# Variability in 24 hour excretion of cyanuric acid: implications for water exposure assessment

Martha Sinclair, Felicity Roddick, Stephen Grist, Thang Nguyen, Joanne O'Toole and Karin Leder

## ABSTRACT

Cyanuric acid (CYA) excretion in urine has been used to estimate the volume of water ingested during swimming and other recreational activities in outdoor pools containing this chemical. These estimates of water ingestion are based on the assumption of 100% excretion within 24 hours, but the supporting evidence for this is scant. While adapting this methodology to investigate other water ingestion scenarios, we observed a high degree of variability in cyanuric acid excretion among experimental subjects, with over 25% of individuals excreting less than 80% of an ingested dose. Use of cyanuric acid to measure inadvertent water ingestion may be a valuable tool to generate data for health risk assessment of non-potable water sources, but our observations indicate that this technique carries an inherent degree of underestimation that should be taken into account when calculating water exposure.

**Key words** | exposure assessment, ingestion, non-potable, recreational water

**Martha Sinclair** (corresponding author)
**Joanne O'Toole**
**Karin Leder**
Department of Epidemiology and Preventive Medicine,
Monash University,
The Alfred Centre, 99 Commercial Road,
Melbourne,
VIC 3004,
Australia
E-mail: *martha.sinclair@monash.edu*

**Felicity Roddick**
**Stephen Grist**
**Thang Nguyen**
School of Civil, Environmental and Chemical Engineering,
RMIT University,
124 La Trobe Street,
Melbourne,
VIC 3001,
Australia

## INTRODUCTION

The application of quantitative microbial risk assessment to health risks associated with recreational water activities requires estimation of the volume of water ingested by people during such pursuits. In the absence of empirical data, guideline documents have used estimates of plausible quantities ingested per hour of swimming or per day with swimming activity (WHO 2003). More recently, empirical data have been generated using measurement of the swimming pool chemical cyanuric acid (CYA) in the urine of volunteer participants as a marker of water ingestion (Dufour *et al.* 2006; Dorevitch *et al.* 2011; Suppes *et al.* 2014). Cyanuric acid is added to outdoor swimming pools to protect the chlorine disinfectant from rapid decomposition by ultraviolet light. When ingested, this compound is not metabolised but is excreted unchanged in the urine. According to published evidence (Allen *et al.* 1982), humans excrete more than 98% of the ingested dose within 24 hours of ingestion. Therefore if 24 hour urine samples (all urine produced in a 24 hour period) are collected from volunteers after a swimming session in an outdoor pool, it is possible to calculate the ingested water volume from the concentration of cyanuric acid in the swimming pool water, the 24 hour urine volume and the measured concentration of cyanuric acid in the urine (with the assumption of 100% excretion).

We decided to explore whether this methodology could be adapted to permit ingestion of small volumes of water in non-potable water exposure scenarios other than swimming. Currently, water quality guidelines for non-potable uses of recycled water are based largely on expert opinion of water volumes accidentally ingested during a range of activities (NRMMC/EPHC/AHMC 2006), with little or no empirical data to support these assumptions. We carried out tests to verify the reported 100% excretion of cyanuric acid within 24 hours, but found that test subjects showed a broad range of excretion values. We describe the further investigation of these findings and their implications for future studies using cyanuric acid to estimate water ingestion.

doi: 10.2166/wh.2015.230

## METHODS

### Ethical approval and recruitment of participants

Free and informed consent of the participants was obtained and the study protocols were approved by the appropriate Committees for the Protection of Human Subjects; RMIT (Royal Melbourne Institute of Technology) University Human Research Ethics Committee (Project 34/09, approved 14 September 2009 and Project 14/13, approved 1 May 2013) and the Monash University Human Research Ethics Committee (Project 2009001617, approved 15 October 2009 and Project 2013000748, approved 23 May 2013). Written informed consent was obtained from participants.

The experimental studies were carried out at the RMIT University city campus in Melbourne, Australia. A total of 26 participants were recruited by advertising to staff and students at RMIT and Monash Universities. Information was provided on a website, and those making enquiries about participation were screened by telephone interview. Volunteers were excluded if they reported a prior medical diagnosis of kidney disease, or a history of significant skin or eye irritation after swimming in outdoor pools. As a precautionary measure routinely applied in chemical exposure studies, female volunteers were advised they should not take part if they were pregnant, although there is no evidence that cyanuric acid is harmful to pregnant women or the developing foetus.

### Known dose excretion tests

Participants were requested not to swim in outdoor pools during the 48 hours before the test or during the 24 hours when urine was collected. At the commencement of the test, participants were asked to empty their bladder, then to drink a solution of 1.0 mg cyanuric acid dissolved in 100 mL of Milli-Q water. Each person was supplied with plastic urine collection bottles, a plastic funnel and a carry bag. Female participants were also provided with a 500 mL plastic jug to assist urine collection. The empty urine collection bottles had previously been weighed and labelled with the participant code number, start time and end time for urine collection. Participants were instructed to collect all urine over the subsequent 24 hours. They were asked to keep urine away from sunlight and direct heat sources, but were not instructed to refrigerate the sample during collection as this was considered impractical. At the end of the 24 hour collection period, the collected urine was returned for analysis. Participants were asked whether they had spilled or forgotten to collect any urine, and if so, to estimate the approximate volume and time of loss. The full urine bottles were weighed and the volume of urine in each was calculated by subtracting the weight of the empty bottle and assuming a urine density of 1.0 g/mL. Returned urine samples were stored at 4 °C until assayed. Fluid intake by participants was not controlled or monitored during the test.

### Cyanuric acid assays

The analytical method for measuring CYA was developed at RMIT University. The method involved: (a) three stage solid phase extraction (SPE) to remove organics and inorganic salts which may interfere with the analysis of CYA; (b) derivatisation of CYA to reduce polarity and increase the volatility of CYA; and (c) gas chromatography/mass spectrometry (GC/MS) analysis.

#### Chemicals

Cyanuric acid (99.5%) was purchased from Merck Millipore, pyridine (anhydrous, 99.8%) and bis(trisilyl)fluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were purchased from Sigma-Aldrich.

#### Matrix source

CYA standards were prepared in a blank urine matrix, as suggested by Panuwet *et al.* (2010). Urine used for calibration plots and blank samples was collected from anonymous donors and stored overnight at 4 °C. The neutral, acidic and basic organics and inorganics in the urine sample which may interfere with the analysis of CYA were removed using SPE prior to use in experiments.

#### SPE procedure

The SPE procedure was carried out under vacuum. Neutral organics were removed using a C18 cartridge (Agilent Bond Elut Mega BE-C18, pre-washed with 10 mL methanol then

10 mL MilliQ water) to which 10 mL of urine sample was added and eluted to waste. The cartridge was then eluted with 15 mL Milli-Q water, the eluate was collected and 5 mL introduced to the SAX cartridge (Agilent Bond Elut SAX which had been pre-washed with 2 mL methanol then 2 mL Milli-Q water) for the removal of organic and inorganic anions and the effluent was collected. The cartridge was then eluted with 1 mL Milli-Q water and the effluent from the SAX cartridge and eluate (total 6 mL) was added to a SCX cartridge (Agilent Bond Elut SCX pre-washed with 2 mL methanol then 2 mL MilliQ water) for removal of interfering cations and the eluate collected. The SCX cartridge was then eluted with 1 mL MilliQ water and the eluate collected such that the final volume from SPE was 7 mL.

### Derivatisation of CYA

An aliquot (500 μL) of the urine extract was evaporated to dryness at 70 °C under $N_2$. Pyridine (200 μL) was added to dissolve the cyanuric acid. BSTFA + TMCS (200 μL 99:1, v/v) was added to form the tri-silyl derivatives and the reaction mixture was heated to 70 °C for 45 min in a sealed 2 mL GC vial.

### GC/MS

Chromatographic separation was carried out on an Agilent 6890/5973 GC/MS with auto sampler using a DB-5 MS 30 m × 0.25 mm column × 0.25 mm film thickness. High purity helium (>99.99%) was used as a carrier gas at a constant flow rate of 1.2 mL/min. The column temperature was initially set at 50 °C for 1 min, then programmed to reach 250 °C at 15 °C/min, and held at 250 °C for 2 min. Selected ion monitoring (SIM) was used to ensure that only peaks with the correct fragment ions were detected (SIM analysis for m/z 345, conducted ion source was 70 eV and injection temperature was 200 °C). The column was backflushed at the end of each run. The approximate retention time of the silyl derivative of CYA (2,4,6-trimethylsilyl-1,3,5-triazine) was 9.81 min.

### Validation of analytical method

Protocols based on the Australian National Association of Testing Authorities (NATA 2009) were employed to validate the developed method. This included running a multi-point calibration curve and performing recovery experiments on spiked urine samples to determine accuracy and precision. A nine point calibration curve covering the range 0.05–1.0 mg/L was prepared using analytical grade CYA (99.5%) in the urine matrix. The linearity of the calibration plot was assessed based on the $R^2$ value of the linear regression plot between the CYA spiked level and peak area across the entire range of calibration curve. The method accuracy and precision was determined by calculating the relative standard deviation (RSD) of triplicate measurements of spiked samples at three concentrations (0.05, 0.1 and 1.0 mg/L).

### Stability of CYA in urine

In order to assess the effect of sample storage prior to analysis, the stability of CYA was determined by analysis of spiked urine samples at three CYA levels (0.5, 0.1 and 1.0 mg/L) which had been stored at 4 °C for 3, 7, 14 and 21 days in a glass-fronted refrigerator (with internal light off). The results were compared with those obtained from samples analysed on day 0.

To determine whether significant loss of excreted CYA may have occurred during the 24 hour urine collection period, urine samples from two participants (A-07 and A-14) were spiked with CYA at concentrations of 0.2, 0.6 and 1.0 mg/L CYA and stored in clear plastic bottles with closed lids at room temperature (20–22 °C). The concentration of CYA was measured in duplicate samples after 0, 24, and 48 hours.

## RESULTS

### Analytical method validation

Linear standard curves were obtained for CYA in urine matrix over the concentration ranges 0.01–10.0 mg/L ($R^2 = 0.999$) and 0–1.0 mg/L ($R^2 = 0.997$, Figure 1), the latter being the effective working range. The results confirmed the Limit of Detection for the method as 0.01 mg/L.

Table 1 shows the results of assay precision tests at three spiking levels including the mean value of peak areas and % RSD, the latter being approximately 10% for this concentration range.

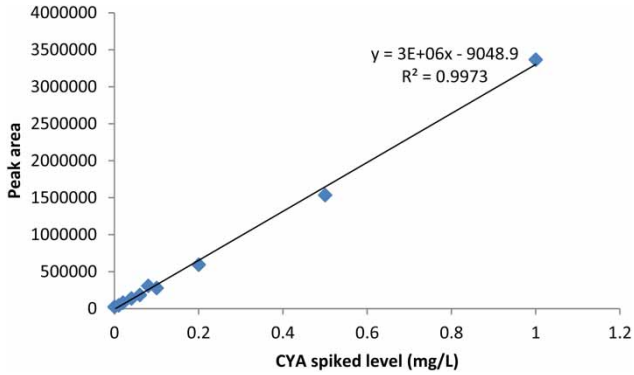


**Figure 1** | Calibration curve for CYA (0–1.0 mg/L) in urine matrix.

**Table 1** | Mean peak area and %RSD of spiked urine samples at three different levels

| Spike level (mg/L) | 0.05 | 0.1 | 1.0 |
|---|---|---|---|
| Replicate 1 (peak area) | 111,479 | 176,774 | 1,366,647 |
| Replicate 2 (peak area) | 91,366 | 167,374 | 1,543,159 |
| Replicate 3 (peak area) | 104,662 | 200,914 | 1,594,813 |
| Mean peak area | 102,502 | 181,687 | 1,501,540 |
| STDEV | 10,229 | 17,301 | 119,641 |
| % RSD | 10 | 10 | 8 |

The recovery of CYA from urine samples spiked at 0.05 mg/L, 0.1 mg/L and 1.0 mg/L was 82%, 92% and 95%, respectively (Table 2). At spike levels of 0.1 and 1.0 mg/L, the error was less than the %RSD of 10% for the method, whereas at 0.05 mg/L it was higher indicating that the measurement becomes less reliable when the CYA concentration approaches the limit of detection (0.01 mg/L). The limit of quantitation of the assay was 0.05 mg/L.

## Stability of CYA in urine

The concentration of CYA in spiked urine samples decreased over the storage period of 21 days at 4 °C, however the changes for 0.05 mg/L, 0.1 mg/L and 1.0 mg/L after 7 days were 0%, 5%, and 7%, respectively, less than the RSD of 10% for the method (Figure 2). Hence, in the range of 0.05 to 1.0 mg/L, CYA may be considered stable when stored for up to 7 days in urine under these conditions.

**Table 2** | Recovery (%) of CYA from spiked urine samples

| Spike level (mg/L) | 0.05 | 0.1 | 1 |
|---|---|---|---|
| Replicate 1 | 84 | 108 | 88 |
| Replicate 2 | 83 | 89 | 94 |
| Replicate 3 | 79 | 80 | 102 |
| Average | 82 | 92 | 95 |
| % Error in accuracy | 18 | 8 | 5 |
| % RSD | 3 | 15 | 7 |

The room temperature stability tests on urine samples from two participants (A-07 and A-14) spiked with CYA at concentrations of 0.2, 0.6 and 1.0 mg/L showed negligible decline in CYA concentrations (less than 5%, within the accepted analytical variation) during storage for 48 hours.

## Known dose excretion tests

Known dose excretion tests were initially performed on three subjects (participant numbers A-01, A-02 and A-03, Table 3) with the intent of confirming 100% excretion as previously reported (Allen *et al.* 1982). However, when all three subjects were found to have excreted less than 100% of the ingested cyanuric acid dose, a further 23 subjects were recruited and tested (participants A-04 to A-15 and B-02 to B-42, Table 1). The age range of participants was 20 to 56 years and the majority (69%) were male.

The total measured amount of cyanuric acid excreted during the 24 hour collection period after ingestion of the 1.0 mg dose ranged from 0.35 mg to 1.05 mg (35% to 105%) among the 26 individuals (Table 3).

Overall, the mean excretion of cyanuric acid was 85.3% (95% confidence interval 77.4– 93.2%) in 24 hours and the median was 94.5%. One participant (B-09) reported incomplete urine collection. The urine loss (one bladder full, assumed to be up to 500 mL), occurred about 23 hours after

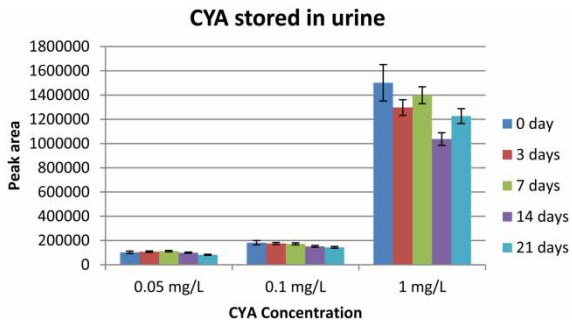


**Figure 2** | The stability of CYA in urine at 4 °C.

**Table 3** | Urinary recovery of 1.0 mg ingested dose of cyanuric acid

| Participant number | Age (years) | Gender | Urine volume (mL) | Total CYA in urine (mg) | % Recovery |
|---|---|---|---|---|---|
| A-01 | 49 | F | 1,498 | 0.71 | 71 |
| A-02 | 47 | M | 2,388 | 0.68 | 68 |
| A-03 | 44 | M | 1,739 | 0.53 | 53 |
| A-04 | 42 | F | 1,453 | 0.84 | 84 |
| A-05 | 42 | F | 2,329 | 0.98 | 98 |
| A-06 | 44 | F | 1,084 | 0.96 | 96 |
| A-07 | 45 | M | 2,201 | 0.86 | 86 |
| A-08 | 54 | F | 1,358 | 0.97 | 97 |
| A-09 | 56 | F | 2,637 | 0.43 | 43 |
| A-10 | 30 | F | 2,058 | 0.96 | 96 |
| A-11 | 26 | F | 1,350 | 1.04 | 104 |
| A-12 | 57 | M | 1,306 | 1.05 | 105 |
| A-13 | 49 | M | 1,263 | 0.96 | 96 |
| A-14 | 27 | M | 911 | 0.91 | 91 |
| A-15 | 30 | M | 1,778 | 0.93 | 93 |
| B-05 | 22 | M | 2,340 | 1.03 | 103 |
| B-09 | 21 | M | 3,142 | 0.60 | 60 |
| B-14 | 20 | M | 2,960 | 0.68 | 68 |
| B-21 | 37 | M | 3,124 | 0.35 | 35 |
| B-23 | 20 | M | 1,194 | 0.85 | 85 |
| B-29 | 29 | M | 1,146 | 1.00 | 100 |
| B-30 | 26 | M | 1,625 | 0.89 | 89 |
| B-34 | 34 | M | 1,120 | 0.96 | 96 |
| B-37 | 39 | M | 1,390 | 1.03 | 103 |
| B-40 | 23 | M | 976 | 1.00 | 100 |
| B-42 | 29 | M | 1,487 | 0.97 | 97 |

ingestion of the CYA dose and was considered to have a minor impact on the CYA recovery for this participant. The relationship between the 24 hour urine volume and the percentage excretion is shown in Figure 3. There was a moderately strong negative association between the 24 hour urine volume and percentage of the ingested dose excreted; *Spearman's rank correlation coefficient* $= -0.53$ ($P = 0.006$).

Human renal function generally declines with age, and since the only prior published data on CYA excretion in humans originated from a study of swimmers aged 9 to 17 years (Allen *et al.* 1982), we examined the relationship between participant age and percentage of CYA excreted to determine whether this might explain the lower excretion

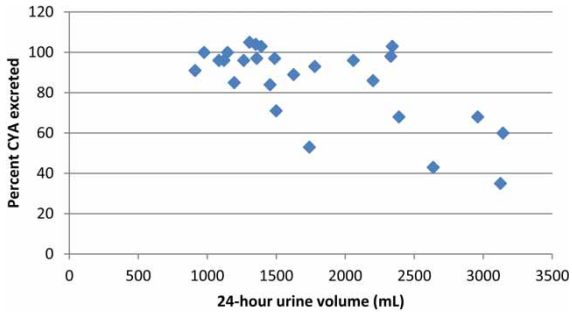


**Figure 3** | Plot of percent CYA excreted versus 24 hour urine volume.

in some members of our study group (Figure 4). This analysis showed no evidence of a significant association; *Spearman's rank correlation coefficient* $= -0.07$ ($P = 0.74$).

## DISCUSSION

In this group of volunteers, 50% (13/26) excreted at least 95% of a 1.0 mg ingested dose of cyanuric acid into urine within 24 hours (essentially complete excretion, taking into account the accuracy of the assay method), but the remainder showed lower levels of excretion. These findings are not consistent with the previous report that humans exhibit 100% urinary excretion of an ingested dose of cyanuric acid within 24 hours, but the extent of published work supporting this premise is very limited (Allen *et al.* 1982). Considerable research on the metabolism of cyanuric acid has been carried out in animals (reviewed by Hammond *et al.* 1986), but the only available human data appear to come from a study by Allen *et al.* (1982) which involved five subjects aged 9 to 17 years. In a controlled test, two of these individuals (ages not stated) ingested a known dose of cyanuric acid and were found to have excreted more than 98% in their urine within 24 hours. The dose of cyanuric acid ingested was not reported.

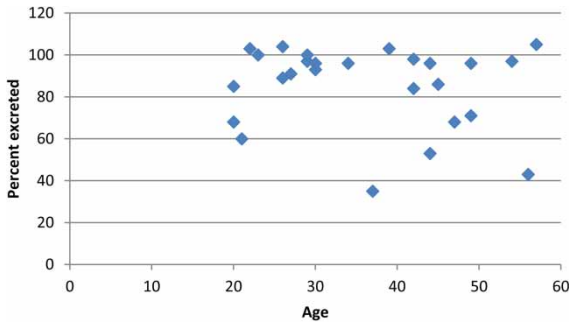


**Figure 4** | Plot of percent CYA excreted versus participant age in years.

On the basis of these results and the CYA excretion kinetics in five subjects who ingested variable amounts of CYA while swimming, it was concluded that cyanuric acid was 'excreted rapidly (half-life about 3 hours) and nearly quantitatively after oral ingestion' (Allen *et al.* 1982). It appears that no further investigation of cyanuric acid excretion in humans has been performed.

Stability tests showed that the variable recovery shown in our subject group could not be explained by loss of CYA during the urine collection period or during refrigerated storage prior to assay. Minimal decline in CYA concentrations in urine stored for up to 48 hours at room temperature have been reported previously (Patel & Jones 2007; Zhang *et al.* 2010). Low recovery of CYA from urine during the extraction and analysis procedure is also unlikely. Our method validation work showed that the percentage recovery of CYA from spiked urine samples declined as CYA concentrations were reduced, with an average 95% recovery at a concentration of 1.0 mg/L, 92% at 0.1 mg/L and 85% at 0.05 mg/L. All of the known dose urine samples from our subjects had calculated CYA concentrations in excess of 0.1 mg/L, suggesting that average recoveries of 92% or more of the actual CYA content should have been expected. Thus, method limitations cannot explain the low percentage recoveries of the ingested dose from some 24 hour urine samples.

There are a number of other possible explanations for our findings, as follows:

- Incomplete absorption of CYA from the gastrointestinal tract. According to a review of available data (Hammond *et al.* 1986) experimental work in rats and dogs showed complete absorption of single oral cyanuric acid doses of 5 mg/kg body weight (equivalent to a 350 mg dose for a 70 kg human). A much higher dose of 500 mg/kg was incompletely absorbed in both animal species. The only data on CYA absorption in humans appear to be those from the two individuals given a known dose as part of the swimming study (Allen *et al.* 1982). Essentially complete (98%) recovery of the administered CYA dose (amount not stated) in urine from both subjects indicated complete gastrointestinal absorption.
- Metabolism of CYA in some participants to a derivative form not detected by the GC/MS assay. No human studies on CYA metabolism appear to exist other than the work of Allen *et al.* (1982), however tests in rats and dogs using $^{14}C$ radio-labelled CYA did not show evidence of metabolism or persistence in tissues (Hammond *et al.* 1986). It has therefore been assumed that humans also do not metabolise CYA.
- Incomplete urine collection by the participants. Only one person among our 26 subjects reported loss of urine during collection, although it is possible that some participants may have lost urine and not reported this. The tendency for lower percentage excretion at higher urine volumes is not consistent with this hypothesis.
- Presence of substances in the urine of some individuals that are not removed by the SPE cleanup procedure and interfere with CYA measurement by GC/MS. There are currently no data to support or refute this possibility.
- Slower excretion of CYA in some individuals. The excretion kinetics of CYA was examined in five people after swimming in an indoor pool (Allen *et al.* 1982) and estimates of the excretion half-life varied from 2.2 to 3.5 hours. Assuming the higher of these values (slower excretion) applies, at least 98% excretion would be expected within 24 hours. However, it is possible that this small study failed to capture the full range of variability in excretion rates among individuals.

One individual in our study (A-14) later completed a second known dose excretion test and demonstrated 99% CYA on this occasion, compared to 91% on the first test. These data are not sufficient to infer whether the percentage of cyanuric acid excreted within 24 hours is relatively constant for each person, or whether an individual might have high excretion on one occasion and lower excretion on another.

## CONCLUSIONS

Our results indicate considerable variability between individuals in the percentage of an ingested dose of cyanuric acid that is excreted in the urine within 24 hours. The percentage excretion was not related to the age of test subjects, but showed a moderately strong negative association with the volume of urine produced over the 24 hour period following ingestion *Spearman's rank correlation coefficient* $= -0.53$ ($P = 0.006$). We have not explored the reason(s) for the observed variability other than excluding the possibilities

relating to urine collection and storage procedures prior to analysis and the sensitivity of the CYA assay methodology. Better characterisation of CYA absorption, metabolism and excretion in humans would require commitment of considerable experimental resources and there is no guarantee that such knowledge would reveal factors that are amenable to changes in participant selection, experimental design or assay methods.

Nevertheless, use of cyanuric acid has potential to generate data on water ingestion during a range of activities, and thus facilitate improved health risk assessment for non-potable water sources, provided the limitations of the methodology are recognised. In our participant group, the assumption of 100% excretion would have resulted in a 15% underestimate of the volume of water ingested. Such an error may have only a minor effect in quantitative risk assessment calculations relative to the uncertainties in other inputs, but since the reasons for individual variation in CYA excretion are unknown, it is unclear whether a greater degree of underestimation might occur in some circumstances. Our observations suggest it would be advisable for future studies to incorporate a randomly selected subgroup of participants to perform known dose excretion tests instead of (or in addition to) water exposure tests in order to ascertain the degree of underestimation associated with calculated ingestion volumes in the particular cohort and experimental conditions under study.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS CONTRIBUTIONS

M.S., K.L. and F.R. designed the study. M.S., J.O.T. and F.R. developed the protocol and recruited participants. S.G. developed the cyanuric acid assay method, S.G. and T.N. performed the experiments and carried out the assays. F.R. supervised the laboratory component, and edited the manuscript. M.S. and T.N. drafted the manuscript. All authors have read and approved the final manuscript.

## ACKNOWLEDGEMENTS

This work was supported by Coliban Region Water Corporation, Melbourne Water, South Australian Water, Sydney Water Corporation and Water Research Australia Limited (Projects 3002 and 3021).

## REFERENCES

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

Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P., Wroblewski, M., Liu, L., Scheff, P. A. & Li, A. 2011 Water ingestion during water recreation. *Water Res.* **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. *J. Water Health* **4**, 425–430.

Hammond, B. G., Barbee, S. J., Inoue, T., Ishida, N., Levinskas, G. J., Stevens, M. W., Wheeler, A. G. & Cascieri, T. 1986 A review of toxicology studies on cyanurate and its chlorinated derivatives. *Environ. Health Persp.* **69**, 287–292.

NATA 2009 *Guidelines for the Validation and Verification of Chemical Test Methods*. National Association of Testing Authorities, Australia, Technical Note 17 – April 2009.

NRMMC/EPHC/AHMC 2006 Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1). National Resource Management Ministerial Council; Environmental Protection and Heritage Council; and Australian Health Ministers Conference.

Panuwet, P., Wade, E. L., Nguyen, J. V., Montesano, M. A., Needham, L. L. & Barr, D. B. 2010 Quantification of cyanuric acid in human urine using high performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* **878**, 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. *J. Chromatog. B* **853**, 360–363.

Suppes, L. M., Abrell, L., Dufour, A. P. & Reynolds, K. A. 2014 Assessment of swimmer behaviors on pool water ingestion. *J. Water Health* **12** (2), 269–279.

WHO 2003 *Guidelines for Safe Recreational Water Environments*. Volume 1, Coastal and fresh waters. World Health Organization, Geneva, Switzerland.

Zhang, M., Li, S., Yu, C., Liu, G., Jia, J., Lu, C., He, J., Ma, Y., Zhu, J. & Yu, C. 2010 Determination of melamine and cyanuric acid in human urine by a liquid chromatography tandem mass spectrometry. *J. Chromatogr. B* **878**, 758–762.

First received 17 June 2015; accepted in revised form 8 October 2015. Available online 30 October 2015