  Journal of Water and Health | 04.4 | 2006

Water ingestion during swimming activities in a pool: A pilot study

Alfred P. Dufour, Otis Evans, Thomas D. Behymer and Ricardo Cantú

ABSTRACT

Chloroisocyanurates are commonly added to outdoor swimming pools to stabilize chlorine disinfectants. The chloroisocyanurates decompose slowly to release chlorine and cyanuric acid. Studies conducted to determine if the chloroisocyanurates might be toxic to swimmers showed that they were not and that ingested cyanuric acid passed through the body unmetabolized. This fact was used to determine the amount of water swallowed during swimming activity. Fifty-three recreational swimmers, using a community swimming pool disinfected with cyanuric acid stabilized chlorine, participated in the study. The participants did not swim on the day before or after the test swim. The swimmers were asked to actively swim for at least 45 minutes and to collect their urine for the next 24 hours. Cyanuric acid was measured in pool water using high performance liquid chromatography and porous graphitic carbon columns with UV detection. The urine sample assay required a clean-up procedure to remove urinary proteins and interfering substances. Results of the study indicate that non-adults ingest about twice as much water as adults during swimming activity. The average amount of water swallowed by non-adults and adults was 37 ml and 16 ml, respectively. The design for this study and the analytical methodology used to assay cyanuric acid in swimming pool water and human urine were effective for measuring the volume of water swallowed during swimming activity.

Key words | cyanuric acid, swimming, water, water ingestion

Alfred P. Dufour (corresponding author)
Otis Evans
Thomas D. Behymer
US Environmental Protection Agency,
National Exposure Research Laboratory,
26 W. Martin Luther King Drive
Cincinnati, OH 45268, USA
Tel.: +1 (513) 569-7330
Fax: +1 (513) 569-7464
E-mail: *dufour.alfred@epa.gov*

Ricardo Cantú
Lilly Research Laboratories,
A Division of Eli Lilly and Company,
Lilly Corporate Center,
Indianapolis, Indiana 46285,
USA

INTRODUCTION

Swimming may be one of the most popular recreational activities in the United States. National surveys show that up to 89 million people swim each year (NSRE 2002).

Many of the natural waters in the U.S. are affected by fecal contamination whose source may be animals or humans. The number of humans who use water resources and subsequently become ill as a result of this type of exposure is unknown. However, it is generally accepted that some swimmers may exhibit gastrointestinal (GI) symptoms after swimming in waters meeting acceptable water quality standards (Federal Register 2004). The route of transmission for the microbial pathogens causing the GI illness is through ingestion. One unknown factor associated with swimming-related illness is how much water is swallowed during swimming activity. Very little empirical evidence is available to indicate how much water is ingested while swimming, but a number of estimates have been suggested in the last 70 years. When calculating an illness level that might be associated with densities of fecal indicator bacteria, Streeter (1951) estimated that swimmers swallowed approximately 10 ml of water during swimming activity. Shuval (1975), in a review of standards associated with bathing beaches, suggested that swimmers ingested about 10 ml of water per bathing day. World Health Organization (2003) guidelines assume that 20 to 50 ml of water is ingested per hour of swimming related activity. None of

doi: 10.2166/wh.2006.017

these estimates were supported by studies that examined water ingestion.

However, Allen *et al.* (1982) conducted a toxicokinetic study of five young competitive swimmers who were exposed to chloroisocyanurate, a compound that is used to stabilize chlorine in outdoor swimming pools. Chloroisocyanurates are used because they decompose very slowly to release chlorine and cyanuric acid. In this study, Allen *et al.* (1982) conducted a number of experiments to show that cyanuric acid could be used to measure water ingestion by swimmers. For two volunteer swimmers, who drank water containing a known amount of cyanuric acid, it was determined that greater than 98% of ingested cyanuric acid could be recovered in 24 hour urine samples. Additionally, in another five subjects who swam in a pool, the cumulative excretion of cyanuric acid was complete in about 20 hours. Furthermore, the dermal absorption of cyanuric acid was shown to be negligible in five subjects who soaked in pool water for 2 hours. Subsequent urine excretion of cyanuric acid in these volunteers, after the two hour soak, was equivalent to swallowing 3 to 3.6 ml of pool water. In the same studies, long-distance swimmers were directed to swim for 2 hours in a pool containing a known concentration of chloroisocyanurate. Following the swimming activity, urine was collected from each swimmer over a 24 hour period. Calculation of the amount of cyanuric acid ingested by each swimmer, and its extrapolation to the volume of water ingested, indicated that the swimmers swallowed an average of about 161 ml of pool water per hour. Consequently, these findings suggested a unique approach to determining the volume of water swallowed by recreational swimmers when participating in swimming activities. Before conducting such a study, improved methodology for measuring cyanuric acid in water and urine had to be developed. The analytical methodology utilized by Allen *et al.* (1982), that had been previously developed by Briggle *et al.* (1981), proved to be cumbersome and difficult to implement. Cantú *et al.* (2001a, b) developed somewhat simpler methodology for assaying the concentration of cyanuric acid in pool water and urine samples. The methodology, used to measure cyanuric acid in pool water samples involved the use of reversed-phase high performance liquid chromatography (RP-HPLC), with porous graphitic carbon columns. The methodology for urine samples required combining solid-phase and liquid-liquid extraction cleanup approaches to pre-concentrate and extract cyanuric acid. This eliminated urinary proteins and other interferences prior to RP-HPLC separation and UV detection. The overall objectives of this pilot study were to 1) determine the efficacy of the study design, 2) evaluate the analytical methodology for measuring cyanuric acid, and 3) get a preliminary indication of the amount of water ingested during recreational swimming activity.

METHODS

Study site

The study was conducted at an outdoor community swimming pool that was disinfected with chloroisocyanate. The disinfectant was added to the water at the beginning of the swimming season and was monitored weekly. Additional disinfectant was added, as needed, to maintain a pool concentration of 30 to 50 mg per liter as suggested by the National Spa and Pool Institute (Tice 1997).

Study protocol

A total of 53 individuals participated in the study. No personal information was collected from the participants. The pool manager recorded the gender and estimated age of each swimmer. An individual older than 18 years of age was listed as an adult and individuals 18 years of age and younger were listed as non-adults. On the day of the swim, each swimmer was requested to void urinating prior to entering the pool. Furthermore, the swimmers were directed to stay in the pool and actively swim for at least 45 minutes. The swimmers were also requested not to swim 24 hours before or after the swimming event.

Sample collection

Pool water samples were collected from four locations around the pool prior to the start of the swimming activities. The sampling locations were on each side of the pool midway between the corners of the pool. The samples (250 ml) were collected at a depth of 25 cm in amber colored plastic storage bottles, refrigerated at 4°C, and analyzed within one week.

Urine samples were collected following the swimming event. Each participant was given a sterile one gallon, large mouth container. The participants were directed to collect their urine over the next 24 hours and return the specimen containers to the pool manager after that period of time.

Assay methodology

The assay methodology for measuring cyanuric acid in pool water samples and urine specimens have been described previously by Cantú *et al.* (2001*a*, *b*). Briefly, the pool water samples were filtered, using an activated cellulose filtration disk, followed by dilution of a suitable aliquot with deionized-distilled water. Samples were then refrigerated at 4°C in an amber colored plastic storage bottle for no more than one week before analysis. A 25 μl aliquot of the sample was directly injected into a porous graphitic carbon column for reversed phase high performance liquid chromatographic analysis (RP-HPLC). Separation of cyanuric acid was achieved using isocratic elution chromatography with a mobile phase of 95% 50 mM phosphate buffer (pH 9.1) and 5% methanol (% v/v) followed by UV detection at 213 nm. The procedure was linear in the range of 1–5 mg/l with a detection limit of 0.1 mg/l. Cyanuric acid was quantified using an external calibration curve. To avoid biological and chemical degradation, the 24 hour urine sample was immediately preserved after collection by the addition of 1 ml of a reagent composed of 10% (v/v) perchloric acid and 1% (w/v) metaphosphoric acid. The urine was cleaned by initially centrifuging 1.5 ml of the preserved sample. Next, 1 ml of the supernatant was loaded onto 3 previously conditioned, stacked, solid phase extraction cartridges. The filtered urine sample was eluted from the cartridges using 4 ml of a 0.05 M hydrochloric acid solution. Subsequently, 2.5 ml of dichloromethane was added to 1.5 ml of the filtered urine, the mixture was vortexed, and the phases were allowed to separate. A 450 μl aliquot of the aqueous phase was then transferred to an HPLC auto-sampler vial and stored at 4°C. Just prior to analysis, the pH of the aqueous phase was adjusted with sodium hydroxide and the sample immediately injected into a porous graphitic column as indicated above for the pool water samples.

The volume of water ingested was calculated from the amount of cyanuric acid measured in the pool water and in the urine sample using the formula:

$$\mathbf{V}^{*}_{\text{water ingested}} = \mathbf{V}_{\text{urine collected}} \times \mathbf{U}_{\text{CA conc.}} / \mathrm{P}_{\text{CA conc.}}$$

where,

$\mathbf{V}^{*}_{\text{water ingested}}$ = volume of pool water ingested
$\mathbf{P}_{\text{CA conc.}}$ = cyanuric acid (CA) concentration in pool water
$\mathbf{V}_{\text{urine collected}}$ = volume of urine collected over a 24 hour period
$\mathbf{U}_{\text{CA conc.}}$ = cyanuric acid (CA) concentration in urine

Data analysis

Statistical evaluation of the data was accomplished using the Wilcoxon Rank Sum Test (Hollander & Wolfe 1973). The small number of individuals in the groups analyzed and the lack of knowledge about the nature of the distribution of the data precluded the use of parametric statistics.

Ethics

Participation in the study was confidential, anonymous and entirely voluntary. Informed consent was obtained from each participant prior to enrollment in the study. Participating adults and non-adults were informed of the objectives of the study and were asked to sign site specific consent forms and assent forms, respectively. Non-adults were also required to have site specific parental informed consent. All participants were compensated for the delivery of a 24-hour urine sample to the pool manager at the designated sample collection site. The consent forms and the study design were reviewed and approved by an Independent Review Board, to ensure adherence to the Food and Drug Administration's Code of Federal Regulations governing human subjects research. It was also reviewed by the U. S. Environmental Protection Agency's Human Subjects Research Review Official (Office of Research and Development) for compliance with EPA regulations on the protection of human subjects.

RESULTS

The distribution of swimmers with regard to gender and age is shown in Figure 1. Twenty-three percent of the participants were adults. Fifty-five percent of the swimmers were female. Of the twelve adults, four were male and eight were female. Twenty-one of the 41 non-adults were female. Since this was a pilot study to test the cyanuric acid assay methodologies and the study protocol, no effort was made to balance the age and gender of the swimmer population distribution.

The distribution of the amount of water swallowed by each swimmer is shown in Figure 2. The range of water volume ingested by non-adults was from 0 to 154 ml. Ninety-seven percent of the non-adults swallowed 90 ml or less. Adults swallowed between 0 and 53 ml of water.

Age and gender comparisons of water ingestion are shown in Table 1. The amount of water swallowed by adults during swimming activity was much less than that swallowed by non-adults. The mean volume of water swallowed by non-adults was 37 ml over a 45 minute period. Adults swallowed an average of 16 ml per 45 minutes of swimming activity. This difference was significant at the 5% level. There also was an appreciable difference in the amount of water swallowed during swimming activity between males and females; however, this difference was not statistically significant. The difference in the volume of water ingested between all males and females was not significant nor was the difference in the amount of water swallowed between adult men and women.

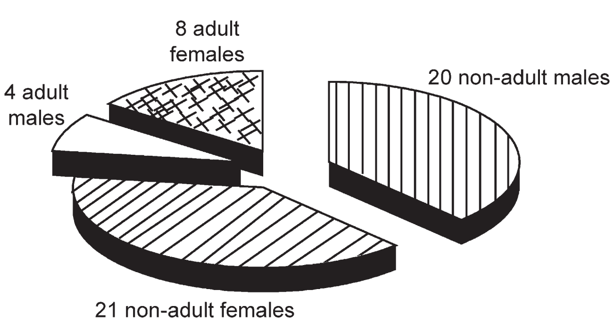

Figure 1 | Distribution of study participants by gender and age group.

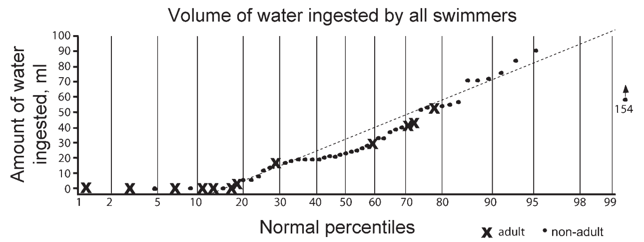

Figure 2 | Distribution of volume of water ingested by study participants during swimming activity.

DISCUSSION

Although this study was conducted to test the design and analytical methods that would be used in a future full field study, some unique results have been observed and are worthy of discussion. This is the first study to empirically determine the amount of water ingested by recreational swimmers and to show that adults swallow considerably less water than non-adults during swimming activity. The results of this study have limitations regarding their translation to natural swimming environments. The ingestion behavior of swimmers in freshwaters is likely to be similar to that of pool swimmers. For instance, freshwater swimmers may frequently immerse their heads under the surface of the water and remain in the water for long periods of time as pool swimmers do. This type of behavior is usually not observed in marine water swimmers. Thus, it would be reasonable to believe that what occurs in pool waters may be transferred to natural freshwater swimmers, but not to saltwater swimmers.

Epidemiological studies that have examined the relationship between swimming-associated health effects and water quality have found that the illness rate in children is higher than that observed in adults (Stevenson 1953; Cabelli *et al.* 1979; Cheung *et al.* 1990;). Speculation about this difference in illness rates has centered on immature immune systems or greater exposure due to accidental ingestion of bathing water. Although both hypotheses are reasonable, the current study would support the hypothesis that children have a higher rate of swimming-associated illness because their exposure to bathing waters is greater. The lack of protective immunity hypothesis, however, cannot be totally eliminated as a possibility because the youngest swimmers in this study were 6 years of age. The

Table 1 | Statistical evaluation of water ingestion by selected groups of swimmers

Comparison groups	Number of particip.	Average water ingestion (ml)	*P*-value	Sig.[a]
Adults	12	16	0.0412	**
Non-adults	41	37		
Males	24	41	0.0668	
Females	29	25		
Boys	20	45	0.1029	
Girls	21	30		
Men	4	22	0.7295	
Women	8	12		

[a]*P*-value after applying the non-parametric Wilcoxon Rank Sum Statistical Test; ** indicates significant difference at 5% level.

exposed populations in the epidemiological water quality studies showing higher illness rates in children contained a much greater number of children that were less than 6 years of age and who would be more likely to fall into that group having underdeveloped immune systems.

A comparison of the water ingested by the non-adult recreational swimmers in the present study with the five young, competitive, long distance swimmers studied by Allen *et al.* (1982) indicates that competitive swimmers swallow significantly more water than recreational swimmers in a 45 minute interval. The competitive swimmers swallowed about three and one-half times more water than the young recreational swimmers in this study (37 ml *vs.* 128 ml).

The analytical methodologies (Cantú 2001*a, b*) that were used in this study provided a satisfactory means of measuring cyanuric acid concentrations in both water and urine. This pilot study has shown that the analytical methodology and study design are sufficient and effective for measuring water ingestion related to swimming activities in a swimming pool. This proof-of-concept pilot study, showing that water ingestion can be measured using cyanuric acid as a non-metabolized marker, will be used as a model for a future study of a large demographically balanced population where the ingestion behaviors can be differentiated with greater certainty than in the present study.

CONCLUSIONS

The results of the pilot study lead to the following conclusions:

- The study design used to determine the amount of water ingested while swimming proved to be effective.
- The assay methods used to measure cyanuric acid in urine and pool water provided the results required to meet study objectives. No significant problems with the methodologies were encountered during the course of the pilot study.
- The ingestion data collected during this study were not remarkable except for the significantly greater amount of water swallowed by non-adults relative to adults. This observed difference, however, should be accepted with a note of caution because of the small number of subjects participating in the study.

DISCLAIMER

The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency review and approved for publication.

The mention of commercial products does not constitute endorsement or recommendation for use by the US Environmental Protection Agency.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

ACKNOWLEDGEMENTS

The authors sincerely thank Mr. David Moore, Village Manager, Mrs. Jennie Tilton, Pool Manager, and the parents and swimming participants from the Village of Greenhills, OH for participating in this study, and Teresa Ruby and Larry Wymer for graphics and statistical support, respectively.

REFERENCES

Allen, L. M., Briggle, T. V. & Pfaffenberger, C. D. 1982 Absorption and excretion of cyanuric acid in long-distance swimmers. *Drug Metabolism* **13**(3), 499–516.

Briggle, 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. *J. Assoc., Off. Anal. Chem.* **64**(5), 1222–1226.

Cabelli, V. J., Dufour, A. P., Levin, M. A., McCabe, L. J. & Haberman, P. W. 1979 Relationship of microbial indicators to health effects at marine bathing beaches. *Amer. J. Public Health* **69**(7), 690–696.

Cheung, W. H. S., Chang, K. C. K., Hung, R. P. S. & Kleevens, J. W. L. 1990 Health Effects of Beach Water Pollution in Hong Kong. *Epidemiology and Infection* **105**, 139–162.

Cantú, R., Evans, O., Kawahara, F. K., Wymer, L. J. & Dufour, A. P. 2001*a* HPLC determination of cyanuric acid in swimming pool waters using phenyl and confirmatory porous graphitic carbon columns. *Anal. Chem.* **73**, 3358–3364.

Cantú, R., Evans, O., Kawahara, F. K., Magnuson, M. L., Shoemaker, J. A., Wymer, L. J., Behymer, T. D. & Dufour, A. P. 2001*b* Simple sample cleanup procedure and high-performance liquid chromatographic method for the analysis of cyanuric acid in human urine. Abstract No. 216: Book of Abstracts, Environmental Chemistry Section, ACS, 221st National Meeting, San Diego American Chemical Society, Washington, D. C.

Federal Register 2004 *Water Quality Standards for Coastal and Great Lakes Recreational Waters*, Vol. 69. Proposed, Rule, No. 131, Friday, July 9, 2004, pp. 41720–41743.

Hollander, M. & Wolfe, D. A. 1973 *Nonparametric Statistical Methods*. John Wiley and Sons, New York.

NSRE (National Survey on Recreation and the Environment) 2002 *The Interagency National Survey Consortium 2000–2002*. USDA Forest Service, Recreation, Wilderness and Demographics Trends Research Group, Athens, GA and the Human Dimensions Research Laboratory University of Tennessee, Knoxville, TN.

Shuval, H. I. 1975 The case for microbial standards for bathing beaches. In: *Discharge of Sewage from Sea Outfalls* (ed. Gameson, A. L. H.), Pergamon Press, Oxford, England, pp. 95–101.

Stevenson, A. H. 1953 Studies of bathing water quality and health. *Amer. J. of Public Health* **43**(5), 529–538.

Streeter, H. W. 1951 *Bacterial Quality Objectives for the Ohio River*. Ohio River Valley Water Sanitation Commission, Cincinnati, OH.

Tice, M. 1997 Pass the Water Test. *Water Technology* **20**(1), 45–50.

WHO (World Health Organisation) 2003 *Guidelines for Safe Recreational Water Environments. Volume 1, Coastal and Fresh Waters*. WHO, Geneva, Switzerland.

Available online May 2006