. I., Fairley, C. K., Andrews, R. M., Bailey, M., Black, J., Dharmage, S.C., Kirk, M. D., 2000. An outbreak of cryptosporidiosis in an urban swimming pool: why are such outbreaks difficult to detect? *Australian and New Zealand Journal of Public Health*. 24 (3), 272-275.
- Hery, M., Hecht, G., Gerber, J. M., Gendre, J. C., Hubert, G., & Rebuffaud, J., 1995. Exposure to chloramines in the atmosphere of indoor swimming pools. *Annals of Occupational Hygiene*. 39 (4), 427-439.
- Hijnen, W. A. M., Suylen, G. M. H., Bahlman, J. A., Brouwer-Hanzens, A., & Medema, G. J., 2010. GAC adsorption filters as barriers for viruses, bacteria and protozoan (oo)cysts in water treatment. *Water Research*. 44 (4), 1224-1234.
- Hlavsa, M. C., Roberts, V. A., Anderson, A. R., Hill, V. R., Kahler, A. M., Orr, M., Garrison, L., Hicks, L., Newton, A., Hilborn, E., Wade, T., Beach, M., Yoder, J. S., 2011. Surveillance for waterborne disease outbreaks and other health events associated with recreational water - United States, 2007-2008 *MMWR Surveillance Summary*. 60, 1-32.

- Hogan, D. A., Vik, A., & Kolter, R., 2004. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Molecular Microbiology*. 54 (5), 1212-1223.
- Insulander, M., Lebbad, M., Stenstrom, T. A., & Svenungsson, B., 2005. An outbreak of cryptosporidiosis associated with exposure to swimming pool water. *Scandinavian Journal of Infectious Diseases*. 37 (5), 354-360.
- International Agency for Research on Cancer (IARC), 1991. IARC Monographs on the evaluation of carcinogenic risks to humans, chlorinated rinking water; chlorinated by-products; some other halogenated compounds; cobalt and cobalt compunds. World Health Organization. 52, 1-35.
- Jacobs, J. H., Spaan, S., van Rooy, G., Meliefste, C., Zaat, V. A. C., Rooyackers, J. M., & Heederik, D., 2007. Exposure to trichloramine and respiratory symptoms in indoor swimming pool workers. *European Respiratory Journal*. 29 (4), 690-698.
- Johnston, K., Bittenbring, C., Bruya, L., Richwine, M. & Youngblood, S., 1999. The encyclopedia of aquatic codes and standards. In *The Encyclopedia of Aquatic Codes and Standards* (ed. K. Johnston). The National Recreation and Park Association, Ashburn, 55. .
- Judd, S. J., & Bullock, G., 2003. The fate of chlorine and organic materials in swimming pools. *Chemosphere*. 51 (9), 869-879.
- Keuten, M. G. A., Schets, F. M., Schijven, J. F., Verberk, J., & van Dijk, J. C., 2012. Definition and quantification of initial anthropogenic pollutant release in swimming pools. *Water Research*. 46 (11), 3682-3692.
- Lee, S. H., Levy, D. A., Craun, G. F., Beach, M. J., & Calderon, R. L., 2002. Surveillance for waterborne-disease outbreaks - United States, 1999-2000. *Morbidity and Mortality Weekly Report Surveillance Summary*. 51 (8), 1-47.
- Leoni, E., Legnani, P. P., Sabattini, M. A. B., & Righi, F., 2001. Prevalence of *Legionella* spp. in swimming pool environment. *Water Research*. 35 (15), 3749-3753.
- Loeb, B. L., 2009. Ozone: Science & engineering thirty years of progress. *Ozone-Science & Engineering*. 31 (5), 379-392.
- Loge, F. J., Emerick, R. W., Thompson, D. E., Nelson, D. C., & Darby, J. L., 1999. Factors influencing ultraviolet disinfection performance part I: Light penetration to wastewater particles. *Water Environment Research*. 71 (3), 377-381.
- Lutz, J. K., & Lee, J., 2011. Prevalence and antimicrobial-resistance of *Pseudomonas aeruginosa* in Swimming Pools and Hot Tubs. *International Journal of Environmental Research and Public Health*. 8 (2), 554-564.
- Massin, N., Bohadana, A. B., Wild, P., Hery, M., Toamain, J., & Hubert, G., 1998. Respiratory symptoms and bronchial responsiveness in lifeguards exposed to nitrogen trichloride in indoor swimming pools. *Occupational and Environmental Medicine*. 55 (4), 258-263.
- Messner, M. J., Chappell, C. L., & Okhuysen, P. C., 2001. Risk assessment for *Cryptosporidium*: A hierarchical Bayesian analysis of human dose response data. *Water Research*. 35 (16), 3934-3940.
- National Sanitation Foundation, 2009. National Sanitation Foundation/American

- National Standards Institute Standard 50: Equipment for Swimming pools, spas, hot tubs and other recreational water facilities. National Sanitation Foundation. 49, 1-9.
- Okhuysen, P. C., Chappell, C. L., Sterling, C. R., Jakubowski, W., & DuPont, H. L., 1998. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infection and Immunity*. 66 (2), 441-443.
- Regli, S., Rose, J. B., Haas, C. N., & Gerba, C. P., 1991. Modeling the risk from *Giardia* and viruses in drinking water. *Journal American Water Works Association*. 83 (11), 76-84.
- Rennecker, J. L., Marinas, B. J., Owens, J. H., & Rice, E. W., 1999. Inactivation of *Cryptosporidium parvum* oocysts with ozone. *Water Research*. 33 (11), 2481-2488.
- Reynolds, K. A., Mena, K. D., & Gerba, C. P., 2008. Risk of waterborne illness via drinking water in the United States. *Reviews of Environmental Contamination and Toxicology*. 192, 117-158.
- Rolfs, R. T., Beach, M. J., Hlavsa, M. C., & Calanan, R. M., 2008. Communitywide cryptosporidiosis outbreak - Utah, 2007. *Jama-Journal of the American Medical Association*. 300 (15), 1754-1756.
- Ruffell, K. M., Rennecker, J. L., & Marinas, B. J., 2000. Inactivation of *Cryptosporidium parvum* oocysts with chlorine dioxide. *Water Research*. 34 (3), 868-876.
- Schets, F. M., Engels, G. B., & Evers, E. G., 2004. *Cryptosporidium* and *Giardia* in swimming pools in the Netherlands. *Journal of Water and Health*. 2 (3), 191-200.
- Schets, F. M., Schijven, J. F., & Husman, A. M. D., 2011. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research*. 45 (7), 2392-2400.
- Shields, J. M., Arrowood, M. J., Hill, V. R., & Beach, M. J., 2009. The effect of cyanuric acid on the disinfection rate of *Cryptosporidium parvum* in 20-ppm free chlorine. *Journal of Water and Health*. 7 (1), 109-114.
- Shields, J. M., Hill, V. R., Arrowood, M. J., & Beach, M. J., 2008. Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *Journal of Water and Health*. 6 (4), 513-520.
- Teunis, P. F. M., Chappell, C. L., & Okhuysen, P. C., 2002. *Cryptosporidium* dose-response studies: Variation between hosts. *Risk Analysis* 22 (3), 475-485.
- Thickett, K. M., McCoach, J. S., Gerber, J. M., Sadhra, S., & Burge, P. S., 2002. Occupational asthma caused by chloramines in indoor swimming-pool air. *European Respiratory Journal*. 19 (5), 827-832.
- USEPA, 1986. Bacteriological water quality criteria for marine and fresh recreational waters. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA-440/5-84-002.
- Villanueva, C. M., Cantor, K. P., Grimalt, J. O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G., Marcos, R., Rothman, N., Real, F., Dosmerci, M., Kogevinas, M., 2007. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *American Journal of Epidemiology*. 165 (2), 148-156.

- Weisel, C. P., Richardson, S. D., Nemery, B., Aggazzotti, G., Baraldi, E., Blatchley, E. R., Blount, B.C., Carlsen, K., Eggleston, P.A., Frimmel, F., Goodman, M., Gordon, G., Grinshpun, S., Heerderik, D., Kogevinas, M., LaKind, J.S., Niewenhuijsen, M.J., Piper, F.C., Sattar, S. A., 2009. Childhood asthma and environmental exposures at swimming pools: State of the science and research recommendations. *Environmental Health Perspectives*. 117 (4), 500-507.
- Weng, S. C., Weaver, W. A., Afifi, M. Z., Blatchley, T. N., Cramer, J. S., Chen, J., & Blatchley, E. R., 2011. Dynamics of gas-phase trichloramine in chlorinated, indoor swimming pool facilities. *Indoor Air*. 21 (5), 391-399.
- Wilkins, J. R., & Comstock, G. W., 1981. Source of drinking water at home and site specific cancer incidence in Washington County, Maryland. *American Journal of Epidemiology*. 114 (2), 178-190.
- World Health Organization, 2003. Guidelines for safe recreational water environments, swimming pool and similar environments. 1, 1-171.
- Yoder, J. S., & Beach, M. J., 2010. *Cryptosporidium* surveillance and risk factors in the United States. *Experimental Parasitology*. 124 (1), 31-39.
- Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V., Roberts, V., Yu, P. A., Hicks, L.A., Alexander, N.T., Calderon, R.L., Roy, S.L., Beach, M. J., 2008. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events - United States, 2005 - 2006 *MMWR Surveillance Summary*. 57, 1-29.
- Zimmer, J. L., Slawson, R. M., & Huck, P. M., 2003. Inactivation and potential repair of *Cryptosporidium parvum* following low- and medium-pressure ultraviolet irradiation. *Water Research*. 37 (14), 3517-3523.

## APPENDIX A – VALIDATION AND STANDARDIZATION OF SWIMMING EXPOSURE AND POOL OPERATIONS QUESTIONNAIRES

Paper was prepared to submit to Water Research

Laura M. Suppes<sup>1</sup>, Kacey C. Ernst<sup>2</sup>, Leif Abrell<sup>3</sup>, Kelly A. Reynolds<sup>4</sup>  
<sup>1,2,4</sup>The University of Arizona Mel and Enid Zuckerman College of Public Health  
<sup>3</sup>The University of Arizona Department of Soil, Water & Environmental Science  
<sup>4</sup>Phone: (520) 626-8230

<sup>1</sup>[suppeslm@email.arizona.edu](mailto:suppeslm@email.arizona.edu) <sup>2</sup>[kernst@email.arizona.edu](mailto:kernst@email.arizona.edu)  
<sup>3</sup>[abrell@email.arizona.edu](mailto:abrell@email.arizona.edu) <sup>4</sup>[reynolds@email.arizona.edu](mailto:reynolds@email.arizona.edu)

Keywords: Exposure, behavior analysis, pool water ingestion, recreational water, swimming pool, questionnaire validation

List of Abbreviations:

AGI – Acute Gastrointestinal Illness  
ARI – Acute Respiratory Illness  
DBP – Disinfection By-product  
ESI – Electrospray Ionization  
NORS – National Outbreak Reporting System  
RWI – Recreational Waterborne Illness  
SAX – Strong Anion Exchange  
SPE – Solid Phase Extraction

### **Abstract**

Swimmer behavior and pool operational risk factors are commonly associated with swimming pool-related acute gastrointestinal outbreaks, but are poorly researched. While epidemiological studies have been employed to examine how pool activities relate to illness, these studies are often criticized for lacking comprehensive assessment of the dynamic factors related to pool water ingestion and pool operation. Research is limited due to inadequate survey tools available for these exposure factors in outbreak investigations. Capacity of the current tools is limited by lack of 1) electronic

administration, 2) validation for self-administration, 3) standardization, and 4) inclusion of activity factors associated with ingestion exposures. Objectives of this study were to develop, critique, and test electronic, self-administered exposure and operations questionnaires; to identify and quantify risk factors of ingestion; to gather behavior and activity data for use in risk assessment; and to validate the questionnaires' ability to determine ingestion by comparing measured with self-reported volumes. Self-administered and electronically distributed swimming behavior and operations questionnaires were developed by implementing a multi-component pilot study. The swimming behavior questionnaire was issued to 126 swimmers in Tucson, Arizona recruited at four pool sites. The questionnaire was completed by 106 participants (84.1%). Demographic, behavior, activity, use-history, and illness information were collected with the "exposure" questionnaire. Twenty-four hour urine samples collected from 46 participants were tested for cyanuric acid to estimate pool water ingestion ("measured" ingestion). Thirty-eight of the 46 participants completed questionnaires. The "operations" questionnaire was issued to pool operators at the four pool sites, and collected pool mechanical, structural, maintenance, operations, bather loading, and water chemistry information. Pool water samples were tested for quality indicators (three indicator organisms and three chemical properties) to validate operations questionnaire responses. The questionnaire accurately captured magnitude of pool water ingested during swimming when measured ingestion was categorized and compared with reported amounts (n=38). The survey ingestion question can be used in place of urinalysis to reduce study costs when ingestion magnitude is a desired outcome. Diving and being a

child ( $\leq 18$ ) were positively associated with measured ingestion, and are thus considered risk factors of ingestion. Activity data collected for use in risk assessment indicates average reported frequency of pool visits per year, time spent at the pool facility per visit, and age at first pool exposure were 72.7 times/year ( $\pm 66.7$ ), 1.57 hrs ( $\pm 1.0$ ), and 2.8 years ( $\pm 2.87$ ), respectively. Urinalysis participants ( $n=38$ ) ingested on average 14.3 mL/hr of pool water ( $\pm 22.8$ ); 24.2 mL/hr ( $\pm 29$ ) if 18 or younger; and 6.3 mL/hr ( $\pm 16.6$ ) if older than 18. This study successfully validated a survey tool for assessing pool water ingestion magnitude, identified risk factors of ingestion, summarized activity data useful for risk assessment, and made exposure and swimming pool operations questionnaires available electronically. A repository for swimmer exposure and pool operational risk factor data provides a centralized and electronically accessible location to begin a database. Increasing swimmer and pool sample sizes and expanding the geographic region where data is collected improves the likelihood risk factor differences across broader demographic groups and venues will be recognized. Exposure scientists and outbreak investigators can use the centralized database to share standardized exposure data and improve risk estimates. Both surveys are available online at the following link: [www.ghi.arizona.edu](http://www.ghi.arizona.edu).

## **1. Introduction**

The annual number of Recreational Waterborne Illness (RWI) outbreaks associated with treated recreational water venues (“pools”) in the U.S. is increasing (pools are defined as swimming pools, spas, interactive fountains, wading pools and dive

pools) (Craun et al., 2005) since the first reporting year in 1978 (Hlavsa et al., 2011; Yoder et al., 2008). Eighty-one of 116 RWI outbreaks reported to the Centers for Disease Control and Prevention (CDC) between 2007 and 2008 resulted in acute gastrointestinal illness (AGI), followed by 24 with skin, and 16 with acute respiratory illness (ARI). Acute Gastrointestinal Illness outbreaks resulted in 13,480 illnesses. While improved reporting likely accounts partially for the noted increase in outbreaks, emergence of *Cryptosporidium* and other pool-related hazards has also contributed. *Cryptosporidium* accounted for 71% of pool-related AGI outbreaks between 2007 and 2008 (Hlavsa et al., 2011). *Cryptosporidium* is a waterborne parasite highly resistant to chlorine (20 mg/L chlorine inactivates *Cryptosporidium* oocysts in 12.75 hrs) (Shields et al., 2008).

Reducing risk of RWI outbreaks caused by biological and chemical hazards requires identification of specific exposures. While epidemiological studies have been employed to examine how pool activities relate to illness, these studies are often criticized for lacking comprehensive assessment of the dynamic factors related to swimmer exposures, pool design and operation. Proper data collection tools are essential to accurately estimate the health effects of swimming, and assure the development of appropriate controls, policies, and regulations.

One primary exposure related to swimming activity is ingestion of water. Previously, the World Health Organization (2003) used questionnaires to estimate swimming ingestion rates and found swimmers reported swallowing 20-50 mL/hr. These values, however, were self-reported and are underestimated when compared to ingestion ranges reported in other studies that applied quantitative measurement techniques. Ingestion can

be quantified using methods that compare cyanuric acid in urine and pool water.

Cyanuric acid is added as a chlorine stabilizer to outdoor pool water, and when ingested, passes through the human body unmetabolized. Controlled studies show 98% of cyanuric acid ingested is excreted in a 24 hr period (Allen et al., 1982). Using this technique, researchers showed swimmers ingested between 0-154 mL/hr, and that children ( $\leq 18$ ) ingested more than adults ( $>18$ ) (Dufour et al., 2006). Information on swimmer activity and behavior was not collected by Dufour et al. (2006), however, as the objective was to test ingestion quantification methodology.

Exposure research on swimmers is limited due to inadequate data collection tools available for outbreak investigators. Capacity of current tools is limited by lack of electronic administration, validation for self-administration, standardization, and inclusion of activity factors associated with ingestion exposures. Recognizing the need for proper swimming pool exposure and operations data collection tools, we built on questionnaires developed by the CDC, U.S. Environmental Protection Agency (USEPA), and academic researchers to improve pool exposure assessment. Study objectives were to develop, critique, and test self-administered exposure and operations questionnaires that could be distributed electronically, to identify and quantify risk factors of ingestion, to gather activity data for use in risk assessment, and to validate the questionnaires' ability to determine ingestion by comparing "measured" (ingestion estimated by urinalysis) with self-reported volumes. Research hypotheses assume no difference between magnitude of measured and reported ingestion, and that young age and boisterous activities, like diving, will be considered risk factors of ingestion.

## **2. Methods**

### **2.1 Literature Review and Questionnaire Development**

The CDC and USEPA websites and peer-reviewed literature were searched for pool outbreak survey tools, tools developed in response to outbreaks, and tools designed to capture swimmer exposures. The CDC National Outbreak Reporting System (NORS) is available for reporting nationwide waterborne disease outbreaks, and includes exposure questions related to recreational water. In-depth survey tools are also available through CDC that collect data on swimmer activity, gastrointestinal symptoms, confounding exposures, pool operations and maintenance, and are designed to be administered by outbreak investigators (Centers for Disease Control and Prevention [CDC], 2013). Surveys intended to collect additional exposure information, such as potential disinfection by-product exposures, were reviewed from the USEPA assessment tool SWIMODEL among others (United States Environment Protection Agency [USEPA], 2013).

Exposure risk factors relative to swimmer behavior and pool maintenance from the CDC surveys, SWIMODEL, and peer-reviewed literature were compiled and organized into draft questionnaires. Three panels were assembled to review the drafts for comprehensiveness and to recommend formatting and included; 1) six experts from the swimming pool industry, 2) an international group of nine microbiologists, exposure scientists, and epidemiologists, and 3) an internal University of Arizona panel of six respiratory health, epidemiology, exposure science, and public health specialists.

Meetings with each panel were held once and last 1-2 hours. Individual communication with panel member by email or phone occurred throughout the questionnaire development process. Questions from the draft were input into DatStat Illume Survey Developer Gateway Version 5.1.1.17347 (Seattle, WA). Questionnaires were further evaluated by the external review panel for errors and comprehensiveness prior to use. Estimates of water ingestion volumes, type of swimming, duration of each type of swimming, swimmer skill level, frequency of deep swimming, observation of a chlorine smell, use of swim aids and diapers, shower use, frequency of pool visits, average visit duration, age of first pool visit, and current respiratory, eye, ear, skin, and gastrointestinal health conditions were collected with the exposure questionnaire. The operations questionnaire collected pool mechanical, structural maintenance, operations, bather loading, and water chemistry information.

The question used in this study to estimate pool water ingestion by “mouthfuls/swim” was developed by Schets et al. (2011). Schets et al. quantified the average volume in one mouthful (27 mL), which allowed measured volumes in the present study to be categorized into “mouthfuls/swim.” For example, participants with measured ingestion between 0-26 mL were categorized as: “1: no water or only a few drops.” The other categories were: one to two mouthfuls; three to five mouthfuls; and six to eight mouthfuls. Categorized measured ingestion volumes were compared with reported volumes using a Chi-squared test to determine differences. The ingestion question was considered reliable if no difference was evident between reported ingestion by child,

adult, male, and female swimmers and measured ingestion values categorized to match reported volumes.

## **2.2 Data Collection**

The exposure questionnaire was issued to 126 swimmers in Tucson, Arizona, recruited at two public pools, and two private pools. The operations questionnaire was issued to the four pool sites. Forty-six (36.5%) participants submitted a 24 hr urine sample to quantify pool water ingestion. Participants who submitted urine were instructed to swim at least 45 minutes. Swimmers who did not submit urine were not restricted to a timeframe, but swam about one hour. Participants accessed the questionnaire either on-site using tablets, electronic or smart phones; or on a personal computer through email.

## **2.3 Questionnaire Validation**

### **2.3.1 Water Quality**

Water quality data were collected to validate questionnaire responses related to illness. Twelve samples per site visit (3 at the pool bottom, and 3 at the pool surface before and after participants entered the water) were collected in sterile 1 L containers for a total of 108 samples (9 site visits x 12 samples/site visit=108). On-site testing was conducted for free, combined and total chlorine, pH, and cyanuric acid using a Lamotte ColorQ Pro-7 spectrophotometric test kit (Chestertown, MD). Off-site assays included fecal coliform, *Pseudomonas aeruginosa*, and heterotrophic plate count bacteria at The University of Arizona Medical Research Building laboratory. Samples for microbial

analysis were neutralized with sodium thiosulfate, held on ice, transported to the laboratory, and processed by membrane filtration within 24 hr. Fecal coliforms, *Pseudomonas aeruginosa*, and heterotrophic plate count bacteria were incubated for 24 hr at 44.5°C on mFC agar (ISO 9308-1:2000); 48 hr at 35°C on *Pseudomonas aeruginosa* CN agar (UNE-EN 12780:2002); and 48 hr at 37°C on tryptic soy agar (EN ISO 6222:1999), respectively.

### **2.3.2 Water Ingestion**

Forty six participants were given a sterile 3 L urine collection container (Fischer Scientific Company, 14-375-248) for a 24 hr post-swimming sample. Participants were excluded if sufficient age-based urine volumes were not provided (Dorevitch et al., 2011). Urine volumes were required to exceed 150 mL, 200 mL, and 300 mL for ages <10, 11-16, and >17, respectively. Pool water samples were collected 30 cm from the surface using 20 mL bottles from 4 locations at each pool, stored on ice, transferred, and stored at 4°C under refrigeration. Pool water and urine were processed at The University of Arizona Medical Research Building and assayed at The University of Arizona's Laboratory for Emerging Contaminants for cyanuric acid following modified methods previously described by Cantu et al. (2000; 2001a) and Smoker and Krynitsky (2008). Briefly, samples were preserved with a 10% perchloric acid (v/v) and 1% metaphosphoric acid (w/v) solution, centrifuged to remove proteins, labeled, covered with tin foil, and held at 4°C under refrigeration.

Urine samples were cleaned by Solid Phase Extraction (SPE) with Strong Anion Exchange (SAX) cartridges (SampliQ Silica, 3 ml, 500 mg; Agilent, 5982-2035) to

remove creatinine and other potential interfering substances and stored at 4°C under refrigeration. Urine and pool water were analyzed by Quattro Premier XE triple-quadrupole mass spectrometer (Waters Corp., Milford, MA) by electrospray ionization in the negative mode (ESI-). Both urine and pool water were analyzed no more than 31 days after preservation. Water ingestion volumes were estimated using a previously developed model by Briggle et al. 1981 and Allen et al. 1982 (Eq. 1). Urine and pool water cleaning and analysis methodology details are described in Suppes et al. (unpublished results).

$$\text{Eq. 1: Water Ingestion (L)} = \left[ \text{[Cyanuric Acid]}_{\text{urine}} \left( \frac{\mu\text{g}}{\text{L}} \right) \div \text{[Cyanuric Acid]}_{\text{pool water}} \left( \frac{\mu\text{g}}{\text{L}} \right) \right] \times \text{Urine Volume (L)}$$

### 2.3 Data analysis

STATA Statistics/Data Analysis Version 11.0 Software (College Station, TX) was used to perform statistical tests. Pearson's correlation, Chi-squared, Fisher's exact, and unpaired t-tests were applied to identify associations between independent variables (age, gender, deep-end swimming, diving, playing, splashing, standing, walking, wading, lap swimming, length of swim, number of pool visits/yr, time spent swimming at any pool visit) and reported and measured ingestion. Reported and measured water ingestion were compared as categorical variables using a Chi-squared test. Associations were considered statistically significant at a p-value <0.05 (95% confidence).

### 3. Results

#### 3.1 Literature Review for Questionnaire Development

The literature search to identify illness risk factors related to pool operation and swimmer behavior showed the following: More than half (52%) of all outbreaks in treated-water venues reported between 1978 and 2000 were due to improper pool maintenance, operation, disinfectant concentrations, and chemistry (Craun et al., 2010). Chemical-related outbreaks from increased DBP concentrations in indoor pool air have been associated with high combined chlorine, poor ventilation, bather attendance, and aerosolizing features like sprayers and fountains (Dang et al., 2010). Even with proper ventilation, outbreaks of ocular and respiratory symptoms from high chloramine concentrations have been documented (Dang et al., 2010). Records from microbial-related outbreaks indicate re-contamination of pools by ill swimmers is common, often with chlorine-resistant parasites like *Cryptosporidium* (Barwick et al., 2000; Yoder et al., 2008). Pool operator testimonies following microbial-related outbreaks indicate a lack of awareness regarding accidental fecal releases (AFRs). Often, AFRs are not reported until weeks after occurring, and are therefore not addressed in time to prevent exposures. *Cryptosporidium* has been identified as the etiological agent in pool outbreaks, despite pool operators maintaining recommended chlorine concentrations and using Ultraviolet light disinfection, which is a secondary prevention measure against *Cryptosporidium* and other waterborne pathogens (Boehmer et al., 2009).

Given the complexity of factors related to pool operations and swimmer behaviors that influence exposures, and the variety of health outcomes that have been associated

with swimming, it is necessary for exposure scientists to have a comprehensive exposure assessment tool that is easy to access and distribute. The CDC recreational waterborne outbreak questionnaires are currently the most comprehensive surveys available for use by outbreak investigators in the U.S. The questionnaires provide a complete assessment of gastrointestinal illness symptoms, potential ingestion exposures, confounding exposures, pool structure, water chemistry, and recirculation equipment operations and maintenance. These questionnaires, however, are not available electronically, are currently not formatted for self-administration, are not validated, and lack questions related to inhalation exposures, indoor air recirculation and ventilation, and other questions that help assess disinfection by-product (DBP) related exposures. As a result, these questionnaires are often modified by local and state health departments when investigating non-AGI outbreaks. Other outbreak-response questionnaires developed by researchers outside of the CDC and published in peer-reviewed literature ask questions that are not on the CDC surveys. To enhance the standard CDC questionnaires we included these specific exposures of interest into our questionnaires. (Table 1).

Table 1: Question and response options from peer-reviewed literature modified and incorporated into the exposure and operations questionnaires. The right column indicates question and answer choice presented to participants. Non-multiple choice questions are followed by bracketed response instructions.

| <b>Source (exposure factor)</b>       | <b>Question and response options</b>   |
|---------------------------------------|--|
| Schets et al., 2011 (water ingestion) | <p>How much water did you swallowed while swimming (estimate)? [check one]</p> <ol style="list-style-type: none"> <li>1. No water or only a few drops</li> <li>2. 1 to 2 mouthfuls (amount in a shot glass)</li> <li>3. 3 to 5 mouthfuls (amount in a coffee cup)</li> <li>4. 6 to 8 mouthfuls (amount in a soda glass)</li> </ol> |

|  |   |
|--|---|
| Font-Ribera et al., 2009 (potential respiratory illness predictor)   | What was your age when you visited a pool for the first time (years)? [fill in]   |
| Bernard et al., 2008 (potential respiratory illness predictor)   | On average, how much time do you spend at the pool per visit (hours)? [fill in]   |
| Bernard et al., 2003; Bernard et al., 2008 (potential respiratory illness predictor)   | On average, how many times in 1 year do you visit any pool facility? [fill in]  |
| Dang et al., 2010; Fantuzzi et al., 2010 (potential predictors of respiratory and ocular illnesses)                            | <p>Are you currently experiencing any of the following respiratory, eye or ear irritation symptoms? [check]</p> <ol style="list-style-type: none"> <li>1. Coughing</li> <li>2. Wheezing</li> <li>3. Chest tightness</li> <li>4. Shortness of breath</li> <li>5. Frequent sneezing</li> <li>6. Itchy, runny nose</li> <li>7. Sore throat</li> <li>8. Backache</li> <li>9. Eye irritation or stinging</li> <li>10. Watery eyes</li> <li>11. Halo vision (halos around lights)</li> <li>12. Blurry or foggy vision</li> <li>13. Blue-gray vision</li> <li>14. Ear infection</li> </ol>   |
| Dang et al., 2010; Levesque et al., 2006 (potential predictors and/or confounders of recreational waterborne illness symptoms) | <p>Other exposures and health conditions [check]:</p> <ol style="list-style-type: none"> <li>1. Smoker (occasional and regular)</li> <li>2. Ex-smoker</li> <li>3. Doctor diagnosed asthma</li> <li>4. Wheezing that limits daily activities</li> <li>5. Hay fever</li> <li>6. Non-drug allergies (other than hay fever)</li> <li>7. Chronic Obstructive Pulmonary Disease (COPD)</li> <li>8. Chronic bronchitis (cough and phlegm for at least months a year)</li> <li>9. Cystic fibrosis</li> <li>10. Sinusitis (a cold that has not improved after ~7 days of coughing, fatigue, fever, headache, sore throat, or nasal congestion)</li> <li>11. Flu (combination of fever, cough, muscle/body aches, sore throat, fatigue, runny/stuffy nose lasting 24 hours to ~7 days)</li> <li>12. Diarrhea anytime in the past 2 weeks (14 days)</li> </ol> |

|  |   |
|--|---|
|  | <ul style="list-style-type: none"> <li>13. Crohn's Disease</li> <li>14. Irritable Bowel Syndrome</li> <li>15. Ulcerative colitis</li> <li>16. Partial removal of stomach or intestines</li> <li>17. HIV/AIDS</li> <li>18. Hepatitis</li> <li>19. Eczema or atopic dermatitis</li> </ul>   |
| Roy et al., 2006 (predictor of Highly Credible Gastrointestinal Illness)               | Has the diarrhea resulted in 3 or more loose stools in the past 24-hours? [check yes, no]   |
| Roy et al., 2006 (predictor of Highly Credible Gastrointestinal Illness)               | Has the diarrhea impaired your daily activities (remained at home or in bed)? [check yes, no]   |
| Dang et al., 2010 (potential predictors of elevated DBPs in air; numbers 3, 10, and 1) | <p>Swimming pool equipment and operations [check]:</p> <ul style="list-style-type: none"> <li>1. Is there a backflow prevention device?</li> <li>2. Is the source/fill water from an approved water supply?</li> <li>3. Is the source/fill water chloraminated?</li> <li>4. Is an automatic chemical feeder used?</li> <li>5. Is an Incidental Fecal Release cleanup policy available at the pool facility?</li> <li>6. Is the Incidental Fecal Release cleanup policy used or followed, if available?</li> <li>7. Is pool water chemistry tested daily?</li> <li>8. Are stabilized chlorine tablets used (tri-chloroisocyanuric)?</li> <li>9. Is cyanuric acid stabilizer manually added to the water?</li> <li>10. Does the pool facility have water agitating features, like sprayers, fountains, jets, etc.?</li> <li>11. Is the filter maintained according to manufacturer specification?</li> <li>12. Is showering mandatory before pool use?</li> <li>13. Is the deck sloped well enough to drain pooled water? (i.e. deck is generally free of standing water)</li> <li>14. Is the water clear? (main drain is visible)</li> <li>15. Is the main drain unblockable or are drains dual in parallel?</li> <li>16. Are bacteriological tests of the water performed?</li> </ul> |

|  |   |
|--|---|
| <p>Dang et al., 2010 (potential predictors of elevated DBPs in air; numbers 9, 15, 17, 18, 19)</p> | <p>Current pool water conditions [fill in]:</p> <ol style="list-style-type: none"> <li>1. Time of water sample collection and testing (ex. "9am")</li> <li>2. Free disinfectant residual (ppm)</li> <li>3. Total disinfectant residual (ppm)</li> <li>4. Combined disinfectant residual (total - free) (ppm)</li> <li>5. pH</li> <li>6. Total alkalinity (ppm)</li> <li>7. Cyanuric acid (ppm)</li> <li>8. Water temperature (°F)</li> <li>9. Air temperature (°F)</li> <li>10. Total Dissolved Solids (ppm)</li> <li>11. Oxidative Reduction Potential (ORP) (mV)</li> <li>12. Pool volume (gal)</li> <li>13. Flow rate (gpm)</li> <li>14. Water turnover rate (hours)</li> <li>15. Bather load (# of people in water)</li> <li>16. Pool filter area (square feet)</li> <li>17. Outdoor air exchange rate (indoor pools only) (hours)</li> <li>18. Room volume (indoor pools only) (square feet)</li> <li>19. Is there noticeable biofilm/slime on pump room equipment, pool or other surfaces?</li> </ol> |
|--|---|

<sup>a</sup>CDC and SWIMODEL questionnaire information not included. The CDC Recreational Water Illness Response Toolkit URL:

<http://www.cdc.gov/healthywater/emergency/toolkit/rwi-outbreak-toolkit.html>.

SWIMODEL URL: <http://www.epa.gov/oppad001/swimodel.htm>.

### 3.2 Reviewer Feedback

Feedback from the review panels identified the need for shorter questionnaires, and the inclusion of questions that assess inhalation exposures, DBP-related illnesses, and indoor pool air quality. Questions about respiratory health and pre-existing conditions, pool ventilation, age at first pool exposure, frequency of pool visits, and olfactory sensitivity to chemicals were included in the questionnaires based on reviewer recommendations. Reviewers also suggested the use of an electronic platform to

eliminate the need for data entry, increase the ease of data analysis and questionnaire administration, and to provide a central repository for exposure and operational risk factor data collected during outbreaks or routinely. Both surveys are available online at the following link: [www.ghi.arizona.edu](http://www.ghi.arizona.edu). Data from completed questionnaires is stored in a secure, central online database.

### **3.3 Data Analysis**

The questionnaire response rate was 84.1% (106/126), as not all swimmers completed the questionnaire who requested the survey by emailed. Reported and measured ingestion results among different age, gender, and activity groups from the questionnaires and urinalysis are summarized in Tables 2 and 3. Divers were selected for comparison based on the hypothesis that diving is a risk factor of ingestion. On average, swimmers reported visiting a pool 72.7 times/year (range: 0-360 times/year, SD: 66.7), spending 1.57 hr/pool visit (range: 0-8 hr, SD: 1.1), visiting a pool for the first time at age 2.7 years (range: 0-22 years, SD: 2.87), and showering before entering the pool for 6.8 minutes (range: 0.16-20 min, SD: 7.6).

Table 2: Age, gender, and activity distributions of questionnaire respondents and urinalysis participants.

|                   | Questionnaire<br>n = 106 (%) | Urinalysis<br>n = 38 (%) |
|-------------------|------------------------------|--------------------------|
| <b>Age</b>        |                              |                          |
| ≤10 years         | 16 (15.1)                    | 4 (10.5)                 |
| ≤18 years         | 52 (49)                      | 17 (44.7)                |
| 11 to 18 years    | 36 (33.9)                    | 13 (34.2)                |
| >10 years         | 90 (84.9)                    | 34 (89.4)                |
| >18 years         | 54 (50.9)                    | 21 (55.2)                |
| <b>Gender</b>     |                              |                          |
| Male              | 55 (51.9)                    | 25 (65.7)                |
| Female            | 50 (47.2)                    | 13 (34.2)                |
| <b>Activity</b>   |                              |                          |
| Lap swimmers      | 21 (19.8)                    | 9 (23.7)                 |
| Leisure swimmers  | 85 (80.2)                    | 28 (73.7)                |
| Deep-end swimmers | 86 (81.1)                    | 28 (73.7)                |
| Diving            | 33 (31.1)                    | 10 (26.3)                |

Table 3: Differences in reported (n=106) and measured (n=38) pool water ingestion among males and females, various age groups, divers and non-divers. Measured ingestion values have been categorized using mouthful volumes characterized by Schets et al. (2011)

|                | No water- few drops |           | 1-2 mouthfuls |          | 3-5 mouthfuls |          |
|----------------|---------------------|-----------|---------------|----------|---------------|----------|
|                | Reported            | Measured  | Reported      | Measured | Reported      | Measured |
| <b>Gender</b>  |                     |           |               |          |               |          |
| Males (%)      | 19 (17.9)           | 21 (55.3) | 24 (22.6)     | 3 (7.8)  | 13 (12.2)     | 1 (2.6)  |
| Females (%)    | 24 (22.6)           | 11 (28.9) | 22 (20.7)     | 2 (5.3)  | 3 (2.8)       | 0 (0)    |
| <b>Age (%)</b> |                     |           |               |          |               |          |
| ≤10            | 3 (2.8)             | 2 (5.2)   | 11 (10.3)     | 1 (2.6)  | 3 (2.8)       | 1 (2.6)  |
| ≤18            | 18 (16.9)           | 12 (31.5) | 27 (25.4)     | 4 (10.5) | 7 (6.6)       | 1 (2.6)  |
| 11-18          | 15 (14.1)           | 10 (26.3) | 17 (16)       | 3 (36.1) | 4 (3.7)       | 0 (0)    |
| >10            | 40 (37.7)           | 30 (78.9) | 37 (34.9)     | 4 (10.5) | 13 (12.3)     | 0 (0)    |
| >18            | 25 (23.5)           | 20 (52.6) | 20 (18.9)     | 1 (2.6)  | 9 (8.5)       | 0 (0)    |
| <b>Diving</b>  |                     |           |               |          |               |          |
| No (%)         | 8 (7.5)             | 23 (60.5) | 9 (8.5)       | 2 (5.3)  | 4 (3.8)       | 0 (0)    |
| Yes (%)        | 35 (33)             | 6 (15.8)  | 6 (16.6)      | 3 (7.9)  | 12 (11.3)     | 1 (2.6)  |

### 3.3.1 Reported Ingestion, Activities and Health

Among the 106 respondents, Chi-2 tests indicated females reported swallowing less water than males ( $p < 0.039$ ), deep-end swimming was associated with higher ingestion volumes ( $p < 0.005$ ), and children did not report ingesting more than adults ( $p < 0.250$ ). Leisure swimming (splashing, playing, diving, wading, standing, and sitting) was practiced more by children ( $\leq 18$ ) and lap swimming by adults using an unpaired t-test ( $p < 0.0115$ ). T-tests also identified lap swimmers swam for shorter durations than leisure swimmers ( $p < 0.0003$ ) (average swim time: 59 min leisure, 41 min lap) and reported visiting swimming pools throughout the year more frequently ( $p < 0.000$ ) (average leisure: 59.3 visits/yr, average lap: 126.4 visits/yr). Child swimmers reported spending more time per pool visit swimming ( $p < 0.0003$ ), but frequency of swimming did not differ between children and adults using t-tests ( $p > 0.6992$ ). Deep-end swimming was practiced more by leisure swimmers (t-test:  $p < 0.0003$ ).

Participants were more likely to report inhaling water by accident (“did you get water up your nose”) if a child ( $p < 0.000$ ), a leisure swimmer ( $p < 0.0230$ ), and if they swam for longer durations ( $p < 0.0191$ ) as indicated by t-tests. Overall, 61 reported getting water up their nose and 26/61 were children. The specific leisure activity associated with inhaling pool water was diving (Chi-2:  $p < 0.006$ ), which was practiced more by males and children (Chi-2:  $p < 0.008$  and t-test:  $< 0.001$ , respectively).

Of the 106 survey respondents, 1 swam with diarrhea; 1 with nausea; 7 responded that they experienced diarrhea in the past two weeks (6.6%); and 9 that they had doctor diagnosed asthma (8.4%). Eight showered before using the pool (7.5%); 59 used goggles

during their swim (55.6%), and 26 used a swim aid (24.5%) (kickboard, life jacket, or arm floats). Swim aids were used by both advanced lap swimmers during workouts, and by beginning swimmers for safety. Participants with asthma reported spending longer durations at pool visits throughout the year (t-test:  $p < 0.0393$ ). The average time/visit reported by participants with asthma ( $n=10$ ) was 2.25 hrs, and 1.5 hr for participants not reporting asthma ( $n=92$ ).

### **3.3.2 Measured Ingestion, Demographics and Activities**

Thirty-eight of 46 participants had useable water ingestion values for analysis. Four did not submit a questionnaire, 1 submitted a urine sample less than the accepted volume threshold, and 3 urine samples had signal-to-noise ratios  $<3$ , which indicates measurement below the UHPLC-MS/MS limit of detection. Ingestion volumes in four age groups (10 years or less, greater than 10 years, 18 years or less, and greater than 18 years) were assessed (Table 4) because the group  $\leq 10$  (“young child”) is often reported as vulnerable to RWIs (Boehmer et al., 2009; Hellard et al., 2000; Hlavsa et al., 2011; Insulander et al., 2005; Puech et al., 2001), and age 18 is the threshold between adult and children. Both child age groups (young child  $\leq 10$ :  $p < 0.0031$ , and child  $\leq 18$ :  $p < 0.0218$ ) ingested more than the older comparison groups indicated by unpaired t-tests (Figure 1). Two adults ingested  $>50$  mL/hr pool water, which is much higher than the average adult ingestion volume (6.3 mL/hr). One was a 45 year old lap swimmer (60.6 mL/hr), and one was a 50 year old leisure swimmer (50.9 mL/hr). The significance between age and ingestion did not change when both were removed from the dataset (t-test:  $p < 0.0014$ ).

Thus, these participants remained in the dataset. The swimmer with the highest ingestion rate (105.5 mL/hr) was 10 years old and also did not affect the age-ingestion relationship when excluded from the dataset (t-test:  $p < 0.0431$ ).

Among the 106 questionnaire respondents, females reported ingesting less water than males, and deep-end swimmers reported ingesting more than the shallow-end comparison group. When the same variables were compared in only those with measured ingestion ( $n=38/106$ ), no difference in reported ingestion was found. These findings validate measured ingestion trends in female, male, deep- and shallow-end swimmers when measured ingestion volumes are converted to categorical variables. Measured pool water ingestion ( $n=38$ ) as a categorical variable did not differ by gender using a Fisher's exact test ( $p > 0.571$ ) or by deep-end swimming ( $p > 0.659$ ). Comparing age and measured ingestion as categories also using a Fisher's exact test showed children did not ingest more than adults ( $p < 0.092$ ), which was the same outcome for age and reported ingestion among the 38 urinalysis participants ( $p > 0.083$ ). This finding was also consistent with results from all 106 child and adult responders. Measured pool water ingestion and ingestion estimated using the questionnaire were not statistically different when measured volumes were categorized and analyzed using a Chi-2 test ( $p > 0.878$ ). These outcomes suggest the survey's ingestion question can be used to estimate pool water ingestion magnitude in place of urinalysis when ingestion magnitude is a desired outcome. Table 5 and Figure 2 illustrate similarities between ingestion volumes reported and measured among urinalysis participants.

Measured pool water ingestion as a continuous variable was positively associated with diving ( $p < 0.0280$ ) and age  $\leq 18$  using unpaired t-tests. Age  $\leq 18$  and diving are therefore considered risk factors of ingestion as initially hypothesized.

Table 4: Pool water ingestion summary in adult ( $>18$ ), child ( $\leq 18$ ), young child ( $\leq 10$ ), young adult  $>10$ , male, female, lap, and leisure swimmers (diving, splashing, playing, wading, sitting, standing).

|                       | Ingestion (mL/hr) |      |      |             |
|-----------------------|-------------------|------|------|-------------|
|                       | n                 | Mean | SD   | Range       |
| All swimmers          | 38                | 14.3 | 22.8 | 0 – 105.5   |
| Adult ( $>18$ )       | 21                | 6.3  | 16.7 | 0 – 60.6    |
| Child ( $\leq 18$ )   | 17                | 24.2 | 28.9 | 0.4 – 105.5 |
| Young child $\leq 10$ | 4                 | 47.6 | 45.7 | 0.4 – 105.5 |
| Young adult $>10$     | 34                | 10.4 | 18.2 | 0 – 60.6    |
| Males                 | 25                | 15.6 | 26.9 | 0 – 105.5   |
| Females               | 13                | 11.9 | 19.4 | 0 – 54.6    |
| Lap swimmers          | 9                 | 8.4  | 19.8 | 0 – 60.6    |
| Leisure swimmers      | 28                | 16.1 | 25.7 | 0 – 105.5   |

Table 5: Urinalysis participant ( $n=38$ ) measured ingestion volumes categorized by mouthful and compared with self-reported mouthfuls. There is no difference between reported and measured ingestion magnitude using a Chi-2 test among the 38 urinalysis participants ( $p > 0.878$ ).

|                    | Self-reported               |                      |                      |                      |   |
|--------------------|-----------------------------|----------------------|----------------------|----------------------|---|
|                    | No water to a few drops (%) | 1 to 2 mouthfuls (%) | 3 to 5 mouthfuls (%) | 6 to 8 mouthfuls (%) |   |
| Measured Ingestion | No water to a few drops     | 11 (28.9)            | 14 (36.8)            | 7 (18.4)             | 0 |
|                    | 1 to 2 mouthfuls            | 1 (2.6)              | 4 (10.5)             | 0                    | 0 |
|                    | 3 to 5 mouthfuls            | 0                    | 1 (2.6)              | 0                    | 0 |
|                    | 6 to 8 mouthfuls            | 0                    | 0                    | 0                    | 0 |

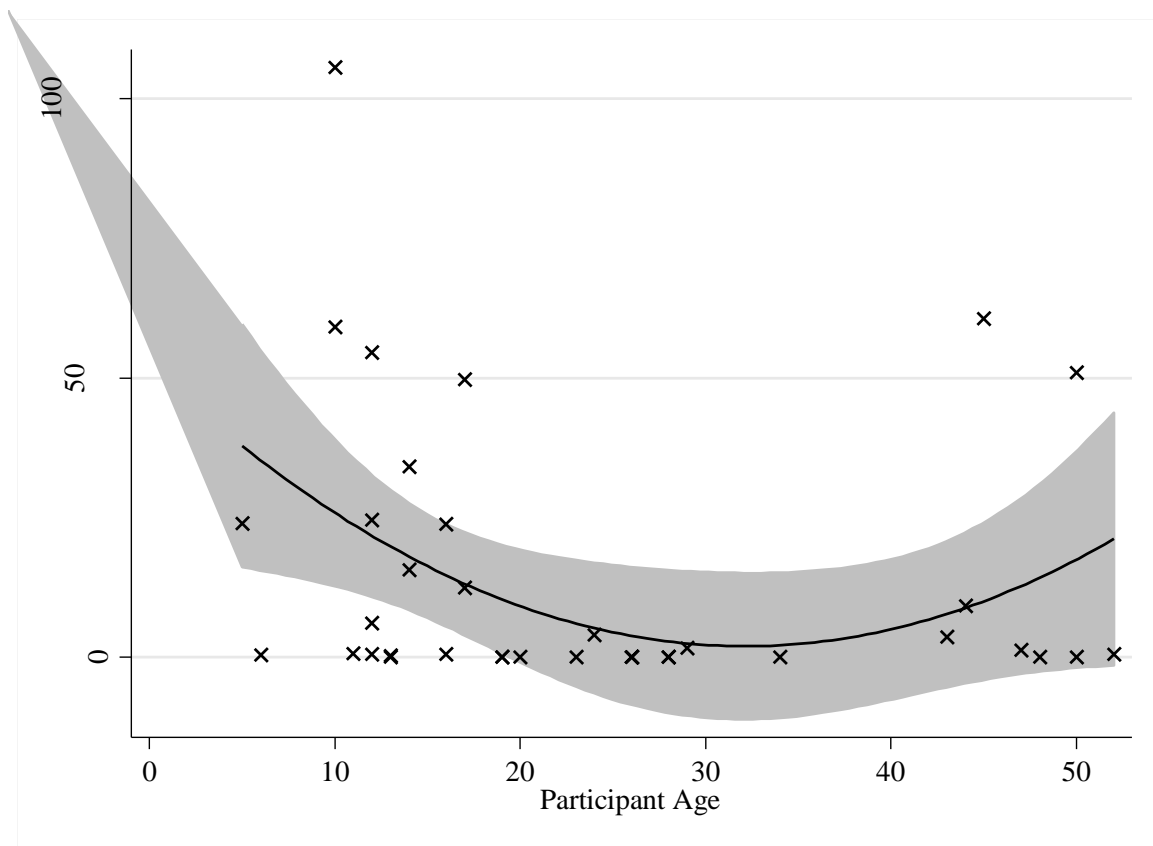


Figure 1: Pool water ingestion (mL/hr) by age. Children ingested more pool water than adults (95 % CI: [-0.8774 – 0.2905]).

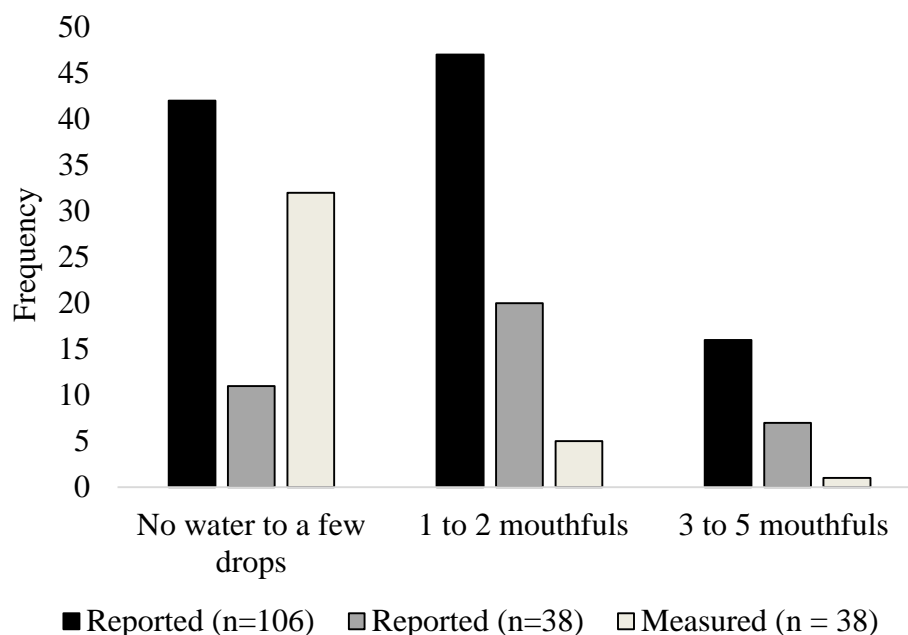


Figure 2: Distribution of reported mouthful volumes by all respondents (n=106) and only urinalysis respondents (n=38). Categorized measured ingestion volumes (n=38) are also shown. No swimmers reported or actually ingested 6-8 mouthfuls, the last question category.

### 3.3.3 Water quality

No associations were found between water quality (free or combined chlorine, pH, and indicator organism concentrations) and reported illnesses, as there was not a large enough sample of pools to compare water quality and reported health outcomes. A positive correlation using Spearman's rank test did exist, however, between cyanuric acid, heterotrophic plate count bacteria, and *Pseudomonas aeruginosa* ( $p < 0.0357$  and  $< 0.0109$ , respectively). Both *Pseudomonas aeruginosa* and fecal coliform bacteria were negatively correlated with free chlorine ( $p < 0.0049$ , and  $< 0.0130$ , respectively).

## **4. Discussion**

### **4.1 Measured Ingestion, Demographics and Activities**

Objectives of this study were to develop, critique, and test electronic, self-administered exposure and operations questionnaires; to identify and quantify risk factors of ingestion; to gather behavior and activity data for use in risk assessment; and to validate the questionnaires' ability to determine ingestion by comparing measured with self-reported volumes. This was accomplished by reviewing and compiling questions from available survey tools, critically reviewing questionnaire drafts, and testing questionnaires at pool facilities where swimmers also submitted a urine sample to quantify ingestion. Results of this study show us that the questionnaire can be used to estimate magnitude of pool water ingestion in swimmers, and can be used in place of urinalysis methods when ingestion magnitude is a desired an outcome. Assessing ingestion magnitude by questionnaire is useful for comparing interventions aimed at reducing pool water ingestion; for assessing swimmer ingestion where cyanuric acid is not present; in outbreak investigations when ingestion exposures have already occurred; and for identifying additional ingestion exposure factors without the expense of urinalysis.

Results from this study also show us that children may be at greater risk of developing RWIs because they ingest higher volumes of pool water. Pool water ingestion is associated with AGI outbreaks, and AGI is often higher in children than in adults (73-83%) (Boehmer et al., 2009; Hellard et al., 2000; Hlavsa et al., 2011; Insulander et al.,

2005; Puech et al., 2001). Children are also more susceptible to gastrointestinal illness because they have underdeveloped immune systems (Buchanan et al., 2000).

Children were identified as an at-risk population because they ingested more pool water than adults. The correlation between pool water ingestion and age  $\leq 18$  is consistent with other research (Dufour et al., 2006). Dufour et al. found on average, children ( $\leq 18$ ) and adult ( $>18$ ) swimmers ( $n=53$ ) ingested 49 and 21 mL/hr of pool water, respectively. Ingestion amounts were lower for this study population (mean of 24.2 mL/hr for participants  $\leq 18$ , and 6.3 mL/hr for participants  $>18$ ), but the trend in ingestion by age is consistent. Lower ingestion values are likely due to assay methodology differences. Dufour et al. applied UV detection and a different SPE urine cleaning process to remove compounds known to interfere with UV detection. Dufour et al. also adjusted the pH of samples to increase UV absorption. Different SPE techniques were applied in this study because UHPLC-MS/MS is more specific than UV detection, and thus can differentiate interfering compounds from cyanuric acid. Recovery of cyanuric acid in this study was 6%. No recovery was reported by Dufour et al. More studies are needed to improve the accuracy of methodology.

No difference in measured or reported pool water ingestion was evident between males and females, or deep- and shallow-end swimmers among the 38 participants with measured ingestion. Differences in reported ingestion were apparent, however, among all 106 respondents. This indicates a larger sample size of participants who complete a questionnaire and submit a urine sample is needed, as reporting differences by gender and activity changed when sample size increased. Differences may have resulted between

genders because the sample size of male and female urinalysis participants (25 males, 13 females) was not large enough to capture perceived ingestion in the general population. Using current data (15/38 swimmers correctly identified the number of pool water mouthfuls ingestion), a power calculation indicates 367 swimmers are needed to produce reliable results. These findings highlight the need for standardized data collection nationally and internationally on swimmer ingestion exposures and risk factors of ingestion.

Although no differences were evident between reported or measured ingestion as mouthful categories among the 38 urinalysis participants, differences were found when measured ingestion was left as a continuous variable, also highlighting the need for more swimming exposure factor data collection. Improvement of the ingestion question is therefore needed to estimate volumes more accurately and beyond magnitude. The current question asks if no water or only a few drops; 1 to 2 mouthfuls (amount in a shot glass); 3 to 5 mouthfuls (amount in a coffee cup); or 6 to 8 mouthfuls (amount in a soda glass) of pool water were swallowed. Developers of the question (Schets et al., 2011) found the average volume of one mouthful to be 27 mL, which was used to categorize measured ingestion volumes in this study to mouthfuls. When 27 mL is substituted for the coded responses, there is not an option representing 6-26 mL because “no water to a few drops” is defined at 0-5 mL by Schets et al., yet both adults and children in this study ingested average volumes within that range. Thus, the ingestion question can be improved by decreasing the sizes of ingestion volume intervals to better estimate lower amounts the majority of swimmers appear to swallow.

Inhaling water through the nasal cavity during recreational swimming is a risk factor for developing Primary Amoebic Meningoencephalitis caused by *Naegleria fowleri* (Carter, 1968; 1970). Sixty-one participants reported inhaling water through the nose, and were more likely to be children, leisure swimmers, and to have swam for longer durations. Diving is the specific leisure activity that likely led to more water inhalation events by children in this study.

Swimmers with diarrhea or gastrointestinal symptoms can contaminate pool water by excreting or shedding enteric pathogens. Participants reported swimming with diarrhea, nausea, and having had diarrhea in the past two weeks. Enteric pathogens associated with RWI outbreaks can be excreted up to 50 days after gastrointestinal symptoms cease, which can lead to pool water contamination by previously-ill swimmers (Chappell et al., 1996). Improvement of tools that aim to educate swimmers about the risks associated with swimming during and after illness is recommended.

## **4.2 Water Quality**

Correlations between high cyanuric acid and indicator organism concentrations are consistent with other research. Cyanuric acid is used in outdoor pools to reduce the photodegradation rates of chlorine, but concentrations above 50 mg/L can inhibit chlorine disinfection efficacy, therefore allowing microbial proliferation when free chlorine levels test at or above recommended concentrations (Andersen, 1965; Shields et al., 2008). It is also expected that higher indicator organism concentrations would be found in lower-chlorine environments, which is consistent with water quality results in this study. No

correlations between water quality and swimmer health (based on a follow-up questionnaire assessing gastrointestinal and respiratory symptoms), or water quality and reported water chemistry, were found due to low numbers of reported illnesses, and low sample number of pools.

Further research is needed to infer any true associations between reporting longer swim durations at pool visits throughout the year and having asthma, as asthmatics often swim because it is regarded as a safe activity for people with respiratory ailments.

## **5. Limitations**

Swimmer sample size was not large enough to capture a distribution of participants developing or swimming with recreational waterborne illnesses. There was also not a large enough sample of pools to compare water quality data with questionnaire responses, but collecting more information from both swimmers and pool operators using the questionnaires can increase the sample size and reliability of these data.

Results are also limited by the underestimated ingestion volumes apparent in this study compared with similar studies. Eight methods for quantifying cyanuric acid in urine, and one available method for pool water, were critically reviewed and considered for use in this study (Cantu et al., 2000; Cantu et al., 2001a; Cantu et al. 2001b; Cantu et al. 2001c; Dorevitch et al., 2011; Smoker and Krinitsky, 2008; Panuwet et al., 2010; Patel & Jones, 2007). All methods applied varying urine cleaning and analysis techniques. Improvements in assay methodology for urine are recommended to increase the validity and consistency of results. The study location may limit the generalizability of results to

the national or international swimming communities. Participants in this study swam on average 72.7 times/year compared to 13 times/year in the Netherlands (Schets et al., 2011). Results may be more consistent with swimmers living in climates similar to Southern Arizona. Expanding the geographic region where data is collected can increase the likelihood risk factor differences across broader demographic groups and venues will be recognized.

## **6. Conclusion**

The objectives of this research were achieved, as the study validated a survey tool for assessing pool water ingestion magnitude, identified risk factors of ingestion, quantified exposure factors useful for risk assessment, and made exposure and pool operation questionnaires available electronically for use by outbreak investigators and exposure scientists. Results of this study could have three important implications for exposure scientists, risk assessors, and government officials:

- The questionnaire can be used to estimate magnitude of pool water ingestion in swimmers in place of urinalysis methods when ingestion magnitude is the desired an outcome,
- Health departments investigating pool-related outbreaks now have electronic, self-administered questionnaires, and
- Exposure scientists now have an electronic tool available for collecting swimmer activity and ingestion risk factor data useful for quantitative risk assessment.

Access to self-administered, electronic questionnaires reduces costs associated with survey administration, data organization, and statistical analysis. Use of the questionnaires nationally and internationally can contribute to a centralized and standardized database of exposure and operations risk factors related to RWI in treated water venues. Increasing swimmer and pool sample sizes and expanding the geographic region where data is collected improves the likelihood risk factor differences across broader demographic groups and venues will be recognized. Both surveys can be used in outbreak investigations or routine data collection and are available online at the following link: [www.ghi.arizona.edu](http://www.ghi.arizona.edu).

## **7. Acknowledgements**

Funding for this research was provided by the National Swimming Pool Foundation and Research Foundation for Health and Environmental Effects. Questionnaire development was made possible with assistance from Kristen Pogreba Brown from the University of Arizona's Foodborne Illness Outbreak Investigation Team. Questionnaires were reviewed by Marlene Gaither, Alfred Bosch, Mary Ostrowski, Martin Keuten, Gabriella Aggazzotti, Guglielmina Fantuzzi, Cristina Villanueva, Franciska Schets, Vincenzo Romano Spica, Que Hales, Sean Assam, Connie Sue Centrella, Beth Hamil, Wayne Ivusich, Bob Williams, Kristen Pogreba-Brown, Mary Kay O'Rourke, Joseph Tabor, Lynn Gerald, and Maia Ingram. Training in video surveillance methods was provided by Paloma Beamer at the University of Arizona's College of Public Health. Meredith Lisse and Leena Patel in the Mel and Enid Zuckerman College of Public Health

assisted with water sample collection and analysis, site and participant recruitment, and urinalysis. The researchers would also like to thank all those who assisted in the review, critique and piloting of the swimmer and operations questionnaire and all of the volunteers who donated their time to participate in this study.

## 8. References

- Allen, L. M., Briggles, T. V., & Pfaffenberger, C. D., 1982. Absorption and excretion of cyanuric acid in long distance swimmers. *Drug Metabolism Reviews* 13 (3), 499-516.
- Andersen, J. R., 1965. A study of the influence of cyanuric acid on the bactericidal effectiveness of chlorine. *American Journal of Public Health and the Nations Health* 55 (10), 1629-1637.
- Barwick, R. S., Levy, D. A., Craun, G. F., Beach, M. J., & Calderon, R. L., 2000. Surveillance for waterborne-disease outbreaks--United States, 1997-1998. *MMWR CDC Surveillance Summary* 49 (4), 1-21.
- Bernard, A., Carbonnelle, S., Michel, O., Higuete, S., de Burbure, C., Buchet, J. P., Doyle, I., 2003. Lung hyperpermeability and asthma prevalence in schoolchildren: unexpected associations with the attendance at indoor chlorinated swimming pools. *Occupational and Environmental Medicine* 60 (6), 385-394.
- Bernard, A., Nickmilder, M., & Voisin, C., 2008. Outdoor swimming pools and the risks of asthma and allergies during adolescence. *European Respiratory Journal* 32 (4), 979-988.
- Boehmer, T. K., Alden, N. B., Ghosh, T. S., & Vogt, R. L., 2009. Cryptosporidiosis from a community swimming pool: outbreak investigation and follow-up study. *Epidemiology and Infection* 137 (11), 1651-1654.
- Briggles, T. V., Allen, L. M., Duncan, R. C. & Pfaffenberger, C. D. 1981. High performance liquid chromatography determination of cyanuric acid in human urine and pool water. *Journal of the Association of Official Analytical Chemists*. 64 (5), 1222-1226.
- Buchanan, R. L., Smith, J. L., & Long, W., 2000. Microbial risk assessment: dose-response relations and risk characterization. *International Journal of Food Microbiology* 58 (3), 159-172.
- Cantu, R., Evans, O., Behymer, T. D., Shoemaker, J. A., Kawahara, F. K., & Dufour, A. P., 2001a. Simple sample clean up procedure and high performance liquid chromatographic method for the analysis of cyanuric acid in human urine. *Abstracts of Papers of the American Chemical Society*, 221, U479-U479.
- Cantu, R., Evans, O., Kawahara, F.K., Wymer, L.J. and Dufour, A.P., 2001b. HPLC

- determination of cyanuric acid in swimming pool waters using phenyl and confirmatory porous graphitic carbon columns. *Analytical Chemistry* 73 (14), 3358-3364.
- Cantu, R., Evans, O. and Magnuson, M.L., 2001c. Rapid analysis of cyanuric acid in swimming pool water by high performance liquid chromatography using porous graphite carbon. *Chromatographia* 53 (7/8) 454-456.
- Cantu, R., Evans, O., Kawahara, F. K., Shoemaker, J. A., & Dufour, A. P., 2000. An HPLC method with UV detection, pH control, and reductive ascorbic acid for cyanuric acid analysis in water. *Analytical Chemistry* 72 (23), 5820-5828.
- Carter, R. F., 1970. Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningo-encephalitis and of the experimental pathological changes induced by it. *The Journal of Pathology and Bacteriology* 100 (4), 217-244.
- Carter, R. F., 1968. Primary amoebic meningoencephalitis: clinical pathological and epidemiological features of six fatal cases. *The Journal of Pathology and Bacteriology* 96 (1), 1-25.
- Centers for Disease Control and Prevention, 2013. Recreational water illness response toolkit. Accessed March, 2013 from:  
<http://www.cdc.gov/healthywater/emergency/toolkit/rwi-outbreak-toolkit.html>.
- Chappell, C. L., Okhuysen, P. C., Sterling, C. R., & DuPont, H. L., 1996. *Cryptosporidium parvum*: Intensity of infection and oocyst excretion patterns in healthy volunteers. *Journal of Infectious Diseases* 173 (1), 232-236.
- Craun, G. F., Brunkard, J. M., Yoder, J. S., Roberts, V. A., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J.M., Beach, M.J., Roy, S. L., 2010. Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews*. 23 (3), 507.
- Craun, G. F., Calderon, R. L., & Craun, M. F., 2005. Outbreaks associated with recreational water in the United States. *International Journal of Environmental Health Research* 15 (4), 243-262.
- Dang, B., Chen, L. L., Mueller, C., Dunn, K. H., Almaguer, D., Roberts, J. L., & Otto, C. S., 2010. Ocular and Respiratory Symptoms Among Lifeguards at a Hotel Indoor Waterpark Resort. *Journal of Occupational and Environmental Medicine* 52 (2), 207-213.
- Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P., Wroblewski, M., Liu, L., Scheff, P.S., & Li, A., 2011. Water ingestion during water recreation. *Water Research* 45 (5), 2020-2028.
- Dufour, A. P., Evans, O., Behymer, T. D., & Cantu, R., 2006. Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water Health* 4 (4), 425-430.
- Fantuzzi, G., Righi, E., Predieri, G., Giacobazzi, P., Mastroianni, K., & Aggazzotti, G., 2010. Prevalence of ocular, respiratory and cutaneous symptoms in indoor swimming pool workers and exposure to disinfection by-products. *International Journal of Environmental Research and Public Health* 7 (4), 1379-1391.

- Font-Ribera, L., Kogevinas, M., Zock, J. P., Nieuwenhuijsen, M. J., Heederik, D., & Villanueva, C. M., 2009. Swimming pool attendance and risk of asthma and allergic symptoms in children. *European Respiratory Journal* 34 (6), 1304-1310.
- Hellard, M. E., Sinclair, M. I., Fairley, C. K., Andrews, R. M., Bailey, M., Black, J., Dharmage, S.C., Kirk, M. D., 2000. An outbreak of cryptosporidiosis in an urban swimming pool: why are such outbreaks difficult to detect? *Australian and New Zealand Journal of Public Health* 24 (3), 272-275.
- Hlavsa, M. C., Roberts, V. A., Anderson, A. R., Hill, V. R., Kahler, A. M., Orr, M., Garrison, L., Hicks, L., Newton, A., Hilborn, E., Wade, T., Beach, M., Yoder, J. S., 2011. Surveillance for waterborne disease outbreaks and other health events associated with recreational water - United States, 2007-2008 *MMWR Surveillance Summary* 60, 1-32.
- Insulander, M., Lebbad, M., Stenstrom, T. A., & Svenungsson, B., 2005. An outbreak of cryptosporidiosis associated with exposure to swimming pool water. *Scandinavian Journal of Infectious Diseases* 37 (5), 354-360.
- Levesque, B., Duchesene, J. F., Gingras, S., Lavoie, R., Prud'Homme, D., Bernard, E., Boulet, L.P., Ernst, P., 2006. The determinants of prevalence of health complaints among young competitive swimmers. *International Archives of Occupational and Environmental Health* 80 (1), 32-39.
- Panuwet, P., Wade, E. L., Nguyen, J. V., Montesano, M. A., Needham, L. L., & Barr, D. B., 2010. Quantification of cyanuric acid residue in human urine using high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 878 (28), 2916-2922.
- Patel, K., & Jones, K., 2007. Analytical method for the quantitative determination of cyanuric acid as the degradation product of sodium dichloroisocyanurate in urine by liquid chromatography mass spectrometry. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 853 (1-2), 360-363.
- Puech, M. C., McAnulty, J. M., Lesjak, M., Shaw, N., Heron, L., & Watson, J. M., 2001. A statewide outbreak of cryptosporidiosis in New South Wales associated with swimming at public pools. *Epidemiology and Infection* 126 (3), 389-396.
- Roy, S. L., Scallan, E., & Beach, M. J., 2006. The rate of acute gastrointestinal illness in developed countries. *Journal of water and health* 4 (2), 31-69.
- Schets, F. M., Schijven, J. F., & Husman, A. M. D., 2011. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research* 45 (7), 2392-2400.
- Shields, J. M., Hill, V. R., Arrowood, M. J., & Beach, M. J., 2008. Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *Journal of Water and Health* 6 (4), 513-520.
- Smoker M., Krynitsky, A., 2008. Interim method for determination of melamine and cyanuric acid residues in foods using LC-MS/MS: 1.0. U.S. Food and Drug Admin LIB. 1, 4422.
- Suppes, L.M., Abrell, L., Dufour, A.P. & Reynolds, K.A., unpublished results.

- Assessment of swimmer head submersion influence on pool water ingestion.  
Journal of Water and Health. Manuscript submitted for publication.
- United States Environmental Protection Agency, 2013. Swimmer exposure assessment model (SWIMODEL). Accessed March, 2013 from:  
<http://www.epa.gov/oppad001/swimodel.htm>.
- World Health Organization, 2003. Guidelines for safe recreational water environments, swimming pool and similar environments 1, 1-171.
- Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V., Roberts, V., Yu, P. A., Hicks, L.A., Alexander, N.T., Calderon, R.L., Roy, S.L., Beach, M. J., 2008. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events - United States, 2005 - 2006 MMWR Surveillance Summary. 57, 1-29.

## APPENDIX B - ASSESSMENT OF SWIMMER HEAD SUBMERSION INFLUENCE ON POOL WATER INGESTION

Paper was prepared to submit to The Journal of Water and Health

Laura M. Suppes<sup>1</sup>, Leif Abrell<sup>2</sup>, Alfred P. Dufour<sup>3</sup>, Kelly A. Reynolds<sup>4</sup>

<sup>1,4</sup>The University of Arizona Mel and Enid Zuckerman College of Public Health P.O.  
Box 245163 Tucson, Arizona 85724, USA, Fax: 520-321-7424

<sup>3</sup>United States Environmental Protection Agency Microbiological and Chemical  
Environmental Research Division, National Exposure Research Laboratory, U.S. EPA  
26 West Martin Luther King Drive, Mail Stop 593, Cincinnati, OH 45268, USA

<sup>2</sup>The University of Arizona Department of Soil, Water & Environmental Science Gould-  
Simspon Building Rooms 828, 848, 1040 East 4th Street Tucson, AZ 85721, USA

<sup>1</sup>[Suppeslm@email.arizona.edu](mailto:Suppeslm@email.arizona.edu), <sup>2</sup>[Abrell@email.arizona.edu](mailto:Abrell@email.arizona.edu),  
<sup>3</sup>[Dufour.Alfred@epamail.epa.gov](mailto:Dufour.Alfred@epamail.epa.gov) <sup>4</sup>[Reynolds@email.arizona.edu](mailto:Reynolds@email.arizona.edu)

### List of Abbreviations:

AGI - Acute Gastrointestinal Illness  
ESI - Electrospray Ionization  
LOD - Limit of Detection  
RWI - Recreational Waterborne Illness  
SAX - Strong Anion Exchange  
S/N - Signal to Noise Ratio  
SPE - Solid Phase Extraction  
UHPLC-MS/MS - Ultra High Pressure Liquid Chromatography - Tandem Mass  
Spectrometry

### Abstract

Enteric pathogens in pool water can be unintentionally ingested during swimming,  
increasing the likelihood of Acute Gastrointestinal Illness. Acute Gastrointestinal Illness

cases in outbreaks are more likely to submerge heads than non-cases, but a true association is unknown because outbreak data are self-reported and can be biased. Head submersion frequency and duration were observed and analyzed for associations with pool water ingestion measured by urinalysis. Frequency of splashes to the face were also quantified. Reliable tools that assess activities associated with pool water ingestion are needed to identify at-risk sub-populations. Objectives were to determine if the observed activities were associated with ingestion, and to test environmental sensor and videography assessment tools. Greater frequency and duration of head submersion were not associated with ingestion, but frequency of splashes to the face, leisurely swimming, and being  $\leq 18$  were. Videography was validated for assessing swimmer head submersion frequency. Results demonstrate a need for more research exploring relationships between pool water ingestion, less frequent head submersion, and skill to clarify why children ingest more pool water than adults. Expanding surveys to include questions on leisure swimming participation and frequency of splashes to the face is recommended to improve exposure assessment during outbreak investigations.

Keywords: Exposure factor, head submersion, pool water ingestion, recreational water, swimming pool, urinalysis

## **Introduction**

Recreational Waterborne Illness (RWI) in the United States was at an all-time high between 2007 and 2008 since reporting began in 1978, with 38 states reporting a total of 134 outbreaks (Hlavsa et al., 2011; Yoder et al., 2008). Ingestion was the most common

source of exposure (60.4%), but direct skin contact (18.7%), inhalation (13.4%), mixed exposure routes (4.5%) and other (3.0%) were also identified. Health outcomes ranged from Acute Gastrointestinal Illness (AGI), respiratory ailments, skin infections and irritations, and hospitalization.

The causative agents in RWIs are as varied as the exposure route, however several trends are notable: 1) the majority of outbreaks (60.4%; 81/134) resulted in AGI; 2) the majority of outbreaks (86.5%; 116/134) and illnesses (96.5%; 13,480/13,966) occurred in treated water venues; and 3) most were caused by the protozoa, *Cryptosporidium* spp. (44.8%; 60/134). Other etiological agents of RWIs between 2007 and 2008 include: Norovirus, *Giardia*, *Leptospira*, *Vibrio* spp., *Campylobacter jejuni*, and *Shigella sonnei*. Swimmer exposure to waterborne enteric pathogens is via the fecal oral route. Accurate assessment of pool water ingestion rates during swimming activities is important for assessing enteric pathogen infection risk. Swimming pool water ingestion can be measured by comparing cyanuric acid concentrations in swimmer urine to pool water (Allen et al., 1982; Briggles et al., 1981; Dufour et al., 2006).

Cyanuric acid is a chemical added to outdoor pool water to prevent photodegradation rates of chlorine, and when ingested, is not metabolized by the human body and is completely excreted within 24 hours (Allen et al., 1982; Andersen, 1965). Cyanuric acid is present in household cleaning materials (bleach, cleansers, dishwashing compounds,

and sanitizers), but is not absorbed or inhaled (Allen et al., 1982; Cantu et al., 2000).

Ingestion during swimming is therefore a primary exposure to cyanuric acid.

Reports following AGI outbreaks at swimming pools show cases are more likely to submerge heads than non-cases (Boehmer et al., 2009; Causer et al., 2006); however, the distribution of head submersion duration and frequency among swimmers is unknown, as the majority of outbreak and exposure assessment data is self-reported and can be biased. Further, activities like lap and leisure swimming may lead to different water ingestion exposures if head submersion frequency and duration differ between activities, or if other micro-activities differ. The objectives of this study were to evaluate risk factors of ingestion and test exposure assessment tools. To achieve this, pool water ingestion was quantitatively measured using urinalysis. Head submersion frequency and duration, and number of splashes to the face, were also quantified by videography and environmental sensor methods. The observed activities were analyzed for associations with pool water ingestion. Other activities suspected of increasing pool water ingestion were noted during videography to make recommendations for future research. Quantitative activity and exposure information were also separated by age and activity for use in future risk assessments.

## **Methods**

### **Measuring Pool Water Ingestion**

Four pool sites and 126 swimmers were recruited. Free and informed consent of the participants or their legal representatives was obtained using a consent form approved by the University of Arizona Human Subjects Research and Institutional Review Board. Participants submitting urine (n=46) were instructed not to swim 24 hr before or after the swimming trial event. Each participant was given a sterile 3 L urine collection container (Fischer Scientific Company, 14-375-248) for a 24 hr post-swimming composite sample. Participants were excluded if sufficient urine volumes based on age were not provided (Dorevitch et al., 2011). Urine volumes for age <10 had to exceed 150 mL; 200 mL for 11-16 years; and 300 mL for >17 years. Pool water samples were collected 30 cm below the surface using 20 mL containers from four locations within each pool, transferred on ice, and stored at 4°C. Pool water and urine were processed within 24 hrs of collection at the University of Arizona Medical Research Building and tested for cyanuric acid within 31 days of collection at the university's Arizona Laboratory for Emerging Contaminants using previously published methods (Cantu et al., 2001a; Cantu et al., 2000; Smoker & Krynitsky, 2008). To preserve samples, 170 µL of pool water or urine was pipetted into a 2 mL microcentrifuge tube. A 10% perchloric acid (v/v) and 1% metaphosphoric acid (w/v) solution was added to each tube. Samples were centrifuged at 13,500 g for 17 min to remove proteins, kept in the dark at 4°C, and processed within 31 days.

Urine samples were cleaned by Solid Phase Extraction (SPE) with Strong Anion Exchange (SAX) cartridges (SampliQ Silica, 3 mL, 500 mg; Agilent, 5982-2035) on a benchtop SPE manifold (Vac Elute 12 Manifold; Agilent, 5982-9110). The cartridges were conditioned by gravity with 2.5 mL of acetonitrile, followed by 2.5 mL of 5% aqueous ammonium hydroxide. Cartridges were loaded by gravity with a mixture of 1.6 mL of 5% aqueous ammonium hydroxide and 1.2 mL of urine sample followed by 2.5 mL of acetonitrile. Cartridges were dried under vacuum (-10 psi) for 30 sec followed by elution of cyanuric acid with 1 mL of 4% formic acid in acetonitrile. Remaining eluent was collected by a final, short vacuum application before analysis by Ultra High Pressure Liquid Chromatography - Tandem Mass Spectrometry (UHPLC-MS/MS). Pool water samples (without SPE) were diluted 1:20 before analysis by UHPLC-MS/MS.

Selected reaction monitoring data were recorded on a Quattro Premier XE triple-quadrupole mass spectrometer (Waters Corp., Milford, MA) by electrospray ionization in the negative mode (ESI<sup>-</sup>). 2.0 µL of pool water and urine extracts were injected into an Acquity UHPLC (Waters Corp., Milford, MA) isocratic mobile phase of 50% (v/v) aqueous acetonitrile at 0.05 mL/min. Each urine and pool water sample was injected twice between blank MilliQ lab water in separate runs. Selected reaction monitoring chromatograms were obtained by monitoring the cyanuric acid transition 128 > 42 in ESI<sup>-</sup> with capillary, cone, and collision energy voltages of 2.9 kV, 22 V, and 12 V, respectively. The ion source temperature was kept at 120°C and desolvation gas temperature was set to 250°C. Nitrogen was used as both cone gas and desolvation gas,

and high purity argon was used as the collision gas ( $9.25 \times 10^{-3}$  mbar). Cyanuric acid peaks eluting at 4.7 min were integrated and quantified using TargetLynx Application Manager software (Waters Corp., Milford, MA). Standard curves were created from a 20 mg/L stock solution of cyanuric acid that was prepared by dissolving 100% cyanuric acid (MP Biomedical 0520871580) in MilliQ water and filtering through a 0.2  $\mu\text{m}$  cellulose filter. A set of eleven calibration standards of 1.0, 2.0, 4.0, 7.0, 15, 31, 62, 125, 250, 500, and 1000  $\mu\text{g/L}$  were prepared by serial dilution with 10% methanol in MilliQ purified water. The UHPLC-MS/MS Limit of Detection (LOD) was defined as having a signal to noise ratio (S/N) greater than 3.0. The method LOD was determined by identifying the lowest concentration recovered from participant urine after blank subtraction.

Quality control measures were taken to ensure accuracy and validity of analytical results. Three controls for pool water, and four controls for urine, were analyzed. Pool water controls included a MilliQ lab water blank; a pool water blank (no cyanuric acid used at the pool); and a 15  $\mu\text{g/L}$  pool water spike. Pool water quality controls were not passed through SAX cartridges. Urine controls were passed through SAX cartridges and included a blank control urine; a blank MilliQ water field control; a blank deionized water with preservative lab control; and a 50  $\mu\text{g/L}$  control urine spike. The 50  $\mu\text{g/L}$  urine spike passed through an SAX cartridge was used to estimate percent recovery of cyanuric acid. The deionized water-preservative control was made at the time urine and pool water were preserved. The blank MilliQ water field control was collected by instructing a non-swimmer to add MilliQ water to a urine collection container over 24 hrs in a volume

approximate to the participant's urination volume per-episode. Another non-swimmer provided a 24 hr urine sample considered the blank urine control.

Ingestion volumes were adjusted using blank subtraction for potential false positives from cyanuric acid carry-over. Participants with average cyanuric acid concentrations in urine lower than blanks were considered measurements less than the method LOD. Urine samples with a  $S/N \geq 3$ , in which no cyanuric acid was detected were also considered measurements lower than the method LOD.

Water ingestion volumes were calculated using cyanuric acid concentrations in urine and pool water (Eq. 1) (Allen et al., 1982; Briggles et al., 1981; Dufour et al., 2006). Urine containers were weighed before and after urine collection to determine gravimetric urine volume. Thus, dilution effects were adjusted by multiplying the ratio of cyanuric acid in urine to pool water by the total volume of urine collected over 24 hrs, during which all cyanuric acid was excreted and collected in the container.

$$\text{Water Ingestion (L)} = \left( \frac{[\text{Cyanuric Acid}]_{\text{urine}} \left( \frac{\mu\text{g}}{\text{L}} \right)}{[\text{Cyanuric Acid}]_{\text{pool water}} \left( \frac{\mu\text{g}}{\text{L}} \right)} \right) \times \text{Urine Volume (L)} \quad (1)$$

### **Videography and Questionnaire**

Sixty-four of 126 swimmers were recorded on video-tapes. Swimmers were excluded (n=62) if swimming off-camera for a portion of the time, not visible at the given camera angle, or not visible because of poor lighting conditions. Each swimmer was equipped

with a color coded headband and associated identification number, and was instructed to state the number into a camera before entering pool water. Two digital cameras with tripods were placed in different locations on pool decks to ensure each swimmer was filmed. After filming, each video was previewed to determine which angle best captured the swimmer's activity, and at what time individual swimmers entered the water. Water entry times of participants on the two videos were recorded. Each swimmer was observed for the entire length of the swim on one video-tape in 10 min consecutive segments by three trained reviewers. Reviewing in ten minute segments was applied to reduce viewer fatigue (Ferguson et al., 2006). Video reviewer training included overviews of pre-determined head submersion and face-splash definitions and a 20 min viewing session with another reviewer to compare results and ensure variability <10% (Ferguson et al., 2006). Head submersion frequency and duration were quantified using a smartphone application (Stopwatch, the Official Timer Eflag Corporation), which records time in hours, minutes, seconds, and milliseconds (submersion duration), and the number of times the stopwatch is started (submersion frequency). Splashes of water to the face were tallied by each reviewer. Four of 64 swimmers (6.3%) were viewed twice to estimate method variability. Total time in water was quantified by viewing videos and used to adjust pool water ingestion volumes (mL/hr), submersion frequencies (frequency/hr), and submersion duration (minutes submerged/hr). Age, gender, and type of swimming activity (splashing, playing, diving, wading, sitting, and lap swimming) were determined by questionnaire (Suppes et al., unpublished results).

### **Environmental Sensors**

The temperature-logging environmental sensors worn by swimmers were developed by attaching i-Button thermometers (Thermochron DS1922L) to the light mounting bracket on modified elastic headbands (1200 Lumens 6 LED 3 Mode Headlamp) (Figure 1). The i-Button is water resistant, has a temperature range from -40 to 85°C; is accurate to  $\pm 0.5^\circ\text{C}$ , and can store up to 8,192 readings logged every 1 to 600 sec. Fifteen headbands were color coded with vinyl tape (Fischer Scientific 19040024) and assigned a number. The i-Buttons were programmed to begin logging a temperature every 3 sec with One-wire Viewer Software (Maxim Integrated) a half hour before swimmers entered the water. Up to 15 swimmers per site visit (10 visits) were provided a color coded headband and identification number after signing a consent form approved by The University of Arizona Human Subjects Research and Institutional Review Board. Temperatures were uploaded using One-wire Viewer Software for analysis. Measurements lower than the air temperature by  $0.5^\circ\text{C}$  were considered a head submersion event. Head submersions were identified by declines in the temperature output. Total decline events  $\geq 0.5^\circ\text{C}$  were counted to quantify head submersion frequency, and the total time (seconds) of each decline event was recorded as the time between head submersion and head emergence and added to the total time of all events to determine head submersion duration. The i-Button was pre-tested in a controlled environment to examine the instrument's ability to record a lower temperature in water.



Figure 1: Environmental sensor

### **Data Analysis**

STATA Statistics/Data Analysis Version 11.0 Software (College Station, TX) was used to perform statistical tests. Pearson's correlation and unpaired t-tests were applied to determine associations between head submersion durations and frequencies and frequencies of receiving a splash to the face, and pool water ingestion in all swimmers, as well as child, adult, lap, and leisure swimmers. A Chi-2 test was used to assess age differences in lap and leisure swimming groups. Swim duration, age, average duration of pool visits throughout the year, and frequency of receiving a splash to the face were included in a stepwise regression model using backwards selection to identify influential variables on pool water ingestion. Videography and environmental sensor methods were analyzed with a paired t-test. Associations were considered statistically significant at a p-value  $<0.05$  (95% confidence).

## **Results**

Thirty-eight of 46 (82.6%) urine samples were usable, and 35 of the 38 participants (92.1%) were analyzable on video. The three swimmers not analyzable by video were either swimming off-camera for a portion of the time, not visible at the given camera angle, or not visible because of poor lighting conditions. The eight swimmers with unusable urine samples either did not submit a questionnaire (no data on age or activity for statistical analysis,  $n=7$ ), or submitted a urine sample below the required volume ( $n=1$ ). Twenty-nine additional swimmers who did not submit urine were observed on video (total viewed,  $n=64$ ) (Table 1). Four of 64 participants did not report age or swim activity type (lap or leisure).

### **Videography Method Validation**

The average head submersion frequency and standard deviation reported by two video reviewers on the same four swimmers was  $134.3 \pm 12.4$  submersions (8.5% variation from the mean). Durations between reviewers differed on average  $\pm 1$  min 19 seconds (average, 8 min 8 sec; 12.8% variation from the mean), and splashes by  $\pm 2.1$  (average, 5.25; 27.8% variation from the mean). Acceptable interobserver variability in exposure assessment (variation between reviewers) by videography is  $<10\%$  (Ferguson et al., 2006).

Table 1: Measured ingestion, videography, and environmental sensor sample sizes by age and activity.

| Data Source                         | All Participants (%) | Adults (%) | Children (%) |
|-------------------------------------|----------------------|------------|--------------|
| All participants                    | 64                   | 26 (40.6)* | 34 (53.1)*   |
| Ingestion                           | 35 (54.7)            | 20 (57.1)  | 16 (45.7)    |
| Videography                         | 64 (100)             | 26 (40.6)* | 33 (53.1)*   |
| Environmental Sensors               | 41 (64.1)            | 16 (39.0)* | 22 (56.7)*   |
| Ingestion and videography           | 35 (54.6)            | 19 (54.3)  | 16 (45.7)    |
| Ingestion and environmental sensors | 24 (37.5)            | 11 (45.8)  | 13 (54.2)    |

\*Four of 64 participants did not report an age on the questionnaire. All participants with measured ingestion values reported age.

### **Environmental Sensors, Videography, and Water Ingestion**

Sixty-four swimmers were recorded on video-tapes, and 35 of 64 had measured ingestion values. Twenty-four participants with measured ingestion values had analyzable environmental sensor data. No associations were found using Pearson's correlation test between pool water ingestion and head submersion frequency or duration measured by videography ( $p > 0.3445$  and  $0.4570$ , respectively), but, swimmers ingesting more water tended to have fewer head submersions (Figure 2), suggesting greater head submersion frequencies and durations are not driving pool water ingestion. Swimmers engaging in less frequent head submersion were suspected to have participated in other micro-activities associated with ingestion not quantified in this research. Based on videography

behavior assessment, there was no visible trend between water ingestion and head submersion durations.

Head submersion and duration measured by environmental sensors showed poor accuracy with videography measurements when compared using a paired t-test. Submersion frequency was under-estimated by the sensors ( $p < 0.000$ ), and duration was over-estimated ( $p < 0.001$ ) relative to the video analysis estimates. Average submersion frequency from videography was 199.1 submersions/hr, and 8.5 submersions/hr from environmental sensors. The average submersion duration per hour of swimming was 7 min, 51 sec from video data, and 22 min 7 sec from environmental sensors (Table 2). Environmental sensor data was therefore not used for identifying ingestion exposure micro-activity factors.

Table 2: Sensor and video analysis method comparison. The environmental sensors over-estimated head submersion duration, and under-estimated head submersion frequency.

|      | Frequency of Head Submersions/hr |                      | Duration of Head Submersions (min:sec/hr) |                      |
|------|----------------------------------|----------------------|---|----------------------|
|      | Videography                      | Environmental Sensor | Videography                               | Environmental Sensor |
| Mean | 199.1                            | 9.6                  | 7:51                                      | 22:07                |
| SD   | ±256.3                           | ±5.2                 | ±7:42                                     | ±17:21               |
| Min  | 4.5                              | 1.37                 | 0:23                                      | 0:23                 |
| Max  | 1222                             | 22.2                 | 32:38                                     | 66:00                |

Two distinct types of swimmers were observed during this study, recreational swimmers who engaged in leisure time activities in the water (splashing, playing, diving, wading,

sitting) and lap swimmers who engaged in serious, vigorous exercise in the water. The former we will describe as leisure swimmers and the later as lap swimmers throughout the text. The most influential parameter identified by stepwise regression on pool water ingestion was frequency of receiving a splash to the face ( $R^2=0.3281$ ), which was higher among leisure swimmers (Pearson's correlation test:  $p < 0.0003$ ). The swimmer with the highest ingestion value (105.5 mL/hr) was 10 years old and received 33 splashes to the face, which was above average (all swimmers on average received 12.5 splashes to the face; min: 0, max: 47, SD: 12.16). The swimmer with the most splashes to the face (47 splashes) ingested 35.1 mL/hr of pool water and was a leisure swimmer. Leisure swimmers were more likely using Pearson's correlation test to have less frequent head submersion ( $p < 0.000$ ), a micro-activity that tended to be associated with higher ingestion rates. A Chi-2 test indicated children were more likely to engage in leisure swimming than adults ( $p < 0.001$ ).

Of all participants, 40 reported leisure swimming, defined as splashing, playing, diving, wading, or sitting (26/40 had measurable cyanuric acid), and 20 were lap swimmers (9/20 had measurable cyanuric acid). An unpaired t-test identified leisure swimmers are more likely to ingest pool water than lap swimmers ( $p < 0.024$ ) (Table 3). Submersion duration did not differ between the groups using an unpaired t-test ( $p > 0.510$ ). The five highest pool water ingestion measurements were all child ( $\leq 18$ ) leisure swimmers. Adults ingested 22.2 mL/hr less than children, which was a significant difference using an

unpaired t-test ( $p < 0.0046$ ). All children with measurable cyanuric acid were leisure swimmers. Ten leisure swimmers were  $>18$ .

Table 3: Pool water ingestion by activity and age group among video-taped participants.

| Group            | n  | Ingestion (mL/hr) |      |             |
|------------------|----|-------------------|------|-------------|
|                  |    | Mean              | SD   | Range       |
| All swimmers     | 35 | 13.7              | 24.0 | 0 – 105.5   |
| Adults           | 19 | 3.5               | 11.7 | 0 – 50.9    |
| Children         | 16 | 25.7              | 29.2 | 0.9 – 105.5 |
| Lap swimmers     | 9  | 1.6               | 3.13 | 0 – 9.19    |
| Leisure swimmers | 26 | 17.8              | 26.6 | 0 – 105.5   |

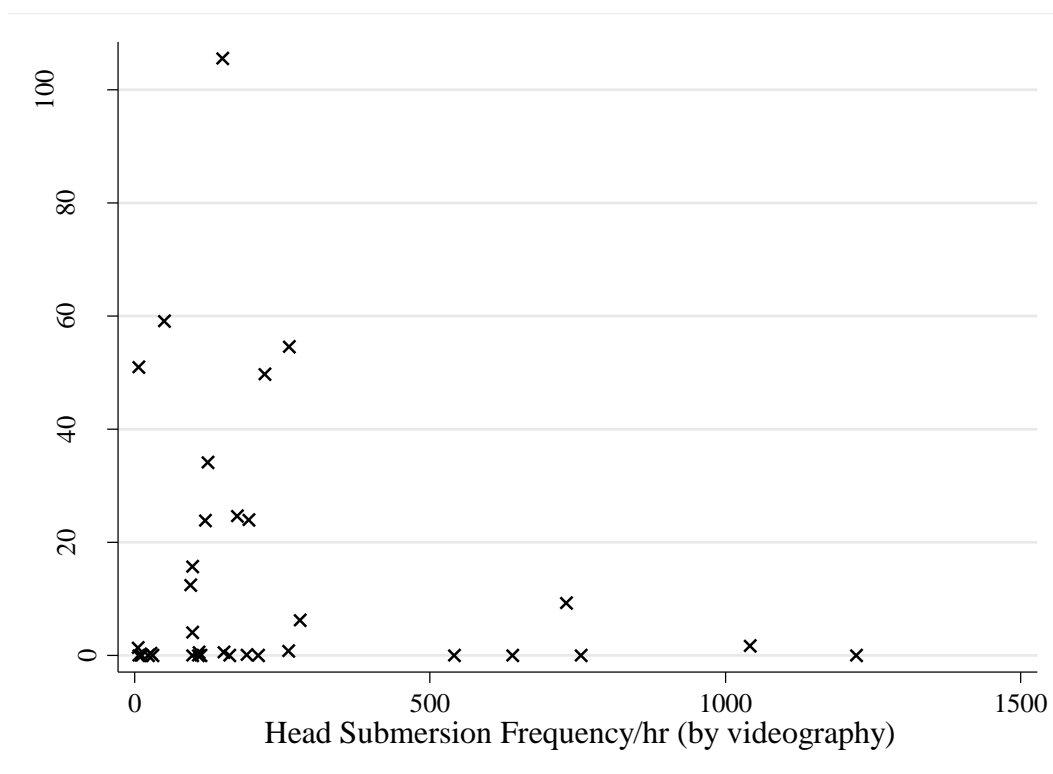


Figure 2: Swimmer head submersion frequency/hr swimming (by videography) and pool water ingestion. Swimmers ingesting pool water tended to have lower head submersion frequencies (95 % CI: [-0.086, 0.067]).

### **Cyanuric Acid Analysis**

A paired t-test indicated cyanuric acid concentrations between injection runs differed ( $p < 0.0420$ ). When average cyanuric acid concentrations carried into blank MilliQ lab water were subtracted from the injection averages of each urine or pool water sample, no statistically significant difference was found using a paired t-test between concentrations ( $p > 0.6237$ ). Thus, the reported ingestion volumes were adjusted for cyanuric acid carry-over.

The UHPLC-MS/MS LOD, 0.263  $\mu\text{g/L}$ , is the lowest measured concentration in urine with a  $S/N \geq 3$ . The method LOD was determined by identifying the lowest calculated ingestion volume after blank subtraction corresponding to a concentration with a  $S/N \geq 3$ , and is 0.05 mL.

Cyanuric acid recovery from pool water was  $103.3 \pm 0.42\%$  based on spiked pool water controls, comparable to efficiencies found by other researchers using similar techniques (Cantu et al., 2001a; Dorevitch et al., 2011). The recovery of cyanuric acid in urine after SPE was 6%, compared to 32.7% by Dorevitch et al. (2011). The majority of cyanuric acid (66.8%) is suspected to have remained in the SAX cartridge following elution steps.

## **Discussion**

### **Environmental Sensors, Video Analysis, and Water Ingestion**

Reliable tools that estimate water ingestion during swimming are needed to assess groups most at-risk of contracting RWI. This study empirically assessed pool water ingestion and associated ingestion volumes with observed activities and behaviors. Novel exposure assessment methods were applied to quantify cumulative time under water, frequency of head submersion, number of splashes to the face, and type of swimming activity.

Associations between these parameters and pool water ingestion measured in lap, leisure, adult and child swimmers were compared using videography, environmental sensing, questionnaire, and urinalysis techniques. Data from both methods were analyzed for significant associations with pool water ingestion, age, and lap and leisure swimming.

Methods used to quantify head submersion frequency by videography were reliable based on interobserver variability rates <10%, the accepted rate in other videography research (Ferguson et al., 2006). Method variation for submersion frequency was 8.5%. Variability in the submersion duration and face-splash quantification methods were higher, however, indicating a need for improvement (variability in swim duration and face-splash quantification were 12.8% and 27.8%, respectively). Given the low variability between submersion counts quantified by reviewers, the videography method can be reliably applied in future research that assesses head submersion frequency. Without quantitative information on behaviors and activities related to head submersion, inferences about pool water ingestion may be inaccurate.

Comparing videography results with environmental sensors showed the sensors over-estimated swimmer time under water and under-estimated head submersion frequency relative to video analysis estimates because the instruments did not record submersion events that lasted <3 seconds. i-Button thermometers were pre-set to read a temperature every three seconds, yet submersions lasting <3 seconds were observed during video analysis. When submersion frequencies are adjusted using a three-fold multiplier, sensor estimates are still under-estimated (26 vs. 199.1 submersions/hr). The instrument was likely not submerged long enough by participants to capture all submersions, despite controlled trials suggesting three second intervals were adequate. Pre-setting the sensors to record temperatures every one second rather than every three may improve the instrument's sensitivity in future studies. The environmental sensor was not effective in this study, but can potentially be applied as a head submersion assessment tool in future swimmer exposure assessment if re-calibrated, as the i-Button thermometers were able to identify temperature differentials between air and water. Improvement of method sensitivity may resolve the instrument's capability to record temperature change when it occurs more often than every three seconds.

Contrary to RWI outbreak reports, less frequent submersion tended to be practiced by swimmers ingesting more pool water. This finding suggests less frequent head submersion should be explored as an ingestion risk factor in future exposure assessments. We recommend additional activities and behaviors be included on outbreak

questionnaires or tools, including engaging in leisure swimming and receiving a splash to the face, as both activities were associated with increased pool water ingestion that was empirically measured.

Leisure swimmers ingested more pool water and submerged heads less frequently than lap swimmers. During videography, analyzers noted leisure swimmers having short and inconsistent submersions, and lap swimmers consistently and frequently submerging heads during activities like the front crawl. Lap swimmers also appeared to hold their breath under water more frequently than leisure swimmers. Some of the most advanced swimmers had the longest submersion durations because they were able to swim a full half-lap without breathing. Higher skill level may therefore be a factor associated longer and more frequent head submersion, but less water ingestion. These behavioral differences help explain why water ingestion was elevated among leisure swimmers who, in contrast to the initial hypothesis, had fewer head submersions than lap swimmers. Information from the activity analysis also helps explain why previous researchers have found children ingest more pool water than adults. These results are useful for designing future exposure assessments that should include inquiries or observations of swimmer age, engagement in leisure swimming, and receipt of splashes to the face.

Water ingestion could also be associated with leisure swimming because all children leisurely swam. No difference was found when adult lap and leisure ingestion rates (children excluded) were compared to test this, suggesting ingestion differences between

all lap and leisure swimmers are attributable to child leisure swimmers. Children are believed to be engaging in “micro-activities” during leisure swimming that lead to pool water ingestion other than greater head submersion frequency and duration. A confirmed micro-activity associated with ingestion was receiving a splash to the face. This association could be due to water entering the mouths of swimmers during a splash, or because there is a relationship between being splashed in the face and participating in boisterous activities that lead to water ingestion. Three other micro-activities observed in children and suspected to lead to ingestion were “bobbing” at the water surface, spouting water for fun, and intentionally allowing pool water to enter the mouth (whether the water was ingested is unknown based on videography, but is suspected based on urinalysis). We hypothesize that “bobbing” at the water surface is an activity within leisure swimming that leads to pool water ingestion. This behavior can be quantified comparing frequency of <1 sec head submersions between age and activity groups, and should be tested as a potential predictor of pool water ingestion in future research. If found to predict ingestion, frequency of <1 sec submersions will serve as a more accurate exposure assessment variable than age, as ingestion differs within child age groups.

Pool water ingestion among young, competitive lap swimmers has been empirically assessed in previous research (Allen et al., 1982). Results from Allen et al. indicate young, competitive swimmers ingest three and one-half times more pool water than non-lap swimmers. This finding is inconsistent with results from the present study, likely because all lap swimming participants were adults. The finding does, however, support

our hypothesis that young swimmers, regardless of engagement in leisure or lap swimming, ingest more water than adults because they are less skilled. Future exposure assessments should compare water ingestion rates between child and adult lap and leisure swimmers to confirm skill as an exposure factor related to ingestion.

This study successfully identified activities and behaviors associated with increased pool water ingestion, validated a videography method for assessing swimmer head submersion frequency, and identified behaviors and characteristics suspected to increase pool water ingestion. Quantifying and comparing pool water ingestion with frequency of spitting, spouting, and allowing pool water into the mouth are also recommended for future research. Assessing skill as an ingestion predictor is also recommended, and can clarify why multiple studies have found children ingest more pool water than adults. Comparing illness probabilities between child, adult, lap, and leisure swimmers by risk assessment is also recommended to identify vulnerable groups and where healthy swimming education efforts should focus.

### **Cyanuric Acid Analysis**

Cyanuric acid concentrations in urine appear to be under-estimated in this study. The recovery analysis following SPE indicates 94% of cyanuric acid was lost during urine cleaning. Cyanuric acid recovered in blank controls suggests carry-over from concentrated samples or spikes can occur during injection cycles. Average cyanuric acid concentrations in blank MilliQ water from each injection cycle were subtracted from

urine samples to adjust for carry-over. Based on these findings, it is believed more cyanuric acid was ingested by study participants than indicated after data processing. This may explain the inconsistency between ingestion volumes reported in this study and the Dufour et al. (2006) study, in which no recovery efficiency or use of blank subtraction was reported (although carry-over into blanks was not reported, developers of the method found cyanuric acid concentrations between sample injections differed 0.3-1.5% (Cantu et al., 2000), suggesting either cyanuric acid carry-over or instrument error occurred). Children ingested 49 mL/hr, and adults ingested 21 mL/hr in the Dufour et al. study, compared to 25.7 and 3.5 mL/hr in the present study. When cyanuric acid in blanks was not subtracted, the average ingestion of pool water among swimmers in this study was 32.1 mL/hr; 59.2 mL/hr for children, and 9.22 mL/hr for adults. The range of ingestion was 0-225 mL/hr, compared with 0-205 mL/hr reported by Dufour et al. Ingestion volumes without blank subtraction are more consistent with previous research, but were not used in the present study due to suspected false-positives/over-estimation of cyanuric acid concentrations from carry-over during UHPLC-MS/MS analysis.

Although a recovery percentage was not reported by Dufour et al., the method developers (Cantu et al., 2000; 2001a) recovered cyanuric acid in urine between 89-112% compared to 6% in this study, likely due to different SPE and analysis techniques. Methods by Cantu et al. (2000) were also only reproducible for quantifying cyanuric acid ranging 500-125,000  $\mu\text{g/L}$ , which does not fit the lower range of cyanuric acid detected in this study (0.263-900  $\mu\text{g/L}$ ). Method sensitivity was higher using UHPLC-MS/MS. The

lowest concentration detected and thus the LOD was 0.263  $\mu\text{g/L}$ , compared to an LOD of 100  $\mu\text{g/L}$  found by Dufour et al. High sensitivity of UHPLC-MS/MS is why pool water was diluted 1:20 in this study. Cyanuric acid detected above 900  $\mu\text{g/L}$  was not considered a reliable measurement, as the highest point on the calibration curve was 900  $\mu\text{g/L}$  in this study. A 1:20 dilution was considered sufficient because cyanuric acid is typically maintained between 50-100 mg/L in pool water (Johnston et al., 1999).

Cantu et al. (2001a) applied three stacked SPE cartridges eluted with hydrochloric acid and dichloromethane. The purpose of using three cartridges was to remove interfering compounds, like creatinine, in urine prior to analysis. A single SPE cartridge was applied in this study because the extent of interfering compound removal by Dufour et al. (applying methods by Cantu et al., 2001a) was not necessary, as tandem mass spectrometry is more specific, as well as more sensitive, than UV detection.

Other researchers using similar methods also experienced problems analyzing cyanuric acid in urine. Problems included low recovery efficiencies, interference from matrix substances, and variability between analysis techniques for Dorevitch et al. (2011). Dorevitch et al. compared cyanuric acid recovery in urine using HPLC-MS/MS and Liquid Chromatography-Diode-array UV detector, and found the HPLC-MS/MS method performed better. Recovery efficiency was  $32.7 \pm 7.1$  %. The researchers applied methods by Cantu et al. (2001b; 2001c) and Smoker and Krynitsky (2008) using a Hypercarb graphite column and electrospray ionization with tandem mass spectrometry, but applied

different urine cleaning methods than our study. Pool water was not diluted because the calibration curve ranged from 0.78-78 mg/L, and thus reliably assessed cyanuric acid concentrations typical in pool water. A 7.0 mL urine sample was cleaned with four Methyl tert-butyl ether elutions by SPE, reconstituted in HPLC grade water, sonicated, filtered, and analyzed by Dorevitch et al. Only 4.2% (27/665) of urine samples were usable due to insufficient removal of interfering substances in urine during SPE. Although the percent cyanuric acid recovery was 26.7% lower in this study, 82.6% of urine samples were usable compared with 4.2% by Dorevitch et al.

The inconsistencies in methods performance between analysis instruments and SPE techniques confirms that more research is needed to develop a reliable, inexpensive method for quantifying cyanuric in urine, and thus pool water ingestion in swimmers. The noted inconsistencies suggest urine processing contributes largely to the success of a method. We learned in this study that combining cyanuric acid extraction and analysis techniques from different methods does not optimize recovery efficiency. In future research, a single method for extraction and analysis should be applied when analyzing urine for cyanuric acid to avoid problems experienced in this and other studies. The advantages of UHPLC-MS/MS in analyzing cyanuric acid should also be studied. No other cyanuric acid quantification methods have achieved an LOD below 1 µg/L. A method able to detect cyanuric acid in the µg/L range allows broader application of ingestion measurement in swimmers who use pools with low cyanuric acid, and thus may improve exposure assessment.

### **Limitations and Recommendations**

The observed difference in ingestion volumes between leisure and lap swimmers in this study may be attributable to the lack of child, lap swimming participants. Observing child lap swimmers and measuring their ingestion is recommended for future research to determine associations between skill, age, lap swimming, and water ingestion.

The percent recovery of cyanuric acid in urine is also a limitation, and could be increased by improving urine cleaning techniques. An inexpensive and rapid SPE method that removes less cyanuric acid from urine is needed for UHPLC-MS/MS analysis because this technique is sensitive and can quantify low levels of cyanuric acid in environmental samples. Improvement of analysis techniques are also needed, as cyanuric acid appeared to carry-over into blank quality controls during UHPLC-MS/MS analysis using a graphite column.

Another limitation was the sensitivity of i-Button thermometers. i-Buttons can be pre-set to collect a temperature every one second instead of every three to improve reliability of the environmental sensors.

Interobserver variability was highest for the method quantifying splashes to the face, followed by head submersion duration. We attempted to control variability by segmenting the analysis of each swimmer into 10 min intervals to reduce viewer fatigue,

and by comparing data collected by two viewers on the same swimmer. Improving the definition of "splash to the face" and reviewer training may reduce interobserver variability. Increasing the number of duplicate analyses, and using the average of the two values, would improve reliability of data quantified by videography.

### **Conclusions**

This study successfully identified activities and behaviors associated with increased pool water ingestion, validated a videography method for assessing swimmer head submersion frequency, and identified behaviors and characteristics suspected to increase pool water ingestion. Less frequent head submersion appears to be associated with greater pool water ingestion rates, and should be explored as an ingestion exposure factor in future research. Outbreak tools should assess leisure activity engagement and number of splashes received to the face among cases and non-cases, as both activities were associated with increased pool water ingestion. Assessing skill as an ingestion predictor is recommended for future swimming exposure assessments to clarify why children ingest more pool water than adults. Quantifying and comparing pool water ingestion with frequency of spitting, spouting, and allowing pool water into the mouth are also recommended for future research. Comparing illness probabilities between child, adult, lap, and leisure swimmers by risk assessment is also recommended to estimate infection risks from swimming in treated recreational water among different age and activity sub-populations. Risk estimates are needed to identify future research needs related to treated recreational water to improve swimmer health and safety.

## Acknowledgements

Funding for this research was provided by the National Swimming Pool Foundation and Research Foundation for Health and Environmental Effects. The questionnaire was developed in association with Kristen Pogreba Brown from the University of Arizona's Foodborne Illness Outbreak Investigation Team. Training in video surveillance methods was provided by Paloma Beamer at the University of Arizona's College of Public Health. Urinalysis method selection and development was aided by Matthew Magnuson with the United States Environmental Protection Agency Office of Research and Development. Video analysis was conducted by Marlee Hernandez and Meredith Lisse, students in The University of Arizona Mel and Enid Zuckerman College of Public Health. Thank you to all swimmer and swimming pool facility volunteers who participated in this study.

## References

- Allen, L. M., Briggles, T. V., & Pfaffenberger, C. D., 1982. Absorption and excretion of cyanuric acid in long distance swimmers. *Drug Metabolism Reviews*. **13**, 499-516.
- Andersen, J. R., 1965. A study of the influence of cyanuric acid on the bactericidal effectiveness of chlorine. *American Journal of Public Health and the Nations Health*. **55**, 1629-1637.
- Boehmer, T. K., Alden, N. B., Ghosh, T. S., & Vogt, R. L., 2009. Cryptosporidiosis from a community swimming pool: outbreak investigation and follow-up study. *Epidemiology and Infection*. **137**, 1651-1654.
- Briggles, T. V., Allen, L. M., Duncan, R. C. & Pfaffenberger, C. D., 198. High performance liquid chromatography determination of cyanuric acid in human urine and pool water. *Journal of the Association of Official Analytical Chemists*. **64**, 1222-1226.
- Cantu, R., Evans, O., Behymer, T. D., Shoemaker, J. A., Kawahara, F. K., & Dufour, A. P., 2001a. Simple sample clean up procedure and high performance liquid chromatographic method for the analysis of cyanuric acid in human urine. *Abstracts of Papers of the American Chemical Society*. **221**, U479-U479.
- Cantu, R., Evans, O., Kawahara, F.K., Wymer, L.J. and Dufour, A.P., 2001b. HPLC

- determination of cyanuric acid in swimming pool waters using phenyl and confirmatory porous graphitic carbon columns. *Analytical Chemistry*. **73**, 3358-3364.
- Cantu, R., Evans, O. and Magnuson, M.L., 2001c. Rapid analysis of cyanuric acid in swimming pool water by high performance liquid chromatography using porous graphite carbon. *Chromatographia*. **53**, 454-456.
- Cantu, R., Evans, O., Kawahara, F. K., Shoemaker, J. A., & Dufour, A. P., 2000. An HPLC method with UV detection, pH control, and reductive ascorbic acid for cyanuric acid analysis in water. *Analytical Chemistry*. **72**, 5820-5828.
- Causser, L. M., Handzel, T., Welch, P., Carr, M., Culp, D., Lucht, R., Mudahar, K., Robinson, D., Neavear, E., Fenton, S., Rose, C., Craig, L., Arrowood, M., Wahlquist, S., Xiao, L., Lee, Y.M., Mirel, L., Levy, D., Beach, M.J., Poquette, G., Dworkin, M. S., 2006. An outbreak of *Cryptosporidium hominis* infection at an Illinois recreational waterpark. *Epidemiology and Infection*. **134**, 147-156.
- Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P., Wroblewski, M., Liu, L., Scheff, P.S., & Li, A., 2011. Water ingestion during water recreation. *Water Research*. **45**, 2020-2028.
- Dufour, A. P., Evans, O., Behymer, T. D., & Cantu, R., 2006. Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water and Health*. **4**, 425-430.
- Ferguson, A. C., Canales, R. A., Beamer, P., Auyeung, W., Key, M., Munninghoff, A., Tse-Wing Lee, K., Robertson, A., Leckie, J. O., 2006. Video methods in the quantification of children's exposures. *Journal of Exposure Science and Environmental Epidemiology*. **16**, 287-298.
- Hlavsa, M. C., Roberts, V. A., Anderson, A. R., Hill, V. R., Kahler, A. M., Orr, M., Garrison, L., Hicks, L., Newton, A., Hilborn, E., Wade, T., Beach, M., Yoder, J. S., 2011. Surveillance for waterborne disease outbreaks and other health events associated with recreational water - United States, 2007-2008. *Morbidity and Mortality Weekly Report Surveillance Summary*. **60**, 1-32.
- Johnston, K., Bittenbring, C., Bruya, L., Richwine, M. & Youngblood, S., 1999. The encyclopedia of aquatic codes and standards. In *The Encyclopedia of Aquatic Codes and Standards* (K. Johnston, ed.). The National Recreation and Park Association, Ashburn, 55.
- Smoker M., Krynitsky, A., 2008. Interim method for determination of melamine and cyanuric acid residues in foods using LC-MS/MS: 1.0. *U.S. Food and Drug Admin LIB*. **1**, 4422.
- Suppes, L.M., Earnst, K.C., Abrell, L., & Reynolds, K.A., unpublished results. Validation and standardization of swimming exposure and pool operations questionnaires. *Water Research*. Manuscript submitted for publication.
- Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V., Roberts, V., Yu, P. A., Hicks, L.A., Alexander, N.T., Calderon, R.L., Roy, S.L., Beach, M. J., 2008. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events - United States, 2005 - 2006 *Morbidity and Mortality Weekly Report Surveillance Summary*. **57**, 1-29.

## APPENDIX C - CRYPTOSPORIDIUM INFECTION RISK IN SWIMMING POOL RECREATIONAL SWIMMERS

Paper was prepared to submit to Water Research

Laura M. Suppes<sup>1</sup>, Robert A. Canales<sup>2</sup>, Charles P. Gerba<sup>3</sup>, Kelly A. Reynolds<sup>4</sup>  
<sup>1,2,4</sup>The University of Arizona Mel and Enid Zuckerman College of Public Health  
<sup>3</sup>The University of Arizona Department of Soil, Water & Environmental Science

<sup>3</sup>Phone: (520) 626-8230

The University of Arizona Mel and Enid Zuckerman College of Public Health  
 P.O. Box 245163 Tucson, Arizona 85724, USA

<sup>1</sup>[supeslm@email.arizona.edu](mailto:supeslm@email.arizona.edu), <sup>2</sup>[rcanales@email.arizona.edu](mailto:rcanales@email.arizona.edu), <sup>3</sup>[Gerba@cals.arizona.edu](mailto:Gerba@cals.arizona.edu)  
<sup>4</sup>[Reynolds@email.arizona.edu](mailto:Reynolds@email.arizona.edu)

Keywords: *Cryptosporidium*, exposure, ingestion, recreational water, risk assessment, swimming pool

List of Abbreviations:

AGI - Acute Gastrointestinal Illness  
 C - Oocyst concentration/L pool water  
 D - Oocyst dose  
 F - Frequency of annual pool visits  
 HGCI - Highly Credible Gastrointestinal Illness  
 LOD - Limit of Detection  
 MAHC - Model Aquatic Health Code  
 P<sub>A.inf</sub> - Probability of per-swim infection  
 P<sub>inf</sub> - Probability of annual infection  
 SPE - Solid Phase Extraction  
 T - Time swimming/annual pool visit  
 UHPLC-MS/MS - Ultra High Pressure Liquid Chromatography - Tandem Mass Spectrometry  
 V - Pool water volume ingestion/hr swimming

### Abstract

Quantitative infection risk estimates from swimming in treated recreational water venues are lacking, and are needed to identify vulnerable populations and activities. Risk

estimates can be useful for identifying future research needs related to treated recreational water quality. Currently, state and local health departments lack standardized swimming pool safety regulations since there is no federal pool code in the U.S. Risk of enteric pathogen infection differs among populations since the magnitude of water ingestion, visit frequency, and swim duration depends on swimmer activity and age. Objectives of this study were to estimate per-swim and annual infection risks from swimming in treated recreational water among different age and activity sub-populations. Risks of *Cryptosporidium* infection in adults (>18), children ( $\leq 18$ ), lap, and leisure swimmers (splashing, playing, diving, wading, standing, and sitting) were estimated using frequency and oocyst concentration data in pool backwash water from the literature, and new and experimental data collected in this study on swimmer ingestion, activity, and pool-use frequency. The average per-swim risk of *Cryptosporidium* infection among swimmers was estimated at  $3.7 \times 10^{-4}$  swimmers/swim event. Among sub-populations, leisure swimmers had the highest estimated risk of infection per-swim event ( $5.2 \times 10^{-4}$  swimmers/swim event), followed by lap swimmers and children (both,  $3.5 \times 10^{-4}$  swimmers/swim event) and adults ( $2.5 \times 10^{-4}$  swimmers/swim event). The annual risk estimates in all swimmers and in each sub-population exceeded the United States Environmental Protection Agency acceptable risk limit for swimming in untreated recreational water (lakes, river, etc.) of  $8 \times 10^{-3}$  swimmers/year. We estimated  $3.3 \times 10^{-2}$  swimmers/year become infected with *Cryptosporidium* in treated recreational water venues. Leisure swimmers had the highest annual risk estimate of  $2.6 \times 10^{-1}$  swimmers/year. Sensitivity analysis identified *Cryptosporidium* concentration as the most

influential variable on infection probability. Results from this study suggest more resources and future research should focus on recreational water quality and swimmer ingestion exposures in treated recreational water venues. Standardizing pool water quality monitoring for *Cryptosporidium*, developing interventions that reduce intentional pool water ingestion and fecal contamination events, and improving removal of oocysts from pool water are recommended. Leisure swimmers were identified as the most vulnerable sub-population, and should specifically be targeted in healthy swimming education campaigns.

## **1. Introduction**

Documented swimming pool-related outbreaks in the United States are increasing (Hlavsa et al., 2011; Yoder et al., 2008). In the most recent surveillance publication of waterborne disease outbreaks associated with recreational water (2007-2008), the Centers for Disease Control and Prevention (CDC) reported 116 pool-related outbreaks that accounted for 13,480 illnesses (Hlavsa et al., 2011). Eighty-one of the 116 outbreaks were associated with Acute Gastrointestinal Illness (AGI) and accounted for 12,411 reported illnesses (diarrhea, abdominal cramps, vomiting, and nausea) (Hlavsa et al., 2011; Dupont et al., 1995). Acute Gastrointestinal Illness can be life threatening in sensitive populations, which represent 20-25% of the U.S. population and includes children <5 (Chen et al., 2002; Dupont et al., 1995; Hunter et al., 2011; Okhuysen et al., 1998; Reynolds et al., 2008). The majority of AGI outbreaks in swimming pools (58 of 81) between 2007 and 2008 were associated with *Cryptosporidium* spp., primarily by

*Cryptosporidium parvum* (referred to as "*Cryptosporidium*" in this document).

Cryptosporidiosis incidence is more than double in children when compared to adults (5.6/100,000 among children 1–4 years of age, and 2.5/100,000 among adults during 2009), and infections predominantly occur following exposures to contaminated pool water (Centers for Disease Control and Prevention [CDC], 2011; Yoder & Beach, 2010). Young children may have greater incidence rates because their immune systems are underdeveloped and they ingest more pool water than adults (Dufour et al., 2006; Suppes et al., unpublished results<sup>b</sup>; Teunis et al., 2002). The incidence of illness may be greater because children ( $\leq 18$ ) participate in swimming activities that lead to pool water ingestion, like playing, splashing, or diving (Suppes et al. unpublished results<sup>a</sup>).

*Cryptosporidium* is the primary cause of AGI outbreaks in pools because oocysts resist chlorine disinfection, are transmitted through the fecal-oral route, are excreted by ill swimmers in high amounts (up to  $10^9$  oocysts/fecal release), and exposure to few oocysts results in disease (Dufour et al., 2006; Teunis et al., 2002; Yoder & Beach, 2010). The resistance of oocysts to chlorine makes *Cryptosporidium* the ideal reference pathogen for assessing AGI risk from swimming in pool water since most pathogens will be inactivated at low chlorine levels without affecting *Cryptosporidium* viability.

Risk estimates among swimming sub-populations can be useful for identifying future research needs related to treated recreational water quality. Currently, state and local health departments lack swimming pool safety regulations standardizing water quality monitoring, water treatment, and pool operations since there is no federal pool code in the U.S., although the CDC are developing a recommended Model Aquatic

Health Code (MAHC). The MAHC consists of guidelines state and local health departments can follow, but are not required to adopt. Risk-based recommendations for water quality improvement are needed to establish standard pool operational guidelines that ensure exposure prevention.

Quantitative infection risk estimates from swimming in treated recreational water venues are needed to identify vulnerable populations and activities. Risk estimates are lacking for U.S. swimmers, who may have different exposures than previously assessed populations since pool visit frequency, swim duration, and magnitude of water ingestion differ regionally. Regulations for untreated recreational water (lakes, river, streams, oceans, etc.) do exist and can be compared with treated recreational risks. A risk comparison between venue types can identify data gaps, and thus where to focus resources and research. The United States Environmental Protection Agency (USEPA) is the federal agency that oversees enforcement of policies related to untreated recreational water quality in the U.S. (Dufour, 1984; United States Environmental Protection Agency [USEPA], 1986). The USEPA acceptable risk of enteric pathogen infection from swimming in untreated fresh water is 8/1,000 and 19/1,000 swimmers/year in marine water (USEPA, 1986). Using frequency and *Cryptosporidium* oocyst concentration data in pool backwash water from the literature (Schets et al., 2004), and new and experimental data collected on swimmer ingestion, activity, and use frequency (Suppes et al., unpublished results<sup>b</sup>), the per-swim and annual risk of *Cryptosporidium* infection in adults (>18), children ( $\leq 18$ ), lap, and leisure swimmers (splashing, playing, diving, wading, standing, and sitting) were estimated. Objectives of this study were to estimate

per-swim and annual infection risks from swimming in treated recreational water among different age and activity sub-populations. To achieve these objectives, *Cryptosporidium* infection risks among sub-populations with different exposures were estimated to identify target groups for education campaigns. Risks among sub-populations with varying exposures were also compared with previous risk estimates to identify geographic- and exposure-related risk differences. Influential variables on infection probability were identified and results were used to make recommendations for improving pool operational controls. Last, infection risks from swimming in treated and untreated recreational water were compared and results were used to make recommendations for future research.

## **2. Methods**

### **2.1 Data collection**

One hundred and twenty-six swimmers were recruited at four pool sites in Tucson, Arizona during the peak swimming months (June-September) and issued a swimming activity questionnaire. Pool water ingestion volumes, frequency of pool visits throughout the year, and duration of pool visits were collected using the questionnaire as beta poisson model variables to estimate annual and per-swim risks of *Cryptosporidium* infection in different age and activity groups (Suppes et al., unpublished results<sup>b</sup>). Age groups were children ( $\leq 18$ ) and adults, ( $> 18$ ), and activity groups were lap and leisure swimmers (leisure swimmers were defined as anyone reporting splashing, playing, diving, wading, standing, and sitting in pool water on the questionnaire). Average

duration of pool visits reported on the questionnaire was used as a surrogate for swim duration per pool visit. Risk differences between child and adult, and lap and leisure swimmers were explored because statistically significant differences at the 95% confidence level between ingestion, pool visit frequency, and/or swim duration have been found within these groups (Suppes et al., unpublished results<sup>b</sup>). Data on *Cryptosporidium* concentrations from 152 backwash samples collected in the Netherlands at 7 pool sites were used as *Cryptosporidium* concentrations in pool water (Schets et al., 2004).

### **2.1.1 Pool Water Ingestion**

Pool water ingestion volumes were estimated in 46 swimmers by analyzing cyanuric acid concentrations in 24 hr post-swimming urine samples (Suppes et al. unpublished results<sup>a</sup>). Participants were excluded if sufficient age-based urine volumes were not provided. Urine volumes were required to exceed 150 mL, 200 mL, and 300 mL for ages <10, 11-16, and >17, respectively. Modified methods by Cantu, et al. (2000; 2001) and Smoker and Krynitsky (2008) were applied to preserve, clean, and analyze urine samples. Briefly, urine was preserved with an acid solution (10% perchloric acid (v/v) and 1% metaphosphoric acid (w/v)), centrifuged, and cleaned to remove interfering substances, specifically creatinine, by Solid Phase Extraction (SPE) (SampliQ Silica, 3 ml, 500 mg; Agilent, 5982-2035). Cyanuric acid in urine and pool water was quantified with Ultra High Pressure Liquid Chromatography Tandem Mass Spectrometry (UHPLC-MS/MS). Concentrations were applied in a model that estimates volume of pool water ingestion (Eq. 1) (Dufour et al., 2006). Ingestion volumes were adjusted for potential false

positives from cyanuric acid carry-over by applying blank subtraction. The method Limit of Detection (LOD) was the lowest calculated ingestion volume after blank subtraction corresponding to a concentration with a MS/MS chromatogram signal to noise ratio (S/N)  $\geq 3$ . Urine with average cyanuric acid concentrations lower than concentrations found in blanks were considered <LOD measurements (n=12). Samples in which no cyanuric acid was detected with a S/N  $\geq 3$  were also considered <LOD measurements (n=1). A 50  $\mu\text{g/L}$  urine spiked sample was passed through SPE to estimate percent recovery of cyanuric acid. Details of this method and study design are discussed by Suppes et al. (unpublished results<sup>a</sup>).

$$\text{Eq. 1: Water Ingestion (L)} = \frac{[\text{Cyanuric Acid}]_{\text{urine}} \left(\frac{\mu\text{g}}{\text{L}}\right)}{[\text{Cyanuric Acid}]_{\text{pool water}} \left(\frac{\mu\text{g}}{\text{L}}\right)} \times \text{Urine Volume (L)}$$

### 2.1.2 Questionnaire and Swimmer Observation

An exposure questionnaire was created in DatStat Illume Survey Developer Gateway Version 5.1.1.17347 (Seattle, WA) to collect information on average annual frequency of pool visits, duration of swim/visit, and swimmer age and activity during the observed swim (Suppes et al., unpublished results<sup>b</sup>). Swim durations during the study were observed, recorded and used to adjust ingestion volumes (mL to mL/hr).

## 2.2 Data analysis

Unpaired t-tests identified variable differences between groups using STATA Statistics/Data Analysis Version 11.0 Software (College Station, TX). Associations were considered statistically significant at a p-value <0.05 (95% confidence).

Quantitative uncertainty analysis using Monte Carlo simulation was applied to obtain a distribution of oocysts ingestion per-swim with R Version 3 (R Studio add-on Version 0.97.248, Free Software Foundation, Inc., Boston, Massachusetts). Each variable was fit to a distribution for use in the oocyst ingestion exposure model, where C = concentration of *Cryptosporidium* oocysts/mL of pool water, V = volume of pool water ingested (mL/hr), T = swim duration/pool visit (hr), and D = oocyst dose/swim event (Eq. 2). Lognormal distributions were applied to all model variables based on goodness of fit tests (Kolmogorov-Smirnov test statistic p-value > 0.05) and information from previous risk assessments of *Cryptosporidium* infection (Pintar et al., 2010; Schets et al., 2011).

$$\text{Eq. 2: } D = C \times V \times T$$

Monte Carlo samples (10,000 iterations) from each variable were multiplied to create a distribution of oocysts ingested per-swim (D) by all swimmers, child swimmers, adults swimmers, and lap and leisure swimmers. Non-detects in concentration and ingestion measurements were accounted for by incorporating the probability of the event not occurring. Spearman's correlation coefficient was used in a sensitivity analysis to assess which model variables contributed most to risk of infection per-swim.

Hypergeometric  $\alpha$  and  $\beta$  model parameters for *Cryptosporidium* dose response were applied in a beta poisson model (Eq. 3) to determine probability of infection per swim ( $P_{inf}$ ) (Haas et al., 1983; Teunis et al., 2002). Hypergeometric and beta poisson models can be used interchangeably when  $\beta \gg 1$  and  $\alpha \ll \beta$  (Teunis et al., 2002). The hypergeometric  $\alpha$  and  $\beta$  parameters meet this condition (0.801 and 56.24, respectively), and were thus used in a beta poisson model.

$$\text{Eq. 3: } P_{inf}(D: \alpha, \beta) = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

After parameters for  $D$  were obtained, the distribution of annual pool visit frequency ( $F$ ) was incorporated into an annual risk model (Eq. 4) (Regli et al., 1991). The variable  $P_{inf}$  represents a distribution of possible per-swim infection probabilities from the initial model. The annual risk model with variable outcome  $P_{A.inf}$  estimates risk of contracting at least one *Cryptosporidium* infection across all pool visits in one year.

$$\text{Eq. 4: } P_{A.inf} = 1 - [(1 - P_{inf})^F]$$

### 3. Results

#### 3.1 Data Collection

The questionnaire was completed by 106 of 126 recruited swimmers, and 38 of 46 urine samples were usable (Table 1). The method LOD was 0.05 mL, and 13/38 (34.2%) swimmers had ingestion values <0.05 mL, including 13/21 adults, 8/29 leisure swimmers,

and 5/9 lap swimmer. These swimmers were thus assumed to have ingested no pool water. All children ingested >0.05 mL. Eighteen of 152 (11.8%) pool water backwash samples were positive for *Cryptosporidium*, ranging from <0.2 to 20.8 oocysts/L (Schets et al., 2004).

Table 1: Questionnaire and urinalysis participant age and activity type

|                  | Questionnaire<br>n=106 (%) | Urinalysis<br>n=38 (%) |
|------------------|----------------------------|------------------------|
| <b>Age</b>       |                            |                        |
| ≤18 years        | 52 (49)                    | 17 (44.7)              |
| >18 years        | 54 (50.9)                  | 21 (55.2)              |
| <b>Activity</b>  |                            |                        |
| Lap swimmers     | 21 (19.8)                  | 9 (23.7)               |
| Leisure swimmers | 85 (80.2)                  | 29 (76.3)              |

Based on similar risk assessments, lognormal distributions were applied to swim duration per pool visit and number of pool visits per year (Pintar et al. 2010; Schets et al. 2011). Schets et al. applied a gamma distribution to *Cryptosporidium* concentration, however, a lognormal distribution was found to fit the Schets et al. data in this study based on a goodness of fit test (Table 2).

Table 2: Model variable, symbol, and sample size, distribution parameters, and goodness of fit test p-values (Kolmogorov-Smirnov test statistic).

| Variable   | Units       | Symbol          | Sample Size | Lognormal Distribution Parameters: Meanlog, SDlog | P-Value             |
|--|-------------|-----------------|-------------|---|---------------------|
| <i>Cryptosporidium</i> oocyst concentration in pool water <sup>a</sup> | oocysts/mL  | C               | 152         | -5.862, 1.123                                     | 0.224 <sup>b</sup>  |
| Pool water ingestion volume  | mL/hr       | V               | 38          | 1.751, 2.036                                      | 0.4914 <sup>b</sup> |
| Pool water ingestion, children   | mL/hr       | V <sub>c</sub>  | 17          | 1.642, 1.575                                      | 0.6404 <sup>b</sup> |
| Pool water ingestion, adults   | mL/hr       | V <sub>a</sub>  | 21          | 1.802, 2.218                                      | 0.3582 <sup>b</sup> |
| Pool water ingestion, lap swimmers                                     | mL/hr       | V <sub>la</sub> | 9           | 2.255, 2.118                                      | 0.3238 <sup>b</sup> |
| Pool water ingestion volume, leisure swimmers                          | mL/hr       | V <sub>l</sub>  | 29          | 1.695, 2.139                                      | 0.4271 <sup>b</sup> |
| Pool visit frequency   | visits/year | F               | 97          | 3.702, 1.335                                      | 0.00165             |
| Time per visit   | hr          | T               | 100         | 0.3008, 0.5654                                    | 0.00024             |
| Time per visit, adults   | hr          | T <sub>a</sub>  | 53          | 0.0891, 0.5084                                    | 0.0043              |
| Time per visit, children   | hr          | T <sub>c</sub>  | 47          | 0.5416, 0.5291                                    | 0.1269 <sup>b</sup> |
| Time per visit, lap swimmers   | hr          | T <sub>la</sub> | 37          | 0.1401, 0.5525                                    | 0.03053             |
| Time per visit, leisure swimmers                                       | hr          | T <sub>l</sub>  | 63          | 0.3803, 0.5648                                    | 0.0077              |

<sup>a</sup>Data re-fit from Schets et al. (2004). All other model variables were collected by Suppes et al. (unpublished results<sup>b</sup>).

<sup>b</sup>Failure to reject the null hypothesis that data are consistent with the specified distribution.

Statistical analysis using unpaired t-tests of questionnaire responses identified children were more likely to ingest pool water than adults ( $p < 0.0218$ ) and to swim for longer durations ( $p < 0.0468$ ). Frequency of swimming did not differ between children and adults ( $p > 0.6992$ ). Leisure swimmers spent more time swimming per visit ( $p < 0.0302$ ) and visited pools less frequently than lap swimmers ( $p < 0.0314$ ), but ingestion rates did not differ between these groups ( $p > 0.3843$ ). Although the difference was not statistically significant, water ingestion by leisure swimmers was higher than lap swimmers (Table 3).

Table 3: Mean, standard deviation, and range of model variables for each sub-population

|                  | Ingestion (mL/hr) |      | Duration (hr/visit) |      | Visit Frequency (visits/yr) |         |       |       |          |
|------------------|-------------------|------|---------------------|------|-----------------------------|---------|-------|-------|----------|
|                  | Mean              | SD   | Range               | Mean | SD                          | Range   |       |       |          |
| All swimmers     | 14.3              | 22.8 | 0 - 105.5           | 1.6  | 1.0                         | 0 - 8   | 72.7  | 66.8  | 0 - 360  |
| Adults           | 6.3               | 16.7 | 0 - 60.6            | 1.4  | 0.11                        | 0 - 4   | 87.4  | 20.9  | 0 - 360  |
| Children         | 24.2              | 28.9 | 0 - 105.5           | 1.9  | 0.24                        | 0.5 - 8 | 76.8  | 15.5  | 0 - 300  |
| Lap swimmers     | 8.4               | 19.8 | 0 - 60.6            | 1.1  | 0.11                        | 0 - 4   | 142.9 | 113.3 | 30 - 360 |
| Leisure swimmers | 16.1              | 25.7 | 0 - 105.5           | 1.7  | 0.13                        | 0 - 8   | 64    | 61.6  | 0 - 200  |

### 3.2 Risk of Infection Per Swim Event

*Cryptosporidium* oocysts collected in pool backwash water by Schets et al. (2004) were assumed to be 100% viable. Viability was thus assumed to be 100% in this study. Our study estimated the risk of infection per-swim of all swimmers at  $3.7 \times 10^{-4}$  swimmers/event. Leisure swimmers had the highest average risk, followed by child and lap swimmers (same risk), and adults (Table 4). Spearman's correlation coefficients showing model variables that contributed most to risk of infection per-swim are in Table 5. The most influential variable among all swimmers and groups was *Cryptosporidium* oocyst concentration, followed by pool water ingestion. Swim duration did not appear to have a strong influence on infection risk.

Table 4: Average, standard deviation, and 95<sup>th</sup>/99<sup>th</sup> percentile per-swim and annual *Cryptosporidium* infection risks from swimming in treated recreational water among sub-populations.

|                  | Infection Risk Per-swim Event |                      |                             | Annual Infection Risk |                      |                             |
|------------------|-------------------------------|----------------------|-----------------------------|-----------------------|----------------------|-----------------------------|
|                  | Mean                          | SD                   | 95 <sup>th</sup> Percentile | Mean                  | SD                   | 95 <sup>th</sup> Percentile |
| All swimmers     | $3.7 \times 10^{-4}$          | $6.4 \times 10^{-3}$ | $1.2 \times 10^{-4}$        | $3.3 \times 10^{-2}$  | $7.7 \times 10^{-2}$ | $1.6 \times 10^{-1}$        |
| Adults           | $2.5 \times 10^{-4}$          | $6.8 \times 10^{-3}$ | $< 2.3 \times 10^{-3}$ (a)  | $2.2 \times 10^{-2}$  | $6.6 \times 10^{-2}$ | $1.1 \times 10^{-1}$        |
| Children         | $3.5 \times 10^{-4}$          | $5.3 \times 10^{-3}$ | $5.7 \times 10^{-4}$        | $2.9 \times 10^{-2}$  | $6.1 \times 10^{-2}$ | $1.3 \times 10^{-1}$        |
| Lap swimmers     | $3.5 \times 10^{-4}$          | $5.3 \times 10^{-3}$ | $1.6 \times 10^{-6}$        | $3.2 \times 10^{-2}$  | $8.3 \times 10^{-2}$ | $1.6 \times 10^{-1}$        |
| Leisure swimmers | $5.2 \times 10^{-4}$          | $9.5 \times 10^{-3}$ | $1.6 \times 10^{-4}$        | $2.6 \times 10^{-1}$  | $3.2 \times 10^{-1}$ | $9.5 \times 10^{-1}$        |

(a) 99<sup>th</sup> percentile risk value

Table 5: Ranked sensitivity analysis comparing Spearman's correlation coefficients. Coefficients describe relationships between independent variables (*Cryptosporidium* concentration, pool water ingestion, and time swimming/visit) and infection risk.

|                  | Concentration | Ingestion/hr | Time/visit |
|------------------|---------------|--------------|------------|
| All swimmers     | 0.796         | 0.181        | -0.011     |
| Adults           | 0.605         | 0.274        | -0.004     |
| Children         | 0.998         | 0.011        | 0.002      |
| Lap swimmers     | 0.630         | 0.244        | -0.001     |
| Leisure swimmers | 0.820         | 0.163        | -0.005     |
| Rank             | 1             | 2            | 3          |

### 3.3 Annual Risk of Infection

Average annual risk of *Cryptosporidium* infection in all swimmers was estimated at  $3.3 \times 10^{-2}$  swimmers/year (33/1,000 swimmers/year) in the present study, and was highest among leisure swimmers, followed by lap, child, and adult swimmers (Table 4). Figure 1 displays mean annual risk of infection for all swimmers and those in the four sub-populations. Both mean and 95<sup>th</sup> and 99<sup>th</sup> percentile risks in all swimmers and sub-populations exceed USEPA acceptable risks of pathogen infection from recreational swimming in untreated fresh (8/1,000 swimmers/year) and marine water (19/1,000 swimmers/year) (USEPA, 1986).

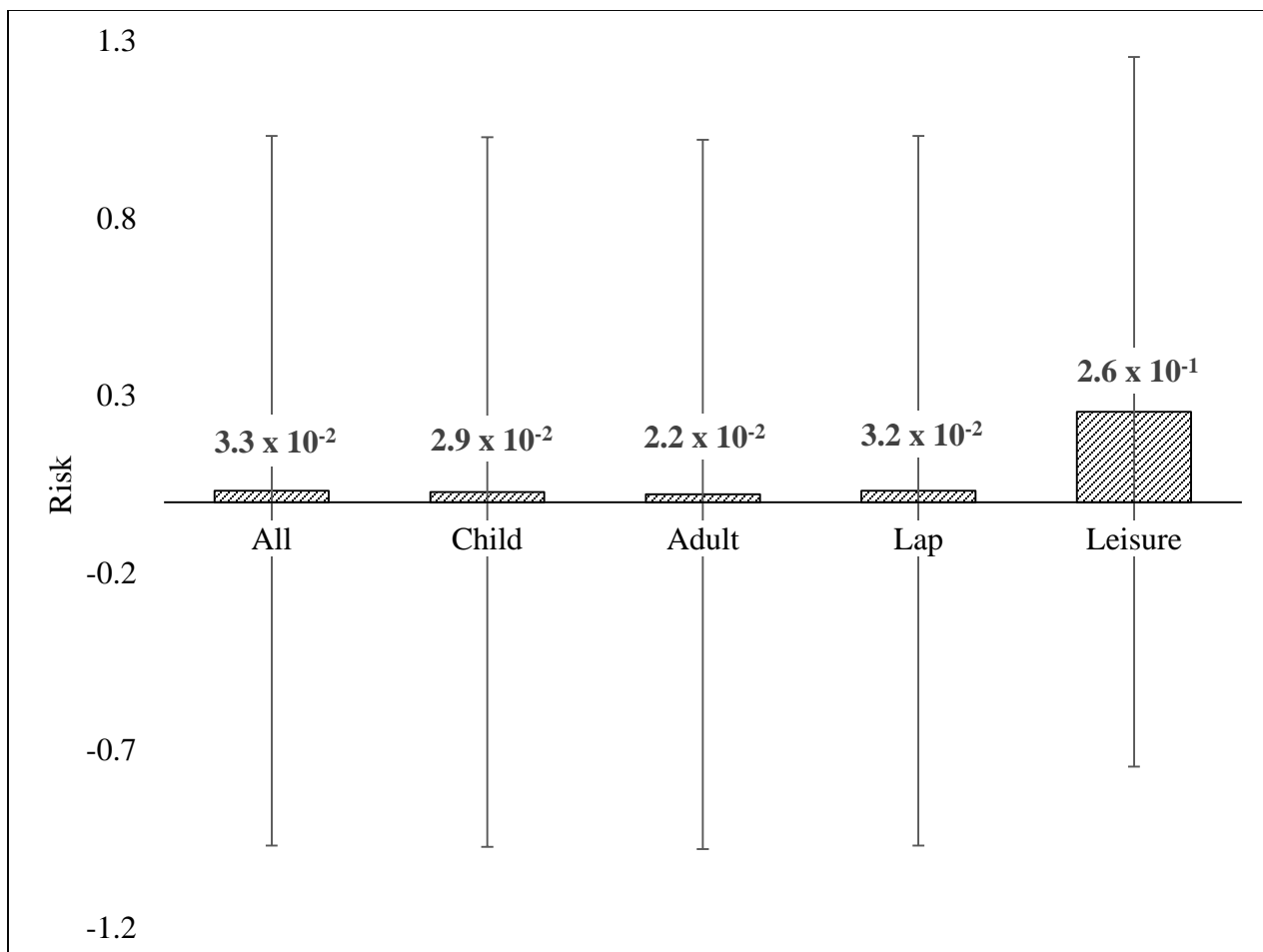


Figure 1: Means (reported on the figure) and standard deviations of annual *Cryptosporidium* infection risk for adult, child, lap, leisure, and all swimmers.

#### 4. Discussion

Comparing per-swim *Cryptosporidium* infection risks across studies highlights the need for routine and standardized monitoring of *Cryptosporidium* oocysts in pool water across the U.S. and internationally. Risk of *Cryptosporidium* infection per-swim was lower in this study compared with estimates by Schets et al. (2011). Schets et al. estimated the probability of *Cryptosporidium* infection in recreational swimmers using pool visit frequency and swim duration data collected in the Netherlands. The same

*Cryptosporidium* concentrations used by Schets et al. were applied in this study since no data on *Cryptosporidium* concentrations in U.S. swimming pools beyond presence/absence are currently available. The risk of *Cryptosporidium* infection per-swim estimated by Schets et al. ranged from  $1.1 \times 10^{-3}$  to  $2.1 \times 10^{-3}$  swimmers/event compared with a mean risk of  $2.5 \times 10^{-4}$  swimmers/event in the present study. The average child ingestion volume (0–15 years) estimated by Schets was 0.63 mL/min, and 0.42 mL/min in adults. In the present study, children (0–18 years) and adults on average ingested 0.403 and 0.105 mL/min, respectively. Considering ingestion was the second most influential variable on risk, the lower risk estimates in this study were likely influenced by the lower ingestion volumes. Per-swim risks estimated in both studies are unique to swimmers in the Netherlands and Southwestern U.S., as risk estimates are based on exposure factor data in addition to oocyst concentrations.

Another research group (Pintar et al., 2010) found comparable mean per-swim risks of *Cryptosporidium* infection in adults and children to this study using an exponential dose response model. Pintar et al. estimated  $2.85 \times 10^{-4}$  adult swimmers/event and  $5.95 \times 10^{-4}$  child swimmer/event are infected (estimates in this study were  $2.5 \times 10^{-4}$  adult swimmers/event, and  $3.5 \times 10^{-4}$  child swimmers/event). *Cryptosporidium* concentrations in pool water collected by Schets et al. (2004), and ingestion data collected by Dufour et al. (2006), were applied by Pintar et al. Dufour et al. found children and adults ingest 0.82 mL/min and 0.35 mL/min, respectively. Given Pintar et al. and Schets et al. (2011) applied similar ingestion rate data, per-swim risk estimates by each group were expected to be similar. Mean per-swim risks estimated by Pintar et al., however, were more

comparable to estimates in this study, likely due to Pintar et al. assuming 13-25% viability of *Cryptosporidium* based on viability of oocysts in reclaimed water. The effect of greater ingestion rates on risk appears to be mitigated by the counter effect of lower percent oocyst viabilities. Assuming 100% viability produces estimates that reflect risks following a fecal accident more accurately than estimates assuming lower viabilities, as oocysts are believed to be 100% viable immediately upon diarrheal excretion (Schets et al., 2004). Risk estimates by Pintar et al. appear to be more representative of background level exposures to oocysts stressed by environmental conditions.

Like this study, results from a sensitivity analysis comparing correlation coefficients by Pintar et al. showed *Cryptosporidium* oocyst concentration/L is the most influential factor on probability of infection. Pintar et al. used *Cryptosporidium* concentration data collected in the Netherland by Schets et al. (2004) as well, even though the primary objective of the research by Pintar et al. was to assess infection risk among Canadian swimmers using swim frequency data collected in Ontario, Canada (average swim frequency was 1.32 swims/person/year). Our study attempted to identify U.S. risks by applying exposure factors specific to U.S. swimmers. Risk estimates in this study, however, may not represent U.S. swimmers because *Cryptosporidium* concentrations in U.S. pools may differ from the Netherlands (oocyst prevalence in U.S. pools is 1.2% compared with 11.8% in the Netherlands) (Shields et al., 2008a; Schets et al., 2004). Likewise, risks estimated by Pintar et al. may not represent Canadian swimmers because *Cryptosporidium* prevalence and concentration may differ in Canadian pools. This demonstrates a need for future research quantifying *Cryptosporidium* concentrations in

U.S. swimming pools and in other regions where more geographically accurate risk assessment is needed.

Currently, no infection risk guidelines exist for treated recreational water in the U.S. The most appropriate risk limits available for comparing our estimates are the USEPA untreated fresh and marine water risks (8/1,000 swimmers/year and 19/1,000 swimmers/year, respectively). Annual risk estimates in the present study exceeded these values. The USEPA annual infection risks were determined in 1982 by correlating non-pathogenic indicator organism concentrations (*E. coli* and enterococci) in recreational water with Highly Credible Gastrointestinal Illness (HCGI) symptoms in swimmers (Dufour, 1984). The etiological agents associated with reported HCGI are unknown. Given the emergence of *Cryptosporidium* and other pathogens associated with outbreaks in recreational water and the advances in risk assessment since the 1980s, developing risk guidelines for swimming in treated water by assessing infection probabilities from specific pathogens should be considered for setting risk-based standards. Using this method, we found risk of *Cryptosporidium* infection in all swimmers at 33/1,000 swimmers/year; 22/1,000 adult swimmers/year; 29/1,000 child swimmers/year; 32/1,000 lap swimmers/year; and 260/1,000 leisure swimmers/year. Annual risk of infection was higher for lap swimmers than children, which is unexpected, as only adults lap swam. This is likely a result of lap swimmers reporting the highest average visit frequency of the sub-populations. Leisure swimmers had the highest annual risk of infection. The large proportion of child leisure swimmers (17/38) are likely influencing this population's annual infection risk. Risk differences between sub-populations show the influence

exposure factors (C, V, T, F) have on infection probability, and highlight the need for further exposure assessment and data collection across swimmer ages, activity groups, and geographic regions.

The sensitivity analysis indicates *Cryptosporidium* concentrations in pool water contribute most to infection risk, followed by water ingestion. Developing more robust engineering and administrative controls and applying these controls on a routine basis are recommended to reduce *Cryptosporidium* concentrations in pool water. Chlorine is the most common disinfectant applied in pools, and concentrations currently recommended for pool water (1-5 mg/L) do not inactivate *Cryptosporidium* oocysts in a timeframe that prevents ingestion exposures. A 15,300 min contact time (*Ct*) (20 mg/L chlorine for 12.75 hrs at pH 7.5) is required for inactivating *Cryptosporidium* oocysts in pool water without cyanuric acid, a chemical used in outdoor pools to reduce photodegradation of chlorine (Andersen, 1965; Shields et al., 2008b). Recent research shows a 67,200 min *Ct* at a lower pH (6.5) and in the presence of 50 mg/L cyanuric acid is necessary to achieve a 3 log reduction of oocysts (Shields et al., 2009). Cyanuric acid can be found in most outdoor pools in concentrations between 50-100 mg/L (Johnston et al., 1999). We recommend high-use leisure pools using cyanuric acid shock-chlorinate pool water and achieve a 67,200 min *Ct* at least twice per week to reduce oocyst concentrations that may be present from unreported fecal contamination events. Shock-chlorinating at least twice per week and achieving a 15,300 min *Ct* in high-use leisure pools without cyanuric acid is recommended. Dumping and refilling wading pool and spa water at least twice per week, and improving Accidental Fecal Release (AFR) identification techniques by

training pool staff and lifeguards to recognize AFRs can help prevent enteric pathogen contamination.

Filtration can effectively remove oocysts  $\geq 3$  logs, but pool water is recirculated through filters only every six hours (Johnston et al. 1999). There is therefore a 6 hour window of time when swimmers can be exposed to *Cryptosporidium* following a contamination event. Ensuring pool water recirculates through filters once every six hours (as required in most pool codes) is essential for ensuring filtration removal efficiencies. Secondary disinfection, like Ultraviolet light (UV) and ozone treatment, can also achieve 3 log removal efficiencies of oocysts, but like filtration, depend on water recirculation rates. A six hour turnover of pool water may be difficult in older pools with small diameter piping and/or biofilm accumulation. More research on swimming pool fluid dynamics is recommended to improve recirculation mechanisms in newly constructed and existing pools. Assessment of water recirculation in older U.S. swimming pools is also recommended to determine pool infrastructure status. Even if pool operators follow closure and shock-chlorination procedures recommended after AFRs, swimmers may still be exposed to *Cryptosporidium* if pump and piping systems are not achieving a six hour turnover rate.

Given the inadequacy of current pool water treatment techniques and the association between infection risk and ingestion, we recommend developing education efforts that teach swimmers to avoid behaviors and activities associated with pool water ingestion, like splashing others in the face (Suppes et al., unpublished results<sup>a</sup>). Public swimming pools often post “pool rules” stating swimmers should not spit or spout water, but this

control measure alone is not adequately preventing ingestion exposures based on results from this study. Swimmers need to be informed by local health departments about risks associated with intentionally ingesting pool water and advised to avoid this behavior. We recommend healthy swimming education materials be developed and distributed in the spring prior to the “pool season” (May–September) that focus on avoiding swimming for two weeks after experiencing diarrhea, intentional pool water ingestion, and splashing others in the face. Health departments can develop and provide literature to swim instructors and pool or facility managers to distribute among pool-users and parents of pool-users. Leisure swimmers have the highest risk of infection among all sub-populations, and thus should be targeted by public awareness messages and in education campaigns.

Comparing annual risk estimates in this study (33/1,000 swimmers/year) with acceptable risk limits established for untreated recreational water (8 and 19/1,000 swimmers/year) demonstrates a need for research in treated recreational water venues. The USEPA Beaches Environmental Assessment and Coastal Health (BEACH) Program requires state or local agencies to monitor water quality at fresh and marine water beaches (USEPA, 2013). If microbial water quality indicator standards are exceeded at the beaches, swimmers are excluded. We recommend a similar monitoring program be developed for treated recreational water. To accomplish this, an indicator organism with chlorine resistant-properties similar to *Cryptosporidium* should be identified, monitored in swimming pools, and maintained at or below a level that ensures an established risk guideline is maintained.

## 5. Limitations

Data on *Cryptosporidium* concentrations in U.S. swimming pools beyond presence/absence are not currently available. Concentrations of *Cryptosporidium* used in this risk assessment were taken from a study conducted in the Netherlands, which may not represent U.S. pool water quality. Only 1.2% of pool water samples (2/160) were positive for *Cryptosporidium* in a U.S. presence/absence study (Shields et al., 2008a), compared with 11.8% of samples in the Netherlands. Results in this study may therefore over-estimate risk. More research is needed to identify *Cryptosporidium* prevalence and concentrations in U.S. treated recreational water venues.

No lap swimmers were children in this study, which is the age group most likely to ingest pool water. Risks estimated for children are therefore specific to child leisure swimmers. Future research should assess determinants of infection probability (V, T, F) in child lap swimmers for a more comprehensive risk assessment of child swimmers.

The study location limits the generalizability of results in national and international swimming communities. Participants in Canada swam 1.3 times/year on average compared to 72.7 times/year in this study (Pintar et al., 2010). Exposure assessments of swimmers across broader age, activity, and geographic ranges are needed.

The methods used to estimate pool water ingestion may have underestimated how much pool water was actually swallowed by participants. The SPE technique applied to urine appeared to remove 94% of cyanuric acid (Suppes et al., unpublished results<sup>a</sup>). Similar difficulties recovering cyanuric acid have been experienced by other researchers (Dorevitch et al., 2011). Dorevitch et al. applied the same analysis method as this study

and recovered on average 32.7% of cyanuric acid from urine. Others applying similar methodologies have not reported recovery efficiencies (Dufour et al., 2006). These findings demonstrate a need for a rapid, more reliable cyanuric acid quantification method. Analyzing cyanuric acid in urine is currently the only quantitative technique for estimating pool water ingestion in swimmers, which is useful data for risk assessment.

## 6. Conclusions

Quantitative infection risk estimates from swimming in treated recreational water venues are lacking, and are needed to identify data gaps and focus areas for future recreational water quality and swimmer exposure research. Based on *Cryptosporidium* infection risks estimated in this study, we recommend the following:

- Quantification of *Cryptosporidium* concentrations in U.S. swimming pools and in other regions where more geographically accurate risk assessment is needed,
- Further exposure assessment and data collection across swimmer ages, activity groups, and geographic regions,
- Research on swimming pool fluid dynamics and current U.S. pool infrastructure status to improve recirculation in newly constructed and existing pools,
- Development of healthy swimming education materials and distribution of materials to leisure swimmers prior to the “pool season” (May–September),

- Implementation of more robust engineering and administrative controls in swimming pool facilities,
- Development of an indicator organism test representative of *Cryptosporidium*, and
- Development of a monitoring program for treated recreational water.

Swimming is a unique activity that can be enjoyed by people of all ages and abilities. Maintaining safe swimming pool environments by implementing operational controls and educating swimmers about risk is an essential role of public health representatives. Results from this study demonstrate a need for more research and resource allocation that improve treated recreational water environments and reduce risks among swimmers.

## **7. Acknowledgements**

Funding for this research was provided by the National Swimming Pool Foundation and Research Foundation for Health and Environmental Effects. Urinalysis was performed in partnership with Dr. Leif Abrell and The University of Arizona Laboratory for Emerging Contaminants. The questionnaire was developed in association with Kristen Pogreba Brown from the University of Arizona's Foodborne Illness Outbreak Investigation Team.

## 8. References

- Andersen, J. R., 1965. A study of the influence of cyanuric acid on the bactericidal effectiveness of chlorine. *American Journal of Public Health and the Nations Health* 55 (10), 1629-1637.
- Centers for Disease Control and Prevention, 2011. Morbidity and Mortality Weekly Report, Summary of Notifiable Diseases - United States, 2009. May 13, 2011 58 (53), 1-100.
- Cantu, R., Evans, O., Behymer, T. D., Shoemaker, J. A., Kawahara, F. K., & Dufour, A. P., 2001. Simple sample clean up procedure and high performance liquid chromatographic method for the analysis of cyanuric acid in human urine. *Abstracts of Papers of the American Chemical Society*, 221, U479-U479.
- Cantu, R., Evans, O., Kawahara, F. K., Shoemaker, J. A., & Dufour, A. P., 2000. An HPLC method with UV detection, pH control, and reductive ascorbic acid for cyanuric acid analysis in water. *Analytical Chemistry* 72 (23), 5820-5828.
- Chen, X. M., Keithly, J. S., Paya, C. V., & LaRusso, N. F., 2002. Current concepts: Cryptosporidiosis. *New England Journal of Medicine* 346 (22), 1723-1731.
- Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P., Wroblewski, M., Liu, L., Scheff, P.S., & Li, A., 2011. Water ingestion during water recreation. *Water Research* 45 (5), 2020-2028.
- Dufour, A. P., 1984. Health effects criteria for fresh recreational waters. Cincinnati, OH: U.S. United States Environmental Protection Agency Research and Development. EPA-600/1-84-004.
- Dufour, A. P., Evans, O., Behymer, T. D., & Cantu, R., 2006. Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water and Health* 4 (4), 425-430.
- Dupont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B., & Jakubowski, W., 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* 332 (13), 855-859.
- Free Software Foundation, Inc., 2013. R Studio Version 0.97.248. Boston Massachusetts Accessed March 13, 2013 from: <http://www.r-project.org/index.html>
- Haas, C. N., 1983. Estimation of risk due to low-doses of microorganisms. *American Journal of Epidemiology* 118 (4), 573-582.
- Hlavsa, M. C., Roberts, V. A., Anderson, A. R., Hill, V. R., Kahler, A. M., Orr, M., Garrison, L., Hicks, L., Newton, A., Hilborn, E., Wade, T., Beach, M., Yoder, J. S., 2011. Surveillance for waterborne disease outbreaks and other health events associated with recreational water - United States, 2007-2008 Morbidity and Mortality Weekly Report Surveillance Summary 60, 1-32.
- Hunter, P. R., de Saylor, M. A., Risebro, H. L., Nichols, G. L., Kay, D., & Hartemann, P., 2011. Quantitative microbial risk assessment of cryptosporidiosis and giardiasis from very small private water supplies. *Risk Analysis* 31 (2), 228-236.
- Johnston, K., Bittenbring, C., Bruya, L., Richwine, M. & Youngblood, S., 1999. The

- encyclopedia of aquatic codes and standards. In *The Encyclopedia of Aquatic Codes and Standards* (ed. K. Johnston). The National Recreation and Park Association, Ashburn, 55.
- Okhuysen, P. C., Chappell, C. L., Sterling, C. R., Jakubowski, W., & DuPont, H. L., 1998. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infection and Immunity* 66 (2), 441-443.
- Pintar, K. D. M., Fazil, A., Pollari, F., Charron, D. F., Waltner-Toews, D., & McEwen, S. A., 2010. A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in ontario. *Risk Analysis* 30 (1), 49-64.
- Regli, S., Rose, J. B., Haas, C. N., & Gerba, C. P., 1991. Modeling the risk from *Giardia* and viruses in drinking water. *Journal American Water Works Association* 83 (11), 76-84.
- Reynolds, K. A., Mena, K. D., & Gerba, C. P., 2008. Risk of waterborne illness via drinking water in the United States. *Reviews of Environmental Contamination and Toxicology* 192, 117-158.
- Schets, F. M., Engels, G. B., & Evers, E. G., 2004. *Cryptosporidium* and *Giardia* in swimming pools in the Netherlands. *Journal of Water and Health* 2 (3), 191-200.
- Schets, F. M., Schijven, J. F., & Husman, A. M. D., 2011. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research* 45 (7), 2392-2400.
- Shields, J. M., Gleim, E. R., & Beach, M. J., 2008a. Prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* in swimming pools, Atlanta, Georgia. *Emerging Infectious Diseases* 14( 6), 948-950.
- Shields, J. M., Hill, V. R., Arrowood, M. J., & Beach, M. J., 2008b. Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *Journal of Water and Health* 6( 4), 513-520.
- Shields, J. M., Arrowood, M. J., Hill, V. R., & Beach, M. J., 2009. The effect of cyanuric acid on the disinfection rate of *Cryptosporidium parvum* in 20-ppm free chlorine. *Journal of Water and Health*. 7 (1), 109-114.
- Smoker M., Krynitsky, A., 2008. Interim method for determination of melamine and cyanuric acid residues in foods using LC-MS/MS: 1.0. U.S. Food and Drug Administration LIB. 1, 4422.
- Suppes, L.M., Abrell, L., Dufour, A.P. & Reynolds, K.A., unpublished results<sup>a</sup>. Assessment of swimmer head submersion influence on pool water ingestion. *Journal of Water and Health*. Manuscript submitted for publication.
- Suppes, L.M., Suppes, L.M., Earnst, K.C., Abrell, L., & Reynolds, K.A., unpublished results<sup>b</sup>. Validation and standardization of swimming exposure and pool operations questionnaires. *Water Research*. Manuscript submitted for publication.
- Teunis, P. F. M., Chappell, C. L., & Okhuysen, P. C., 2002. *Cryptosporidium* dose-response studies: Variation between hosts. *Risk Analysis* 22 (3), 475-485.
- U.S. EPA, 1986. Bacteriological water quality criteria for marine and fresh recreational waters. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA-440/5-84-002.

- U.S. EPA, 2013. Beaches Environmental Assessment and Coastal Health Program. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. Accessed March 13, 2013 from: [http://water.epa.gov/type/oceb/beaches/beaches\\_index.cfm](http://water.epa.gov/type/oceb/beaches/beaches_index.cfm)
- Yoder, J. S., & Beach, M. J., 2010. *Cryptosporidium* surveillance and risk factors in the United States. *Experimental Parasitology* 124 (1), 31-39.
- Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V., Roberts, V., Yu, P. A., Hicks, L.A., Alexander, N.T., Calderon, R.L., Roy, S.L., Beach, M. J., 2008. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events - United States, 2005 - 2006 *MMWR Surveillance Summary*. 57, 1-29.