Chemical contaminants in swimming pools: Occurrence and health risk assessment

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CHEMICAL CONTAMINANTS IN SWIMMING POOLS: OCCURRENCE AND HEALTH RISK ASSESSMENT

By

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A thesis in fulfilment of the requirements for the degree of

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School of Civil and Environmental Engineering

Faculty of Engineering

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ORIGINALITY STATEMENT

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ABSTRACT

A wide variety of chemical substances may be present as trace contaminants in swimming pools. These include chemicals which may be formed as by-products of swimming pool disinfection processes, as well as chemicals, which may be derived from bathers, such as from bodily excretions or wash-off of cosmetics or lotions. In other circumstances, chemicals may have been present in the fill water used to fill swimming pools, or may be leached from bathing equipment such as flotation devices. Bathers may be exposed to trace chemical contaminants in swimming pools via a variety of exposure routes including accidental ingestion, inhalation and dermal absorption. However, the range of chemicals present, their concentrations and potential levels of exposure have scarcely been investigated.

The aim of this research was to investigate the concentrations of anthropogenically-derived chemicals in swimming pools and to provide a risk assessment corresponding to the chemicals detected. Various types of swimming pools were analysed including indoor pools, outdoor pools, spa pools and seawater pools.

Swimming pool water samples were analysed for 30 pharmaceuticals and personal care products and 7 *N*-nitrosamines. Caffeine, ibuprofen and three *N*-nitrosamines were detected in swimming pool water samples. Daily monitoring of caffeine revealed high variations throughout the day roughly reflecting bather loads.

A rapid and reliable analytical method was developed for the analysis of five organophosphate flame retardants (PFRs) in water using isotope dilution gas chromatography tandem mass spectrometry. The method was applied to investigate the occurrence and source of PFRs in swimming pools. Laboratory experiments were carried out to investigate the potential leaching of PFRs from commonly used swimming equipment to identify the sources of PFRs in swimming pools.

A quantitative risk assessment revealed that exposure health risk to these chemicals via swimming pools were generally very low and below commonly applied health risk benchmarks.

The potential application of fluorescence as an online monitoring tool in swimming pools was assessed by investigating the relationships between fluorescence signals at various excitation and emission wavelengths and changes in water quality over time.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

µSPE	micro solid phase extraction
2,6-DBBQ	2,6-dibromo-(1,4)benzoquinone
2,6-DCBQ	2,6-dichloro-1,4-benzoquinone
4-MBC	4-methyl-benzylidene camphor
AOPs	advanced oxidation processes
AOX	absorbable organic halogen
BAN	bromoacetonitrile
BCAA	bromochloroacetic acid
BCAN	bromochloroacetonitrile
BCDMH	bromochlorodimethylhydantoin
BCNM	bromochloronitromethane
BDCAA	bromodichloroacetic acid
BFA	body fluid analogues
BMDBM	butyl methoxy dibenzoylmethane
BNM	bromonitromethane
BP-3	benzophenone-3
CAN	chloroacenotonitrile
CDBAA	chlorodibromoacetic acid
C-DBPs	carbonaceous disinfection by-products
CDMA	chlorinated dimethylamine
$CHBr_3$	bromoform
$CHBrCl_2$	bromodichloromethane
$CHCl_3$	chloroform
$CHClBr_2$	dibromochloromethane
CHO	Chinese hamster ovary
CI	chemical ionization
CZE	capillary zone electrophoresis
DAI	direct aqueous injection
DBAA	dibromoacetic acid
DBAN	dibromoacetonitrile
DBNM	dibromonitromethane
DBPs	disinfection by-products
DCAA	dichloroacetic acid
DCAN	dichloroacetonitrile
DCM	dichloromethane
DEET	*N*,*N*-diethyl-meta-toluamide
DMDBBQ	2,3-dibromo-5,6-dimethyl-(1,4)benzoquinone
DOM	dissolved organic matter
ECD	electron capture detection
EEM	excitation-emission matrix
EGMO	electrochemically generated mixed oxidants

EHPABA	2-ethylhexyl 4-(dimethylamino) benzoate
EI	electron ionisation
EPA	Environmental Protection Agency
ES	2-ethylhexyl salicylate
ESI	electrospray ionization
FD	fluorescence detection
FID	flame ionization detection
GC	gas chromatography
GC-MS/MS	gas chromatography tandem mass spectrometry
HAAs	haloacetic acids
HANs	haloacetonitriles
HBQs	halobenzoquinones
HLB	hydrophilic lipophilic balance
HMS	homosalate
HNMs	halonitromethanes
HPLC	high-performance liquid chromatography
HQ	hazard quotient
HS	headspace
IARC	International Agency for Research on Cancer
*i*PrP	isopropylparaben
ITMS	ion trap mass spectrometry
LC	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
LC-UV-DAD	liquid chromatography and photodiode array detection
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limits of quantification
MBAA	monobromoacetic acid
MCAA	monochloroacetic acid
MDLs	method detection limits
med	median
MIMS	membrane introduction mass spectrometry
MQLs	method quantification levels
MRM	multiple reaction monitoring
MS	mass spectrometry
MSD	mass-selective detection
MTBE	methyl *tert*-butyl ether
NCl_3	tri-chloramine
NDBuA	*N*-nitrosodibutylamine
N-DBPs	nitrogenous disinfection by-products
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NDPA	*N*-nitrosodipropylamine

NMEA	*N*-nitrosomethylethylamine
NMor	*N*-nitrosomorpholine
NPip	*N*-nitrosopiperidine
NPyr	*N*-nitrosopyrrolidine
NR	not reported
OC	octocrylene
OCR	2-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate
ODPABA	octyl dimethyl-*p*-aminobenzoate
OMC	octyl-*p*-methoxycinnamate
PAC	powdered activated carbon
PAT	purge and trap
PBS	2-phenyl-1H-benzimidazole-5-sulfonic acid
PCPPs	pharmaceutical and personal care products
PCPs	personal care products
PFRs	organophosphate flame retardants
PPCPs	pharmaceuticals and personal care products
RfD	reference dose
S/N	signal-to-noise ratio
SAHC	South Australian Health Commission
SCGE	single cell gel electrophoresis
SDME	single drop microextraction
SPE	solid phase extraction
SPME	solid phase microextraction
TBAA	tribromoacetic acid
TBOEP	tris(2-butoxyethyl) phosphate
TNBP	tributyl phosphate
TCAA	trichloroacetic acid
TCAN	trichloroacetonitrile
TCEP	tris(2-chloroethyl) phosphate
TCNM	trichloronitromethane
TCIPP	tris(1-chloro-2-propyl) phosphate
TDCIPP	tris(1,3-dichloro-2-propyl) phosphate
THMs	trihalomethanes
TOC	total organic carbon
TPHP	triphenyl phosphate
TriCBQ	2,3,6-trichloro-(1,4)benzoquinone
UPLC	ultra performance liquid chromatography
US EPA	United States Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organization

LIST OF PUBLICATIONS

International Refereed Journals

1. Teo TLL, Coleman HM, Khan SJ. (2015) Chemical contaminants in swimming pools: Occurrence, implications and control, Environment International 76 (2015) 16-31. doi: 10.1016/j.envint.2014.11.012

2. Teo TLL, McDonald JA, Coleman HM, Khan SJ. (2015) Analysis of organophosphate flame retardants and plasticisers in water by isotope dilution gas chromatography-electron ionisation tandem mass spectrometry. Talanta 143:114-120. doi: 10.1016/j.talanta.2015.04.091

3. Teo TLL, Coleman HM, Khan SJ. (2015) Occurrence and daily variability of pharmaceuticals and personal care products in swimming pools, Environmental Science and Pollution Research 1-10, doi: 10.1007/s11356-015-5967-4

4. Teo TLL, Coleman HM, Khan SJ. Presence and select determinants of organophosphate flame retardants in public swimming pools, *in preparation for submission to Talanta.*

Conference Proceedings / Presentations

1. T.L.L. Teo, H.M. Coleman, S.J. Khan. Occurrence of chemical contaminants in swimming pools, In: 3rd SETAC Australasia Conference: Melbourne 2013. Conference Handbook. *3rd SETAC Australasia Conference: Melbourne 2013*, Melbourne, VIC, Australia, (74) 1-3 October, 2013.

2. T.L.L. Teo, H.M. Coleman, S.J. Khan. Chemical contaminants in swimming pools In: Programme and Abstract Book: Micropol & Ecohazard 2013. *Micropol & Ecohazard 2013: 8th IWA Specialized Conference on Assessment and control of micropollutants and hazardous substances in water*, Zurich, Switzerland, (P16-P17). 16-20 June, 2013.

CHAPTER 1 INTRODUCTION

This chapter has been published in part in the following journal paper:

Teo TLL, Coleman HM, Khan SJ. (2015) Chemical contaminants in swimming pools: Occurrence, implications and control. Environment International 76:16-31.

1.1 Background

Given the many health benefits associated with swimming, it has become a popular sport for people of all ages. Treatment of pool water along with the advancement of engineering and design aspects of swimming pools has made swimming possible throughout the whole year. Although the process of disinfecting swimming pool water varies locally, the World Health Organization (WHO, 2006) has outlined a 'typical pool' water treatment process (see Figure 1.1).

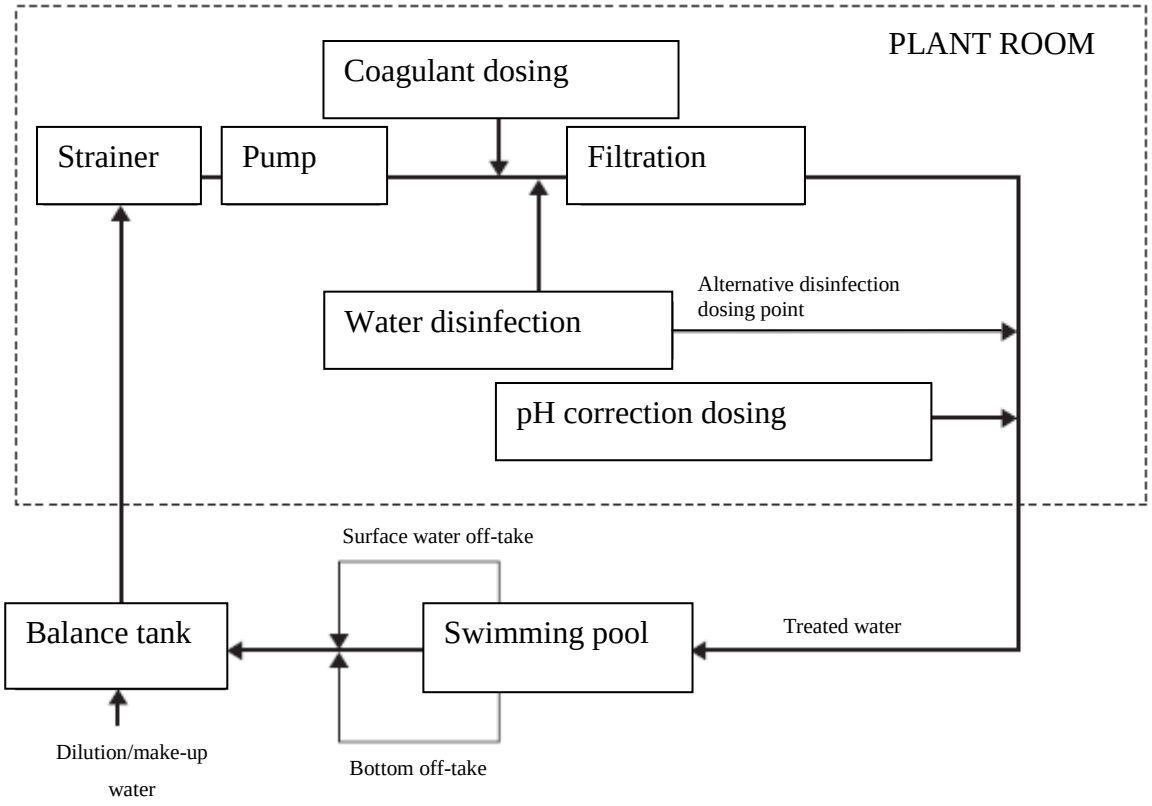


Figure 1.1 Schematic diagram of a 'typical pool' water treatment process (WHO, 2006)

Chlorine-based disinfectants are commonly employed for microbial disinfection in swimming pools worldwide (WHO, 2006; Lee *et al.*, 2010). Chlorination provides rapid and long-lasting disinfection of swimming pool water. In chlorinated seawater swimming pools, bromine is the predominant disinfectant as the naturally occurring bromide ions in seawater react with free chlorine (hypochlorous acid) undergoing rapid oxidation to form hypobromous acid (Xue *et al.*, 2008). The use of ozone and UV disinfectants have been adopted in some cases, although generally they are used together with either chlorine or bromine for the provision of a residual disinfectant

(WHO, 2006). Disinfectants that have been used for swimming pool disinfection are summarised in Table 1.1.

Table 1.1 Disinfectants used in swimming pools

Chlorine based	Chlorine gas Calcium/sodium/lithium hypochlorite Dichloro isocyanurates Trichloro isocyanurates
Bromine based	Bromochlorodimethylhydantoin (BCDMH) Sodium bromide + oxidizer
Others (usually in combination with chlorine/bromine based)	Ozone Ultraviolet (UV) Chlorine dioxide Iodine (Potassium iodide)
New/emerging disinfectants	Magnesium salts Sodium bromide + oxidizer Ozone + hydrogen peroxide

Continuous organic loading from swimmers and the need to maintain a set amount of free chlorine in swimming pools tend to require higher chlorine doses for disinfection compared to drinking water. According to the guidelines set by the WHO, the concentration of free chlorine should not exceed 3 mg/L for public/semi-public swimming pools and 5 mg/L for hot tubs with pH levels maintained between 7.2-7.8 (WHO, 2006). While these guidelines are recommended by the WHO, the practices adopted for the disinfection of swimming pools may be considerably more variable.

A disadvantage to chlorination is that it has the potential to produce a wide range of disinfection by-products (DBPs) through the reaction with organic and inorganic matter (Rook, 1974; Richardson *et al.*, 2007; Tanju Karanfil *et al.*, 2008). The organic matter concentrations may be highly variable due to continuous loading introduced into the pool by swimmers, the environment and from the water supplied to the pool. Much of the research focusing on chemical contaminants in swimming pools has concentrated on the occurrences of DBPs (Zwiener *et al.*, 2007; Richardson *et al.*, 2010; Chowdhury *et al.*, 2014). Simulated laboratory experiments have also been carried out to study the chlorination reactions of DBPs in swimming pools (Kim *et al.*, 2002; Kanan and Karanfil, 2011). These studies reported that higher loadings of organic matter introduced by swimmers (body fluids, skin, hair, lotions) contributed to higher levels of

DBPs. Parabens (Terasaki and Makino, 2008; Alcudia-León *et al.*, 2013), ultraviolet (UV) filters (Zwiener *et al.*, 2007; Vidal *et al.*, 2010) found in personal care products (PCPs) such as lotions and sunscreens and more recently, *N*,*N*-diethyl-meta-toluamide (DEET), caffeine and tris(2-carboxyethyl)phosphine (TCEP) (Weng *et al.*, 2014) have also been detected in swimming pools. Furthermore, concerns are emerging over the possibility that these chemicals may react with the disinfectants used, transforming them into by-products which may be more harmful than the unchanged PCPs (Bottoni *et al.*, 2014). There is evidence to suggest that exposure to some of these chemicals may lead to health risks (Villanueva *et al.*, 2007a; Kogevinas *et al.*, 2010).

The priority of the disinfection process in swimming pools is to maintain microbial water quality in order to inhibit the spread of infections and diseases. However, with an increasing range of chemical contaminants being detected in swimming pools, increasing attention is being paid to the potential significance of exposure by bathers to these chemicals. This may affect the way that disinfection is applied or other aspects of swimming pool operation and management. Most countries do not have a specified regulatory limit for DBPs or any other type of chemicals in swimming pools. However, the German Standard (2012) has set a maximum level of 20 µg/L for total trihalomethanes (THMs) in swimming pool waters. Also in France a maximum limit of 100 µg/L for total THMs has been recommended for all public swimming pools (ANSES, 2012).

Swimming pool users continuously introduce organic matter to swimming pools through the excretion of body fluids (urine and sweat) and from the washing-off of personal care products (cosmetics and sunscreens) during swimming. As a result, a wider range of chemical contaminants may be present in swimming pools. Through this study, the occurrence of various chemical contaminants and a health risk assessment of the major chemical contaminants found in swimming pool water were determined. The concentrations of chemicals present in swimming pools may provide an alternative indicator to swimming pool water quality, providing insights to contamination sources.

1.2 Research objectives

The objectives of this research were as follows:

1. Review the existing literature to provide an understanding of the state of the science regarding trace chemical contaminants in swimming pools, and identify key knowledge gaps;
2. Develop an analytical method for the analysis of organophosphate flame retardants (PFRs) in swimming pool water by gas chromatography tandem mass spectrometry (GC-MS/MS);
3. Investigate the sources of PFRs in swimming pools through laboratory-based leaching studies;
4. Investigate the presence and concentrations of chemicals in swimming pools, including pharmaceuticals and personal care products (PPCPs), *N*-nitrosamines and PFRs. This would involve sampling from a variety of swimming pools including freshwater indoor pools, outdoor pools, spa pools and seawater pools;
5. Investigate the use of fluorescence spectroscopy as a real-time monitoring tool for swimming pool water;
6. Conduct a health risk assessment on the chemicals identified in this study based on the Australian EnHealth Environmental Health Risk Assessment Guidelines.

Overall, the results of this research will provide detailed and valuable information with regards to the concentrations of chemical contaminants in swimming pool water, their sources, the possible use of fluorescence spectroscopy to monitor swimming pool water quality and provide an assessment of the risks (if any) posed to public health.

1.3 Overview of chapters

The thesis is presented in 10 chapters:

Chapter 1 provides a brief introduction, the background and objectives of this research and the structure of the thesis.

Chapter 2 provides a comprehensive literature review of the chemicals that have previously been reported in swimming pools. A range of factors influencing the fate of chemicals and the control measures that can be implemented to reduce their occurrence in swimming pools are discussed. The possible health risks to swimmers when exposed to these chemicals during swimming are also considered.

Chapter 3 describes the sampling procedures and analytical methods used for swimming pool water analysis. Liquid chromatography tandem mass spectrometry (LC-MS/MS) and GC-MS/MS were used for the analysis of chemical contaminants. The development of a suitable fluorescence method for measuring fluorescent dissolved organic matter in swimming pools is also presented.

Chapter 4 describes the development and optimisation of a GC-MS/MS analytical method for the simultaneous determination of five PFR compounds in water. This chapter also reports the method performance and applicability in various environmental waters.

Chapter 5 reports on the occurrence and concentrations of PFRs in various swimming pools. Results from the leaching experiments carried out in the laboratory on commonly used swimming equipment are also presented.

Chapter 6 reports on the concentrations and daily variability of caffeine and ibuprofen concentrations in various swimming pools.

Chapter 7 presents the occurrence and concentrations of seven *N*-nitrosamines compounds in chlorinated public indoor and outdoor swimming pools and seawater pools.

Chapter 8 details the use of fluorescence to monitor organic loading in swimming pools. The potential use of an online fluorescence monitoring tool to monitor the changes in water quality in swimming pools is also discussed.

Chapter 9 presents a quantitative assessment on the potential health risks associated with the exposure to the chemicals detected in this study based on the Australian EnHealth Environmental Health Risk Assessment Guidelines.

Chapter 10 summarises the conclusions from this study and presents recommendations for future research.

CHAPTER 2 CHEMICAL CONTAMINANTS IN SWIMMING POOLS: OCCURRENCE, IMPLICATIONS AND CONTROL – A REVIEW

This chapter has been published in the following journal paper:

Teo TLL, Coleman HM, Khan SJ. (2015) Chemical contaminants in swimming pools: Occurrence, implications and control. Environment International 76:16-31.

2.1 Introduction

A range of trace chemical contaminants have been reported to occur in swimming pools (Zwiener *et al.*, 2007; Terasaki and Makino, 2008; Chowdhury *et al.*, 2014; Teo *et al.*, 2015). Possible sources of chemical contaminants in swimming pools include contamination from pool users themselves (bodily excretions, lotions, cosmetics, etc.), the fill water used where trace chemicals may already be present, and reactions between disinfectants and the daily fluxes of organic contribution corresponding to levels of patronage. Organic matter in swimming pools may be highly variable due to continuous loading introduced into the pool by swimmers, the environment, and from the water supplied to the pool. Additionally, the recirculation of swimming pool water may lead to the accumulation of various chemical contaminants over time. On the other hand, recirculation may lead to enhanced elimination of some chemicals by volatilisation.

The following sections reviews the various types of chemicals reported to have been detected in swimming pools. A range of factors, such as the treatment processes which affect the fate of chemical contaminants occurring in swimming pools are discussed. The possible risks these chemicals may pose to swimmers when exposed during swimming are also considered.

2.2 Chemical health concerns

Exposure to trace chemical contaminants in swimming pools may affect the health of swimmers. The overall *in vitro* chronic cytotoxicity of swimming pool water analysed using Chinese Hamster Ovary (CHO) cells found that swimming pool water was significantly more toxic than tap water (Plewa *et al.*, 2011). However, another *in vitro* toxicity study using the Ames-Test determined that swimming pool waters have the same mutagenicity potential as chlorinated drinking water (Richardson *et al.*, 2010).

The overall genotoxicities of swimming pools treated with a range of disinfectants were evaluated using the CHO cells and single cell gel electrophoresis (SCGE) assay which measures the level of genomic DNA damage and the carcinogenic potential (Liviac *et al.*, 2010). As the constituents of pool water responsible for the observed genotoxicity is unknown, this study sought to measure total bather chemical load exposure for overall genotoxicity effects. It was determined that swimming pool water was more genotoxic

by this assay compared to chlorinated tap water. Swimming pools disinfected with brominated disinfectants showed the highest genotoxic effects suggesting that the type of disinfectant used had an impact on the toxicity of pool water. The addition of bromochlorodimethylhydantoin (BCDMH) is one method of bromine disinfection in large, heavily used swimming pools and is often used in tablet or granular form South Australian Health Commission (SAHC) (1991); World Health Organization (WHO) (2006). BCDMH reacts with water to produce hypobromous acid, hypochlorous acid and dimethylhydantoin. The disadvantage of using BCDMH is the need to monitor dimethylhydantoin by a qualified laboratory as there is no poolside test kit available (WHO, 2006). However, the advantages of using BCDMH are that it is a relatively safe chemical, does not regularly need pH correction and is easy to dose (WHO, 2006). Swimming pools disinfected with BCDMH were approximately four times more genotoxic than pools disinfected with chlorine-based and UV disinfectants. Further studies on the same water samples tested by Liviac *et al.* (2010) confirmed that swimming pool water was significantly more cytotoxic than the chlorinated tap water (Plewa *et al.*, 2011). Another study using Hep-G2 cells (SCGE assay) reported that the constituents of swimming pool water extracts with non-volatile, lower molecular weight compounds consisting of more than 30% total organic carbon (TOC) and absorbable organic halogen (AOX) were most genotoxic (Glauner *et al.*, 2005b).

Concentrates derived from swimming pools exposed to sunlight (outdoor pools) were reported to be five times less genotoxic than indoor pools despite similar TOC and total chlorine residual levels and it was suggested that environmental conditions of outdoor pools potentially increased volatilisation of chemical contaminants thus reducing its genotoxicity (Liviac *et al.*, 2010). Kogevinas *et al.* (2010) associated swimmers exposure to DBPs during swimming with genotoxicity biomarkers. Total THMs (chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$)) concentration in pool water was reported at 46 µg/L while air samples collected near the pool vicinity had total THM levels of about 74 $\mu g/m^3$. THM levels were then measured in blood, urine and exhaled air collected from 49 adults before and after swimming. In exhaled air samples of swimmers, THM levels were found to be seven times higher after swimming compared to before swimming. Micronucleated lymphocyte frequency and urine mutagenicity increased after

swimming which was associated with higher concentrations of brominated THMs in exhaled breath. Swimming was not associated with DNA damage in peripheral blood lymphocytes measured using the SCGE assay. Also, no significant association with changes in micronucleated urothelial cells, another measure of DBPs genotoxicity, was observed. Overall, it was determined that exposure only to brominated THMs in swimming pools was associated with increased genotoxicity markers indicating that brominated DBPs are linked to higher genotoxicity compared to chlorinated DBPs.

An epidemiological study identified an association between increased incidences of bladder cancer risk with long-term THMs exposure from the ingestion of drinking water and dermal absorption and inhalation while showering, bathing and swimming in pools (Villanueva *et al.*, 2007a). Lifetime data on water consumption and water-related habits were collected from 1219 individuals with 1271 controls and linked to THM levels in the study area. Results show that subjects with THM exposure through drinking water had an odds ratio of 1.35 (95% confidence interval: 0.92, 1.99). The duration of shower or bath weighted by residential THM level was 1.83 (95% confidence interval: 1.17, 2.87) while swimming in pools was associated with an odds ratio of 1.57 (95% confidence interval: 1.18, 2.09).

Haloacetonitrile (HAN) contributions were the highest in the overall toxicological effect of swimming pool water compared to other DBPs such as THMs and haloacetic acids (HAAs) (Hansen *et al.*, 2012a). HANs from swimming pool water extracts especially dibromoacetonitrile (DBAN) and bromochloroacetonitrile (BCAN) showed high cytotoxic and genotoxic responses in biological assays (Kramer *et al.*, 2009). Also, the toxicity of halonitromethanes (HNMs), in particular trichloronitromethane (TCNM) and bromonitromethane (BNM), tested using human cells were found to be genotoxic and cytotoxic (Liviac *et al.*, 2009). The brominated compound (BNM) showed higher genotoxicity response than the chlorinated compound (TCNM).

An association between repeated DBP exposure during swimming and the increase of impaired respiratory health have been reported (Font-Ribera *et al.*, 2010). Additionally, individuals regularly exposed to swimming pool air, such as pool attendants and elite swimmers, reported greater respiratory symptoms, suspected to be due to chloramine

exposure (Thickett *et al.*, 2002; Lévesque *et al.*, 2006; Jacobs *et al.*, 2007). Furthermore, chloramines were found to be the potential causes of skin and eye irritation in swimmers (Hery *et al.*, 1995; Kaydos-Daniels *et al.*, 2008; Parrat *et al.*, 2012). Florentin *et al.* (2011) concluded that there are potential health risks from chemical exposures to DBPs in swimming pools due to various reports of skin and eye irritation, respiratory symptoms and increased toxic risk from DBPs during swimming. Most DBPs that have been detected in swimming pools are unregulated.

The presence of sunscreen agents and parabens in swimming pools may further increase the health risk during swimming as some of those compounds exhibit hormonal activity (Schlumpf *et al.*, 2004; Golden *et al.*, 2005). Previous authors have concluded that further research is needed to evaluate potential health risk not only from DBPs but also from other chemicals occurring in swimming pool such as sunscreen agents and other chemicals from PCPs (Zwiener *et al.*, 2007; Terasaki and Makino, 2008). Additionally, concerns have been raised that degradation of these chemicals may produce by-products that are more toxic than their parent compound and may be of more relevance to the health of swimmers (Díaz-Cruz and Barceló, 2009; Terasaki *et al.*, 2009).

Most studies have only investigated the potential health effects and toxicity of a certain class of DBPs. However, with many chemical contaminants occurring in swimming pools, their combined health effects to swimmers may be more significant and further studies would be required to investigate potential mixture effects that may occur in swimming pools.

2.3 Disinfection By-Products (DBPs)

Chlorination has the potential to produce a wide range of disinfection by-products (DBPs) through the reaction with organic and inorganic matter as is well established from studies on disinfection of drinking water (Rook, 1974; Richardson *et al.*, 2007; Tanju Karanfil *et al.*, 2008). The two main groups of organic precursor which lead to the formation of DBPs in swimming pools are (1) organic matter from the fill water and (2) body substances from swimmers such as urine, sweat and skin lipids. Increased contact time between disinfectants and the organic precursors in swimming pools lead to higher levels of DBP formation (Kanan and Karanfil, 2011). The formation of DBPs

has been correlated with the organic loadings from swimmers indicating that swimmers are a key source of DBP precursors in a swimming pool environment (Kanan and Karanfil, 2011; Kim and Han, 2011).

Since the detection of THMs in swimming pool water in 1980 (Beech *et al.*, 1980), most of the existing research conducted has concentrated on chlorinated DBPs including THMs and HAAs (Erdinger *et al.*, 2004; Wang *et al.*, 2014b). There are now over 700 DBPs that have been identified in disinfected waters, mainly chlorinated drinking waters (Malliarou *et al.*, 2005; Richardson *et al.*, 2007).

THMs are among the commonly detected DBPs in both disinfected drinking waters and swimming pool waters with chloroform being the most documented THM. International studies on the occurrence of THMs and the methods used to detect them in swimming pools are summarised in Table 2.1. However, the THM levels from studies using headspace gas chromatographic analysis with unreported headspace temperatures may not reflect accurate levels of THMs in swimming pools because reports have shown that this method has led to the overestimation of THM levels due to the decarboxylation of related compounds into THMs at elevated temperatures (60°C) (Cammann and Hübner, 1993; Takahashi *et al.*, 2003). Most of the studies presented in Table 2.1 have been conducted on chlorinated indoor swimming pools. Chloroform was detected much more frequently in chlorinated swimming pools compared to the other THMs. Chloroform accounted for about 97% of the total THMs ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$) found in 54 swimming pools which were investigated over a one year period (Simard *et al.*, 2013). Chloroform had the highest concentration among THMs in ten out of the eleven swimming pools that were sampled over a 6 month period (Table 2.1) (Weaver *et al.*, 2009). Bromoform dominated in the outstanding pool which was interpreted as likely to be due to residual bromide ions from previous bromine disinfection practices prior to the study. Similar results were reported where levels of bromoform in pools increased due to higher levels of bromide from bromine disinfection (Lourencetti *et al.*, 2012).

Table 2.1 Concentrations of trihalomethanes (THMs) in swimming pools

Country	Pool type	Disinfection method	THMs concentration (μg/L)				Detection method	Reference
			$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$		
U.S	Outdoor	Chlorine	390 (max)	120 (max)	83 (max)	8 (max)	GC-ECD	Beech *et al.* (1980)
Sweden	NR	Chlorine	50-100				GC-ECD	Norin and Renberg (1980)
		Bromine				400	GC-ECD	
Germany	Covered	Chlorine	43-980	0.1-150	0.1-140	<0.1-88	GC-ECD	Lahl *et al.* (1981)
France	NR	Chlorine	<0.5-665			<0.5-45	GC-ECD	Chambon *et al.* (1983)
	NR	Bromine	1-14			180-600	GC-ECD	
Italy	NR	Chlorine	62-180	6-10	0.8-2.0	<LOD[a]	HS-GC-ECD	Aggazzotti and Predieri (1986)
Canada	Spa	Chlorine	15-370				GC-MS	Benoit and Jackson (1987)
	Spa	Bromine				37-3600	GC-MS	
Italy	Indoor	Chlorine	9-180				HS-GC-ECD	Aggazzotti *et al.* (1995)
Germany	Indoor	Chlorine	3-28	0.7-6	0.03-7	0.02-2	HS-GC-ECD	Cammann and Hübner (1995)
Italy	Indoor	Chlorine	25-43	2-3	0.5-10	0.1	GC-MS	Aggazzotti *et al.* (1998)
Greece	Indoor	Chlorine	4-26	0.3-7	0.5-3	0.07-1	PAT-GC	Golfinopoulos (2000)
Canada	Indoor	Chlorine	18-80				HS-GC-ECD	Lévesque *et al.* (2000)
Australia	Indoor	Chlorine	20-85	0.2-2	<LOD[a]	<LOD[a]	NR	Kelsall and Sim (2001)
		Chlorine/Ozone	13-24	0.1-0.9	<LOD[a]	<LOD[a]	NR	
		Bromine/Ozone	<LOD[a]	0.3-0.5	0.8-1.2	100-160	NR	
Italy	Indoor	Chlorine	33.2 (mean)	4 (mean)	2 (mean)	0.4 (mean)	HS-GC-ECD	Fantuzzi *et al.* (2001)
U.K	Indoor	Chlorine	45-212	2-23	0.7-7	0.7-2	GC-ECD	Chu and Nieuwenhuijsen (2002)
Germany	Indoor	Chlorine	7-25				GC-ECD	Erdinger *et al.* (2004)
Poland	Indoor	Chlorine	10-41	0.7-6	0.4-2		DAI-GC-ECD	Kozłowska *et al.* (2006)
Spain	Indoor	Chlorine	95-145	2			HS-GC-MS	Caro and Gallego (2007)
U.S	Indoor	Chlorine	70-140				MIMS	Li and Blatchley III (2007)

	Outdoor	Chlorine	0.1				MIMS	
France	Indoor	Chlorine	47-82	5-12	1-5	1-2	GC-MS	Villanueva *et al.* (2007b)
Thailand	Outdoor	Chlorine	10-37	9-18	5-23	<0.07-7	GC-ECD	Panyakapo *et al.* (2008)
Taiwan	Indoor	Chlorine	44-74				GC-MS	Hsu *et al.* (2009)
Germany	Indoor	Chlorine	up to 19	up to 9	up to 10	up to 9	GC-MS	Kramer *et al.* (2009)
Korea	Indoor	Chlorine	0.2-100	<0.2-11	<0.2-6	<0.2	GC-MS	Lee *et al.* (2009)
	Indoor	Ozone/Chlorine	0.2-65	<0.2-6	<0.2-3	<0.2	GC-MS	
	Indoor	EGMO	7-56	2-27	<0.2-30	<0.2-36	GC-MS	
U.S	Pool 1	NR	41-150	<LOD[a]-22	0.2-55	<LOD[a]-68	MIMS	Weaver *et al.* (2009)
	Pool 2	NR	22-160	<LOD[a]-21	<LOD[a]-3	<LOD[a]-8	MIMS	
	Pool 3	NR	<LOD[a]-65	<LOD[a]-26	<LOD[a]-5	<LOD[a]-5	MIMS	
	Pool 4	NR	2-54	<LOD[a]-31	<LOD[a]-7	<LOD[a]-7	MIMS	
	Pool 5	NR	<LOD[a]-45	<LOD[a]-11	<LOD[a]-2	<LOD[a]-4	MIMS	
	Pool 6	NR	7-81	<LOD[a]-38	16 -77	8-310	MIMS	
	Pool 7	NR	23-170	<LOD[a]-150	<LOD[a]-25	<LOD[a]-24	MIMS	
	Pool 8	NR	17-130	<LOD[a]-0.1	0.2-8	<LOD[a]-6	MIMS	
	Pool 9	NR	1-300	<LOD[a]-12	<LOD[a]-6	<LOD[a]-22	MIMS	
	Pool 10	NR	13-300	<LOD[a]-120	<LOD[a]-9	<LOD[a]-18	MIMS	
	Pool 11	NR	4-170	<LOD[a]-55	<LOD[a]-27	<LOD[a]-8	MIMS	
Spain	Indoor	Chlorine	8-21	9-27	7-23	3-17	GC-MS	Richardson *et al.* (2010)
	Indoor	Bromine	0.1-0.3	0.2-0.7	2-3	52-64	GC-MS	
U.S	Indoor	Chlorine	25-200	1-28	<1-10	<1-1	GC-ECD	Kanan (2010)
Korea	Indoor	Chlorine	<0.2-46	<0.2-7	<0.2	<0.2	GC-MS	Lee *et al.* (2010)
	Indoor	Ozone/Chlorine	<0.2-21	<0.2-3	<0.2	<0.2	GC-MS	
	Indoor	EGMO	<0.2-40	<0.2-34	<0.2-32	<0.2-18	GC-MS	
France	Indoor	Chlorine	<5-73	0.6-15	<0.5-4	<0.5-2	GC-MS	Bessonneau *et al.* (2011)
France	Indoor seawater	Chlorine	0.01-0.3	0.05-1	3-64	29-930	HS-GC-MS	Parinet *et al.* (2011)

Portugal	Indoor	Chlorine	18-520				GC-ECD	Sá *et al.* (2011)
Canada	Indoor	Chlorine	10-46				GC-ITMS	Catto *et al.* (2012)
Spain	Indoor	Chlorine	9-20	9-25	7-23	3-16	GC-MS	Lourencetti *et al.* (2012)
	Indoor	Bromine	0.08-0.3	0.2-0.6	2-3	52-61	GC-MS	
Portugal	Indoor	Chlorine	6-120	1-22	1-10	1-6	HS-SPME-GC-ECD	Silva *et al.* (2012)
Portugal	Indoor	Chlorine	17-400	<34	<39	<36	HS-SPME-GC-ECD	Maia *et al.* (2014)
Australia	Outdoor	Chlorine	65-84	2-3	0.3	<0.1	GC-ECD	Yeh *et al.* (2014)

$CHCl_3$: chloroform, $CHBrCl_2$: bromodichloromethane, $CHClBr_2$: dibromochloromethane, $CHBr_3$: bromoform, EGMO: electrochemically generated mixed oxidants

GC: gas chromatography, ECD: electron capture detection, HS: headspace, MS: mass spectrometry, PAT: purge and trap, DAI: direct aqueous injection, MIMS: membrane introduction mass spectrometry, ITMS: ion trap mass spectrometry

NR: Not reported

[a]Limit of detection (LOD) not mentioned in paper

The most common HAAs reported in swimming pools are monochloroacetic (MCAA), dichloroacetic (DCAA), trichloroacetic (TCAA), monobromoacetic (MBAA) and dibromoacetic (DBAA) acids (Legay *et al.*, 2010). HAAs are less volatile compared to THMs (Lee *et al.*, 2010). This may lead to a higher accumulation of HAAs over time in swimming pools. The occurrences of HAAs in swimming pools are presented in Table 2.2. DCAA and TCAA have been found to be the most abundant HAAs detected in swimming pools corresponding to about 93% of the total HAAs detected in the 54 swimming pools studied which consisted of indoor and outdoor pools in Québec City (Canada) (Simard *et al.*, 2013). Similarly, DCAA and TCAA dominated in all seven pools tested consisting of indoor, outdoor and children's pools with both compounds being accounted for up to 95% of the total HAAs measured (Yeh *et al.*, 2014). This trend was also observed in swimming pools in China and the United States (Wang *et al.*, 2014b).

Table 2.2 Concentrations of haloacetic acids (HAAs) in swimming pools

Country	Pool type	Disinfection method	HAAs concentration (µg/L)									Detection method	Reference
			MCAA	DCAA	TCAA	MBAA	DBAA	BCAA	BDCAA	CDBAA	TBAA		
Spain	NR	Chlorine	25	69	42	7	15					SPE-CZE	Martinez *et al.* (1999)
Switzerland	NR	Chlorine	11-120	1-240	17-95							GC-MS	Berg *et al.* (2000)
Spain	NR	Chlorine	4 (mean)	45 (mean)	150 (mean)	<0.4	2.8 (mean)	11 (mean)	61 (mean)	33 (mean)	19 (mean)	HS-SPME-GC-ITMS	Sarrión *et al.* (2000)
Spain	NR	NR	15-1000	<0.8	1000-1700	<0.8	<1.3	<0.1	210-910	<0.4-62	<0.2-15	SPE-LC-ESI-MS	Loos and Barceló (2001)
Korea	Indoor	Chlorine		14-250	20-630							GC-MS	Lee *et al.* (2010)
	Indoor	Ozone/Chlorine		<0.3-32	1-86							GC-MS	
	Indoor	EGMO		2-99	1-410							GC-MS	
Spain	NR	Chlorine	33-40	100-120	60-180	<0.1	1.7-2.2	<0.02	3.6-7.4	<0.12	<0.4	HS-GC-MS	Cardador and Gallego (2010)
U.S	Indoor	Chlorine		52-6800	76-1900		<1-25	1-180	8-110		<1	GC-ECD	Kanan (2010)
Spain	Indoor	Chlorine	9-36	60-120	85-170							HS-GC-MS	Cardador and Gallego (2011)
	Outdoor	Chlorine	20-34	130-170	99-150							HS-GC-MS	
France	Indoor seawater	Chlorine	1-96	1-9	3-87	4-160	11-1100	5-220	1-20	36-240	4 -430	GC-ECD	Parinet *et al.* (2011)
Canada	Indoor	Chlorine		48-190	54-200			0.4-3.0	<1.6-24			GC-ECD	Catto *et al.* (2012)
Portugal	NR	Chlorine	<0.3-3	29-84	29-76	<0.3	0.3-0.7					SPE-LC-MS/MS	Prieto-Blanco *et al.* (2012)
Portugal	Indoor	Chlorine	0.6-13	0.4-54	0.5-73	0.5-20	0.1-12[a]	0.4-25		0.2-0.9	0.4-0.9	HS-SPME-GC-	Sá *et al.*

												ECD	(2012)
Saudi Arabia	NR	NR	47-49	11-35	<2.2-13	9-25	16	6.8-7.1				µSPE-UPLC-UV	Nsubuga and Basheer (2013)
U.S	Outdoor	NR		310-1330	370-1140								Wang *et al.* (2014b)
	Indoor	NR		50-2040	20-2970								
	Spa	NR		50-750	40-530								
China	Outdoor	Chlorine		44-195	33-98								
	Indoor	Chlorine		5-60	6-90								
Australia	Various	Chlorine	<0.5-120	230-2400	110-2600	<0.5	<0.5	<0.5	<0.5-22	<0.5		GC-ECD	Yeh *et al.* (2014)

MCAA: monochloroacetic acid, DCAA: dichloroacetic acid, TCAA: trichloroacetic acid, MBAA: monobromoacetic acid, DBAA: dibromoacetic acid, BCAA: bromochloroacetic acid, BDCAA: bromodichloroacetic acid, CDBAA: chlorodibromoacetic acid, TBAA: tribromoacetic acid
SPE: solid phase extraction, CZE: capillary zone electrophoresis, GC: gas chromatography, MS: mass spectrometry, HS: headspace, SPME: solid phase microextraction, ITMS: ion trap mass spectrometry, LC: liquid chromatography, ESI: electrospray ionization, ECD: electron capture detection, µSPE: micro solid phase extraction, UPLC-UV: ultra performance liquid chromatography-ultraviolet detection
NR: Not reported, [a]DBAA+BDCAA

Halobenzoquinones (HBQs) have only relatively recently been identified as DBPs in drinking water (Zhao *et al.*, 2010). More recently Wang *et al.* (2013) reported the presence and concentrations of HBQs in ten indoor swimming pools which were treated with either chlorine or chlorine/UV disinfectants. HBQs detected were 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,3,6-trichloro-(1,4)benzoquinone (TriCBQ), 2,3-dibromo-5,6-dimethyl-(1,4)benzoquinone (DMDBBQ) and 2,6-dibromo-(1,4)benzoquinone (2,6-DBBQ). 2,6-DCBQ was the most abundant HBQ detected in all 10 pools at concentrations 100 times higher than in chloraminated tap water (Wang *et al.*, 2013). It was also reported that lotions and sunscreens introduced into pools by swimmers are a possible source of HBQ precursors and may increase the formation of HBQs in swimming pool waters. Four lotions and four sunscreens were dissolved in water and exposed to a sodium hypochlorite solution to compare the formation potential of HBQs from various PCPs (Wang *et al.*, 2013). 2,6-DCBQ was detected in all of chlorinated waters containing PCPs. TriCBQ which was detected in swimming pools were also detected in two of the chlorinated samples of sunscreens. This study concluded that the different ingredients in PCPs affected the formation of HBQs and PCPs which contain more aromatic structure ingredients are more likely to produce HBQs. Benzoquinone compounds have been reported to increase in toxicity with halogen substitution (Bull *et al.*, 2011). The predicted chronic lowest observed adverse effect levels and *in vitro* toxicity for HBQs have indicated that HBQs may be considerably more toxic than regulated DBPs such as THMs, and could be carcinogenic (Bull *et al.*, 2011; Wang *et al.*, 2014a).

Tri-chloramine (NCl_3), nitrate, chloral hydrate, chlorate, chlorite and bromate have also been detected in swimming pools. Levels of NCl_3 in swimming pool water samples were reported to be about 100 µg/L as Cl_2 (Li and Blatchley III, 2007). Mean concentrations of NCl_3 (as Cl_2) in eleven swimming pool waters ranged from 10 – 150 µg/L (Weaver *et al.*, 2009). NCl_3 concentrations in the air directly above swimming pools are generally higher than in pool water due to the volatility of NCl_3. In the air, NCl_3 levels ranged between 130 – 1300 µg/m^3 at six indoor swimming pools measured between 0.3 – 1.5 m above the water (Jacobs *et al.*, 2007). Average concentrations of NCl_3 at 290 and 80 µg/m^3 in air samples from indoor chlorinated and brominated pools respectively measured between 1 m from the pool water level, but was below the limit

of detection (<100 µg/L) in swimming pool waters indicating that most of NCl_3 is volatised into the air (Richardson *et al.,* 2010). The average concentrations of NCl_3 in the air taken at 0.25 m and 1.5 m from the water surface in 15 indoor chlorinated swimming pools was reported at 190 µg/m^3 (Bessonneau *et al.,* 2011). Regulations on certain air pollutants such as NCl_3 may be of significance to avoid unwanted health effects and provide a comfortable environment to swimming pool patrons. A limit value of 500 µg/m^3 for NCl_3 for indoor air quality at swimming pools has been proposed based on findings that no irritating effects were reported below this level (Hery *et al.,* 1995). Also, the Pennsylvania Department of Health in the US has recommended regulations keeping the levels of combined chlorine below 0.2 ppm to control chloramine levels (Pennsylvania Department of Health and Bureau of Community Health Systems, 2008).

Nitrate concentrations in 101 chlorinated pools were reported at an average of 8.6 mg/L while the tap water used as filling water had average nitrate concentrations of less than 0.1 mg/L (Beech *et al.,* 1980). Similarly, Lee *et al.* (2010) determined that nitrate concentrations in pool waters were much higher than in tap water (which had a mean of 1.5 mg/L) with nitrate concentrations between 6.6 – 24 mg/L for chlorinated pools, 1.2 – 22 mg/L for ozone/chlorine treated pools and 11 – 49 mg/L for electrochemically generated mixed oxidants (EGMOs) treated pools. This was mainly attributed to the oxidation of organic nitrogen compounds derived from swimmers such has hair, sweat and urine. Urine is likely an important source of nitrate as the two nitrogen atoms of the urea molecule are oxidized by chlorine to form nitrate (Samples, 1959; Blatchley III and Cheng, 2010). While nitrate can be formed as an oxidative DBP, other routes may also contribute to the formation of nitrates. Nitrates may be formed through biological formation in swimming pools. Despite chlorination, biofilms are present in swimming pools providing an environment for biological activity (Goeres *et al.,* 2004). Biological filtration used in some swimming pools to reduce urea may also produce nitrate. Furthermore, nitrate is a stable end product from the photodecay of NCl_3 (Blatchley III and Cheng, 2010). If control of nitrate concentrations is required, an effective strategy is frequent dilution with fresh water to limit accumulation (Judd and Bullock 2003).

Chloral hydrate was detected in 5 chlorinated pool samples but not in brominated pools (Richardson *et al.*, 2010). Chloral hydrate levels of 5 – 35 µg/L in 30 chlorinated pools, <0.1 – 10 µg/L in 30 in ozone/chlorinated pools and <0.1 – 23 µg/L in 26 EGMO pools were reported (Lee *et al.*, 2010). In this study, chloral hydrate contributed 10%, 8%, and 7% of the total measured DBP loads in waters treated with chlorine, ozone/chlorine and EGMO respectively. Chloral hydrate is potentially carcinogenic with increased incidences of liver tumours reported in mice when administered orally through drinking water (Daniel *et al.*, 1992; Leakey *et al.*, 2003). Nonetheless, the cancer risk to humans has not been determined (Haselkorn *et al.*, 2006).

Chlorate levels in chlorinated swimming pools have been reported to be highly variable with average concentrations of 16 mg/L (Beech et al. 1980). In this study, chlorate was undetectable in 20 pools while another 12 pools had chlorate levels exceeding 40 mg/L. It was speculated that the low chlorate concentrations could be a consequence of the pools being regularly maintained at pH 7.2 – 7.8 since chlorate formation decreases under alkaline conditions (Beech et al 1980). This study was conducted over 30 years ago and may not represent contemporary swimming pool management practice. Chlorate was detected in all swimming pools in a later study which consisted of chlorinated and ozonated pools (Michalski and Mathews, 2007). Higher levels of chlorate were detected in the chlorinated pools with levels between 21 – 32 mg/L while significantly lower levels of about 3 mg/L were observed in ozonated pools. In the same study, chlorite was also detected only in the chlorinated pools at levels between 0.3 – 2.5 mg/L. A study which evaluated chlorate and chlorite levels in swimming pools with various treatments including sodium dichloroisocyanurate, sodium hypochlorite, sodium hypochlorite and UV radiation, found that chlorate was detected in most of the samples between 25 – 270 µg/L while chlorite was undetectable above a detection limit of 25 ug/L (Ribeiro *et al.*, 2011). Similarly, chlorate was predominant in all 24 investigated indoor swimming pool waters with average concentrations of 3.7 mg/L whereas chlorite was only detected in one swimming pool sample at approximately 20 µg/L (Righi *et al.*, 2014). The main concern with exposure to chlorite and chlorate is oxidative damage to red blood cells (Couri *et al.*, 1982; WHO, 2008).

Bromate is an inorganic substance typically found in bromide containing waters treated with ozone (Von Gunten and Hoigne, 1994). Of five chlorinated swimming pools and two ozonated swimming pools that were tested, bromate was only detected in the ozonated pools at levels 80 and 500 µg/L (Michalski and Mathews, 2007). Also, bromate levels ranging from 10 – 48 µg/L were detected in three out of the 24 chlorinated indoor pools tested but was not present in the fill water which mostly used chlorine dioxide as the main disinfectant (Righi *et al.*, 2014). The presence of bromate as an impurity in hypochlorite solutions have been reported (Garcia-Villanova *et al.*, 2010). This study observed bromate median concentrations of about 1 g/L in more than 80% of 40 hypochlorite solutions tested, which may be of significance in swimming pools due to their common usage for disinfection purposes. Bromate has been classified as possibly carcinogenic to humans (Group 2B) (IARC, 1999).

In swimming pools, a study to compare the occurrences of DBPs using chlorination and bromination disinfection processes detected over 100 DBPs including the identification of many new DBP compounds which have not been previously identified in swimming pool water or drinking water before (Richardson *et al.*, 2010). These "new" DBPs may be formed from nitrogen containing precursors such as those which may be present in urine and sweat introduced by swimmers.

There has been considerable interest in nitrogenous DBPs (N-DBPs) with the results of toxicological studies showing that some N-DBPs may be considerably more genotoxic, cytotoxic and carcinogenic than carbonaceous DBPs (C-DBPs) (Muellner *et al.*, 2007; Richardson *et al.*, 2007). N-DBPs are formed when organic nitrogen compounds react with disinfectants. Although much less frequently reported compared to THMs and HAAs, N-DBPs have been detected in the aquatic environment and more recently, in swimming pool waters (Richardson *et al.*, 2010). N-DBPs detected in swimming pools include HANs, HNMs and *N*-nitrosamines.

Dissolved organic nitrogen compounds have been found to be the main precursors of N-DBPs (Shah and Mitch, 2011). As a result, formation of N-DBPs in swimming pools is highly probable with high nitrogen content from sweat and urine (Kim and Han, 2011). Although generally occurring at lower levels compared to THMs and HAAs, the higher

toxicity levels of N-DBPs may have more adverse effects to human health. The cytotoxities of HNMs and HANs were found to be around two orders of magnitude greater than HAAs, with genotoxicity levels ranked HNMs>HANs>HAAs (Muellner *et al.*, 2007). HANs that have been identified in swimming pool water include bromoacetonitrile (BAN), dichloroacetonitrile (DCAN), BCAN, DBAN and trichloroacetonitrile (TCAN) (Richardson *et al.*, 2010). A summary of HAN levels detected in swimming pools is presented in Table 2.3. DCAN was the most frequently detected HAN compounds in swimming pool water.

Table 2.3 Concentrations of haloacetonitriles (HANs) in swimming pools

Country	Pool type	Disinfection method	HANs concentration (µg/L)						Detection method	Reference
			DCAN	DBAN	BCAN	TCAN	CAN	BAN		
U.S.	Indoor	Chlorine	10-20						MIMS	Li and Blatchley III (2007)
	Outdoor	Chlorine	30						MIMS	
Germany	Indoor	Chlorine	up to 20	up to 6	up to 12	up to 15	<LOD[a]	up to 13	LLE-GC-MS	Kramer *et al.* (2009)
U.S.	Pool 1	NR	2-21						MIMS	Weaver *et al.* (2009)
	Pool 2	NR	5-18						MIMS	
	Pool 3	NR	4-14						MIMS	
	Pool 4	NR	4-11						MIMS	
	Pool 5	NR	2-40						MIMS	
	Pool 6	NR	6-31						MIMS	
	Pool 7	NR	7-87						MIMS	
	Pool 8	NR	2-24						MIMS	
	Pool 9	NR	0.6-45						MIMS	
	Pool 10	NR	7-44						MIMS	
	Pool 11	NR	2-47						MIMS	
U.S.	Indoor	Chlorine	4-47	<1-5	<1-13	<1-1	1-3	<1-1	GC-ECD	Kanan (2010)
Korea	Indoor	Chlorine	0.5-12	<0.1-1	<0.2-2	<0.2			GC-ECD	Lee *et al.* (2010)
	Indoor	Ozone/Chlorine	0.2-3	<0.1-0.8	<0.2-0.6	<0.2			GC-ECD	
	Indoor	EGMO	<0.1-8	<0.1-7	<0.2-9	<0.2			GC-ECD	

DCAN: dichloroacetonitrile, DBAN: dibromoacetonitrile, BCAN: bromochloroacetonitrile, TCAN: trichloroacetonitrile, CAN: chloroacenotonitrile, BAN: bromoacetonitrile, EGMO: electrochemically generated mixed oxidants

MIMS: membrane introduction mass spectrometry, LLE: liquid-liquid extraction, GC: gas chromatography, ECD: electron capture detection, MS: mass spectrometry, NR: Not reported

[a]Limit of detection (LOD) not mentioned in paper

Table 2.4 Concentrations of halonitromethanes (HNMs) in swimming pools

Country	Pool type	Disinfection method	HNMs concentration (µg/L)			Detection method	Reference
			TCNM	BNM	BCNM		
Germany	Indoor	Chlorine	up to 7			LLE-GC-MS	Kramer *et al.* (2009)
U.S.	Indoor	Chlorine	<0.7-2	<0.7-2	0.8-11	GC-ECD	Kanan (2010)
Spain	NR	Chlorine	0.4-2			HS-SDME-GC-MS	Montesinos *et al.* (2011)
Spain	NR	Chlorine	0.4-2			HS-GC-MS	Montesinos and Gallego (2012)

TCNM: trichloronitromethane, BNM: bromonitromethane, BCNM: bromochloronitromethane,
LLE: liquid-liquid extraction, GC: gas chromatography, ECD: electron capture detection, MS: mass spectrometry, HS: headspace
NR: Not reported

HNMs found in swimming pools include TCNM, BNM, bromochloronitromethane (BCNM) and dibromonitromethane (DBNM) (Kanan, 2010; Richardson *et al.*, 2010). HNM concentrations in swimming pools from previous studies are summarised in Table 2.4. TCNM is the most commonly reported HNM in swimming pools. BCNM was found to have the highest concentration at 11 µg/L among the three different HNMs (Kanan, 2010). Very few studies have been conducted on HANs and HNMs compared to the more traditional DBPs. Tables 2.3 and 2.4 show that HAN and HNM concentrations are generally lower (<0.1 – 87 µg/L) when compared to THMs and HAAs (<0.1 – 6800 µg/L) (See Tables 2.1 and 2.2).

Table 2.5 Concentrations of *N*-nitrosamines in swimming pools

Country	Pool type	Disinfection method	*N*-Nitrosamine concentration (ng/L)						Detection method	Reference
			NDMA	NDEA	NMor	NPyr	NPip	NDBuA		
U.S	Indoor	Chlorine	32 (med)				<2	<2	GC-MS/MS	Walse and Mitch (2008)
	Indoor	UV/chlorine	≈10				<2	<2	GC-MS/MS	
	Outdoor	Chlorine	5 (med)				<2	<2	GC-MS/MS	
	Hot tubs	Chlorine	310 (med)				<2	<2	GC-MS/MS	
Spain	NR	NR	<0.2-6	<0.1-1.4		<0.2-4.5			GC-MS	Jurado-Sánchez *et al.* (2010)
U.S.	Indoor	Chlorine	2-83						GC-MS/MS	Kanan (2010)
Italy	Indoor	Chlorine	<1	<1	<1	53-127	<1	<1	GC/CI/MS	Pozzi *et al.* (2011)
Korea	Indoor	Chlorine	0.7-210	1.5-53	0.25-34				HPLC-FD	Kim and Han (2011)

NDMA: *N*-nitrosodimethylamine, NDEA: *N*-nitrosodiethylamine, NMOR: *N*-nitrosomorpholine, NPyr: *N*-nitrosopyrrolidine, NPiP: *N*-nitrosopiperidine, NDBuA: *N*-nitrosodibutylamine

UV: ultraviolet, med: median, GC: gas chromatography, MS: mass spectrometry, CI: chemical ionization, HPLC: high-performance liquid chromatography, FD: fluorescence detection

NR: Not reported

N-nitrosamines are a group of N-DBPs, with some known to have carcinogenic effects (Radomski *et al.*, 1978; Fishbein, 1979; Patterson *et al.*, 2012). *N*-nitrosamines which have been reported in swimming pools include *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosomorpholine (NMor), *N*-nitrosopyrrolidine (NPyr), *N*-nitrosopiperidine (NPip) and *N*-nitrosodibutylamine (NDBuA). Table 2.5 lists the concentrations of those *N*-nitrosamines reported in different studies. Higher levels of *N*-nitrosamines may be expected in swimming pools compared to drinking water due to the continuous loading of amine precursors from bathers from urine and sweat (Walse and Mitch, 2008; Kim and Han, 2011).

2.4 Factors influencing DBPs formation and persistence

Various management aspects adopted by swimming pools may influence the formation and persistence of DBPs. Key factors that may affect formation and persistence include the fill water, type of swimming pools, disinfection/treatment process, temperature, pH, exposure to sunlight and wind and bather rate.

2.4.1 Fill water / Type of swimming pools

Some chemicals found in swimming pools are dependent upon the fill water. For example, a study of eight seawater pools in France treated with chlorine-based disinfectants found that the formation of brominated DBPs dominated due to seawater containing high levels of bromide (Parinet *et al.*, 2011). Bromoform and DBAA were the most prevalent with levels from 74 – 930 µg/L and 11 – 1100 µg/L respectively (Parinet *et al.*, 2011). This observation is similar to that of another study conducted on chlorinated outdoor saline pools in Miami where bromoform had the highest concentrations among the four chlorine/bromine THMs (Beech *et al.*, 1980). Bromoform was accounted for around 98% of the total THM concentration where the total THM concentrations were observed at 660 µg/L. Similarly, bromoform dominated the THM composition in brominated swimming pools (Lourencetti *et al.*, 2012). In addition, humic substances which are present in fill water may produce higher levels of DBPs when chlorinated (Singer, 1999). Furthermore, DBPs may already be present in chlorinated fill waters used for swimming pools.

DBP concentrations are also influenced by the type of pool, indoor or outdoor. The relative concentration of THMs, compared to other DBPs was observed to be lower in outdoor pools, compared to indoor pools (Zwiener *et al.*, 2007). This was assumed to be a consequence of wind-enhanced volatilisation of THMs from outdoor pools. However, in a recent study, THMs and HAAs in outdoor pools were detected at levels double the amount in indoor pools (Simard *et al.*, 2013). This was attributed to outdoor pools having additional exposure to the external environment such as air particles, grass, soil, leaves, rain and insects which may lead to more DBP precursors in the pool (Simard *et al.*, 2013). This study also reported higher values of turbidity, conductivity and TOC in outdoor pools indicating relatively poor water quality. The formation of THMs has also been shown to increase under UV radiation (Liu *et al.*, 2006). As such, THM levels in outdoor pools will depend upon the relative rates of formation and volatilisation, which may vary among locations and seasons.

Indoor pools have indicated higher levels of NDMA (44 ng/L) compared to outdoor pools (7 ng/L) at the same temperature suggesting that NDMA levels may be reduced from UV photolysis (Walse and Mitch, 2008). In the same study, another swimming pool with a retractable roof recorded NDMA levels averaging between the levels detected in the indoor and outdoor pools. As pool covers may prevent volatilisation of DBPs, this would lead to DBPs accumulating when the pools are covered. The release of these accumulated volatile DBPs would start once the cover is removed which could explain the NDMA levels averaging between the levels detected in the indoor and outdoor pools. Conversely, recreational pools with water attractions, such as diving boards and slides, could affect the levels of DBPs as they would have more intense water-air contact due to splashing, and therefore enhanced release of volatile DBPs.

2.4.2 Disinfection method and treatment process

The type of disinfectant used also affects the type of DBPs generated. Concentrations of total DBPs (THMs, HAAs, HANs and chloral hydrate) were 180 µg/L, 33 µg/L and 140 µg/L for pools treated with chlorine, ozone/chlorine and EGMO respectively (Lee *et al.*, 2010). In all the indoor pools tested, total HAA levels were highest followed by total THMs, chloral hydrate and total HANs regardless of the disinfection method used which may be due to HAAs being less volatile. Higher concentrations of brominated

DBPs ($CHCl_2Br$, $CHClBr_2$, bromoform, BCAN and DBAN) were recorded in EGMO-disinfected pools, due to the presence of bromide ions from impure sodium chloride salt used during the EGMO disinfection process. High purity sodium chloride salts are commercially available and may be used to minimise brominated DBP formation in EGMO disinfected swimming pools. Also, brominated HAAs were dominant in a spa where bromine containing disinfectants were used (Wang *et al.*, 2014b). Similar results were obtained when bromide was added to synthetic pool water to study its effects on DBPs (Kanan, 2010). Although generally chloroform was detected in higher concentrations in chlorinated pools among the THM compounds, studies that investigated swimming pools which used bromine as a disinfectant found that concentrations of bromoform greatly increased whereas concentrations of chloroform were reduced (Chambon *et al.* (1983), Benoit and Jackson (1987), Richardson *et al.* (2010) Lourencetti *et al.* (2012)). This is consistent with established bromine chemistry from drinking water disinfection practice (Watson *et al.*, 2014).

Nitrate concentrations between 4 – 9 mg/L were detected in five chlorinated pools while two chlorinated/ozonated pools had nitrate in the range of 16 – 26 mg/L (Michalski and Mathews, 2007). These results are similar to those reported by Lee *et al.* (2010) where ozonated pools tended to have higher nitrate concentrations. This could be due to the oxidation of ammonia to nitrate by ozone (Singer and Zilli, 1975). Alternatively, nitrate may be produced by biological nitrification in subsequent carbon filtration processes.

The combined use of UV and chlorine decreased the concentration levels of NDMA as pools that incorporated UV treatment showed lower NDMA concentrations (Walse and Mitch, 2008). This, however, is dependent on the initial *N*-nitrosamine and precursor concentrations along with the applied UV dose as research have found that UV disinfection may simultaneously degrade *N*-nitrosamines and also form new *N*-nitrosamines in swimming pools (Soltermann *et al.*, 2013). Soltermann et al determined that the use of UV in waters where NDMA precursors are present, namely chlorinated dimethylamine (CDMA) and monochloramine, increased NDMA formation with a 1 – 2% molar yield based on initial CDMA concentration. It has also been reported that NDMA formation was UV dose dependent with the maximum NDMA concentration occurring between UV doses of 250 – 850 mJ/cm^2. Accordingly, UV treatment can be

effective for *N*-nitrosamine degradation when pool water contains relatively high *N*-nitrosamine concentrations compared to chloramines and chlorinated secondary amines but it is not without risk of inducing a net *N*-nitrosamine formation. UV irradiation of swimming pool waters at a wavelength of 222 nm and 254 nm also led to the formation of some other N-DBPs consisting of DCAN and cyanogens chloride and elimination of others such as chloramines (Weng *et al.*, 2012).

It was found that chloroform and $CHCl_2Br$ concentrations significantly increased but bromoform and $CHClBr_2$ concentrations decreased in swimming pools treated with medium pressure UV lamps at a wavelength of 254 nm (Cassan *et al.*, 2006). The increase of chloroform and $CHCl_2Br$ concentrations may be due to the increase of active chlorine by photolysis of some combined chlorine (Cassan *et al.*, 2006). Similar to the increased reactivity of organic matter from fill water by UV treatment, it was proposed that UV radiation may also increase the reactivity of organic matter from anthropogenic sources towards chlorination leading to additional formation of THMs. Also, an observed reduction of the brominated THMs in this study has been attributed to bromoform having a band absorption within the UV spectral lamp range and lower energy required to break the bromine-carbon bond compared to chlorine-carbon bonds. This process may lead to the further production of chloroform and $CHCl_2Br$.

The amount of disinfectants added to swimming pools can have an impact on the formation of DBPs. For example, the combined use of ozone/UV with lower chlorine doses resulted in lower levels of NDMA in swimming pool water (Walse and Mitch, 2008). Chlorinated pools had the most HAAs detected compared to pools treated with ozone/chlorine and EGMO (Lee *et al.*, 2010). This might be due to the amount of chlorine in the chlorinated swimming pools as formation of HAAs has been reported to be higher compared to THMs with high chlorine dose/residual (Singer, 1994). Similarly, lower chlorine residuals were reported to be the cause of lower HAA levels detected in swimming pools in China compared with swimming pools in the United States (Wang *et al.*, 2014b). Except for THM formation potential which increased slightly after advanced oxidation processes (AOPs), elevated ozone dose and longer reaction times gave increased elimination rates for DBPs measured as adsorbable organic halogens (Glauner and Frimmel, 2006). Laboratory experiments have shown

that concentrations of HBQs increased with higher chlorine dosage (Wang *et al.*, 2013). This could be due to the higher reactivity generated by high chlorine doses to chlorinate phenols into HBQs (Zhao *et al.*, 2010). The relative mass of disinfectants added to swimming pools can have an impact on the formation of DBPs. However, the amount of pollutants generated in a pool and the mass of disinfectants applied used in each case would need to be taken into consideration before direct comparisons can be made. Unfortunately, this information is rarely reported.

Disinfection methods used produce varying amounts of DBPs in swimming pools. Hence, the ideal disinfection process would be one which achieves effective disinfection with minimal production of DBPs with known health risks. Based on the information gathered in Tables 2.1 – 2.5, there is evidence to support that the use of chlorine at reduced concentrations with the addition of other treatment methods such as UV or ozone leads to lower overall DBP concentrations in swimming pools. Nonetheless, the selection of disinfection processes must always maintain an appropriate focus on effective pathogen control. Compromising pathogen control to achieve reduced DBP formation would be a very poor public health outcome.

Effective treatment such as frequent backwashing and shock chlorination of swimming pool water can reduce the amount of pollutants in the pools which will subsequently reduce the amount of disinfectants needed to maintain the quality of pool water. Thus, effective treatment will reduce the formation potential of DBPs leading to a decrease in DBP concentration.

2.4.3 Temperature

The occurrences of some groups of DBPs in swimming pools are also affected by the temperature of the pools. Significantly higher NDMA levels (430 ng/L) were detected in hot tubs (41°C) compared to swimming pools at lower temperatures (Walse and Mitch, 2008). The same study reported that one hot tub which had similar temperatures (23°C) and levels of amine precursors to indoor pools had comparable NDMA levels to those indoor pools. This suggests that the higher temperature in the hot tubs lead to higher NDMA levels. This might be due to higher temperatures increasing the rate of the nitrosation process (Rostkowska *et al.*, 1998). Another study on whirlpool spas did

not produce higher THM formation at 40°C (Benoit and Jackson, 1987). The heat and agitation of the waters could have contributed to the volatilisation of THMs resulting in lower levels of THMs. In general, higher temperature swimming pools (32 – 34°C) generate higher levels of THMs, HAAs, HANS, HNMs and NDMA (Kanan, 2010; Hansen *et al.*, 2012a). Higher levels of THMs and HAAs were also reported in heated outdoor pools compared to unheated pools (Simard *et al.*, 2013). Laboratory-based simulation studies also showed that levels of THMs and HNMs doubled at 40°C compared to at 26°C and HAA concentrations increased by 60% (Kanan, 2010).The increase of DBP levels at higher temperatures may also be due to the higher release of pollutants. Higher temperatures in swimming pools lead to the increased release of sweat from bathers, even without exercise (Keuten *et al.*, 2014). Thus, more sweat-sourced organics will be available to react with disinfectants in swimming pools with higher temperatures.

2.4.4 pH

The effect of pH (between pH 6.0 – 8.0) on the formation of DBPs in swimming pools has been investigated (Hansen *et al.*, 2011; Hansen *et al.*, 2012a; Hansen *et al.*, 2012b; Hansen *et al.*, 2013). Three of those studies (Hansen *et al.*, 2011; Hansen *et al.*, 2012a; Hansen *et al.*, 2013) using body fluid analogues (BFAs), which are composed of organic compounds and amino acids to simulate organic matter release of bathers, found that the formation of THMs increased with increasing pH. It has been suggested that a higher pH could increase the hydrolysis of precursors of THMs such as trihalopropanones, trihaloacetonitriles and trihaloacetaldehydes resulting in high THM formations (Adin *et al.*, 1991; Nikolaou *et al.*, 2004). In contrast, HANs formation decreased with increasing pH and formation of HAAs remained fairly constant over the pH range. The effect of pH on the formation of DBPs during chlorination was investigated using filtered particles collected from a microsieve filter on a hot tub which showed that THM and HAA formation were reduced with decreasing pH whereas HANs formation decreased with increasing pH (Hansen *et al.*, 2012b). The decrease of HANs at higher pH is likely due to their decomposition to form HAAs at pH conditions higher than 7.0 which also accounts for the higher levels of HAAs at higher pH. Due to the instability of HANs, hydrolysis of HANs can yield the corresponding HAAs (Glezer *et al.*, 1999) and also hydrolyze the corresponding carboxylic acids and release THMs

in the process (Koch and Volker, 1996). It has also been reported that swimming pool water genotoxicity increased for pH lower than 6.7 possibly due to the higher levels of HANs at lower pH (Hansen *et al.*, 2013). Among the three classes of DBPs studied (THMs, HAAs and HANs), Hansen reported that HANs contributed exclusively to the genotoxicity of swimming pool water (Hansen *et al.*, 2011).

Similar findings were reported where synthetic pool water was used to study the effects of different operational parameters in swimming pools (Kanan, 2010). The formation of THMs, HAAs and HNMs tested at pH levels of 6.0, 7.0 and 8.0 increased with increasing pH. THMs and HAAs formation reduced to roughly 40 – 60% when pH was reduced to 6.0 compared to pH at 8.0. An increase of 30% of HNM formation was reported at pH 8.0 compared to pH 6.0.

Table 2.6 gives a summary of the effects of pH, temperature and UV on DBPs in swimming pools.

Table 2.6 Summary of the effect of pH, temperature and UV on DBPs in swimming pools

	Effect on DBP formation	**Reference**
Increase in pH ($6 \leq pH \leq 8$)	• THMs, HNMs increase • HAAs increase / no change • HANs decrease	Kanan (2010), Hansen *et al.* (2011), Hansen *et al.* (2012a), Hansen *et al.* (2012b), Hansen *et al.* (2013)
Increase in temperature (<26°C)	• NDMA, THMs, HAAs, HANs, HNMs increase	Walse and Mitch (2008), Kanan (2010), Hansen *et al.* (2012a), Simard *et al.* (2013)
UV	• $CHCl_3$, $CHBrCl_2$, DCAN increase • $CHClBr_2$, $CHBr_3$, chloramines decrease	Cassan *et al.* (2006), Weng *et al.* (2012)

2.4.5 Operation and management

The operation and management of a swimming pool would also affect the levels of DBPs. Significant factors may include frequency of swimming pool water replacement, frequency of filter backwashing and frequency of shock chlorination procedures. Swimming pool coverage could lead to reduced entry of DBP precursors to swimming pools, but may also lead to reduced loss of some volatile DBPs. With effective ventilation, the levels of volatile DBPs such as chloramines may be minimised. Swimming pools with higher temperature require more effective ventilation to account for increased volatilisation.

The turnover period, which is the time taken for a volume of water equivalent to the volume of the entire pool to pass through the filters and treatment plant and back to the pool, typically ranges between 2 – 8 hours (SAHC, 1991; WHO, 2006). However, the refreshment rate of swimming pools is much longer. This may lead to the accumulation of contaminants, which are not removed during treatment, especially non-volatile DBPs. Thus, the most effective way to minimise DBP concentrations may be to reduce the precursors before they have a chance to react with the disinfectants. Frequent water refreshment may also be an effective means of controlling contaminant accumulation in the pool. However, the effectiveness of this strategy would be limited by the DBP-formation potential of the refreshment water.

2.4.6 Organic loading / Bather load

DBP formation in swimming pools has been correlated with the organic loadings originating from swimmers (Zwiener *et al.*, 2007; Kanan and Karanfil, 2011; Kim and Han, 2011). An investigation into the changes of DBP levels during a national swimming competition reported that some DBPs such as chloroform, NCl_3 and DCAN increased over the course of the competition (Weng and Blatchley III, 2011). An investigation of the role of various precursors in the formation of DBPs in swimming pool water demonstrated that organic matter from tap water contributed to higher THM formation potentials than HAA formation potentials in swimming pools (Kanan and Karanfil, 2011). Conversely in the same study, organic matter originating from human BFAs contributed to higher HAA formation potential compared to THMs. However, skin lipids being the main carbon source in swimming pools may possibly be the main

contributor to the production of carbonaceous DBPs (Keuten *et al.*, 2014). Some studies using BFAs lack this carbon source and their role in the formation of DBPs have thus not been fully investigated (Judd and Bullock, 2003; Zwiener *et al.*, 2007; Kanan and Karanfil, 2011).

2.5 Personal care products (PCPs)

PCPs are used externally on the human body and many such substances are known to be returned to the environment in an unaltered state (Ternes *et al.*, 2004; Peck, 2006). PCPs include disinfectants, fragrances, insect repellents, preservatives and UV filters (Brausch and Rand, 2011). The PCPs of concern in swimming pool water include sunscreens and parabens (preservatives present in cosmetics).

2.5.1 Sunscreens / Ultraviolet (UV) filters

UV filters are compounds added to sunscreens to prevent ultraviolet rays from penetrating through to the skin and are composed of organic and inorganic compounds which absorb and reflect UV light. In addition to their use in sunscreen lotions they are used in a variety of other personal care products including hair sprays, lipsticks and shampoos (Vidal *et al.*, 2010).

With their increasing usage, the presence of sunscreen active ingredients in the environment is a growing concern due to their potential ability for endocrine disruption in some cases (Caliman and Gavrilescu, 2009). Some sunscreen agents containing benzophenone-3 (BP-3), homosalate (HMS), 4-methyl-benzylidene camphor (4-MBC), octyl-*p*-methoxycinnamate (OMC) and octyl dimethyl-*p*-aminobenzoate (ODPABA) exhibit estrogenicity (Schlumpf *et al.*, 2001; Morohoshi *et al.*, 2005; Kunz *et al.*, 2006) and antiandrogenicity (Ma *et al.*, 2003; Suzuki *et al.*, 2005; Kunz and Fent, 2006) as shown by *in vitro* and *in vivo* analysis.

Organic sunscreen agents commonly consist of aromatic compounds conjugated with carbonyl groups. These compounds are mostly lipophilic and studies have shown that they have the ability to bioaccumulate in fish (Balmer *et al.*, 2005). Four sunscreen agents were detected in fish - 4-MBC, BP-3, OMC and octocrylene (OC) at concentrations of 166, 123, 72 and 25 ng/g in lipid respectively (Balmer *et al.*, 2005).

Sunscreen agents have also been detected in human urine (BP-3) (Felix *et al.*, 1998), breast milk (OMC, OC, 4-MBC, HMS, BP-3 and ODPABA) (Schlumpf *et al.*, 2010) and semen (ODPABA) (León-González *et al.*, 2011) following application of sunscreen products to skin.

Contamination of swimming pools with sunscreen agents occurs mainly through washing off from the skin of swimmers who have used sunscreen products (Stokes and Diffey, 1999; Wright *et al.*, 2001). Furthermore, as sunscreen agents are also excreted via urine (Felix *et al.*, 1998; Kim and Choi, 2014), this is possibly another source of sunscreen agent contamination in swimming pools. Five UV filters have been identified – BP-3, OMC, 2-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate (OCR), 2-phenyl-1H-benzimidazole-5-sulfonic acid (PBS) and MBC – in outdoor swimming pools where the highest concentration of 40 μg/L was detected in children's pools (Zwiener *et al.*, 2007). Adult pools were reported to have roughly ten times lower concentrations of sunscreen compounds compared to children's pools in the same study.

Further occurrences of UV filters in different water matrices reported in various studies have been compiled and are presented in Table 2.7. All of the studies listed are method development studies to quantify certain compounds of sunscreen agents in various water samples. The only reported study of sunscreen agents in swimming pools is by Zwiener *et al.* (2007). Most UV filters are seen to occur at high concentrations in swimming pools compared to other water matrices (see Table 2.7) which may be due to high dilution rates of rivers, lakes and oceans compared to swimming pool water which is recycled. Furthermore, some UV filters are biodegradable (Kupper *et al.*, 2006; Liu *et al.*, 2012b). Thus, the presence of a residual disinfectant in swimming pools may restrain bioactivity compared to natural water bodies. Some UV filters are known to partition strongly to the surface layer of water bodies (Poiger et al. 2004). Thus, the depth of sample collection may have an effect on the amount of UV filters being detected. Furthermore, their occurrence on the surface layer may conceivably reduce sunlight penetration thus decreasing the rates of any photolytic reactions in the water body. However, information on the fate of UV filters in swimming pools is still limited and potential effects on other DBPs have yet to be determined. Average rates of water ingestion by adults and children in swimming pools have been estimated to be 4 and 26

mL/h, respectively (Suppes *et al.*, 2013). If chemical contaminants are more concentrated at the pool water surface, enhanced exposure is likely. It is expected that UV filters would occur in higher concentrations during summer months and in outdoor swimming pools due to the increased use of sunscreens by swimmers.

Table 2.7 Concentrations of UV filters in different water matrices

Country	Matrix	UV filters concentration (ng/L)								Detection method	Reference
		BP-3	**4-MBC**	**OMC**	**BMDBM**	**IMC**	**OCR**	**ODPABA**	**PBS**		
Greece	Swimming pool (µg/L)	2.4-3.3						<0.9-2		SPME-GC-MS-FID	Lambropoulou *et al.* (2002)
	Seawater (µg/L)	<1.7						<0.9			
Greece	Swimming pool	4.2-5.7	5.4-6.9	3-4.5	<24					LC-UV-DAD & GC-MS	Giokas *et al.* (2004)
	Seawater	1.8	<0.7	<0.9	<24						
Greece	Bathing waters	6.5-8.2	13-20	7.4-11	<1.3 (µg/L)					LC-UV-DAD & GC-MS	Giokas *et al.* (2005)
Slovenia	Swimming pool	103-400	<150-330				<270			GC-MSD	Cuderman and Heath (2007)
	River	<54-110	<180				34-35				
	Lake	<28-85	<140				<17-31				
Germany	Baby pool (µg/L)	1.2	10	7			25		16		Zwiener *et al.* (2007)
	Swimmer pool (µg/L)		0.6	1.8			7		0.7		
	Non-swimmer pool (µg/L)		1.4	2.7			11		2		
Spain	Public pool (µg/L)	<0.11	<0.2			0.7	<3	<0.07		IL-SDME-LC-UV	Vidal *et al.* (2010)
	Private pool (µg/L)	<0.11	<0.06			<0.16	<3	<0.07			
	Beach (µg/L)	<0.11	<0.06			<0.16	<3	<0.07			
	River (µg/L)	<0.11	<0.06			<0.16	<3	<0.07			

BP-3: benzophenone-3, 4-MBC: 4-methylbenzylidene camphor, OMC, octyl methoxycinnamate, BMDBM: butyl methoxy dibenzoylmethane, IMC: isoamyl methoxycinnamate, OCR: octocrylene, ODPABA: octyl dimethyl-*p*-aminobenzoate, PBS: 2-phenyl-1H-benzimidazole-5-sulfonic acid

SPME: solid phase microextraction, GC: gas chromatography, MS: mass spectrometry, MSD: mass-selective detection, FID: flame ionization detection, LC-UV-DAD: liquid chromatography and photodiode array detection, IL-SDME-LC-UV: Ionic liquid-based single-drop microextraction liquid chromatography-ultraviolet spectrophotometry detection

2.5.2 Degradation of sunscreen agents / UV filters

Studies have shown that some sunscreen agents in aqueous matrices undergo degradation from exposure to sunlight (photodegradation) (Sakkas *et al.*, 2003; Rodil *et al.*, 2009a) and reactions with chlorine (Serpone *et al.*, 2002; Negreira *et al.*, 2008; Nakajima *et al.*, 2009; Virkutyte *et al.*, 2012). These degradation processes may produce by-products which may be more harmful than their parent compounds (Giokas *et al.*, 2007; Díaz-Cruz and Barceló, 2009). Toxicity studies carried out on mouse cells found certain degradation products of UV filters to be toxic (Kockler *et al.*, 2012). Mouse lymphoma cell line was used to determine the potential toxicity of degradation by-products of OMC and butyl methoxy dibenzoylmethane (BMDBM) and it was found that solutions of OMC with sun exposure were more toxic than those without sun exposure whereas BMDBM showed no significant effect (Butt and Christensen, 2000). Similar findings were reported where BMDBM had no significant difference in toxicity levels in mouse cells before and after UV irradiation (Kockler *et al.*, 2012). The same study found that, in contrast, the toxicity of OMC increased after UV irradiation which may be attributed to the toxicity of the degradation products.

The stabilities of UV filters in aqueous matrices were dependant on the pH, the concentration of chlorine used and the structures of the UV filters (Negreira *et al.*, 2008; Nakajima *et al.*, 2009). The stability of 2-ethylhexyl salicylate (ES), 2-ethylhexyl 4-(dimethylamino) benzoate (EHPABA) and BP-3 were investigated and it was determined that EHPABA and BP-3 were less stable in water samples containing higher levels of chlorine (Negreira *et al.*, 2008). In another study, ODPABA was seen to react quickly in a chlorinated aqueous solution at pH 7 whereas OMC had a slower reaction rate under the same conditions (Nakajima *et al.*, 2009).

The photostabilities of six UV filters were examined and the results showed that BP-3, OC and 4-MBC were highly stable during irradiation (Rodil *et al.*, 2009a). Conversely, degradation occurred for the other three UV filters consisting of ethylhexyl methoxycinnamate (EHMC), isoamyl methoxycinnamate (IMC) and ODPABA. The rate of ODPABA photodegradation was found to vary in different aquatic environments (sea, swimming pool and distilled water) when exposed to simulated solar irradiation (xenon lamp) and under natural light (Sakkas *et al.*, 2003). It was determined that the

presence of dissolved organic matter in the waters hindered the rate of photolysis with lower transformation rates observed in seawater and swimming pool water when compared to distilled water. Possible reasons for this reaction given in this study include i) the dissolved organic matter and ODPABA compete for available photons, reducing the direct photochemical reaction of ODPABA, ii) suspended particles in the waters inhibit the penetration of light beneath the surface and iii) a partial binding between the dissolved organic matter and ODPABA by hydrophobic partitioning or weak van der Waals forces making this fraction unavailable to photolysis. By-products were detected in all waters with additional degradation by-products detected in swimming pool water possibly due to the further chlorination reactions of the parent compound and other intermediates which confirms that sunscreens may be an important source of DBPs in swimming pools.

Although by-products of UV filters in swimming pool waters have been investigated, research in this area is still relatively sparse. A comprehensive review on various studies concerning degradation products of sunscreen agents in chlorinated waters determined that further research should focus on a wider range of regulated UV filters since current studies have only focused on a small group of these compounds (Santos *et al.*, 2012). Also, more research is needed to determine the amount of UV filters released from swimmers and possible ways to reduce this.

2.5.3 Parabens

Parabens, also known as *p*-hydroxybenzoic esters, are a class of compounds most commonly used as antimicrobial preservatives in the production of PCPs (Terasaki *et al.*, 2009). Frequently used parabens include methylparaben, ethylparaben, propylparaben, butylparaben, benzylparaben, isobutylparaben and isopropylparaben. Due to their presence as a key ingredient in many PCPs and the high daily usage of PCPs, parabens are constantly released into the aquatic environment. Methylparaben, ethylparaben and propylparaben have been detected in river waters in South Wales (UK) with methylparaben being the most frequently detected compound at concentrations up to 150 ng/L (Kasprzyk-Hordern *et al.*, 2008). Concentrations of methylparaben were observed from <0.5 – 1100 ng/L and propylparaben from <0.1 – 2100 ng/L in an urban river in South China (Peng *et al.*, 2008). Methylparaben (9 – 26

ng/L) and propylparaben (0.6 – 16 ng/L) were also detected in various environmental waters in Ria de Aveiro, Portugal (Jonkers *et al.*, 2010). Seven parabens in urban streams were reported in Japan with the highest concentration observed for methylparaben at 670 ng/L (Yamamoto *et al.*, 2011).

It has been suggested that some of these compounds such as butylparaben, isobutylparaben and benzylparaben possess estrogenic activity (Routledge *et al.*, 1998; Golden *et al.*, 2005). The toxicity levels of parabens and their by-products tested on aquatic organisms showed that parabens toxicity increased with chlorination and the chlorinated by-products showed more toxicity than their parent compounds (Terasaki *et al.*, 2009).

The reactions of parabens in chlorinated laboratory grade water and tap water samples was investigated by spiking known amounts of paraben compounds into the water matrix of one set and by adding a PCP containing parabens in another set (Canosa *et al.*, 2006). Several chlorinated by-products were detected in all scenarios in both chlorinated waters after a set reaction period. Brominated by-products of parabens were further identified in the chlorinated tap water samples which were reported to be caused by traces of bromide being present in tap water. From this study, it was found that the parent compounds of parabens degraded at a much higher rate in samples with free chlorine concentrations over 0.4 mg/L and at pHs 7.3 and 8.0. Furthermore, the presence of free chlorine enhanced the photodegradation rate of parabens when exposed to UV light, increasing the formation of halogenated by-products (Alvarez-Rivera *et al.*, 2014). Consequently, more halogenated by-products of parabens may possibly be present in swimming pools since pools have higher chlorine concentrations than drinking water. Parabens are regularly used in cosmetics and lotions and therefore have the potential to be introduced by bathers.

The parent compound, benzylparaben was detected at levels up to 28 ng/L and isopropylparaben (*i*PrP), a dichlorinated by-product was detected at levels up to 25 ng/L in swimming pools (Terasaki and Makino, 2008). The chlorinated by-product of methylparaben was also detected at concentrations below 10 ng/L. This study shows that formation of by-products from PCPs do occur in swimming pool waters which calls

for further research to investigate if there are adverse effects from these by-products on humans through exposure from swimming.

2.6 Control measures for reducing chemical contaminants in swimming pools

With the understanding of how chemical contaminants are occurring in swimming pools, preventive measures can be taken to reduce their levels and thus increase swimming pool water quality. Incorrect behaviours are widespread among pool users and there is little awareness on following swimming pool rules to reduce microbial and chemical contaminants such as having a pre-swim shower or not urinating in the pool (Pasquarella *et al.*, 2014). Unhygienic behaviours of swimmers can lead to a significant amount of anthropogenic pollution in swimming pools (Keuten *et al.*, 2014). Therefore, increasing swimmer awareness of the importance of hygienic behaviour in swimming pools might be an effective first step to reducing pollutants in the pool. Educating swimmers and providing information on the occurrences of chemical contaminants would increase swimmers awareness to take preventive steps. For example, as most PCP compounds are originating from swimmers, informing swimmers to reduce the application of PCPs on their bodies before entering the pool so as to prevent wash off could be an effective way of reducing PCP levels.

Effective hygiene practices by pool users such as showering prior to entering the pool could significantly decrease the formation of DBPs in swimming pools (Chowdhury *et al.*, 2014). A pre-swim shower could significantly reduce both chemical and microbial anthropogenic pollution, which will very likely minimise DBP formation in the pool and chlorine demand (Keuten *et al.*, 2012). Shower experiments conducted in the laboratory and in the field showed that the majority of skin-borne pollutants were eliminated within the first 60 seconds of showering. The same study further showed that the use of swim caps further reduced the release of TOC (19%) and total nitrogen (70%). Hence, implementing mandatory pre-swim shower and the use of swim caps could be effective for the further reduction of chemical contaminants in swimming pools. Furthermore, the availability and physical layout of pool facilities is important. Swimming pool facility designers may ensure that showers are installed and are conveniently accessible to bathers approaching the pool. Consideration should also be given to the availability of hot water (as opposed to just cold water) in showers since

this may also lead to increased shower use. Also, pool managers should be aware that they can influence the amount of pollutants swimmers release in their pools. Reducing the water temperature for example will reduce the sweat production.

Adopting new treatment processes for swimming pools such as the use of activated carbon treatment, AOPs and membrane filtration could further improve the quality of swimming pool water with different studies showing promising outcomes (Glauner *et al.*, 2005a; Glauner *et al.*, 2005b; Glauner and Frimmel, 2006; Zwiener *et al.*, 2007). The addition of membrane filtration to swimming pool disinfection process was investigated and it was determined that high pressure membranes with low-molecular weight cut offs down to 200 g/mol were required for effective DBPs removal and decreasing genotoxicity in swimming pool water (Glauner *et al.*, 2005b). The performance of a hybrid process to treat swimming pool water where activated carbon was added as a second step to remove lower molecular weight organic compounds passing through the ultrafiltration membranes was studied (Barbot and Moulin, 2008). This study which used combined chlorine as the general term for all chlorinated DBPs showed that the hybrid process would be efficient in maintaining the quality of pool water and decreasing the combined chlorine concentrations in swimming pools. Furthermore, the use of filtration with powdered activated carbon (PAC) was found to be an effective treatment for HANs removal in swimming pools (Kramer *et al.*, 2009). Analysis of swimming pool water treated with activated carbon showed reduced genotoxic effect and lower concentrations of DBPs compared to untreated pool water (Kramer *et al.*, 2009). Implementing biological filtration in the operations of swimming pools could also be an effective option to remove biodegradable DBP precursors such as urea and other anthropogenic compounds. This option could provide a low cost alternative to remove DBP precursors. Although the use of biological filters in swimming pools seems to be in conflict with the hygienic environment of a swimming pool, there is evidence that such processes are widely and effectively implemented in some countries (Keuten *et al.*, 2009). As new regulations in Germany have already implemented the use of activated carbon for swimming pool water treatment, the long term performance of three types of granular activated carbon consisting of a low-activated carbon and two catalytically enhanced activated carbon in pool water treatment were investigated (Uhl and Hartmann, 2005). This study reported the decrease

of removal efficiency over time for free chlorine, combined chlorine, dissolved organic carbon, spectral absorption coefficient, absorbable organic carbon and most DBPs. Further research is required to investigate the optimal operating parameters of activated carbon for long term use in swimming pool treatment. Much research previously undertaken for drinking water will be relevant for determining factors such as filter saturation times.

Ozonation and AOPs could be advantageous additions for the disinfection process of swimming pool water to reduce DBPs and their precursors (Glauner *et al.*, 2005a). The effectiveness of ozone/hydrogen peroxide treatment compared to ozonation showed potential with higher elimination rates of DBPs and their precursors in swimming pool water. This was reported to be due to the non-selective oxidation of organics by hydroxyl radical attack of AOPs. Shorter reaction time (3 min) for the AOP treatment was needed to achieve maximum elimination of TOC content compared to ozonation. The study also showed that the addition of membrane filtration together with AOPs increased the elimination of DBPs and its precursors by up to 80%. Another study reported similar results where ozone/hydrogen peroxide had a more effective reduction of DBPs compared to ozonation and ozone-UV treatments (Glauner and Frimmel, 2006).

The use of ozone/hydrogen peroxide treatment and filtration through granular activated carbon in swimming pools could potentially reduce the concentrations of chemical contaminants not only DBPs and its precursors but also a wider range of other chemicals as applied in environmental water studies (Esplugas *et al.*, 2007; Snyder *et al.*, 2007; Chen and Wang, 2012; Sato *et al.*, 2014). Additionally, AOPs have been shown to successfully degrade PCP compounds such as parabens. Butylparaben in aqueous solution degraded at a high rate under UV radiation and hydrogen peroxide treatment (BŁędzka *et al.*, 2010) while methylparaben in wastewater was easily removed by UV photodegradation even without additional oxidants (Sánchez-Martín *et al.*, 2013). Thus, the use of ozone, hydrogen peroxide and/or UV may possibly be advantageous in reducing PCP compounds in swimming pools. With the use of AOPs, DBPs are not removed but are chemically changed and by products would still be present. It should be investigated whether these new AOP by-products might be more or

less harmful than their parent compounds. The by-products can further react with each other or with chlorine or other pollutants, thus the overall effect of these techniques is difficult to predict.

Further studies are needed to verify if these new treatment methods would provide better treatment than the usual swimming pool treatment processes. The addition of these treatment processes will significantly increase the capital and operational costs of swimming pools due to higher energy usage and carbon footprint. The use of activated carbon would be expensive as the carbon may become saturated on the order of 20 to 40 days and would need to be renewed on a monthly basis. The use of PAC might be an effective treatment solution, but could only be used in combination with sand filtration and coagulation or ultrafiltration. The use of PAC would enforce more frequent filter backwashes and thus increase water and energy consumption. High pressure membrane processes also require high energy consumption and are much more complex than traditional treatment methods. For example, the integrity of reverse osmosis membranes is highly sensitive to oxidants such as chlorine. This limitation would likely present significant challenges for the implementation of this technology in a swimming pool environment. A cost-benefit assessment would need to be undertaken to evaluate the feasibility of the addition of these treatment processes to swimming pool operation. As these treatment methods are expensive, it should be first established that the chemical contaminants occurring in swimming pools are a hazardous risk to pool users.

2.7 Conclusions

Research on chemical contaminants in swimming pools has focused on the DBPs traditionally regulated in drinking water such as THMs and HAAs. Variation of DBP levels appears to occur due to many factors including the number of swimmers, variable swimming pool management practices, treatment steps and type of pools. HAAs generally occur in swimming pools at a higher concentration than THMs as they are less volatile and tend to accumulate. With recent studies showing that N- DBPs such as HANs, HNMs and *N*-nitrosamines are more toxic, further studies on N-DBPs may be of more significance in swimming pools compared to C-DBPs and research is needed to ascertain their potential health risk to swimmers when exposed over a period of time. Various operational and treatment parameters in swimming pools such as fill water

used, type of disinfectants, chlorine dosage, temperature and pH were seen to affect the type of DBPs occurring in swimming pools. Higher levels of brominated DBPs can be expected to occur in swimming pools which have high bromide content, such as those using seawater or salt addition. Additionally, UV filters and parabens from PCPs are potential endocrine disrupting chemicals and their occurrences in swimming pools come from anthropogenic sources. Their degradation in swimming pool waters through the reactions with disinfectants or sunlight irradiation may produce by-products which are potentially more toxic than their parent compounds. Studies on these compounds in swimming pools are still in their infancy and available data are limited. Research is needed to identify the degradation products formed and to determine which of those have significant toxicity. Furthermore, the toxicity of PCP compounds in relation to well-established DBPs such as THMs and HAAs in swimming pool needs to be identified. A wider range of chemicals originating from the use of PCPs may be present in swimming pools and needs to be identified. The possible risk of exposure of PCPs and their by-products to swimmers in swimming pools can then be determined. As DBP levels in swimming pools are higher than those occurring in drinking water, the significance of these levels to the overall exposure and health of swimmers should be evaluated. From a health perspective, in order to assess the potential health risk of chemicals in swimming pools, a broader range of chemicals need to be identified and taken into account.

CHAPTER 3 ANALYTICAL METHODS AND EXPERIMENTAL PROCEDURES

3.1 Introduction

Various analytical techniques have been previously used for the determination and quantification of trace organic chemical contaminants. The use of chromatography is among the standard technique used due to its high sensitivity and specificity in detecting individual chemicals and combined with mass spectrometry, provides detailed information on compounds for identification.

This chapter details the experimental procedures used for conducting this research. The analytical methods used in assessing the various chemical contaminants consisting of 30 PPCPs, 7 *N*-nitrosamines and 5 PFRs in swimming pools are described. Based on the outcome of an extensive literature review (Chapter 2), it was concluded that a detailed study into the occurrence of a wider range of chemical contaminants, namely PPCPs, *N*-nitrosamines and PFRs, in swimming pool was required. These chemicals were therefore selected for this study. PPCPs were analysed using LC-MS/MS and *N*-nitrosamines and PFRs were analysed using GC-MS/MS. The physicochemical properties of the targeted chemicals are presented in Table 3.1.

A fluorescence excitation-emission matrix (EEM) method was developed for the analysis of swimming pool water. Since swimming pool waters are highly chlorinated, therefore the fluorescence signals may be significantly quenched leading to low fluorescence output. The data collected from the fluorescence EEMs analysis were used to identify suitable fluorescence regions for the online monitoring of swimming pool water. Based on these results, the suitability and applicability for online monitoring of swimming pool water were assessed.

Table 3.1 Physicochemical properties of targeted chemicals contaminants

Target compounds	MW	Log D (pH 7, 25 °C)[a]	Mass solubility (pH 7, 25 °C)[a]	pKa[a]	Vapour pressure at 25 °C (Torr)[a,b]	Henry's Law Constant at 25 °C (atm m^3 mol^{-1})[c]
PFRs						
TNBP	266	3.83	Sparingly Soluble (0.64 g/L)	n/a	4.09E-03	3.19E-06
TCEP	286	1.47	Slightly Soluble (7.4 g/L)	n/a	1.08E-04	2.55E-08
TCIPP	328	2.53	Slightly Soluble (1.0 g/L)	n/a	5.25E-05	5.96E-08
TDCIPP	431	3.27	Sparingly Soluble (0.12 g/L)	n/a	negligible	2.61E-09
TPHP	326	4.59	Sparingly Soluble (7.2E-3 g/L)	n/a	negligible	3.98E-08
N-nitrosamines						
NDMA	74	-0.57	Very Soluble (284 g/L)	-3.63±0.70	4.56	1.20E-06
NDEA	102	0.52	Soluble (62 g/L)	-3.14±0.70	1.66	1.73E-06
NMor	116	-0.59	Very Soluble (130 g/L)	-5.72±0.20	0.13	2.13E-10
NMEA	88	0.01	Very Soluble (134 g/L)	-3.39±0.70	4.10	1.44E-06
NDPA	130	1.54	Soluble (13 g/L)	-3.18±0.70	0.35	3.46E-06
NPyr	100	-0.09	Soluble (45 g/L)	-3.14±0.20	0.23	1.99E-07
NDBuA	158	2.56	Slightly Soluble (2.8 g/L)	-3.14±0.70	0.03	9.96E-06
PPCPs:						
Amitriptyline	277	2.28	Slightly Soluble (4.4 g/L)	9.18±0.28	negligible	6.85E-08
Atenolol	266	-2.09	Very Soluble (999 g/L)	9.43±0.10	negligible	1.37E-18
Caffeine	194	-0.63	Soluble (58 g/L)	0.52±0.70	negligible	3.58E-11
Carbamazepine	236	1.89	Sparingly Soluble (0.22 g/L)	-0.49±0.20	negligible	1.08E-10
Clozapine	327	3.23	Sparingly Soluble (0.024 g/L)	7.33±0.20	negligible	9.29E-15
Diazepam	285	2.80	Sparingly Soluble (0.051 g/L)	3.40±0.10	negligible	3.64E-09
Dilantin	252	1.41	Sparingly Soluble (0.20 g/L)	-2.81±0.40	n/a	1.02E-11

Enalapril	377	-0.14	Very Soluble (700 g/L)	5.43±0.39	negligible	3.34E-16
Fluoxetine	309	1.15	Soluble (34 g/L)	10.05±0.10	negligible	8.90E-08
Hydroxyzine	375	2.15	Slightly Soluble (4.5 g/L)	6.62±0.10	negligible	3.86E-17
Ibuprofen	206	0.94	Soluble (68 g/L)	4.41±0.10	1.39E-04	1.52E-07
Meprobamate	218	0.70	Slightly Soluble (8.5 g/L)	-1.09±0.70	negligible	1.85E-10
Omeprazole	345	2.35	Sparingly Soluble (0.030 g/L)	4.72±0.40	negligible	3.04E-19
Paracetamol	151	0.47	Soluble (15 g/L)	1.72±0.50	negligible	6.42E-13
Primidone	218	0.83	Slightly Soluble (1.5 g/L)	-1.07±0.40	negligible	1.94E-10
Risperidone	411	1.53	Sparingly Soluble (0.16 g/L)	8.07±0.10	negligible	2.17E-16
Sulfamethoxazole	253	-0.22	Slightly Soluble (2.8 g/L)	1.39±0.10	negligible	9.56E-13
Triamterene	253	1.03	Sparingly Soluble (0.041 g/L)	6.28±0.10	negligible	1.86E-18
Trimethoprim	290	0.27	Slightly Soluble (1.0 g/L)	7.04±0.10	negligible	2.39E-14
Verapamil	455	2.08	Sparingly Soluble (0.64 g/L)	8.97±0.50	negligible	8.79E-15
Bisphenol A	228	3.64	Sparingly Soluble (0.071 g/L)	10.3±0.10	negligible	9.16E-12
Gemfibrozil	250	2.07	Soluble (11 g/L)	4.75±0.45	negligible	1.19E-08
Ketoprofen	254	0.19	Soluble (58 g/L)	4.23±0.10	negligible	2.12E-11
Naproxen	230	0.73	Soluble (15 g/L)	4.84±0.30	negligible	3.39E-10
Nonylphenol	220	6.14	Sparingly Soluble (0.020 g/L)	10.2±0.15	negligible	5.97E-06
Propylparaben	180	2.88	Slightly Soluble (1.2 g/L)	8.23±0.15	9.30E-04	6.37E-09
Simvastatin	419	4.72	Sparingly Soluble (4.6E-3 g/L)	13.5±0.40	negligible	2.81E-10
Simvastatin hydroxy acid	436	1.79	Slightly Soluble (3.8 g/L)	4.31±0.10	negligible	9.68E-14
Triclocarban	316	6.07	Sparingly Soluble (1.0E-4 g/L)	-0.34±0.50	negligible	4.52E-11
Triclosan	290	5.28	Sparingly Soluble (1.3E-3 g/L)	7.80±0.35	negligible	4.99E-09

[a] Values obtained from Scifinder American Chemical Society
[b] Vapour pressure less than 10^-5 considered negligible
[c] US EPA (2016) Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11

3.2 LC-MS/MS method for analysis of pharmaceutical and personal care products

The analysis of PPCPs using LC-MS/MS was based on an adaptation of a published method (Vanderford and Snyder, 2006). Target compounds analysed in this study consisted of 30 PPCPs. Direct isotope labelled internal standard were used where available as detailed in Table 3.1. The PPCPs targeted in this study were selected based on their frequency of detection in environmental waters, common usage and the availability of analytical standards. Bisphenol A which is used as a monomer in commercially available products and is not a PPCP was included in the list of target chemicals due to its persistence in environmental waters and endocrine disrupting properties.

Table 3.2 List of PPCP compounds

Analytes	**Internal standard**	**Uses**
ESI positive mode:		
Amitriptyline	Amitriptyline-D6	Anti-depressant
Atenolol	Atenolol-D7	Beta-blocker
Caffeine	Caffeine-D9	Stimulant
Carbamazepine	Carbamazepine-D10	Anti-seizure
Clozapine	Clozapine-D4	Anti-schizophrenia
Enalapril	Enalapril-D5	Enzyme inhibitor
Diazepam	Diazepam-D5	Muscle relaxant
Dilantin	Dilantin-D10	Anti-convulsant
Fluoxetine	Fluoxetine-D5	Anti-depressant
Hydroxyzine	Hydroxyzine-D8	Antihistamine
Meprobamate	Meprobamate-D3	Anti-anxiety
Omeprazole	Omeprazole-D3	Proton pump inhibitor
Paracetamol	$^{15}N^{13}C$-paracetamol	Analgesic, Anti-inflammatory
Primidone	Primidone-D5	Anti-convulsant
Risperidone	Risperidone-D4	Antipsychotic
Sulfamethoxazole	Sulfamethoxazole-D4	Antibiotic

Triamterene	Triamterene-D5	Diuretic agent
Trimethoprim	Trimethoprim-D9	Antibiotic
Verapamil	Verapamil-D6	Calcium channel blocker
ESI negative mode:		
Bisphenol A	Bisphenol A-D6	Plastisizer
Gemfibrozil	Gemfibrozil-D6	Anti-cholesterol
Ibuprofen	Ibuprofen-D3	Analgesic, Anti-inflammatory
Ketoprofen	Ketoprofen-D3	Analgesic, Anti-inflammatory
Naproxen	Naproxen-D3	Analgesic, Anti-inflammatory
Nonylphenol	Nonylphenol-D4	Surfactant
Propylparaben	N/A	Anti-microbial
Simvastatin	Simvastatin-D6	Anti-lipidemic
Simvastatin hydroxy acid	Simvastatin hydroxy acid-D6	Simvastatin metabolite
Triclocarban	Triclocarban-D4	Anti-microbial
Triclosan	Triclosan-D3	Anti-microbial

N/A Not available

3.2.1 Materials

$^{15}N^{13}C$-paracetamol and D5-diazepam were purchased from Cambridge Isotope Laboratories Inc., USA. D4-sulfamethoxazole, D6-trimethoprim, D4-risperidone, D5-enalapril, D6-simvastatin, D6-simvastatin hydroxy acid, D3-triclosan, D5-triamterene, D3-meprobamate and D8-hydroxyzine were purchased from Toronto Research Chemicals Inc., Canada. D6-amitriptyline, D7-atenolol, D7-bisphenol A, D9-caffeine, D10-carbamazepine, D4-clozapine, D10-dilatin, D5-fluoxetine, D6-gemfibrozil, D3-ibuprofen, D3-ketoprofen, D3-naproxen, D3-omeprazole, D5-primidone, D4-triclocarban and D6-verapamil were purchased from Dr. Ehrenstorfer GmbH, Germany. D4-nonylphenol was purchased from ISOTEC, Sigma Aldrich. For propylparaben no direct isotopically labeled were available, therefore quantification for these compounds

were based on external calibration only. Methyl *tert*-butyl ether (MTBE) and all target analytes were obtained from Sigma Aldrich except fluoxetine, risperidone, simvastatin hydroxy acid were purchased from Toronto Research Chemicals Inc., Canada.

HPLC grade methanol was purchased from Ajax Finechem (Tarron Point, Australia). Ultrapure water was produced using a Direct-Q filtering system from Milipore (North Ryde, NSW, Australia). Kimble culture tubes (13 mm I.D. x 100 mm) and a Thermo Speedvac concentrator (model no. SPD121P) were purchased from Biolab (Clayton, Vic, Australia). Oasis hydrophilic lipophilic balance (HLB) solid phase extraction cartridges (6 mL, 500 mg) were purchased from Waters (Rydalmere, NSW, Australia).

Stock standard solutions and isotope labelled standards of PPCPs were prepared in methanol (1 g/L, 10 mL) in amber vials and then further serial diluted with methanol to obtain working standard solutions of lower concentrations. All standard solutions were stored at 4 °C. Calibration standards were prepared by serial dilution in methanol from working stocks.

3.2.2 Solid phase extraction

In preparation for analysis, SPE was undertaken to concentrate and isolate compounds of interest. Figure 3.1 shows the SPE set up in the laboratory.

Figure 3.1 Solid phase extraction

The Oasis HLB SPE cartridges were used for the analysis of PPCPs which were preconditioned sequentially prior to extraction with MTBE (5 mL), methanol (5 mL) and ultrapure water (10 mL). SPE was then carried out using the pre-conditioned cartridges with 1 L of each of the spiked samples being drawn through under vacuum at a rate not exceeding 5 mL/min. Laboratory blank samples using ultrapure water were simultaneously extracted and analysed for each batch of sample. After loading, the SPE cartridges were rinsed with ultrapure water before drying under a gentle flow of nitrogen until visibly dried. Loaded cartridges were stored at 4 °C in sealed bags until elution and analysis. Analytes were eluted from the cartridges with MTBE (5 mL) and methanol (5 mL) into 20 mL glass tubes and then concentrated under a stream of nitrogen to approximately 100 µL. The evaporated extracts were reconstituted with methanol to bring the final volume to 1 mL. Finally, the extracts were transferred to 2 mL GC vials for instrumental analysis.

3.2.3 Liquid chromatography tandem mass spectrometry

Isotope dilution LC-MS/MS was used to analyse swimming pool water samples for PPCPs. Analytes were separated using an Agilent (Palo Alto, CA, USA) 1200 series high performance liquid chromatography (HPLC) system (Figure 3.2) equipped with a 150 x 4.6 mm, 5 µm particle size, Luna C18 (2) column (Phenomenex, Torrence CA, USA).

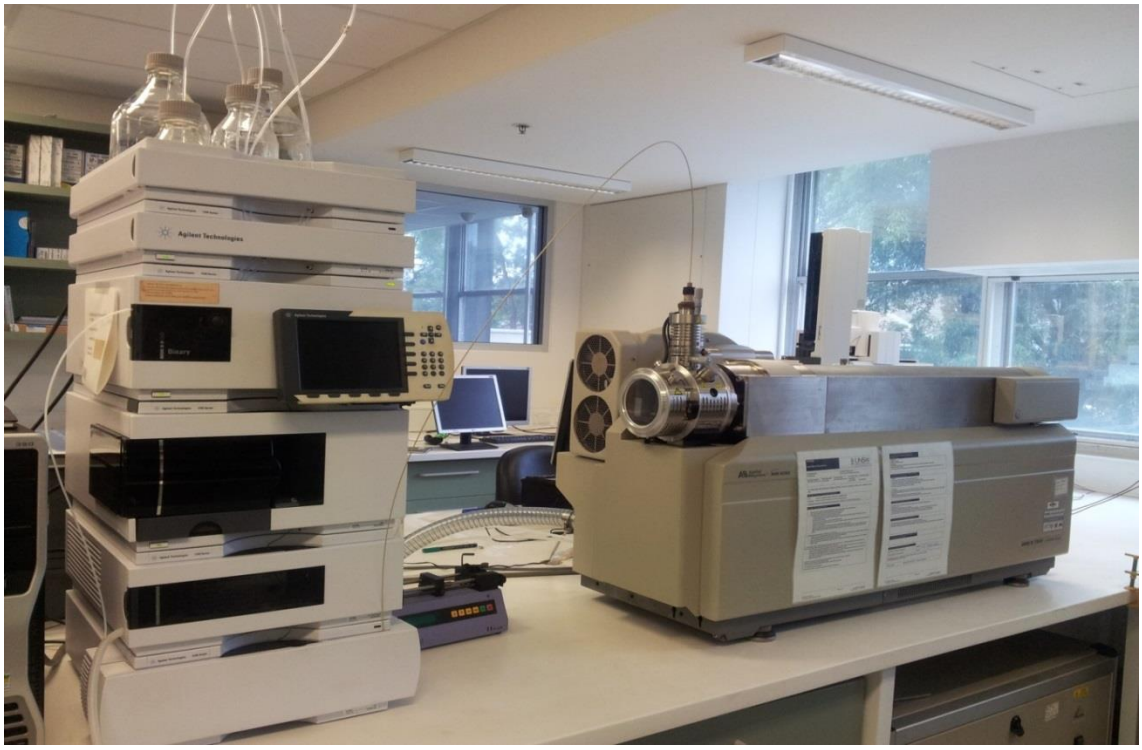


Figure 3.2 High performance liquid chromatography tandem mass spectrometry

The mobile phase gradient consisted of 5 mM ammonium acetate in water (A) and 100% methanol (B) at a flow rate of 800 µL/min. Two LC-MS/MS method were used for analysis; positive mode electrospray ionization (ESI+) and negative mode electrospray ionization (ESI-). For ESI positive analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 50% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 2 min. For ESI negative analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 60% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 3 min. A 5 min equilibration step

at 10% B was used at the beginning of each run. The sample injection volume was set at 10 µL for both methods.

Detection was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbo-V ion source employed in both positive and negative electrospray modes. Isotopically labelled standards for each target compound except for propylparaben were used as surrogate standards for accurate quantification. Additionally, multiple reaction monitoring (MRM) for two mass transitions were monitored for univocal confirmation of analyte detection. Only the first transition was used for quantitation. One mass transition for the labelled internal standard was monitored. Relative retention times of the analyte and isotopically labelled internal standard were also monitored to ensure correct identification.

3.2.4 Calibration and detection limits

Standard solutions of all analytes were prepared at 1, 5, 10, 50, 100 and 200 ng/mL in methanol. Isotopically labeled internal standards (50 ng) were also added to each calibration standard. A relative response ratio of analyte/internal standard over a 1 – 200 ng concentration range was generated enabling quantitation with correction for losses due to ion suppression and incomplete SPE recovery. All calibration curves had a correlation coefficient of 0.99 or better. The limits of quantification (LOQs) for targeted analytes were determined by the second lowest quantifiable concentration in the calibration curves with signal-to-noise (S/N) ratio of greater than 10. In the few cases where traces of contaminants were observed in the sample 'blanks', the LOQs were increased 10 times above these levels.

3.3 GC-MS/MS method for analysis of *N*-nitrosamines

This analytical method used was developed at UNSW and has been fully optimised and validated (McDonald *et al.*, 2012). In this method, seven *N*-nitrosamine compounds consisting of *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBuA), *N*-nitrosopyrrolidine (NPyr) and *N*-nitrosomorpholine (NMor) were targeted and analysed.

3.3.1 Materials

NDMA, NMEA, NDEA, NDPA, NDBuA, NPyr, NMor, dichloromethane (DCM) (spectroscopic grade), methanol (HPLC grade) and Supelclean coconut charcoal SPE cartridges were purchased from Supelco (St Louis, MO, USA).

NDMA-D6, NMEA-D3, NDEA-D10, NDPA-D14, NDBuA-D18, NPyr-D8 and NMor-D8 were purchased from CDN isotopes (Pointe-Claire, Quebec, Canada).

Ultrapure water was produced using a Direct-Q filtering system from Milipore (North Ryde, NSW, Australia). Kimble culture tubes (13 mm I.D. x 100 mm) and a Thermo Speedvac concentrator (model no. SPD121P) were purchased from Biolab (Clayton, Vic, Australia).

Stock standard solutions and isotope labelled standards of *N*-nitrosamines were prepared in methanol (1 g/L, 20 mL) in amber vials and then further serial diluted with methanol to obtain working standard solutions of lower concentrations. All standard solutions were stored at 4 °C. Calibration standards were prepared by serial dilution in methanol from working stocks.

3.3.2 Solid phase extraction

The target analytes were extracted using coconut charcoal SPE cartridges (6 mL, 2 g). The SPE cartridges were conditioned by sequentially eluting DCM (6 mL), methanol (6 mL) and ultrapure water (12 mL). Using a vacuum manifold, samples were loaded onto the cartridges at 5 mL/min, after which the cartridges were rinsed with 6 mL of ultrapure water and dried with a stream of nitrogen for 45 min. Laboratory blank samples using ultrapure water were simultaneously extracted and analysed for each batch of sample. Loaded cartridges were stored in the dark at 4 °C in sealed bags under nitrogen until elution and analysis. Analytes were eluted from the cartridges with DCM (4 x 3 mL) into 20 mL glass tubes. 100 mL of toluene was added to the glass tubes to minimize evaporative losses of analytes during solvent removal. The resulting extract was concentrated under a stream of nitrogen to approximately 1 mL and transferred to 2 mL GC vials for instrumental analysis.

3.3.3 Gas chromatography tandem mass spectrometry

Samples were analysed on an Agilent 7890A gas chromatograph coupled with an Agilent 7000B triple quadrupole mass spectrometer (Figure 3.3).

Figure 3.3 Gas chromatography tandem mass spectrometry

Chromatographic separation of the analytes was achieved using an Agilent DB-1701P, (30 m x 0.25 mm, 0.25 mm film thickness) column. Ultra high purity helium was used as carrier gas at a flow rate of 1.2 mL/min. The GC inlet was held at a temperature of 280 °C degrees and used in split-less mode equipped with a single tapered deactivated inlet liner (4 mm, Agilent Technologies). An injection volume of 1 µL was used and the oven temperature program was: 50 °C held for 1 min then raised to 80 °C at a rate of 10 °/min, increased to 180 °C at 15 °C/min, increased to 260°C at 35 °C/min and held for 5 min (total run time: 14 min). GC-MSMS interface temperature was kept at 260 °C. Mass spectrometric ionisation was undertaken in electron ionisation (EI) mode with an EI voltage of 70 eV and a source temperature of 280 °C. Using MRM data acquisition

mode with the gain set to 100 for all analytes, two transitions were monitored for all analytes and surrogate standards. Collision energies were optimised for each transition. Details are given in Table 3.2.

Table 3.3 GC-MS/MS method parameters

Segment start time	Analytes and isotope standards	Retention time (min)	MRM transitions (m/z)	Collision energy (V)	Dwell time (ms)
4.30	NDMA	04.56	74.0 → 44.1	3	20
			74.0 → 42.1	7	10
	NDMA-D6	04.55	80.0 → 50.1	3	20
			80.0 → 48.1	7	10
	NMEA	05.62	88.0 → 71.0	3	20
			88.0 → 43.0	5	10
	NMEA-D3	05.60	91.0 → 74.0	3	20
			91.0 → 46.0	5	10
	NDEA	06.44	102.0 → 85.0	5	20
			102.0 → 56.1	10	10
	NDEA-D10	06.39	112.1 → 94.1	5	20
			112.1 → 62.0	10	10
8.20	NDPA	08.38	130.1 → 113.0	0	20
			130.1 → 43.0	10	10
	NDPA-D14	08.31	144.0 → 126.1	0	20
			144.0 → 50.1	10	10
	NMor	08.72	116.0 → 86.0	0	20
			116.0 → 56.1	10	10
	NMor-D8	08.70	124.0 → 94.0	0	20
			124.0 → 62.0	10	10
	NPyr	08.90	100.0 → 70.0	5	20
			100.0 → 55.0	5	10
	NPyr-D8	08.86	108.0 → 78.1	5	20
			108.0 → 62.1	7	10
10.0	NDBuA	10.26	158.0 → 141.1	3	20

		158.0 → 99.0	5	10
NDBuA-D18	10.17	176.2 → 158.0	0	20
		176.2 → 110.0	5	10

Two MRM transitions of a single precursor ion were monitored for each target compound. Analysis of the acquired data was undertaken using Agilent MassHunter software. The confirmed identification of a target compound was only established once the analysis met all of the identification criteria. These included the observed presence of the two expected transitions at the same retention time, the area ratio of two transitions within a range of 20% variability with respect to the mean area ratio of all calibration solutions, and a consistent analyte-surrogate relative retention time as that of calibration solutions with relative standard deviation of less than 0.1 min.

3.3.4 Calibration and detection limits

Internal calibration using isotope dilution was used for all analytes. The calibration and detection limits procedure for *N*-nitrosamines is similar to the LC-MS/MS method (refer to Section 3.2.5).

3.4 GC-MS/MS method for analysis of organophosphate flame retardants

The analysis of organophosphate flame retardants was carried out using isotope dilution GC-MS/MS. The PFRs investigated were tributyl phosphate (TNBP), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCIPP), tris(1,3-dichloro-2-propyl) phosphate (TDCIP) and triphenyl phosphate (TPHP). The full method development and validation is presented in Chapter 4.

3.5 Fluorescence EEMs analysis and method development

In this study, fluorescence EEMs were measured in a 4 mL volume quartz cuvette with 1 cm path length (Starna, Australia) using a Varian Cary Eclipse Fluorescence Spectrometer (Figure 3.4).

Figure 3.4 Fluorescence spectrophotometer

Raw EEMs were measured at excitation wavelengths between λ_{ex} = 200 – 450 nm in 5 nm increments and emission wavelengths of 250 – 600 nm in 5 nm increments. The excitation and emission slit widths were set at 10 nm and the photomultiplier tube (PMT) voltage of 800 V was used. Scan speed of 9600 nm/min was used. No sample preparation was undertaken prior to running EEM analysis.

Cuvettes were rinsed with ultrapure water and then further rinsed with the sample before analysis. This process was repeated for every new sample. Ultrapure water samples were analysed in the cuvettes before and intermittently during analysis to ensure the cleanliness of the cuvettes.

The Raman value of high purity water in a sealed cuvette (Varian, Australia) was measured before every analysis to test for instrument drift and changes in lamp output. The Raman spectra was obtained from an average of 5 scans acquired at an excitation

wavelength of 348 nm and emission wavelength between 380 nm and 410 nm with slit widths of 5 nm. The PMT voltage of 800 V and scan speed of 600 nm/min was used.

3.6 Free chlorine

Free chlorine concentrations were determined with the Hach DPD Method 8021 using n,n-diethyl-*p*-phenylenediamine sulfate on a HACH pocket colorimeter II (HACH, Australia).

CHAPTER 4 DEVELOPMENT AND VALIDATION OF A METHOD FOR THE ANALYSIS OF ORGANOPHOSPHATE FLAME RETARDANTS

This chapter has been published in the following journal paper:

Teo TLL, McDonald JA, Coleman HM, Khan SJ. (2015) Analysis of organophosphate flame retardants and plasticisers in water by isotope dilution gas chromatography-electron ionisation tandem mass spectrometry. Talanta 143:114-120.

4.1 Introduction

Gas chromatography (GC) and liquid chromatography (LC) are the most common techniques employed to analyse PFRs in water (Quintana *et al.*, 2008). GC is most commonly used coupled with mass spectrometry (Andresen *et al.*, 2004; Meyer and Bester, 2004). Methods by LC- tandem MS have also been reported (Bacaloni *et al.*, 2007; Martínez-Carballo *et al.*, 2007; Rodil *et al.*, 2009b; Wang *et al.*, 2011). GC-MS has good selectivity and provides the option of isotopic dilution. However, some PFRs, especially the aliphatic triesters, undergo unfavourable fragmentation which can make identification of compounds difficult (Quintana *et al.*, 2008). Furthermore, the use of GC may lead to tailing peaks of some PFRs such as tris(2-butoxyethyl) phosphate (TBOEP) (Quintana *et al.*, 2008). LC-MS/MS can offer good selectivity and sensitivity but often suffer from the disadvantages of ion suppression, matrix interferences and incomplete separation of some compounds (Rodil *et al.*, 2005; Bacaloni *et al.*, 2007; Van der Veen and de Boer, 2012). Previous studies using LC-MS/MS have reported method detection limits (MDLs) between 0.8 – 7 ng/L for surface waters (Bacaloni *et al.*, 2007; Martínez-Carballo *et al.*, 2007; Wang *et al.*, 2011) and 7 – 20 ng/L for wastewaters (Martínez-Carballo *et al.*, 2007; Rodil *et al.*, 2009b).

The use of GC-MS/MS for the detection of PFRs has not been as widely reported as other methods of analysis. GC-MS/MS is able to provide improved selectivity, precision and detection limits in complex environmental matrices, compared to GC-MS. The improved selectivity of MS/MS is due to the ability to monitor the precursor and product ion transitions, thus selecting against background noise and leading to analyte quantitation at low levels in the presence of complex sample matrices. GC-MS/MS has been used to quantify TCEP, TCIPP, TDCIPP and TPHP in environmental water samples (Cristale *et al.*, 2012). The MDLs obtained in this study were 40 ng/L (TCEP), 4 ng/L (TCIPP), 20 ng/L (TDCIPP) and 10 ng/L (TPHP) and the method was applied to assess water from a drinking water treatment plant. There are no reports of using GC-MS to analyse for PFRs which incorporate the use of direct isotope dilution for each of the targeted compounds. This is essential to account for analyte losses during sample preparation and potential matrix effects which provides for accurate quantification. No published methods have investigated the applicability of isotope dilution to swimming pool waters and seawaters.

In this chapter, a simple, sensitive and selective method was developed for the simultaneous determination of five commonly detected PFRs based on SPE followed by isotope dilution gas chromatography tandem mass spectrometry. The PFRs investigated include an alkyl phosphate (TNBP), three chlorinated alkyl phosphates (TCEP, TCIPP, TDCIPP) and an aryl phosphate (TPHP). The chemical structures of target analytes used in this study are presented in Table 4.1. The method performance and applicability in various water matrices specifically ultrapure water, tap water, surface water, secondary effluent, swimming pool water and seawater were also investigated.

4.2 Materials and methods

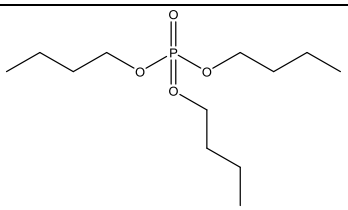
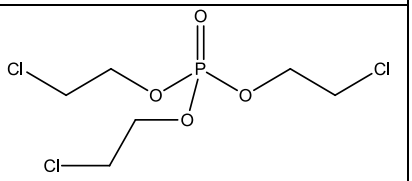
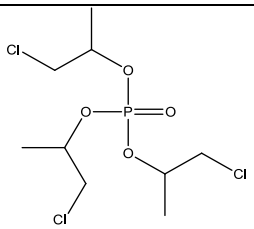
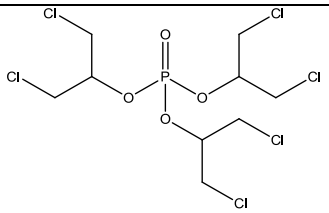
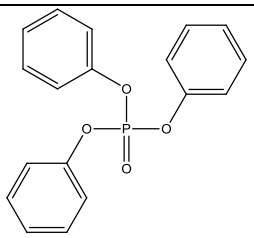
4.2.1 Materials and reagents

TNBP, TCEP, TCIPP, TDCIPP, TPHP and MTBE were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Tris(1-chloro-2-propyl) phosphate-D18, tris(1,3-dichloro-2-propyl) phosphate-D15 and triphenyl phosphate-D15 were purchased from TRC Inc. (Ontario, Canada). Tributyl phosphate-D27 was purchased from Novachem (Collingwood, Vic, Australia). Tris(2-chloroethyl) phosphate -D12 was purchased from Sapphire Bioscience (Waterloo, NSW, Australia). HPLC grade methanol and spectroscopic grade DCM were purchased from Ajax Finechem (Tarron Point, Australia). Ultrapure water was produced using a Direct-Q filtering system from Millipore (North Ryde, NSW, Australia).

Kimble culture tubes (13 mm I.D. x 100 mm) and a Thermo Speedvac concentrator (model no. SPD121P) were purchased from Biolab (Clayton, Vic, Australia). Oasis HLB solid phase extraction cartridges (6 mL, 500 mg) were purchased from Waters (Rydalmere, NSW, Australia).

Stock standard solutions and isotope labelled solutions of organophosphate flame retardants were prepared in methanol (1 g/L, 10 mL) in amber vials and then further serial diluted with methanol to obtain working standard solutions of lower concentrations. All standard solutions were stored at 4 °C. Calibration standards were prepared by serial dilution in methanol from these working stocks.

Table 4.1 Molecular structure of investigated flame retardants and their corresponding isotope labelled standards

Compounds		**MW of target analytes (MW of corresponding isotope labelled standards)**	**Structure**
Alkyl phosphate	tributyl phosphate (TNBP)	266.3 (293.5)	
Chlorinated alkyl phosphate	tris(2-chloroethyl) phosphate (TCEP)	285.5 (297.6)	
	Tris(1-chloro-2-propyl) Phosphate (TCIPP)	327.6 (345.7)	
	Tris(1,3-dichloro-2-propyl) Phosphate (TDCIPP)	430.9 (445.9)	
Aryl phosphate	Triphenyl phosphate (TPHP)	326.3 (341.4)	

4.2.2 Sample collection and preparation

All samples were collected and prepared in clean 500 mL amber glass bottles. Tap water was collected from a regular potable water tap at UNSW. Surface water was collected from a pond in an urban Sydney park and secondary effluent was collected from a water recycling plant in Sydney. Swimming pool water was collected from a university pool and seawater was collected from a rock pool located on a beach in Sydney. Swimming pool samples were quenched with approximately 1 g/L $Na_2S_2O_3$ to eliminate any residual chlorine. All samples prepared for quantitative analysis including blanks were spiked with 50 µL of a 1 mg/L stock of isotope labelled standards. Isotope labelled compounds were used as surrogate standards to correct for matrix effects, SPE recovery variability and instrumental variations. Direct analogue isotopic standards were used for all targeted compounds. Spiked water samples were extracted without any further treatment or processing within 24 h of collection.

4.2.3 Solid phase extraction

Target compounds were extracted using Oasis HLB SPE cartridges which were preconditioned prior to extraction with MTBE (5 mL), methanol (5 mL) and ultrapure water (10 mL). 500 mL of each water samples spiked with all analytes and isotopic standards were drawn through the pre-conditioned cartridges under vacuum at a rate not exceeding 5 mL/min. The loaded cartridges were then rinsed with 10 mL ultrapure water before drying under a flow of nitrogen until visibly dried (approximately 30 minutes). If required, loaded cartridges were stored at 4 °C in sealed bags prior to elution. Analytes were eluted from the cartridges with methanol (5 mL) and MTBE (5 mL) into 20 mL Kimble culture tubes. The extracts were then concentrated under a stream of nitrogen to approximately 1 mL using a Turbovap LV (Calliper Life Sciences, Hopkinton, MA, USA) at 35 °C. The evaporated extracts were transferred to 2 mL amber GC autosampler vials for instrumental analysis.

4.2.4 Gas chromatography-tandem mass spectrometry

Analyses of samples were carried out on an Agilent 7890A gas chromatograph coupled with an Agilent 7000B triple quadrupole mass spectrometer. The GC injection port was operated in splitless mode with the inlet temperature maintained at 280 °C. An injection

volume of 1 µL was used. An Agilent HP5-MS-ultra inert (length: 30 m, I.D.: 0.25 mm, film thickness: 0.25 µm) column was used to separate the analytes. Ultra high purity helium was used as the carrier gas with a constant flow of 1.2 mL/min.

The GC oven temperature was initiated at 50 °C and held for 1 min, increased by 20 °C/min to 200 °C, then further increased to 280 °C at 35 °C/min and held at 280 °C for 2 min with a total run time of 13 min. Mass spectrometric ionisation was undertaken in EI mode with an EI voltage of 70 eV and a source temperature of 280 °C. The GC-MS/MS interface was maintained at 270 °C. The triple quadrupole MS detector was operated in MRM mode with the gain set to 100 for all analytes.

In order to identify the most suitable transitions for MRM, all analytical standards and internal standards were initially analysed in scan mode to identify suitable precursor ions in MS 1 with a scan range of *m/z* 30 to *m/z M* + 10 (where *M* is the mass of the compounds of interest). The identified precursor ions were then fragmented in the collision cell by performing a product ion scan using the same mass range and scan time. Different collision energies were used to optimise product ion intensity for each transition. Analytes were separated into 3 discrete time segments for MRM monitoring. All samples were run with a solvent delay of 5 min and with dwell times between 10 – 30 ms depending on the time segment to achieve approximately 5 – 10 cycles across each peak for good quantification. All ions were monitored at wide resolution (1.2 amu at half height). Two transitions for each analyte and the internal standard were monitored with the first transition used for quantification and the second used for confirmation of molecular identification. The transitions for all analytes and isotope standards, the specific dwell times and optimised collision energies for the method are presented in Table 4.2.

Table 4.2 GC-MS/MS method parameters

Segment start time (min)	**Analytes and isotope labelled standards**	**Retention time (min)**	**MRM transitions**	**Dwell time (ms)**	**Collision energy (V)**
05.00	TNBP	09.20	211 → 155	30	5
			211 → 99	30	5
	TNBP-d27	09.11	231 → 167	30	5

			231 → 103	30	15
09.60	TCEP	09.78	249 → 187	20	5
			249 → 125	20	10
	TCEP-d12	09.73	261 → 196	20	5
			261 → 131	20	10
	TCIPP	09.96	277 → 201	20	5
			277 → 125	20	10
	TCIPP-d18	09.89	293 → 212	20	5
			293 → 131	20	10
11.00	TDCIPP	11.90	381 → 271	10	5
			381 → 159	10	10
	TDCIPP-d15	11.84	394 → 280	10	5
			394 → 164	10	10
	TPHP	12.20	326 → 233	10	15
			326 → 170	10	20
	TPHP-d15	12.16	341 → 243	10	10
			341 → 180	10	20

4.2.5 Identification and quantification

Two MRM transitions of a single precursor ion were monitored for each compound. Analysis of the acquired data was undertaken using Agilent MassHunter software. The confirmed identification of a target compound was only established once the analysis met all of the identification criteria. These included the observed presence of the two transitions at the same retention time, the response ratio of two transitions within a range of 20% variability with respect to the mean area ratio of all calibration solutions and a consistent analyte-surrogate relative retention time as that of calibration solutions with relative standard deviation of less than 0.1 min.

A chromatogram of the quantifier peaks for all five analytes from a swimming pool sample is depicted in Figure 4.1. Quantitative determination of the target analytes was undertaken using internal calibration with isotope dilution. Calibration curves were constructed using no less than six of the eight calibration standards (1.0, 5.0, 10, 50, 100, 200, 500, 1000 ng/mL). 50 ng/mL of isotopically labelled internal standards were

added to all calibration solutions which is equivalent to the mass added to samples prior to SPE and were made up to 1 mL in DCM in GC auto-sampler vials. The calibration points for each of the analytes were fitted to linear regressions and the calibration curve regression correlation coefficients were ensured to be at least 0.99 for all sample batches.

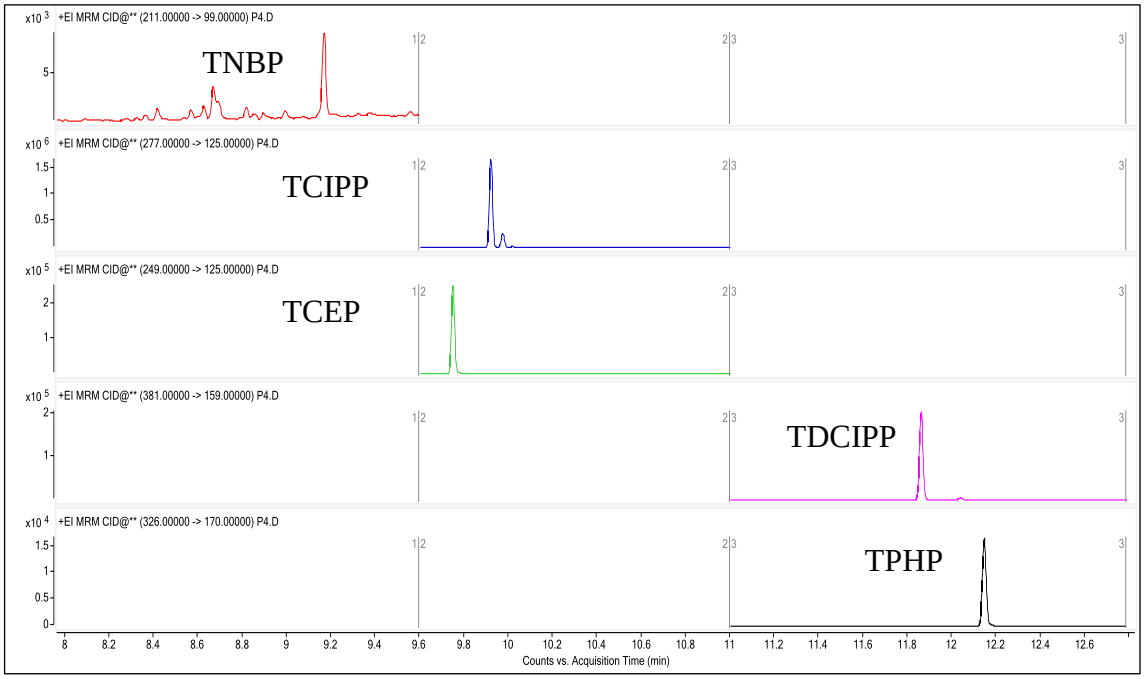


Figure 4.1 MRM chromatograms of quantifier peaks from a swimming pool sample

4.2.6 Method validation studies

Method recoveries of the individual flame retardant compounds were validated in each water matrices which were spiked at 10 ng/L and 500 ng/L concentrations and subjected to the full method procedure. 50 ng of internal standards were added to each sample before undergoing SPE.

Similarly, SPE absolute recoveries were determined by spiking ultrapure water, swimming pool water and secondary effluent samples at both low (10 ng/L) and high (500 ng/L) concentrations. However for absolute recoveries, isotope standards were

only added to the SPE extract after the elution step for direct relative comparison to assess the loss of the targeted analytes during SPE extraction.

The potential losses of analyte occurring during the evaporation step by Turbovap LV were also assessed. Triplicate tubes of 10 mL of elution solvents (5 mL each of methanol and MTBE) spiked with 50 ng of all analytes and 50 ng isotope standards were vacuum dried and reconstituted to about 1 mL with DCM. Another triplicate set was prepared containing only 50 ng of target analytes and vacuum dried. Isotope standards were only added after the evaporation step for the second set. The percentage recoveries of target analytes for both sets were then compared.

Instrument stability was assessed on an intra-day and inter-day basis. A 100 ng/mL standard in DCM was analysed three times a day over two days. The variation in the peak area of each analyte was compared from each analysis. This variation was expressed as the coefficient of variation (C_v), which is defined as the ratio of the standard deviation σ to the mean μ.

The overall stability of the whole method in each matrix was assessed by processing two triplicates spiked at 500 ng/L over two days. The instrument stability does not include corrections from isotope dilution while the method stability calculation does.

4.2.7 Method detection limit (MDL)

The MDLs were assessed in five types of water matrices: ultrapure water, tap water, surface water, secondary effluent and swimming pool water according to Method 1030C from the Standard Methods for the Analysis of Water and Wastewater (Rice *et al.*, 2012). For each water matrix, seven replicates were spiked with the target analytes at concentrations close to the expected MDLs which was determined as the concentrations that can achieve a signal-to-noise ratio (S/N) of 3. The samples were also spiked with the isotopic standards at the same concentrations used in the calibration standards, then extracted and analysed. The seven samples were divided into two batches which were extracted and analysed on different days (three samples on one day and four samples on another day) to better represent day-to-day variability.

MDLs were calculated by multiplying the standard deviation of the seven replicates by Student's *T* value of 3.14 (one-side T distribution for six degrees of freedom at the 99%

level of confidence). Where the calculated MDLs were greater than the actual spiked concentration of any target analytes, a further seven replicates spiked with higher concentrations were analysed to calculate a revised MDLs for those analytes. Alternatively, where the calculated MDLs were 5 or more times smaller than the actual spiked concentrations, a further seven replicates spiked with lower concentrations were analysed to calculate revised MDLs. This procedure was repeated until MDLs of all target analytes were determined with a signal-to-variability ratio within the bounds of the above criteria. The method quantification levels (MQLs) were calculated as 10 times the standard deviation of the same seven replicate samples or the second lowest calibration level, whichever was the highest.

4.3 Results and discussion

4.3.1 SPE recovery experiments

The method recoveries for low (10 ng/L) and high (500 ng/L) spikes of the targeted compounds in ultrapure water, tap water, secondary effluent, surface water, swimming pool water and seawater are presented in Tables 4.3.

Table 4.3 Method recoveries of analytes in various water matrices from a spiking concentration of 10 ng/L and 500 ng/L, μ ± σ %, (*n* = 3)

Analytes	**Ultrapure water**		**Tap water**		**Secondary effluent**	**Surface water**	**Swimming pool water**	**Seawater**	
	10 ng/L	**500 ng/L**	**10 ng/L**	**500 ng/L**	**500 ng/L**	**500 ng/L**	**500 ng/L**	**10 ng/L**	**500 ng/L**
TNBP	119±7	113±13	106±4	101±1	86±2	101±0	116±4	*	100±16
TCEP	135±8	125±10	126±8	110±0	127±1	108±1	113±2	118±3	*
TCIPP	*	112±2	*	*	*	132±1	113±4	*	*
TDCIPP	98±5	103±4	108±4	98±1	134±3	90±2	103±6	110±7	102±13
TPHP	119±5	105±3	120±3	81±1	84±2	85±1	104±7	104±4	92±20

* High matrix concentrations

The results show that the use of isotope dilution adequately corrected for any losses during sample preparation, matrix effects and instrument variation leading to accurate quantification of all targeted compounds in all tested matrices. High matrix concentrations of four PFRs (TNBP, TCEP, TDCIPP and TPHP) were observed in surface water, secondary effluent and swimming pool water and thus accurate recoveries could not be determined at the 10 ng/L concentration for these compounds. The matrix concentrations could also account for the high recoveries (>110%) at the 500 ng/L concentration. The matrix concentrations observed in those water matrices are presented in Table 4.4. The matrix concentrations in surface waters observed in this study are comparable to previous studies with concentrations reported at 25 – 110 ng/L (TNBP), 13 – 130 ng/L (TCEP), 20 – 200 ng/L (TCIPP), up to 50 ng/L (TDCIPP) and 40 ng/L (TPHP) (Andresen *et al.*, 2004; Martínez-Carballo *et al.*, 2007). In wastewater, concentrations have been reported from <11 – 810 ng/L (TNBP), 43 – 1600 ng/L (TCEP), 270 – 1400 ng/L (TCIPP), 19 – 1400 ng/L (TDCIPP) and <7 – 170 ng/L (TPHP) (Martínez-Carballo *et al.*, 2007).

Table 4.4 Matrix concentrations of target analytes in swimming pool water, surface water and secondary effluent (ng/L)

Analytes	**Swimming pool water**	**Surface water**	**Secondary effluent**
TNBP	6	21	17
TCEP	190	116	490
TCIPP	1653	891	1139
TDCIPP	259	26	52
TPHP	15	6	9

TCIPP was observed to have two peaks which is due to its various isomers with the most intense peak corresponding to tris(2-chloroisopropyl) phosphate (Marklund *et al.*, 2003). This peak was used for quantification throughout the whole experiment. High matrix concentrations of TCIPP were observed in all sample matrices including in ultrapure water making the process of method validation problematic for this compound (further discussed in Section 4.3.3). Results discussed henceforth do not take TCIPP into consideration. The results obtained for method recoveries were similar for all the compounds in water matrices which did not have high matrix concentrations. In

ultrapure water and tap water, method recoveries ranged between 98% – 135% (max σ = 8%) at 10 ng/L spike and 103% – 125% (max σ = 13%). These results are in agreement with Cristale *et al.* (2012) where reported recoveries for TCEP, TCIPP, TDCIPP and TPHP were between 88% – 121% in ultrapure water. At 500 ng/L spike for seawater, method recoveries for TNBP, TCEP, TDCIPP and TPHP were in the range between 85% – 108% (max σ = 2%). For surface water, secondary effluent and swimming pool water for all compounds where matrix concentrations were not so significant, method recoveries at 500 ng/L spike were 81% - 134% (max σ = 20%). Recoveries for TCEP were observed to be slightly higher than the other compounds and this could be due to background traces in the SPE cartridges (Chen *et al.*, 2012).

The results of the recovery experiment undertaken to assess analyte losses during evaporation are presented in Table 4.5. Percentage recoveries for the set with isotope standards added before drying were much higher compared to the set where isotope standards were added after drying. This indicates that evaporative losses occur and the incorporation of isotope standards is essential to account for losses during sample preparation.

Table 4.5 Recoveries of analytes obtained after evaporation, μ ± σ %, (*n* = 3)

Analytes	**Recoveries during evaporation**	
	Isotope standards added before drying	**Isotope standards added after drying**
TNBP	97 ± 1	79 ± 5
TCEP	95 ± 1	66 ± 7
TCIPP	115 ± 3	103 ± 2
TDCIPP	84 ± 3	84 ± 2
TPHP	90 ± 6	70 ± 7

Table 4.6 SPE absolute recoveries of analytes in various water matrices from spiking concentration of 10 ng/L and 500 ng/L, $\mu \pm \sigma$ %, (n = 3)

Analytes	**Ultrapure water**		**Swimming pool water**	**Surface water**
	10 ng/L	**500 ng/L**	**500 ng/L**	**500 ng/L**
TNBP	46 ± 3	42 ± 3	44 ± 3	23 ± 15
TCEP	54 ± 11	52 ± 3	62 ± 2	25 ± 15
TCIPP	311 ± 15	50 ± 5	179 ± 3	58 ± 13
TDCIPP	45 ± 3	45 ± 6	69 ± 4	22 ± 17
TPHP	48 ± 3	48 ± 7	43 ± 3	20 ± 13

The results for the absolute SPE recovery experiments are presented in Table 4.6. Relatively poor recoveries were observed for analytes which did not have significant matrix concentration. Absolute SPE recoveries in ultrapure water ranged from 42% – 54% at both low and high spikes. High matrix concentrations in swimming pool water and surface water samples were observed therefore the absolute SPE recovery for those two compounds could not be accurately quantified at the 10 ng/L spike. When spiked at 500 ng/L, absolute recoveries for TNBP, TCEP, TDCIPP and TPHP in swimming pool water were between 43% – 69% while surface water ranged between 20% – 25%. The low recoveries could be due to the low partitioning capabilities of the PFRs particularly for the more polar PFRs such as TCEP and TCIPP making them highly soluble in water and less likely to partition into non-polar media. Another possibility could be due to the adsorption or absorption of the compounds to plastic materials during sample preparation. Furthermore, non-complete elution and evaporative losses during the concentration step could account for the low absolute recoveries. The variability and poor recoveries emphasises the importance of isotope dilution to compensate for losses for an optimised SPE recovery correction in diverse matrices. Peak tailings were minimised by keeping the GC column and injector liner clean.

4.3.2 Method validation

The linear calibration range for each of the target compounds was determined from their identified MDLs to 1000 ng/L. The determined MDL and MQL values for the various water matrices tested in this study are presented in Table 4.7.

All MDLs were in the range of 0.3 – 24 ng/L in ultrapure water, tap water, seawater surface water, secondary effluent and swimming pool water. The MDLs determined in this study were significantly lower than those obtained in Cristale *et al.* (2012) who reported values between 4 – 40 ng/L for TCEP, TCIPP, TDCIPP and TPHP. Due to high matrix concentrations in surface water, secondary effluent and swimming pool water (Table 4.4), MDLs and MQLs were estimated using a less robust method. The MDLs were estimated by using the S/N values of the measured matrix concentrations in surface water, secondary effluent and swimming pool water and extrapolating that value down to a corresponding concentration with S/N = 3 and the MQL values were estimated by extrapolating to S/N = 10.

Table 4.7 MDLs and MQLs of target analytes in various water matrices, ng/L (*n* = 7)

Analytes	**Ultrapure water**		**Tap water**		**Seawater**		**Surface water***		**Secondary effluent***		**Swimming pool water***	
	MDL	**MQL**	**MDL**	**MQL**	**MDL**	**MQL**	**MDL**	**MQL**	**MDL**	**MQL**	**MDL**	**MQL**
TNBP	2	6	2	6	4	13	0.8	3	2	6	2	5
TCEP	2	5	1	5	1	3	14	47	24	80	10	34
TCIPP*	6	20	3	9	3	11	3	10	22	74	1	4
TDCIPP	3	9	7	24	2	7	3	9	4	12	18	59
TPHP	2	7	1	3	3	9	0.5	2	0.5	2	0.3	1

* MDLs and MQLs were estimated by extrapolating the S/N values of the measured matrix concentrations to a corresponding concentration with S/N = 3 and S/N = 10 respectively

Similarly, this method was applied to estimate MDLs and MQLs for TCIPP due to the problems associated with high matrix concentrations (refer to Section 4.3.3). MDLs and MQLs obtained from this method produce more conservative values. The results of instrument and method stability assessments are presented in Table 4.8. The coefficients of variability ($C_v = \sigma/\mu$) for instrument stability on an intra-day basis ranged between 0.14 – 0.19. Instrument stability on an inter-day basis was observed from 0.20 – 0.31 which is slightly higher when compared to intra-day. For the full method analysis of spiked tap water and secondary treated effluent, the C_v was found to be lower for both intra-day and inter-day basis for analytes with direct isotopically labelled standards. These ranged between 0.03 – 0.20. Slightly higher variations were observed for secondary effluent samples compared to tap water. Overall, the coefficients of variation were less than 1% which indicates a high level of reproducibility. Furthermore, these results emphasise the need for the isotopic dilution process to ensure a high level of analytical reproducibility.

Table 4.8 Coefficient of variations $C_v = \sigma/\mu$ for instrument and method stability of target analytes in various water matrices

Analytes	**Instrument stability**[a]		**Method stability**[b]			
	Standard 100 ng/mL		**Tap water 500 ng/L**		**Secondary effluent 500 ng/L**	
	Intra-day (*n* = 3)	**Inter-day (*n* = 6)**	**Intra-day (*n* = 3)**	**Inter-day (*n* = 6)**	**Intra-day (*n* = 3)**	**Inter-day (*n* = 6)**
TNBP	0.16	0.20	0.03	0.10	0.17	0.15
TCEP	0.19	0.23	0.07	0.05	0.10	0.09
TCIPP	0.18	0.21	0.03	0.09	0.07	0.12
TDCIPP	0.18	0.31	0.03	0.04	0.14	0.14
TPHP	0.14	0.28	0.08	0.07	0.20	0.18

[a] Instrument stability not corrected by isotope dilution
[b] Method stability includes correction by isotope dilution

4.3.3 Blank contamination of TCIPP

TCIPP levels were determined in all water samples including ultrapure water. Other than TCIPP, the other PFRs were below the detection limit in the blank samples. Investigations to determine the source of TCIPP were carried out. Triplicates of three different sources of ultrapure water were collected and subjected to the full method process. Triplicates of blank cartridges which were just conditioned and eluted were also analysed at the same time. Due to repeated detection of TCIPP in ultrapure water from different sources, high-purity (LC-MS CHROMASOLV) grade water was purchased from Sigma Aldrich (Castle Hill, NSW, Australia) and analysed. These results are presented in Table 4.9.

Table 4.9 TCIPP concentrations in ultrapure water, high-purity grade water and blank cartridges ($\mu \pm \sigma$)

Sample (*n* = 3)	**TCIPP (ng/L)**
Ultrapure water source 1	40 ± 3
Ultrapure water source 2	66 ± 6
Ultrapure water source 3	70 ± 7
High-purity grade water	13 ± 0
Blank cartridges	2 ± 1

Ultrapure water samples from all three sources contained high levels of TCIPP (40 – 70 ng/L) while high-purity grade water had matrix concentrations of 13 ng/L. Analysis of the blank cartridges showed low traces of TCIPP. TCIPP was not detected in the organic solvents used during the method process and thus were eliminated as a possible source of TCIPP. In order to avoid false positive results of TCIPP in water samples, a higher limit of reporting is required and a set of several blanks should be processed together with each batch samples. The concentrations are reported after taking the blank levels into consideration.

Several studies have reported the contamination of some PFRs such as TNBP, TBOEP, TDCIPP, TPHP and TCIPP in procedural blanks during water, air and sediment samples analysis (Marklund *et al.*, 2005a; García-López *et al.*, 2009; Regnery and Püttmann, 2009; Rodil *et al.*, 2009b; García-López *et al.*, 2010). The polymeric materials used in the water purification systems have been suggested as a possible source of contamination (García-López *et al.*, 2010). TCIPP and TNBP have also been previously reported to leach from SPE plastic cartridges (Chen *et al.*, 2012). TCIPP has also been detected in the air in various indoor environments including laboratory air, contamination of TCIPP in the blanks could possibly occur during sample preparation (Marklund *et al.*, 2005a). As PFRs are often used in commercially available items, contamination is often unavoidable. The source of PFRs contamination requires further study. However, steps to minimise their levels should be taken such as wearing gloves at all times, avoiding the use of plastic materials where possible, pre-cleaning all glassware and SPE tubes with solvents before use and analysing procedural blanks for every sample batch (Quintana *et al.*, 2008; García-López *et al.*, 2009; Chen *et al.*, 2012;

Brandsma *et al.*, 2013). Each of these measures was applied in the development of this analytical method to ensure contamination was minimised.

4.3.4 Application to environmental samples

This SPE-GC-MS/MS method was applied for the analysis of PFRs in surface water, secondary effluent and swimming pool water to demonstrate the feasibility of the method. It can be seen from Table 4.4 that TNBP, TCEP, TCIPP, TDCIPP and TPHP were detected in all three water matrices. The concentrations of PFRs detected in surface water and secondary effluent in this study were comparable to previous studies where the concentrations of PFRs ranged from 0.3 – 250 ng/L in surface waters (Andresen *et al.*, 2004; Bollmann *et al.*, 2012) and from 19 – 52000 ng/L in wastewaters (Marklund *et al.*, 2005b; Martínez-Carballo *et al.*, 2007). PFRs in swimming pool water samples were between 6 – 1700 ng/L in this study. To the best of the authors' knowledge, this is the first report of the analysis of PFRs in swimming pools.

4.4 Conclusions

An analytical method with a rapid analysis time of 13 min was developed to analyse for five organophosphate flame retardants in different water matrices. The use of isotope dilution ensures the accurate quantification of targeted analytes, accounting for analytical variability which may have been introduced during sample collection, extraction, chromatography, ionisation or mass spectrometric detection. MDLs ranging between 0.3– 24 ng/L in various water matrices were achieved making the determination of PFRs at low levels possible. A higher limit of reporting for TCIPP should be estimated based on the levels of contamination in the procedural blanks. Method validation has confirmed the stability of this method and is effective for the analysis of a variety of environmental water matrices. Further research is needed to determine the sources of contamination of PFRs in blank samples which will lead to a more precise analysis of PFRs in the environment.

CHAPTER 5 PRESENCE AND SELECT DETERMINANTS OF ORGANOPHOSPHATE FLAME RETARDANTS IN PUBLIC SWIMMING POOLS

This chapter is in preparation for submission to Talanta journal.

5.1 Introduction

Organophosphate compounds are widely used as flame retardants and plasticizers. Due to their prevalent usage, organophosphate flame retardants (PFRs) have been detected globally in the environment including in indoor and outdoor air (Hartmann *et al.*, 2004; Marklund, 2005), indoor dust (Van den Eede *et al.*, 2011), surface water (Andresen *et al.*, 2004; Martínez-Carballo *et al.*, 2007; Regnery and Püttmann, 2010), ground water (Fries and Püttmann, 2001), rainwater (Regnery and Püttmann, 2009), snow (Marklund *et al.*, 2005c; Regnery and Püttmann, 2009) and drinking water (Bacaloni *et al.*, 2007; Stackelberg *et al.*, 2007).

Some PFRs are potential endocrine disruptors and may pose a health risk to humans (Meeker and Stapleton, 2010; Dishaw *et al.*, 2011; Liu *et al.*, 2012a; Zhang *et al.*, 2014). For example, PFRs might be associated with altered thyroid levels and reduced sperm quality in men (Meeker and Stapleton, 2010). Contact dermatitis from TPHPs has also been reported (Camarasa and Serra-Baldrich, 1992). PFRs that were reported to have a significant estrogenic effect include TPHP, tricresyl phosphate and TDCIPP. Furthermore, some chlorinated PFRs are considered to be potentially carcinogenic (Van der Veen and de Boer, 2012). With the occurrence of PFRs in the environment, humans are exposed to these chemicals with PFRs being detected in human milk (Sundkvist *et al.*, 2010; Kim *et al.*, 2014), human plasma (Shah *et al.*, 2006), human hair and nails (Liu *et al.*, 2015) and their metabolites in human urine (Reemtsma *et al.*, 2011; Van den Eede *et al.*, 2015). Although the toxicity of PFRs to humans is as yet unknown, environmental exposure to these chemicals may lead to possible adverse health effects and their occurrence in swimming pools may potentially be one of many sources of exposure to humans. Swimmers may be exposed to PFRs in swimming pools via a variety of exposure routes including accidental ingestion, inhalation and dermal absorption.

Studies have shown that PFRs can leach out or diffuse from various materials including, but not limited to, items made out of plastic (Kim *et al.*, 2006; Choi *et al.*, 2009). PFRs are used and have been detected in various plastic materials including furniture foam and polyurethane foam (Stapleton *et al.*, 2009; Stapleton *et al.*, 2011). In swimming pools, equipment made from plastic materials is constantly used in the form of kickboards and swimsuits among others. It is conceivable that the leaching of PFRs

from these materials into swimming pools is possible and could explain their occurrence in swimming pools although further research is needed to confirm this. Furthermore, PFRs may also already be present in the fill water used for swimming pools, thus contributing to their presence in the pool. As swimming pool water is continuously recirculated, the accumulation of these compounds may occur over time.

The aim of this study was to investigate the occurrence of five PFRs consisting of TNBP, TCEP, TCIPP, TDCIPP and TPHP in swimming pools. Various types of chlorinated public swimming pools were sampled including indoor pools, outdoor pools and spa pools. Laboratory simulation studies were conducted to investigate the potential leaching of PFRs into swimming pools from commonly used swimming equipment.

5.2 Sample preparation and analysis

5.2.1 Sample collection

Swimming pool water samples were collected from five locations in Sydney, NSW, Australia. The swimming pools selected to undergo sampling were based on the swimming pool operators' agreement to participate in the study. A total of 15 chlorinated public swimming pools were sampled which consisted of indoor pools, outdoor pools and spa pools. Swimming pool fill waters were also collected where possible. In total, fill water samples were collected from three of the locations – Location A, C and D, all of which were sourced from reticulated municipal drinking water. Fill water samples from Location A and D were collected from the pump room while fill water samples from Location C was collected from a tap on location. Characteristics and the number of swimmers at the time of sampling for each swimming pool are presented in Table 5.1.

Table 5.1 Details of chlorinated swimming pools

Location	Sample description	Pool characteristics				No. of swimmers at time of sampling			
		Temperature (°C)	pH	Disinfection	Water source	Adult male	Adult female	Children	TOTAL
A	Indoor heated	28	7.4-7.6	UV Sodium hypochlorite	Tap water	4	10	10	24
B	Indoor main pool	29	7.2-7.8	Sodium hypochlorite	Tap water	7	3	-	10
	Indoor training pool	32				2	8	14	24
	Indoor spa	28				2	7	-	9
C	Indoor training pool	32	7.5-7.6	UV (indoor pools only)	Tap water	7	20	30	57
	Indoor competition pool	28		Sodium hypochlorite			school carnival		
	Outdoor 50m pool	25					school carnival		
	Outdoor wading pool	25				-	3	10	13
	Outdoor children pool (shaded)	25				-	-	6	6
D	Outdoor 22m	25	7.6-7.8	Sodium hypochlorite	Tap water	-	-	-	-
	Outdoor 50m	25				2	2	-	4
	Outdoor children pool (shaded)	25				-	-	-	-
E	Indoor 50m	26	7.2-7.6	UV	Tap water	5	3	-	8
	Indoor wading/spa	30		Sodium hypochlorite		1	1	8	10
	Indoor training pool	32				2	9	10	21

For each swimming pool, three grab samples were collected in 1 L amber glass bottles without headspace from three various locations around the pool as a representation of the whole pool and to account for some variability within the pool. All samples were then quenched with approximately 1 g/L sodium thiosulphate ($Na_2S_2O_3$) to eliminate residual chlorine before the bottles were tightly sealed with a screw cap and transported directly to the laboratory.

Samples were spiked with a solution containing 50 ng of an isotopically labeled version of each analyte for accurate isotope dilution quantification. Spiked samples were extracted using solid phase extraction without any further treatment or processing within 24 h of collection and spiking.

Swimming pool water samples were subjected to SPE and instrumental analysis as detailed in Chapter 4. As PFRs are often used in commercially available items, contamination is often unavoidable. Steps to minimise their levels have been taken in this study, such as wearing gloves at all times, avoiding the use of plastic materials where possible, all glassware and SPE tubes were pre-cleaned with solvents before use and analysing procedural blanks for every sample batch. The LOQs for the five PFRs are TNBP (5 ng/L), TCEP (5 ng/L), TCIPP (50 ng/L), TDCIPP (5 ng/L) and TPHP (5 ng/L).

5.2.2 Leaching experiments

Laboratory experiments were undertaken to investigate the potential leaching of PFRs from two brands of kickboards and one swimsuit over a period of time. The kickboards and swimsuit tested were representatives and were chosen based on their commercial availability in shops in Sydney. The kickboards were made from rigid high density foam while the swimsuit consisted of nylon, polyamide, elastane and polyester materials. Samples were prepared by soaking the materials for 15, 30, 180 and 1440 minutes (24 hours) in 1 L amber glass bottles. Each material was cut into small pieces (roughly about 1 cm^2) and weighed to a total of 1 g for swimsuits and 2 g for kickboards. These were then placed into glass bottles which were then filled with chlorinated water (sodium hypochlorite added to ultrapure water) which had a free available chlorine concentration of approximately 4 ppm. Triplicate samples were prepared for each time set. A separate set of triplicate samples were prepared by soaking

the materials in just ultrapure water for 180 and 1440 minutes to investigate the effect of free chlorine on the leaching of PFRs, if any. Control samples were also prepared in triplicates for 180 and 1440 minutes, each with ultrapure water and chlorinated ultrapure water in glass bottles. Following the allocated soaking time, each sample was transferred to 500 mL glass bottles (leaving behind to solid materials), quenched with approximately 1 g/L $Na_2S_2O_3$ and spiked with 50 ng of isotope standards before undergoing solid phase extraction and analyses.

5.3 Results and discussion

5.3.1 Occurrence in swimming pool waters

The results obtained from the analysis of PFRs in swimming pools are presented in Table 5.2. All five PFRs were detected above the LOQs in swimming pool waters. However, the PFRs were all below the LOQs in the fill water samples eliminating this as their source. Of the five PFRs, TNBP was observed with the lowest concentrations and was detected only in the indoor pools with concentrations between 5 – 27 ng/L. TCEP was detected in all the indoor swimming pools in the range of 20 – 290 ng/L and in three out of the six outdoor pools with concentrations from 7 – 82 ng/L. Concentrations of TCEP were significantly higher (over 3 times) than that in the indoor pools. The concentrations of TCIPP were generally much higher compared to the other PFRs with concentrations ranging between 62 – 1180 ng/L in all of the swimming pools, except two outdoor pools which were below the LOQs. TDCIPP was detected in all of the swimming pools (indoor and outdoor) at concentrations between 10 – 670 ng/L. TPHP was only detected in the indoor pools at levels from 8 – 132 ng/L.

Table 5.2 Concentrations of PFRs in swimming pools reported as mean ± standard deviation (n = 3)

Location	Sample description	Chemical concentration (ng/L)				
		TNBP	**TCEP**	**TCIPP**	**TDCIPP**	**TPHP**
A	Indoor heated	<5	127±10	476±18	132±10	9±1
	Fill water	<5	<5	<50	<5	<5
B	Indoor main pool	11±1	145±15	665±57	213±21	59±5
	Indoor training pool	27±1	191±4	1180±44	335±15	132±7
	Indoor spa	5±0	20±3	177±14	102±6	25±2
C	Indoor training pool	10±1	293±17	1110±108	465±30	21±3
	Indoor competition pool	5±0	69±3	363±24	138±4	16±1
	Outdoor 50 m pool	<5	7±0	<50	19±1	<5
	Outdoor wading pool	<5	82±1	239±7	330±5	<5
	Outdoor children pool (shaded)	<5	76±6	216±8	312±28	<5
	Fill water	<5	<5	<50	<5	<5
D	Outdoor 22 m	<5	<5	<50	10±0	<5
	Outdoor 50 m	<5	<5	62±43	10±2	<5
	Outdoor children pool (shaded)	<5	<5	87±26	10±0	<5
	Fill water	<5	<5	<50	<5	<5
E	Indoor 50 m	<5	62±2	445±5	189±5	8±0
	Indoor wading / spa	<5	135±7	1010±51	508±25	22±2
	Indoor training pool	5±0	126±3	831±17	670±19	27±1

The levels of PFRs in swimming pools could be affected by the number of people using the pools and also the type of activity carried out in the pool. The lower levels of PFRs in the outdoor pools in Location D could be due to the low usage at the time of sampling. The indoor training pools in Location B, C and E generally had higher concentrations of PFRs compared to the other swimming pools in the same locations. This might be due to the higher number of swimmers using the training pools. Furthermore, there is likely to be a higher usage of kickboards or flotation devices in the training pools, which may be significant if these are found to be a source of PFRs. TDCIPP and TCIPP are commonly used as an additive in polyurethane foam and other plastics which could explain their higher detection in swimming pool waters compared to the other PFR compounds (Stapleton *et al.*, 2009). In Location C, although the indoor competition pool and outdoor 50 m pool were in a similar state of high usage (for a school swimming carnival), the PFR levels in the indoor competition pool were significantly higher compared to the outdoor 50 m pool. Overall, higher concentrations of PFRs in indoor swimming pools compared to outdoor swimming pools were observed in this study.

Some PFRs, especially the non-halogenated PFRs, undergo photodegradation when exposed to sunlight (Regnery and Püttmann, 2010; Bollmann *et al.*, 2012), which may explain the lower levels of PFRs in the outdoor pools. In Location C, the concentrations of PFRs in the outdoor wading and children pool (both shaded) were comparable to the concentrations of PFRs in the indoor pools while the outdoor 50 m pool (without shades) showed markedly lower concentrations of PFRs. This further suggests that the shades prevented sunlight penetration, hence prohibiting the photodegradation of PFRs. TNBP, TCEP and TCIPP are the most volatile compared to the other PFR compounds with their occurrence common in indoor air at higher concentrations (Reemtsma *et al.*, 2008). This could further explain their occurrence only in indoor swimming pools and at a higher concentration compared to outdoor swimming pools as these compounds would be more susceptible to wind-enhanced volatilisation and evaporation in outdoor pools. Higher temperatures may also promote evaporation especially for the more volatile non-halogenated PFRs (Bollmann *et al.*, 2012), which could explain the lower concentrations of TNBPs in heated indoor swimming pools compared to the other PFRs.

PFRs have been widely reported in indoor dust and they have been found to be more abundant in an indoor environment due to their significant use in building materials and electrical appliances (Hartmann *et al.*, 2004; Reemtsma *et al.*, 2008; Stapleton *et al.*, 2011; Ali *et al.*, 2012). With the persistence of PFRs occurring in indoor air, it is plausible that PFRs may be entering the pool through settled dust particles. This could contribute to further contamination of PFRs in indoor swimming pool water. Further studies are required to investigate the significance of PFRs in dust as a source of PFRs in swimming pools.

The concentrations of PFRs detected in swimming pools in this study are comparable to those detected in environmental waters such as surface waters and wastewaters. PFRs in surface water have been reported from 25 – 110 ng/L (TNBP), 13 – 130 ng/L (TCEP), 20 – 200 ng/L (TCIPP), up to 50 ng/L (TDCIPP) and 40 ng/L (TPHP) (Andresen *et al.*, 2004; Martínez-Carballo *et al.*, 2007) while in wastewater, concentrations have been reported from <11 – 810 ng/L (TNBP), 43 – 1600 ng/L (TCEP), 270 – 1400 ng/L (TCIPP), 19 – 1400 ng/L (TDCIPP) and <7 – 170 ng/L (TPHP) (Martínez-Carballo *et al.*, 2007). Furthermore, concentrations of TCIPP has been observed to be amongst the highest PFRs occurring in the aquatic environment (Marklund *et al.*, 2005b; Martínez-Carballo *et al.*, 2007; Bacaloni *et al.*, 2008; Regnery and Püttmann, 2010), which is similar to the results obtained in this study.

The presence of these PFRs in swimming pools suggests that swimmers are exposed to these chemicals during swimming. Furthermore, some PFRs have been found to be recalcitrant to degradation during wastewater treatment (Meyer and Bester, 2004; Marklund *et al.*, 2005b). The highly persistent behavior of PFRs may lead to higher accumulated levels of PFRs over time in swimming pools. In addition, the degradation of PFRs through sunlight irradiance may lead to the occurrence of by-products which may potentially be more toxic. Limiting the time spent in swimming pools may be one way to limit the exposure to these prevalent chemicals. Further monitoring of these chemicals in swimming pools may be needed to investigate if swimming pools are a significant source of PFRs exposure to humans.

5.3.2 Laboratory leaching experiments

Laboratory experiments revealed no discernible leaching from either of the two rigid high density foam kickboard brands (all PFRs below the LOQs for all samples). The results of the leaching experiments for the swimsuit are presented in Figure 5.1.

TNBP, TCIPP and TCEP were all detected in the aqueous samples after soaking the swimsuit pieces in both ultrapure water and chlorinated ultrapure water. In the set with chlorinated water, these three PFR compounds were observed to increase in concentration when the swimsuits were soaked throughout the day (Figure 5.1-A). The results also show that within the same allocated soaking time, the concentrations of PFRs were similar in the set where the swimsuit pieces were soaked in ultrapure water and in chlorinated water (Figure 5.1-B). This suggests that chlorine does not have a significant effect on the leaching of PFRs. The PFRs were all below the LOQs in the control samples. These results indicate that swimsuits are likely a source of PFRs in swimming pools. However, it should be noted that the sample size for the leaching experiments are small and are only indicative thus, the relative significance of this source will require further investigations of a wider range of sample set and other possible sources. As TDCIPP and TPHP were not detected in the leaching experiments of either the kickboards or swimsuit, it is likely that other sources are contributing to the occurrence of these two compounds in swimming pools. Furthermore, based on the results of PFRs occurring in swimming pools, PFR concentrations were observed to be generally higher in swimming pools heated at a higher temperature. Therefore, further research is required to investigate the effect of temperature on the leaching of PFRs in swimming pools as higher temperatures could enhance the leaching of PFRs from swimsuits.

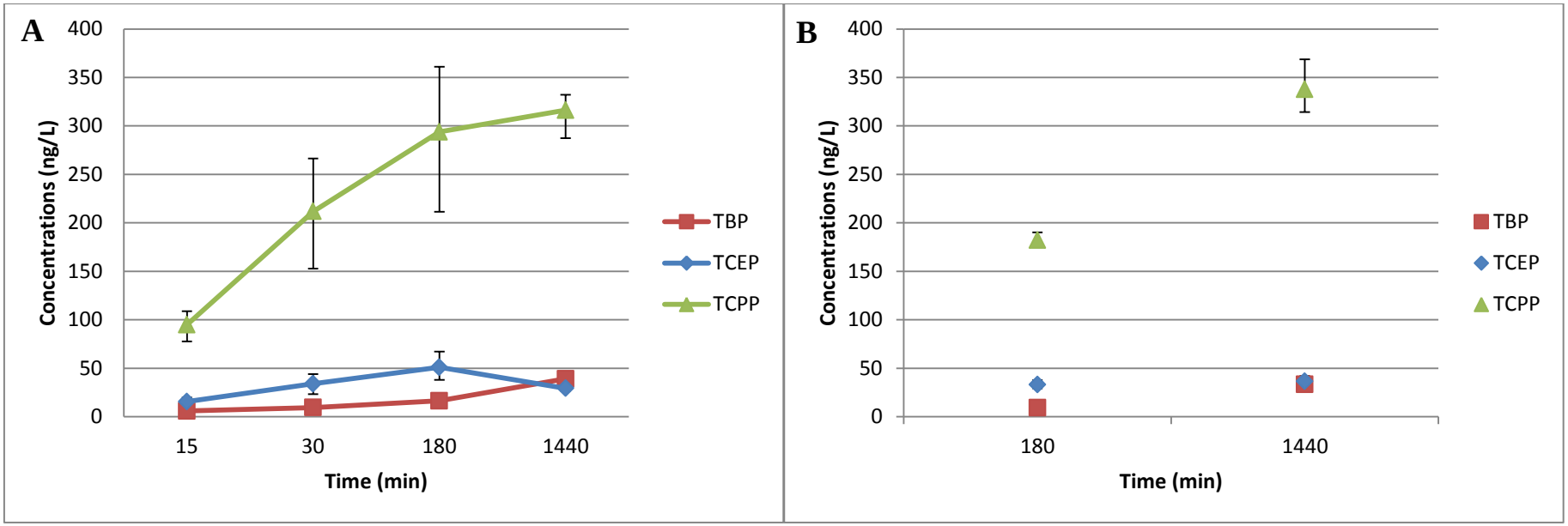


Figure 5.1 Concentrations of PFRs leached from swimsuits in A: Chlorinated water and B: Ultrapure water over a day

5.4 Conclusions

Five PFRs consisting of TNBP, TCEP, TCIPP, TDCIPP and TPHP were detected in chlorinated public swimming pool waters. Their concentrations were comparable to those reported in environmental surface waters and municipal wastewaters. Higher concentrations of PFRs were observed in indoor swimming pools compared to outdoor swimming pools. Fill waters to these swimming pools did not appear to be a significant source of these PFRs. It was determined that PFRs are leaching from swimsuits. The concentrations of PFRs increased as the swimsuits were soaked over a period of time, up to 24 hours. Swimsuits are possibly one of many sources of PFRs in swimming pools. Other materials or swimming equipment used in swimming pools may also contribute to the occurrence of PFRs. Furthermore, as PFRs are highly persistent in the environment, further research may identify a broader range of sources of PFRs in swimming pool water. People are exposed to PFRs by a wide range of exposure routes and the relative significance of exposure to swimming pool users is currently unknown.

CHAPTER 6 OCCURRENCE AND DAILY VARIABILITY OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN SWIMMING POOLS

This chapter has been published in the following journal paper:

Teo TLL, Coleman HM, Khan SJ. (2015) Occurrence and daily variability of pharmaceuticals and personal care products in swimming pools, Environmental Science and Pollution Research 1-10.

6.1 Introduction

Pharmaceuticals and personal care products (PPCPs) have been detected globally in various environmental waters such as surface waters (Yoon *et al.*, 2010; Esteban *et al.*, 2014), groundwater (López-Serna *et al.*, 2013; Peng *et al.*, 2014), drinking waters (Benotti *et al.*, 2009; Padhye *et al.*, 2014) and more recently, in swimming pool waters (Weng *et al.*, 2014; Teo *et al.*, 2015). These chemicals are commonly detected in nanogram-per-litre (ng/L) to microgram-per-litre (µg/L) concentrations in the environment.

Swimming pool users continuously introduce organic matter to swimming pools through the excretion of body fluids (urine and sweat) and from the washing-off of personal care products (cosmetics and sunscreens) during swimming. The excretion of urine into swimming pools has been roughly estimated to be about 25 – 80 mL per swimmer (Gunkel and Jessen, 1988; Erdinger *et al.*, 1997). As a result, it is possible that commonly ingested chemicals such as pharmaceuticals could be transported to the swimming pool water matrix as they are co-excreted with these bodily fluids. The application of personal care products by swimmers would also contribute to the presence of these contaminants in swimming pools. Furthermore, concerns have been expressed that these chemicals may react with swimming pool disinfectants to produce by-products that may be more harmful to swimmers than the introduced chemicals themselves (Bottoni *et al.*, 2014).

Reports on the occurrences of PPCPs in swimming pools are not common. Only one recent study has reported the presence of *N*,*N*-diethyl-*m*-toluamide (DEET), caffeine, and tri(2-chloroethyl)-phosphate (TCEP) in swimming pools of the 32 PPCPs that were analysed (Weng *et al.*, 2014). Chlorination reaction experiments carried out by Weng *et al.* (2014) showed that some PPCPs were highly likely to be degraded by chlorination while others which are less reactive to chlorine are prone to accumulation due to the constant recirculation of pool water. A wider range of commonly used PPCPs may be occurring in swimming pools. The aim of this study was to assess the presence and daily variability of PPCPs in various swimming pools including freshwater indoor pools, outdoor pools, spas and seawater pools. Furthermore, this study sought to evaluate whether the presence of PPCPs could be used as surrogate indicators to monitor bodily excretion levels in swimming pools and hence, risks of exposure to other

potentially more harmful substances. The levels of chemical contaminants occurring in swimming pools and in fill water samples were compared.

6.2 Sample collection and analysis

The same swimming pool water and fill water samples from the PFRs analysis were used for the analysis of PPCPs in swimming pools. The description of swimming pool water sample collection along with the characteristics of each chlorinated swimming pool is detailed in Section 5.2.1 (Chapter 5). In addition to the 15 chlorinated swimming pools, four seawater swimming pools situated by the sea in Sydney were also sampled in this study. These seawater pools do not undergo chemical disinfection, but are maintained and cleaned periodically. Characteristics and the number of swimmers at the time of sampling for each seawater swimming pool are presented in Table 6.1. For each pool, three grab samples were collected in 1 L amber glass bottles without headspace. In order to account for some variability within the swimming pool, three locations around the pool were selected as sampling points as a representation of the whole pool.

Table 6.1 Details of seawater swimming pools

Location	Sample description	Pool characteristics	No. of swimmers at time of sampling			
		Water source	Adult male	Adult female	Children	TOTAL
F	Seawater pool 1	Seawater	10	4	19	33
G	Seawater pool 2	Seawater	2	3	-	5
H	Seawater pool 3	Seawater	3	-	-	3
I	Seawater pool 4	Seawater	4	1	-	5

Of the 15 chlorinated swimming pools, the swimming pool at Location A (see Table 5.1) was selected to undergo daily monitoring. For this experiment, three grab samples were taken three times daily throughout the day for over six days. For consistency, the samples were taken from the same three various locations around the pool for each sampling event. All samples were quenched with approximately 1 g/L $Na_2S_2O_3$ to eliminate any residual chlorine before the bottles were sealed tightly with a screw cap and transported directly to the laboratory.

Samples were spiked with a solution containing 50 ng of an isotopically labeled version of each analyte for accurate isotope dilution quantification. Spiked samples were extracted using SPE without any further treatment or processing within 24 h of

collection and spiking, and subjected to instrumental analysis using isotope dilution LC-MS/MS as described in Section 3.2 (Chapter 3). The method recoveries of each target compound in pool water were obtained by spiking 50 ng/L concentrations of target analytes and 50 ng/L of internal standards before undergoing SPE. The LOQs and percentage recoveries of all analytes are presented in Table 6.2.

Table 6.2 LOQs and method recoveries of target compounds

Analytes	LOQ (ng/L)	Method recovery (%)		
		Swimming pool water	Seawater pools	Ultrapure water
ESI positive mode:				
Amitriptyline	5	134	74	101
Atenolol	5	109	62	125
Caffeine	10	212*	91	119
Carbamazepine	5	100	73	101
Clozapine	5	130	72	104
Diazepam	5	103	76	103
Dilantin	5	90	8	92
Enalapril	5	106	72	109
Fluoxetine	5	154	86	114
Hydroxyzine	5	114	77	112
Meprobamate	5	104	70	119
Omeprazole	5	118	70	106
Paracetamol	5	117	84	106
Primidone	5	105	67	103
Risperidone	5	115	86	125
Sulfamethoxazole	5	103	68	104
Triamterene	5	96	72	101
Trimethoprim	5	99	68	96
Verapamil	5	105	71	112
ESI negative mode:				
Bisphenol A	20	84	80	153
Gemfibrozil	5	104	70	108
Ibuprofen	5	161*	66	94
Ketoprofen	10	112	80	110
Naproxen	5	86	72	96
Nonylphenol	10	124	66	101
Propylparaben	10	23	55	40
Simvastatin	5	117	35	82
Simvastatin hydroxy acid	5	109	74	112
Triclocarban	10	116	79	95
Triclosan	5	129	81	91

Limit of quantification (LOQ) is defined as the concentration of an analyte giving a signal to noise ratio greater than 10.

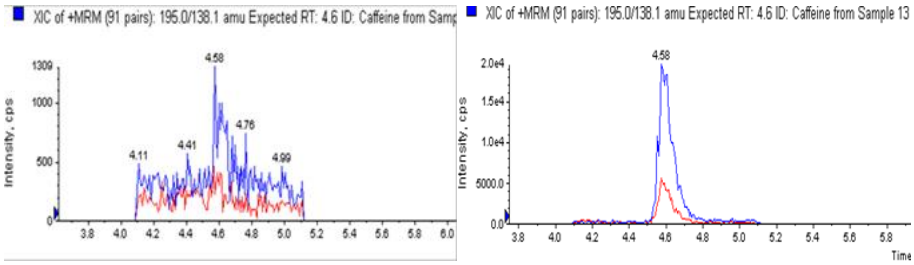
*The high recoveries are due to the matrix concentrations

6.3 Results and discussion

Among the 30 PPCPs, only caffeine and ibuprofen were detected above the LOQ in swimming pool water samples. Figure 6.1 shows the MRM transitions for caffeine and ibuprofen in the fill water and swimming pool water at Location A.

a b

Caffeine



Ibuprofen

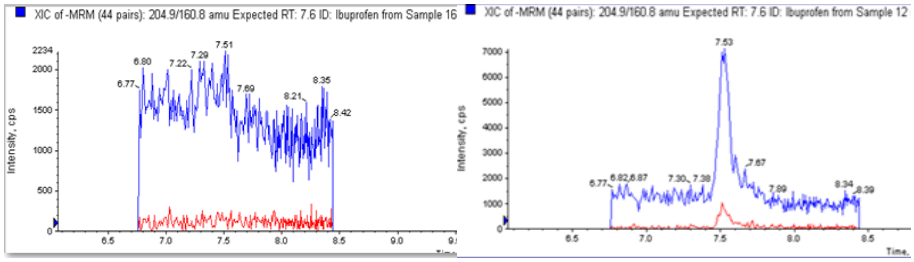


Figure 6.1 Overlay of two MRM transitions for caffeine and ibuprofen for Swimming Pool Location A (a: fill water, b: swimming pool water). The blue transition was used for quantification and the red transition was used for identification confirmation.

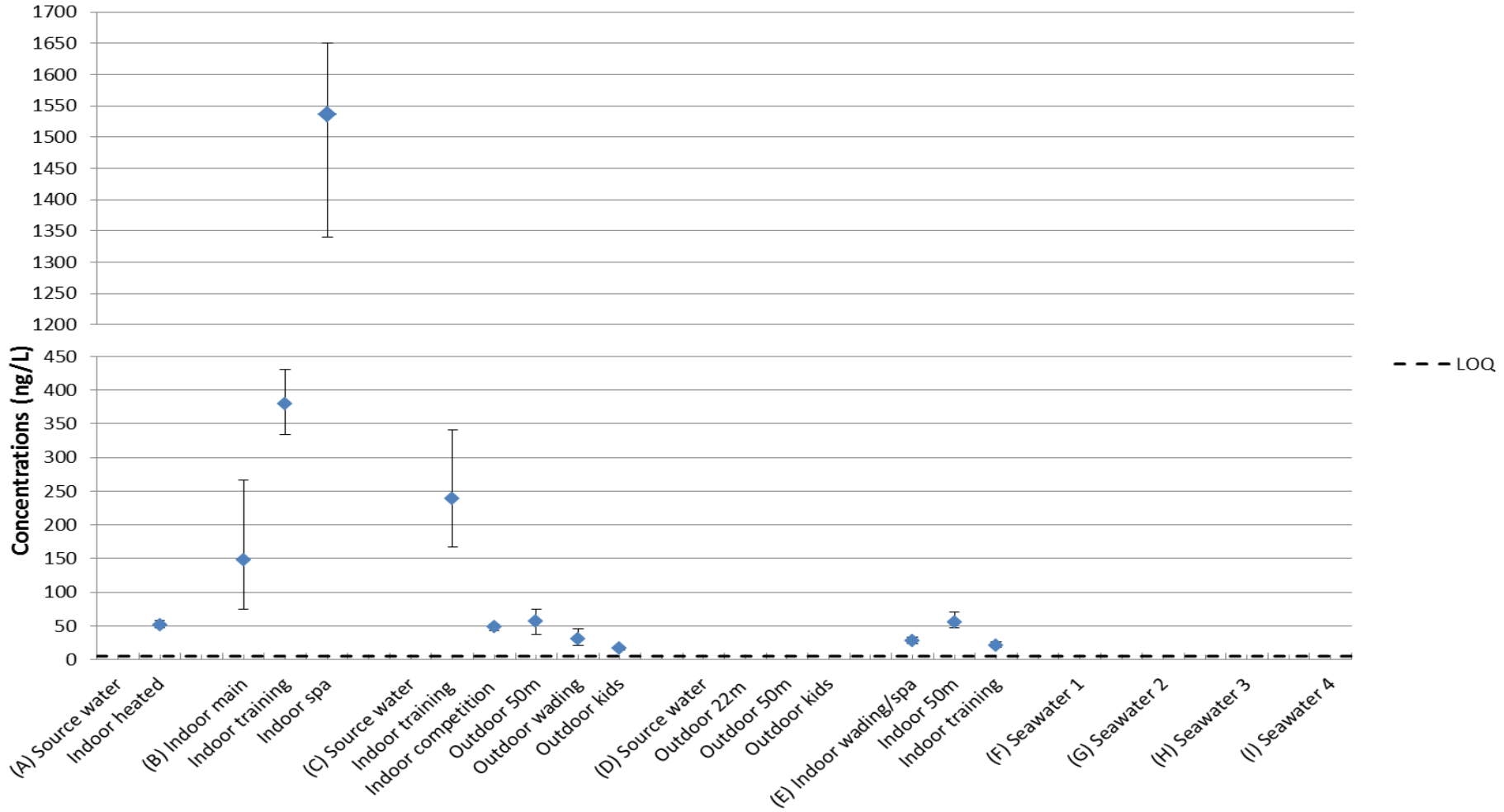


Figure 6.2 Mean concentrations of caffeine in swimming pools. Error bars represent the observed range of triplicate sample

Caffeine and ibuprofen were below the LOQs in all of the fill water samples. This implies that contamination is occurring within the swimming pools themselves and is due to human-derived sources. As swimming pool fill water is generally sourced from tap water, it can be expected that the level of PPCPs in the fill water are below the LOQs. The analysis on fill water samples were carried out to confirm that fill water do not contribute to the occurrence of PPCPs in swimming pools and to exclude fill water as a possible source for subsequent detections in swimming pools. As caffeine and ibuprofen were only detected in swimming pool water samples and not in the fill water, it is speculated that these two compounds were introduced into the pool water matrix through swimmers' excretion of body fluids such as accidental urinary excretion or sweat.

The concentrations of caffeine detected in swimming pools are presented in Figure 6.2. Caffeine was detected in all of the indoor pools at concentrations ranging between 20 – 1540 ng/L. The highest concentration of caffeine (1540 ng/L) was observed in an indoor spa pool. This could be due to the smaller volume of the spa and higher usage compared to other-sized pools. Outdoor pools in Location C had caffeine concentrations from 16 – 56 ng/L while caffeine was below 5 ng/L in all of the outdoor pools at Location D. The caffeine concentrations detected in this study are comparable to those that were detected in Weng *et al.* (2014). Caffeine is known to undergo slow photodegradation under sunlight with half-lives of roughly 12 days under sunlight (Buerge *et al.*, 2003; Zhang *et al.*, 2013) and is highly stable during chlorination (Glassmeyer and Shoemaker, 2005; Weng *et al.*, 2014). Thus, a possible reason for the low levels in Location D could be due to the low usage of the pools at the time of sampling. However, although the outdoor 50 m pool and indoor competition pool at Location C were in a period of high use (for a school swimming carnival), levels of caffeine detected in those pools were lower. The school carnival involves mostly children using the pools. This suggests that the demographic variability of swimming pool users may potentially be a significant factor in influencing the occurrence of caffeine in swimming pools. Furthermore, the type of activity carried out in the pool may also be significant. As a school carnival usually involves races, swimmers are usually in the pool for a shorter period of time compared to swimmers taking a leisurely swim therefore, the amount of bodily excretion occurring may conceivably be less.

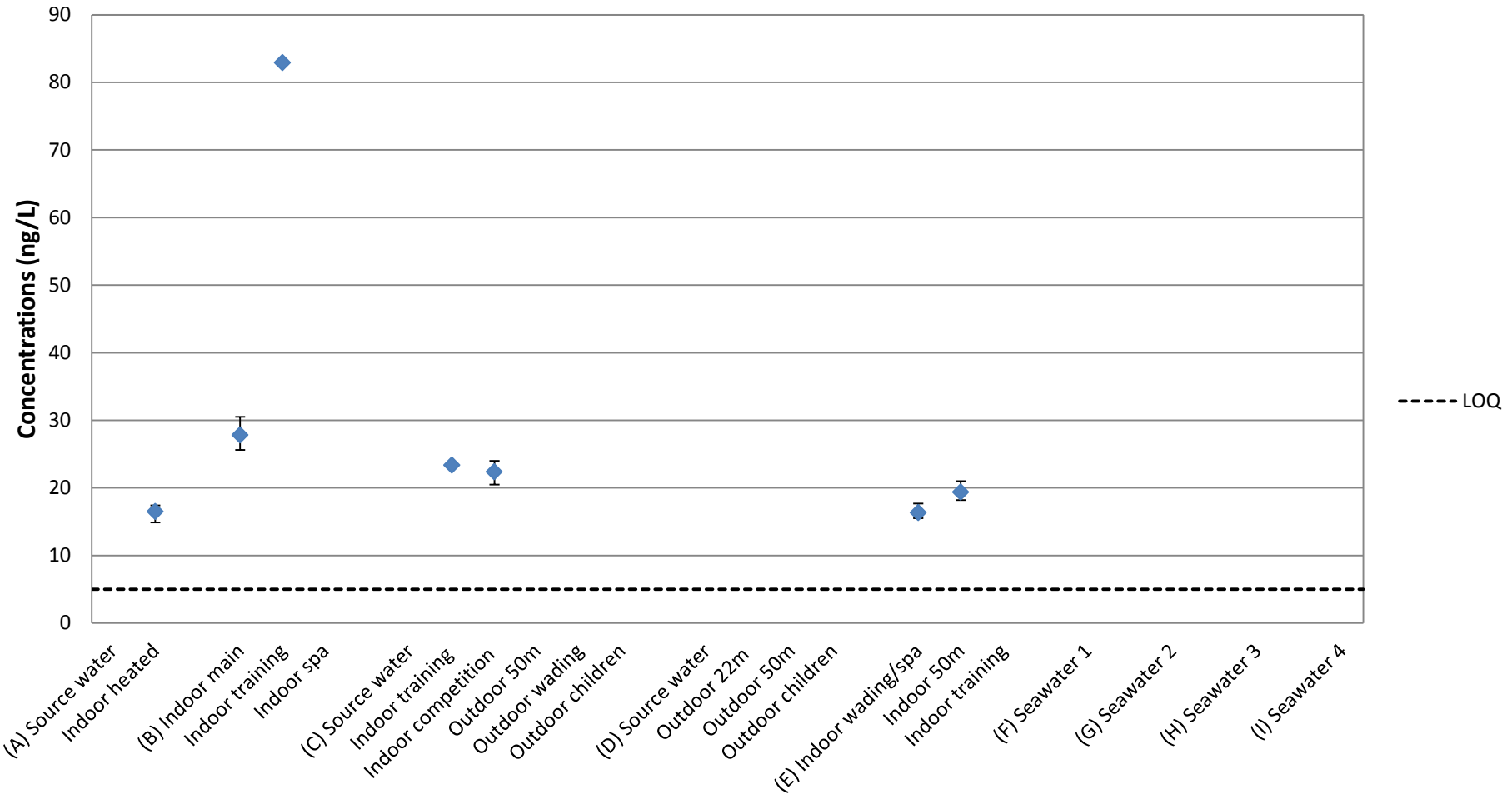


Figure 6.3 Mean concentrations of ibuprofen in swimming pools. Error bars represent the observed range of triplicate samples

The concentrations of ibuprofen observed in swimming pools are shown in Figure 6.3. Ibuprofen was detected at levels from 16 – 83 ng/L in 7 of the 8 indoor swimming pools sampled. The indoor spa pools at Location B had levels of ibuprofen below 5 ng/L. The spa pools were highly aerated and were at a higher temperature which could potentially enhance the degradation of ibuprofen leading to lower detection levels. Ibuprofen was below 5 ng/L in all of the outdoor pools in Location D and also in Location C, although there were similar bather loads in both the indoor and outdoor pools in Location C. Similar to caffeine, ibuprofen is slow to degrade in chlorinated drinking water and under sunlight (Packer *et al.*, 2003; Simazaki *et al.*, 2008; Weng *et al.*, 2014). Thus, other factors may be responsible for its lower detection frequency in the outdoor swimming pools such as the pool user demographics and the type of activity occurring in the swimming pools prior to and during sampling. As with caffeine in swimming pools in Location D, the lower number of swimmers could also account for the lower levels of ibuprofen. Furthermore, ibuprofen is an analgesic drug which, compared to caffeine, is only used by a relatively small proportion of the population at any particular time. This factor would be expected to lead to a more limited occurrence in swimming pools, compared to caffeine. Since caffeine and ibuprofen are similar in that they are slow to degrade during chlorination and under exposure to sunlight, the overall higher detection levels of caffeine compared to ibuprofen is likely due to greater consumption and excretion of caffeine.

In all the seawater swimming pool samples, all PPCPs were below the LOQs. The tides occurring at the time of sample collection likely influenced the low levels of PPCPs as they were undetectable even in Location F where there were a considerable number of swimmers. In the other three seawater pools, the low usage of the pool at the time of sampling could also explain the undetectable levels of PPCPs.

It is plausible that other PPCPs may have been introduced in swimming pools but were not detectable due to their transformation through reactions with the disinfectants used in the pools as it was reported that more than 90% of chlorine-susceptible PPCPs such as acetaminophen and naproxen degraded within 6 hours of chlorination (Weng *et al.*, 2014). Further investigations into the chlorination reaction pathways of PPCP compounds may be necessary to better understand the fate of these chemicals in swimming pool waters. In addition, as most pharmaceuticals are ingested and

metabolised before excretion, the occurrence of pharmaceutical metabolites may be of more significance compared to their parent compounds. Further investigations may be warranted to detect transformation products of PPCPs and their potential harm to human health. The significance of exposure to PPCP chemicals in swimming pools has generally not been thoroughly investigated. However, it is conceivable that they may be relatively high contributors to the overall environmental exposure of some chemicals for regular swimming pool users.

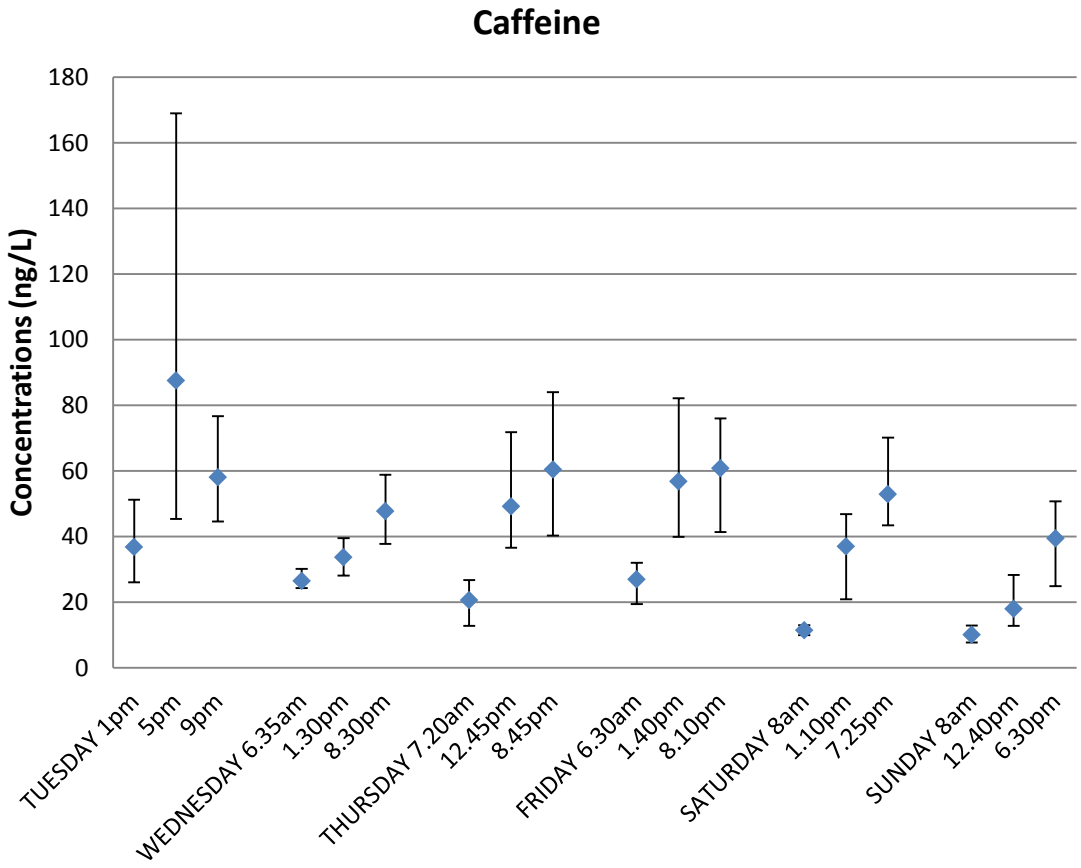


Figure 6.4 Variations in caffeine concentrations over 6 days. Error bars represent the observed range of triplicate samples

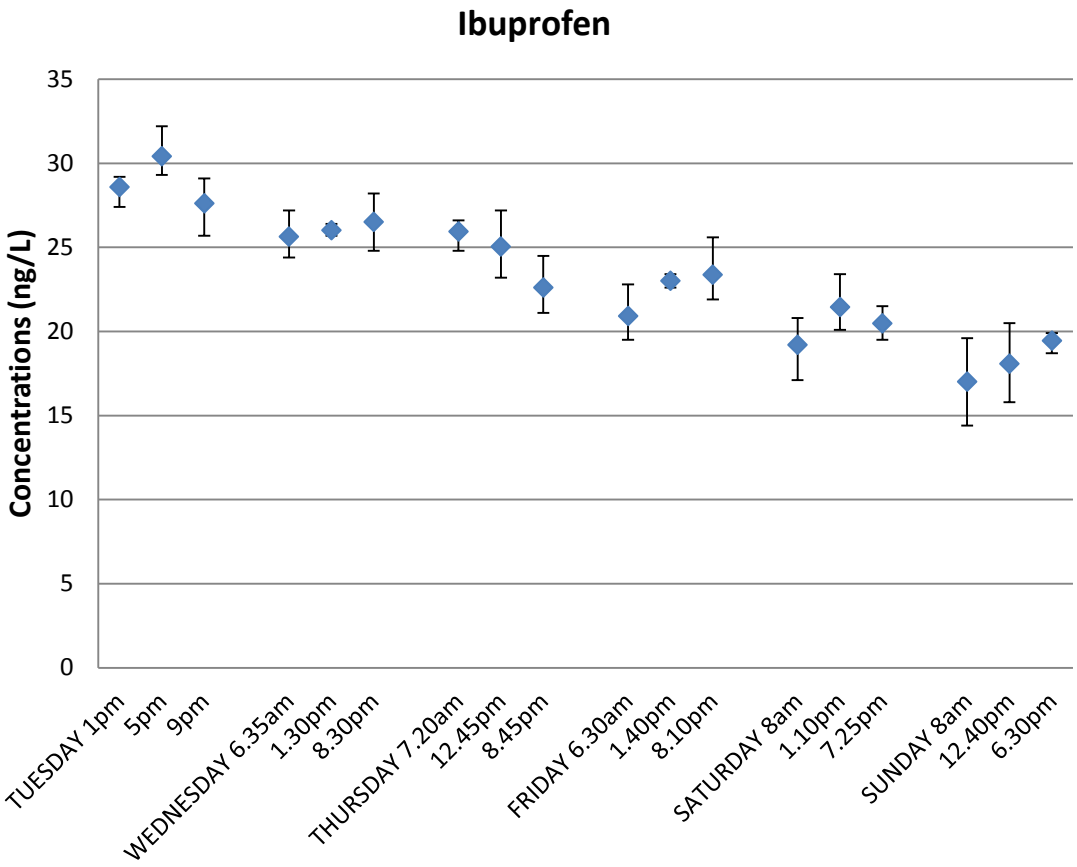


Figure 6.5 Variations in ibuprofen concentrations over 6 days. Error bars represent the observed range of triplicate samples

The daily changes to the levels of caffeine and ibuprofen from a 6-day monitoring study at one swimming pool are presented in Figures 6.4 and 6.5 respectively. The changes to caffeine concentrations are much greater compared to the changes in ibuprofen concentrations throughout this period. The levels of caffeine are observed to be lower at the start of each day and increases throughout the day with the highest concentration occurring during the last sampling of the day for most cases. This is possibly due to caffeine accumulating with higher pool usage as the day progresses. Furthermore, this observation may also be due to the time of day that caffeine is consumed and then metabolised and excreted via urine. Although both caffeine and ibuprofen are metabolised before excretion via urine and only small amounts (about 3%) are excreted in their original forms (Mills *et al.*, 1973; Tang-Liu *et al.*, 1983), both of these compounds were detectable above the LOQs in this study similar to findings presented by Weng *et al.* (2014). This trend suggests that monitoring of caffeine might potentially be a useful indicator reflecting bather loads in swimming pools. The same trend was not observed for ibuprofen. The decline of ibuprofen concentrations in Figure 6.5 suggests that ibuprofen was initially introduced and subsequently degraded/removed in swimming pools.

Pharmaceuticals and caffeine have previously been proposed as possible water monitoring surrogate indicators for sewage contamination in environmental waters (Seiler *et al.*, 1999; Fono and Sedlak, 2005; Williams *et al.*, 2013; Khan *et al.*, 2014). Caffeine, in particular, is a useful indicator due to its ubiquitous occurrence in the environment. The same idea could be applied in swimming pool water where caffeine may be used as a potential indicator of anthropogenic contamination as caffeine was detected frequently in swimming pools and roughly reflect bather loads in this study. Furthermore, caffeine is not readily susceptible to degradation during chlorination and in the presence of sunlight (Buerge *et al.*, 2003; Glassmeyer and Shoemaker, 2005; Gibs *et al.*, 2007; Weng *et al.*, 2014). Further detailed studies are required to investigate the strength of the relationship between caffeine concentrations and the amount of body excretions.

6.4 Conclusions

This study provides insights into the concentrations and variability of PPCPs in various types of swimming pools. Caffeine was detected in 12 chlorinated swimming pools

(indoor pool, outdoor pool and spa pools) in the range of 16 – 1540 ng/L. Ibuprofen was detected in 8 out of the 15 swimming pools tested in the range of 16 – 83 ng/L. Caffeine and ibuprofen were not detected in any of the fill water samples. This suggests that the chemicals that were present in these swimming pools were from bather-related sources. PPCPs were undetectable in seawater swimming pools. The results obtained in this study demonstrate that caffeine and ibuprofen can potentially reflect amounts of bodily excretions to swimming pools since these chemicals are likely to be markers of accidental urinary excretions during swimming. Six-day monitoring of caffeine revealed that caffeine concentrations changes significantly throughout the day. The levels of chemical contaminants were likely to be affected by many factors such as bather rate, the types of swimming pools, types of activities carried out in swimming pools, demographics of pool users, exposure to sunlight and the type of disinfection used such as the incorporation of UV disinfection. Measurement of these chemicals has the potential to provide quantitative indications of the quantities of human excreted substances in the pool. To achieve that outcome, further research is required to fully understand the strength of the relationships (and confounding factors) between the concentrations of these chemicals and actual human excretion levels.

CHAPTER 7 OCCURRENCE OF *N*-NITROSAMINES IN SWIMMING POOLS

7.1 Introduction

N-nitrosamines are a group of non-halogenated DBPs occurring in chlorinated drinking waters (Asami *et al.*, 2009; Templeton and Chen, 2010). There are growing health concerns and regulations for the occurrence of these compounds in water especially in drinking water systems as some of them are known to be highly carcinogenic (Lijinsky and Epstein, 1970; Nawrocki and Andrzejewski, 2011; Patterson *et al.*, 2012). In recent years, the research interest on DBPs has expanded from regulated DBPs such as THMs and HAAs to *N*-nitrosamines after toxicological studies have shown that *N*-nitrosamines are considerably more genotoxic, cytotoxic and carcinogenic (Richardson, 2005; Richardson *et al.*, 2007). The US EPA (2012) has listed *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosopyrrolidine (NPyr) and *N*-nitrosodibutylamine (NDBuA) are listed as probable carcinogens to humans (Group B2) as there is sufficient evidence of carcinogenicity from animal studies. With public concern increasing regards the risks associated with exposure, studies on *N*-nitrosamines have been an area of growing interest in the last decade.

The detection of several *N*-nitrosamine compounds such as NDMA in swimming pools indicate that swimming pools are possibly another source of human exposure to *N*-nitrosamines (Walse and Mitch, 2008; Kim and Han, 2011). The high organic loading and continuous disinfection in swimming pools increases the rate of formation of *N*-nitrosamines leading to higher concentrations in swimming pools compared to drinking water. There is limited research on concentrations of *N*-nitrosamines in swimming pool water.

This chapter presents the results from the analysis of seven *N*-nitrosamine compounds namely NDMA, NMEA, NDEA, NDPA, NDBuA, NPyr and NMor in (indoor and outdoor) chlorinated swimming pools and seawater pools. A comparison of the concentrations occurring in swimming pools and fill waters is also presented.

7.2 Sample collection and analysis

Triplicate sets of swimming pool water samples and fill water samples were collected as described in Chapter 5. Samples were collected from 15 chlorinated swimming pools consisting of indoor pools, outdoor pools and spa pools while seawater pool samples

were collected from four locations in Sydney, Australia. The characteristics and the number of swimmers for each swimming pool at the time of sampling were the same as presented in Table 5.1 (Chapter 5) and Table 6.1 (Chapter 6). Collected samples were quenched with 1 g/L $Na_2S_2O_3$ to eliminate any residual chlorine and subjected to SPE and instrumental analysis using isotope dilution GC-MS/MS as described in Section 3.3 (Chapter 3). LOQs for the seven *N*-nitrosamines were 1 ng/L. The method recoveries for each *N*-nitrosamine compound in swimming pool water were obtained by spiking 50 ng/L concentrations of target analytes and 50 ng/L of internal standards before undergoing SPE (Table 7.1).

Table 7.1 Method recoveries for *N*-nitrosamines

***N*-nitrosamines**	**Method recovery (%)**		
	Swimming pool water	**Seawater pools**	**Ultrapure water**
NDMA	98	101	82
NMEA	100	97	96
NDEA	106	98	92
NDPA	87	97	96
NMor	97	100	90
NPyr	65	95	77
NDBuA	90	100	91

7.3 Results and discussion

Three *N*-nitrosamines, specifically NDMA, NDEA and NMor were detected above the LOQ and they were observed to occur only in the indoor pools. Their concentrations in fill water samples, all of which were sourced from reticulated municipal drinking water, were all below the LOQ. This suggests that *N*-nitrosamines were formed or deposited within the swimming pools themselves. None of the outdoor pools had detectable levels of *N*-nitrosamines, even those which were in high use. A possible reason for the low detection of *N*-nitrosamines in outdoor pools could be due to their degradation under sunlight as *N*-nitrosamines are susceptible to photodegradation (Tuazon *et al.*, 1984; Stefan and Bolton, 2002). This was similar to findings presented by Walse and Mitch (2008).

In this study, NDMA, NDEA and NMor were detected in all pools in Location B which did not use UV disinfection. They were also detected at higher concentrations than the pools at other locations which had used UV disinfection. Research has shown a decrease in *N*-nitrosamine levels when UV disinfection is used with chlorine (Walse and Mitch, 2008). Research has also shown that UV disinfection may also lead to the formation of new *N*-nitrosamines depending on the initial concentrations of precursors such as chlorinated dimethylamine and monochloramine (both of which are present in swimming pool water) and applied UV dose (Soltermann *et al.*, 2013). It was reported that UV treatment can be effective in degrading *N*-nitrosamines if their concentrations are higher than its precursors, while additional NDMA was formed between UV doses of 250 – 850 mJ/cm^2.

The concentrations of NDMA observed in swimming pool waters are presented in Figure 7.1. NDMA was detected in 9 out of the 15 chlorinated swimming pools ranging between 2 – 9 ng/L, all of which were from the indoor swimming pools. The highest concentration was detected in the indoor training pool at Location B. Aside from the lack of UV disinfection in this swimming pool location, the higher levels of NDMA in the indoor training pool could also be due to the higher number of swimmers at the time of sampling. The other two pools at the same location had lower NDMA concentrations and lower number of swimmers. The higher usage of pools would result in higher organic loadings leading to the presence of more precursors for reactions with the disinfectants used in the pools. The NDMA levels detected in this study were significantly lower than those detected by Walse and Mitch (2008) (max 44 ng/L) and Kim and Han (2011) (max 208 ng/L) but were comparable to Jurado-Sánchez *et al.* (2010) (max 6 ng/L) and Yeh *et al.* (2014) where NDMA levels in pools were below the 5 ng/L detection limit.

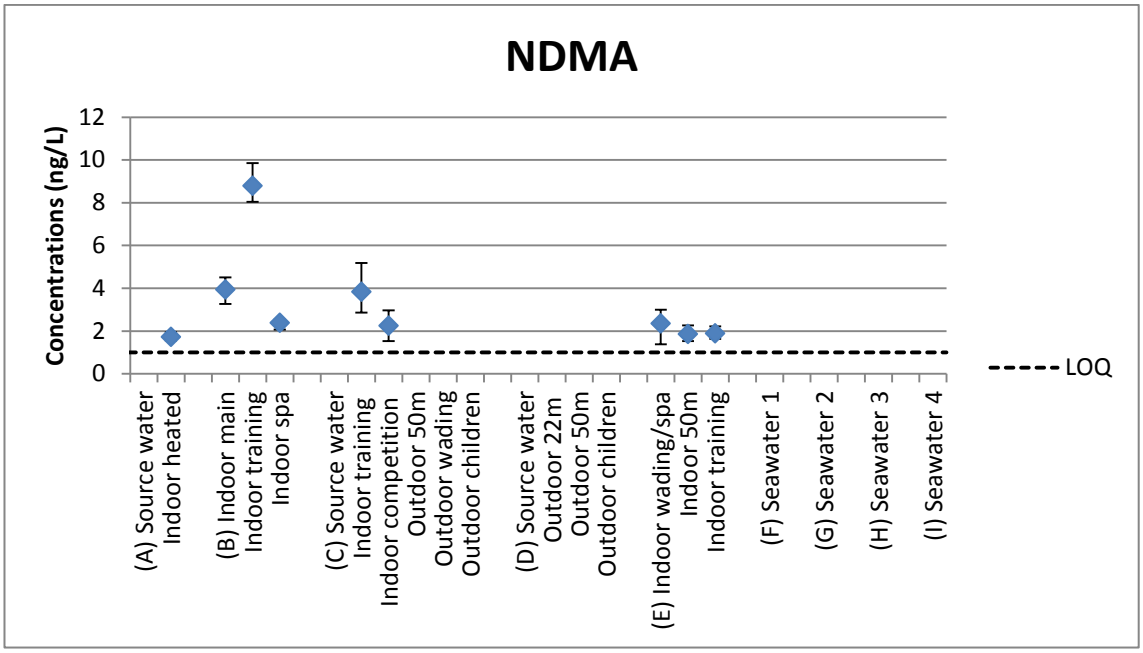


Figure 7.1 Mean concentrations of NDMA in swimming pool waters. Error bars represent the observed range of triplicate samples

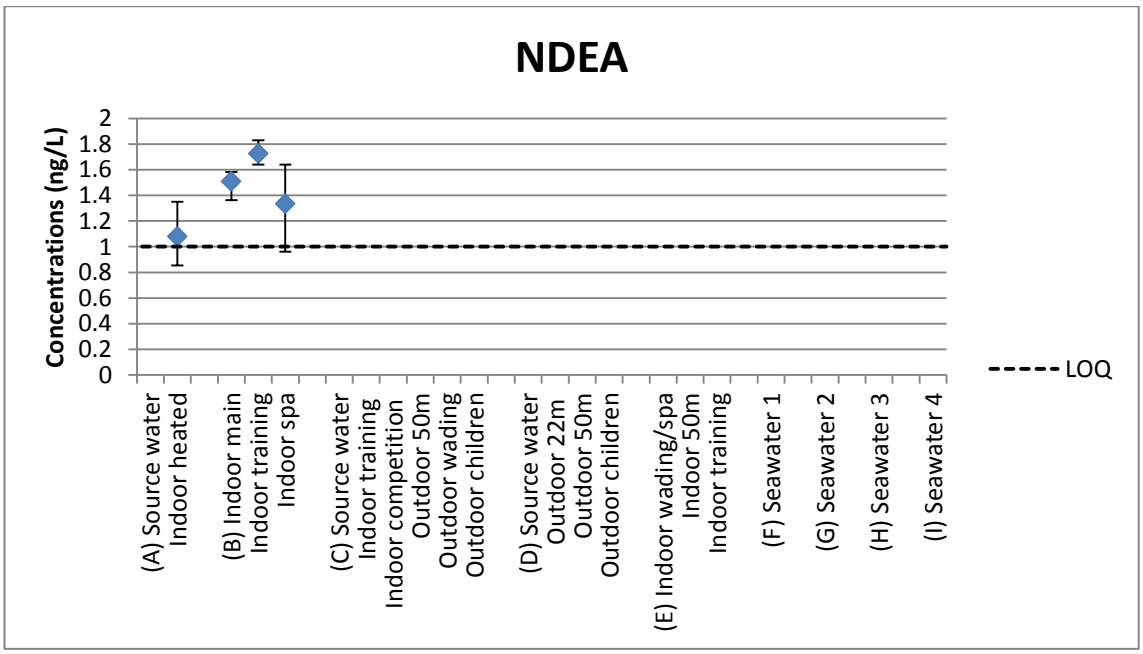


Figure 7.2 Mean concentrations of NDEA in swimming pool waters. Error bars represent the observed range of triplicate samples

The concentrations of NDEA detected in swimming pools are shown in Figure 7.2. NDEA was observed in the pools at Location A and B at concentrations slightly higher than the LOQs (2 ng/L). This is agreement with work by Jurado-Sánchez *et al.* (2010) who detected concentrations of approximately 1 ng/L by GC-MS. This was considerably lower than the concentrations detected by Kim and Han (2011) where NDEA analysed using HPLC-FD, ranged from 1.5 – 53 ng/L. In all of these studies, the overall NDEA concentrations were lower than those of NDMA. This is most likely due to the presence of NDMA precursors in swimming pool water such as dimethylamine and trimethylamine which are constituents of urine and sweat (Walse and Mitch, 2008), therefore leading to more significant concentrations of NDMA.

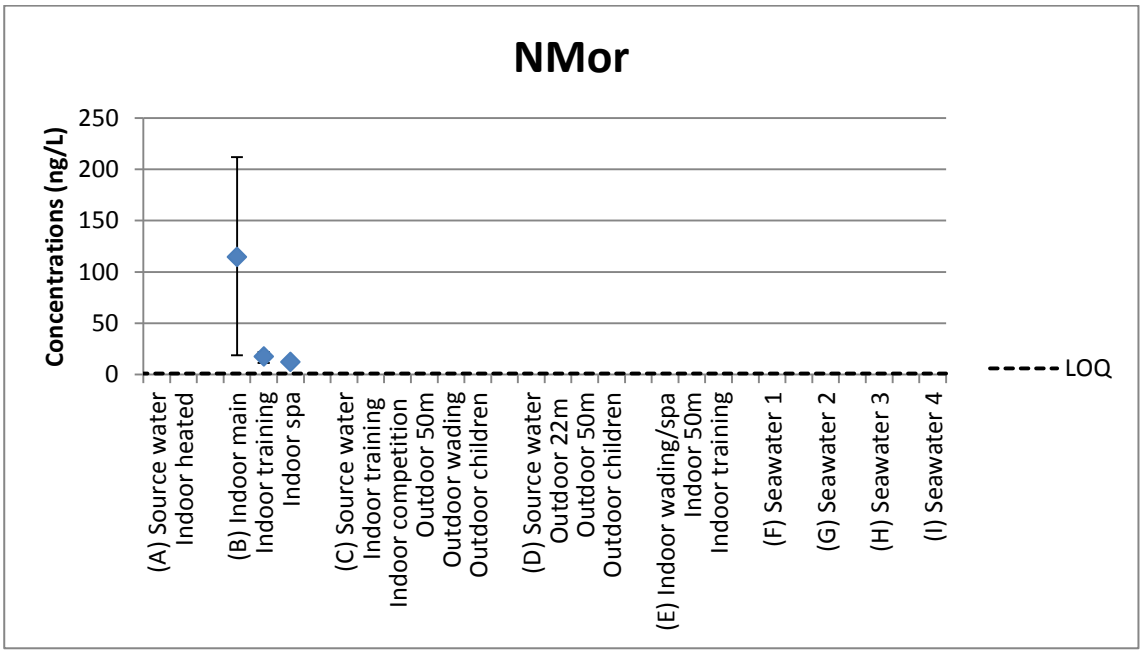


Figure 7.3 Mean concentrations of NMor in swimming pool waters. Error bars represent the observed range of triplicate samples

The concentrations of NMor, presented in Figure 7.3, showed that NMor was only detected in the swimming pool water at Location B in the range of 12 – 114 ng/L. The highest concentration of NMor was detected in the indoor main pool which had 10 swimmers in the pool at the time of sampling. NMor levels were lower or below the LOQ in the other indoor pools which had higher bather loads during sampling compared

to the indoor main pool in Location B, suggesting the influence of other factors for the occurrence of NMor. Compared to the indoor pools at Location A, C and E, the pools at Location B did not incorporate UV treatment which suggests that treatment methods may impact the levels of NMor. The NMor concentrations detected in this study were generally higher than those reported by Kim and Han (2011) where the NMor levels ranged from 0.25 – 34 ng/L. In general, NMor recorded the highest concentration among the three *N*-nitrosamines in this study.

Aside from being components of DBPs in swimming pool water, bather-derived sources could also be contributing to the occurrence of NDMA and NMor in swimming pools. Previous research has shown that these compounds occur in commercially available cosmetics and toiletries (Spiegelhalder and Preussmann, 1984). This may also explain the higher concentrations of NMor in swimming pools as higher concentrations of NMor were detected in the cosmetic products tested compared to NDMA although the frequency of detection of NDMA was higher.

The occurrence of bather-derived chemicals such as PPCPs in swimming pools may further enhance the formation of *N*-nitrosamines as research has reported that some PPCPs with amine groups can act as precursors for the formation of *N*-nitrosamines during chloramine disinfection (Shen and Andrews, 2011). This may further contribute to the concentrations of *N*-nitrosamine precursors in swimming pools. Furthermore, high bather loads in swimming pools would lead to the introduction of more precursor compounds in swimming pools. Body fluids from swimmers especially the nitrogenous components have been shown to lead to an increase in formation of *N*-nitrosamines (Walse and Mitch, 2008). Therefore, the number of swimmers using the pools is another significant factor in the occurrence of *N*-nitrosamines in swimming pools.

Other *N*-nitrosamines have been reported in chlorinated swimming pools such as NPyr, NDBuA and NPip (Walse and Mitch, 2008; Jurado-Sánchez *et al.*, 2010; Pozzi *et al.*, 2011). In addition, NPyr was the only *N*-nitrosamine compound detected from 53 –127 ng/L in the five indoor swimming pools that were tested in Italy (Pozzi *et al.*, 2011). Further research may be needed to elucidate why certain *N*-nitrosamine compounds are present in some swimming pools while others are not.

In all the seawater pool samples, *N*-nitrosamines were below the LOQs. Unquantifiable levels of *N*-nitrosamines in seawater pools are most likely due to the lack of oxidizing disinfectants, which would normally be required for their formation. Other factors such as the composition of seawater which has high bromide content may also have an impact on the formation of *N*-nitrosamines in seawater pools. It was reported that formation of NDMA from tertiary alkylamines was inhibited in the presence of bromide during chlorination (Chen *et al.*, 2010).

7.4 Conclusions

N-nitrosamines consisting of NDMA, NMEA and NMor were detected only in the indoor chlorinated swimming pools. NMor was observed to have the highest concentration at 114 ng/L among the three compounds detected possibly due to additional bather-derived sources. *N*-nitrosamines were below the LOQs for fill water samples indicating that these compounds are occurring within the swimming pools. The concentrations of *N*-nitrosamines are affected by many factors such as the amount of organic loading in swimming pools, the types of swimming pools, exposure to sunlight and the type of disinfection used/fill water. Further research is needed to investigate the formation pathways of *N*-nitrosamines in swimming pools and what portion of bather-derived organic matter contribute to the level of these compounds in the pool. Additional treatment methods such as the use of UV disinfection may help to reduce the concentrations of *N*-nitrosamines in swimming pools. Swimming pools may be another important route of *N*-nitrosamines exposure to humans and further research is needed to investigate the overall contributions through swimming.

CHAPTER 8 USE OF FLUORESCENCE TO MONITOR ORGANIC LOADING IN SWIMMING POOLS

Part of the work presented in this chapter has been carried out as part of an Honours project entitled "**Sensitivity of using fluorescence excitation to detect organic loading in different types of swimming pool water**" for which I was the project leader.

The work which was carried out as part of the Honours thesis under my training and guidance includes:

1. Investigating the variability of dissolved organic matter within a swimming pool.
2. Investigating the correlations between relevant fluorescence peaks and the addition of bather-derived organic matter to swimming pool water through laboratory-based experiments.

The remaining parts of the chapter were fully carried out by me as part of this research study. These include:

1. Development of a fluorescence method for measuring fluorescent dissolved organic matter in swimming pools.
2. Investigation of the quenching effects on fluorescence signals when quenching agents were used to remove residual chlorine during sampling.
3. Sampling and analysis of various water samples including swimming pool water, fill water and tap water for fluorescence EEMs comparison and to ascertain the most relevant peak for swimming pool water monitoring.

8.1 Introduction

Dissolved organic matter (DOM) in swimming pools may be highly variable due to continuous organic loading introduced by the fill water supplied to the pool and by pool users. Anthropogenically-derived DOM is constantly introduced into swimming pools through the excretion of body fluids (urine and sweat) and from the washing-off of personal care products (cosmetics and sunscreens) during swimming. Human-derived trace organic chemicals that have been detected in this study such as pharmaceuticals would further contribute to higher levels of DOM in the pool. DOM in swimming pools is a source of DBP formation, some of which are known to be harmful to human health. The presence of DOM in swimming pool water therefore impacts the water quality of the pool.

Fluorescence spectroscopy involves exciting molecules by photons absorbance to various vibrational states and measuring the emitted photon frequencies when the molecules reach their stable state. The data obtained portrayed through three-dimensional EEMs can be used to identify and quantify fluorescent DOM components based on specific peaks and their fluorescence intensity (Chen *et al.*, 2003). The EEM spectrum is used to distinguish different types of fluorescent DOM occurring at specific regions in the EEM (Coble, 1996; Baker, 2001). Excitation-emission regions have been distinguished by a number of peaks: Peak A ($\lambda_{ex/em}$ = 237-260/400-500 nm) and Peak C ($\lambda_{ex/em}$ = 300-370/400-500 nm) as 'humic-like' peaks, Peak T_1 ($\lambda_{ex/em}$ = 275/340 nm) and Peak T_2 ($\lambda_{ex/em}$ = 225-237/340-381 nm) as tryptophan-like/protein-like and Peak B ($\lambda_{ex/em}$ = 225-237/309-321 nm) as 'tyrosine-like' (Coble, 1996; Hudson *et al.*, 2007).

The use of EEM fluorescence spectroscopy as a monitoring tool has been widely applied to various aquatic systems such as natural waters (Coble, 1996; Baker, 2001), municipal wastewaters (Vasel and Praet, 2002; Hudson *et al.*, 2007), recycled waters (Henderson *et al.*, 2009) and more recently in swimming pool waters (Seredyńska-Sobecka *et al.*, 2011). A fluorescence peak occurring at $\lambda_{ex/em}$ = <240, 310/360 nm was found to be specific to swimming pool water likely derived from a mixture of swimming pool microbial activity products and humic-like substances (Seredyńska-Sobecka *et al.*, 2011). Chlorination has been known to quench fluorescence intensity (Henderson *et al.*, 2009). As swimming pools are constantly chlorinated, the impact of chlorination leading to decreased overall fluorescence intensity in swimming pools is

seen to be an ideal situation to monitor the excessive organic loading through fluorescence (Seredyńska-Sobecka *et al.*, 2011).

A quick and reliable monitoring system to monitor the effectiveness of water treatment in swimming pools would facilitate the assurance of a healthy and safe environment. The use of fluorescence spectroscopy to monitor DOM fluorescence provides a potentially sensitive, rapid and simple approach to detect water quality changes in swimming pools. The aim of this study was to assess the potential application of fluorescence as an online monitoring tool in swimming pools by investigating the relationships between fluorescence signals at various excitation and emission wavelengths and changes in water quality over time. The sensitivity of using fluorescence to detect changes in organic loadings in swimming pools was further investigated.

8.2 Experimental design and analysis

The procedures undertaken to investigate the potential use of fluorescence for swimming pool water monitoring are detailed in the following sections:

8.2.1 Sample collection and analysis

Swimming pool water samples were collected from the same five locations described in Section 5.2.1 (Chapter 5) in 50 mL polypropylene tubes with a total of 15 chlorinated swimming pools sampled. In addition, fill water samples used at the swimming pool location were collected where possible. Water samples were analysed immediately after collection as described in Section 3.5 (Chapter 3). The impact of quenching agents such as $Na_2S_2O_3$, which were added to the water samples to remove residual free chlorine to stop further chlorine reactions, on the fluorescence intensity was investigated. Tap water samples were also collected from a regular potable water tap at UNSW and subjected to analysis in order to compare their differences with swimming pool water samples during fluorescence analysis. Hence, the fluorescence peaks most relevant to swimming pool water for monitoring can be ascertained.

8.2.2 Quenching effects of quenching agents

Swimming pool water samples quenched with the quenching agent $Na_2S_2O_3$ showed that the use of $Na_2S_2O_3$ quenched the fluorescence signals in swimming pool water samples during the EEMs analysis. The quenching effects of other quenching agents comprising of sodium sulphite (Na_2SO_3) and ascorbic acid ($C_6H_8O_6$) were further tested in tap water to assess their effects on fluorescence signals when added to water samples (Figure 8.1).

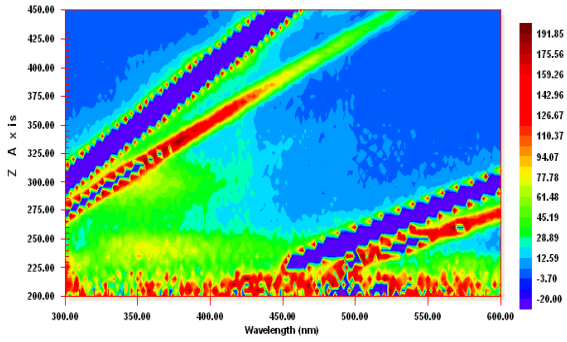
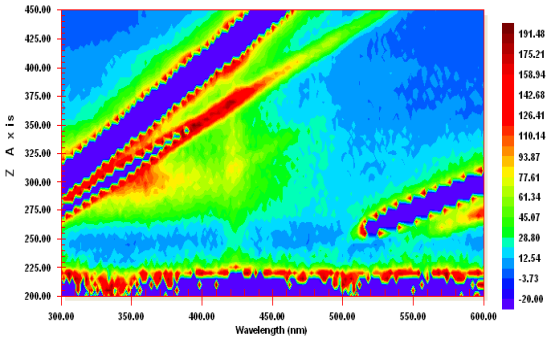
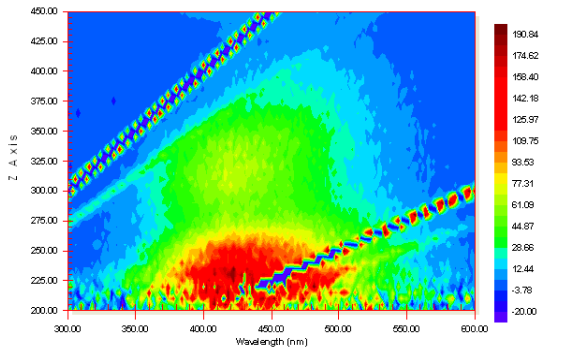
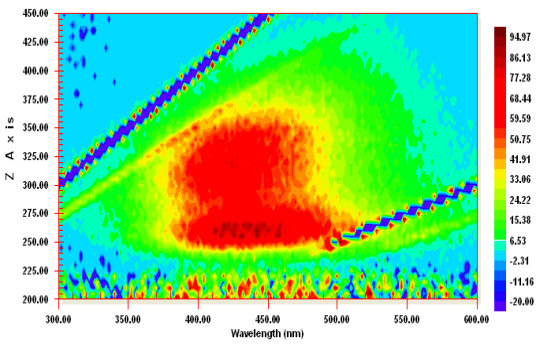
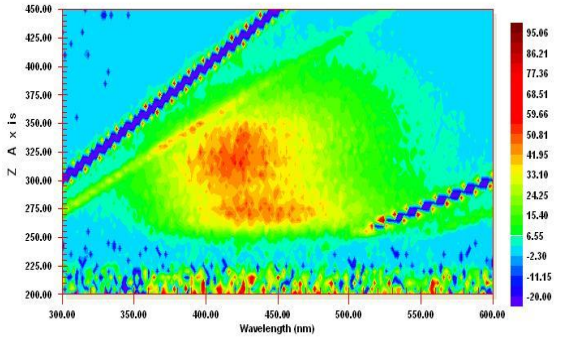
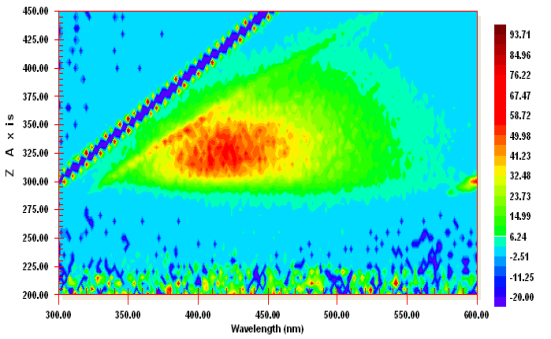
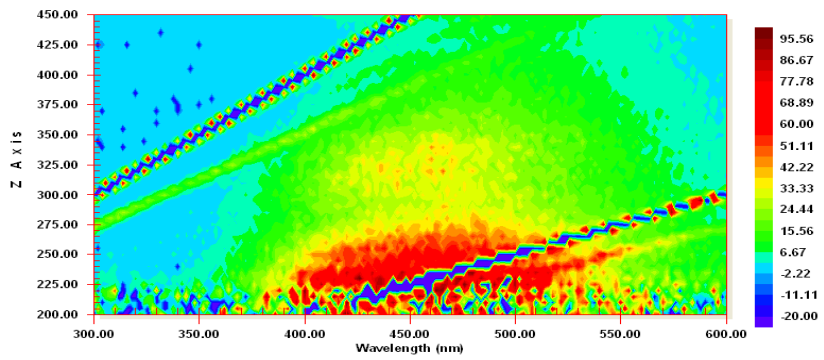
Pool water not quenched	Pool water quenched with $Na_2S_2O_3$
	
Tap water not quenched	**Tap water quenched with Na_2SO_3**
	
Tap water quenched with $Na_2S_2O_3$	**Tap water quenched with $C_6H_8O_6$**
	

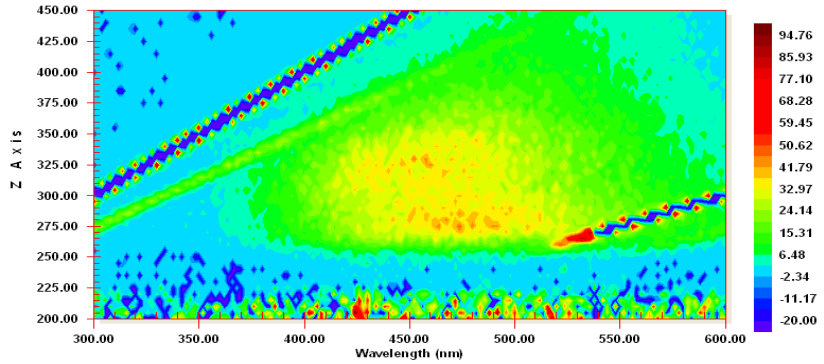
Figure 8.1 Raw fluorescence EEMs of tap water quenched with different quenching agents

The quenching effect of the quenching agents were also tested on water samples made up of humic acid (a fluorescence standard) as the fluorescence peak for humic acid occurs in the lower excitation range (<260 nm). Ultrapure water with the addition of humic acid was quenched with $Na_2S_2O_3$ and Na_2SO_3 and analysed (Figure 8.2).

1. Ultrapure water + humic acid



2. Ultrapure water + humic acid quenched with $Na_2S_2O_3$



3. Ultrapure water + humic acid quenched with Na_2SO_3

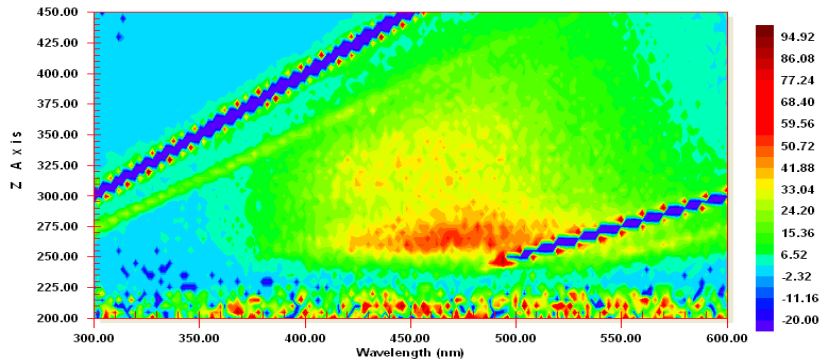


Figure 8.2 Raw fluorescence EEMs of ultrapure water added with humic acid and quenched with different quenching agents

The EEMs of all samples quenched with a quenching agent showed that the fluorescence signals were significantly quenched especially at excitation wavelengths of <250 nm. Therefore, swimming pool water samples collected for fluorescence analysis were not quenched and were analysed directly without further treatment.

8.2.3 Bather-derived organic loading experiments

Laboratory experiments were undertaken to investigate the relevant fluorescence peaks when bather-derived organic matter in the form of urine were added to swimming pool water samples. Increasing volumes of urine were added to swimming pool water samples taken from the indoor training pool at Location C and analysed. Water samples for analysis were made up from swimming pool water and varying concentrations of urine as shown in Table 8.1.

Table 8.1 Concentrations of urine in swimming pool water

Urine volume (µL)	Pool water volume (mL)	Percentage of urine (%)
50	300	0.017
60	300	0.020
70	300	0.023
80	300	0.027
90	300	0.030
100	300	0.033

8.3 Results and discussion

The results from the fluorescence analysis to investigate the most relevant peak for monitoring swimming pool water quality are presented and discussed in the following sections:

8.3.1 Comparison of EEMs in various water matrices

In order to assess the potential application of fluorescence as an online monitoring tool in swimming pools, the fluorescence signals at various excitation and emission wavelengths of swimming pool water were first analysed using a Varian Cary Eclipse Fluorescence Spectrophotometer with the method described in Section 3.5 (Chapter 3)

to obtain fluorescence EEMs. Water samples including ultrapure water, tap water and fill water collected from the swimming pool location were also analysed for comparison.

Peak-picking technique was used to determine the most appropriate wavelength for monitoring swimming pool water. Peak-picking involved the visual comparison of EEMs and selecting appropriate excitation-emission wavelength pairs based on intensity and frequency of occurrence. Raw EEMs of ultrapure water, tap water, fill water and swimming pool water from Location C are shown in Figure 8.3.

Visual comparisons of these results show that the EEMs spectra for swimming pool water were significantly different from tap water and fill water. Peaks C_1 and A which are humic-like peaks, are more distinct in tap water and fill water samples compared to swimming pool water samples. Peaks T_1 and T_2 which are protein-like peaks dominate in swimming pool water. These protein-like peaks have been associated with anthropogenic DOM and microbial origins commonly related to sewage contamination (Baker and Inverarity, 2004; Hudson *et al.*, 2007). Therefore, these peaks may be more relevant for monitoring in swimming pool water with the constant organic load from swimmers. As the fill water collected at the swimming pool location is sourced from reticulated municipal drinking water, the EEMs fluorescence characteristics are similar to that with the tap water samples. The fluorescence EEMs spectrum of swimming pool water samples in this study also showed the fluorescence peak $\lambda_{ex/em}$ = <240, 310/360 nm reported to be unique to swimming pool waters (Seredyńska-Sobecka *et al.*, 2011) with the highest intensity seen to occur in the indoor training pool (Figure 8.3). The EEMs analyses of swimming pool water from four other locations show a similar trend. The full EEMs spectra for each of the swimming pool are presented in Appendix A.

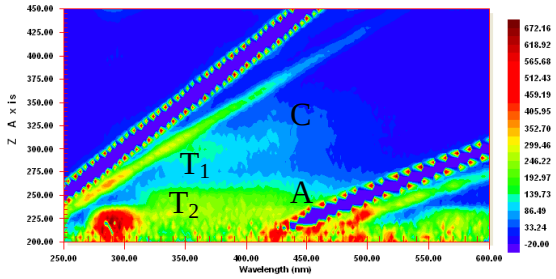
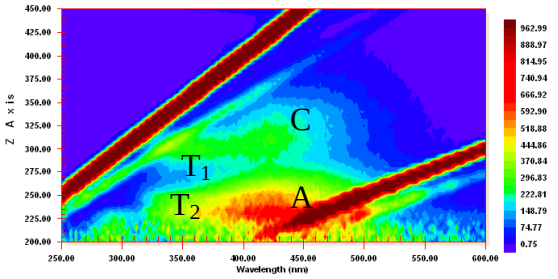
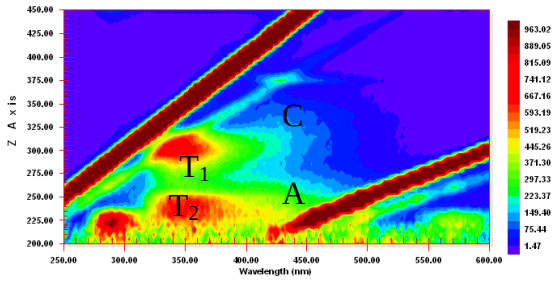
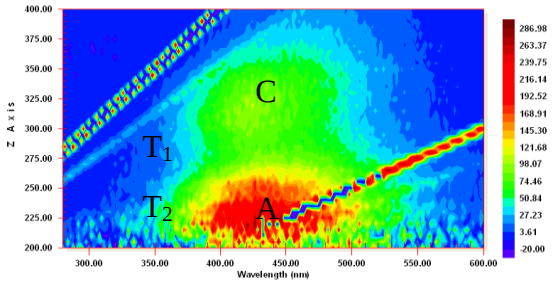
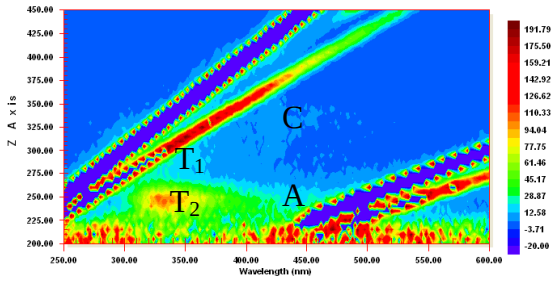
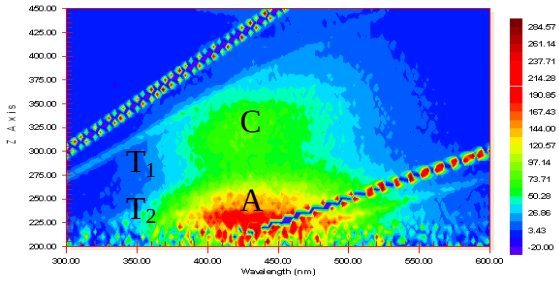
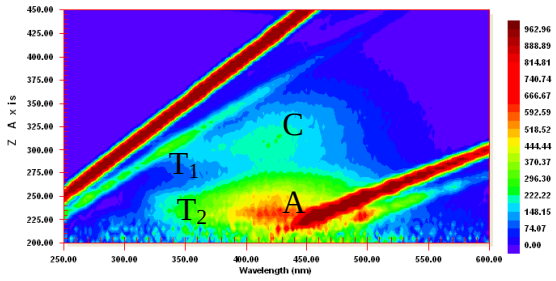
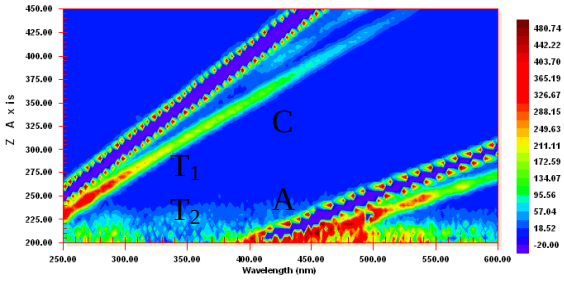
Indoor competition pool	Outdoor wading pool
	
Indoor training pool	**Fill water**
	
Outdoor 50m pool	**Tap water**
	
Outdoor children pool (shaded)	**Ultrapure water**
	

Figure 8.3 Raw fluorescence EEMs of various types of water samples consisting of swimming pool water and fill water (from Location C), tap water and ultrapure water

8.3.2 Variability of DOM within a swimming pool

The EEMs analysis of swimming pool water collected from three various locations around a swimming pool show significant DOM variability depending on the location where swimming pool water was collected. A comparison of the fluorescence intensity for specific peaks at each sampling location is presented in Figure 8.4. The fluorescence intensity were compared based on selected wavelengths consisting of Peak T1 ($\lambda_{ex/em}$= 285/350 nm), Peak T2 ($\lambda_{ex/em}$= 230/350 nm), Peak C ($\lambda_{ex/em}$=340/426 nm) and Peak A ($\lambda_{ex/em}$=240/426 nm).

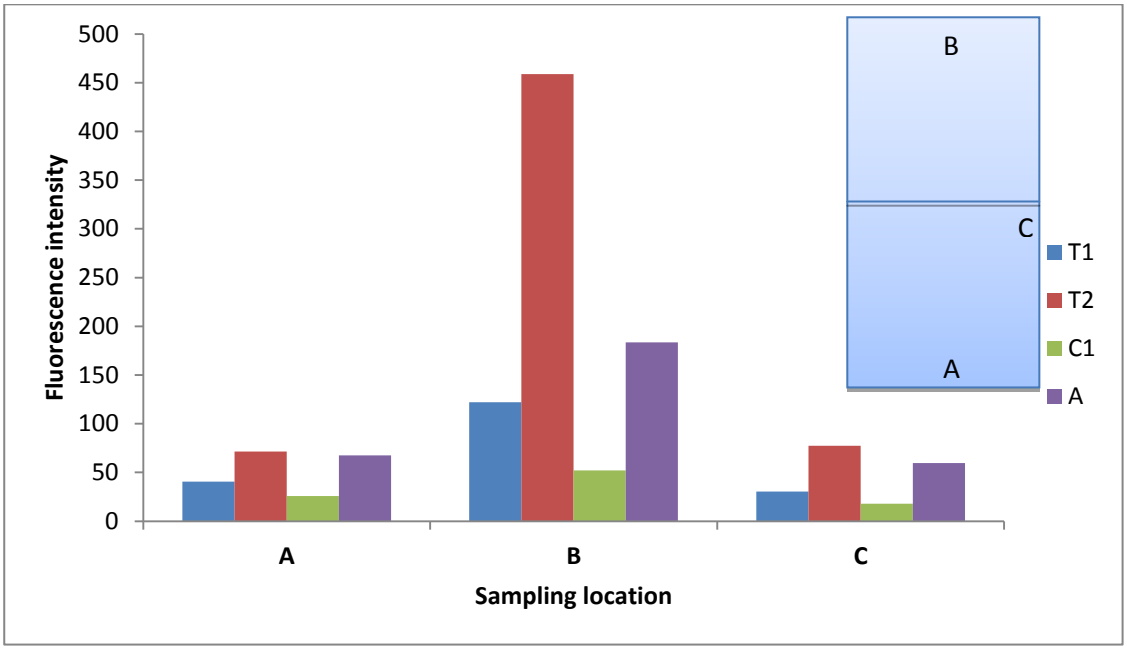


Figure 8.4 Comparison of fluorescence intensity from three locations around the pool for peaks T_1, T_2, C_1 and A. Figure inset shows the location of water samples collected around the pool

For samples collected at Location A which was closest to where freshly disinfected water is discharged from, the EEMs spectra showed overall fluorescence intensity to be low. At the sampling point at the deepest end of the pool (B), higher fluorescence intensity was observed compared to samples taken at Location A. This is possibly due to the accumulation of DOM as pool water at that point was at the furthest end to the treatment plant and water in that region would be disinfected at a slower rate. Pool water was collected into the drainage system at Location C for treatment. At this sampling point, the fluorescence intensity was similar to the intensity at Location A.

This is possibly due to the frequent flow of fresh pool water resulting in the dilution of DOM in that area. These results show that there is a high degree of DOM variability within a swimming pool itself depending on where samples were taken from. Generally, peak T_2 was observed to have the highest fluorescence intensity and the most significant variability compared to the other peaks at all three sampling locations. Furthermore, the ratios of peak T_2 when compared to the other peaks were much greater (Figure 8.5).

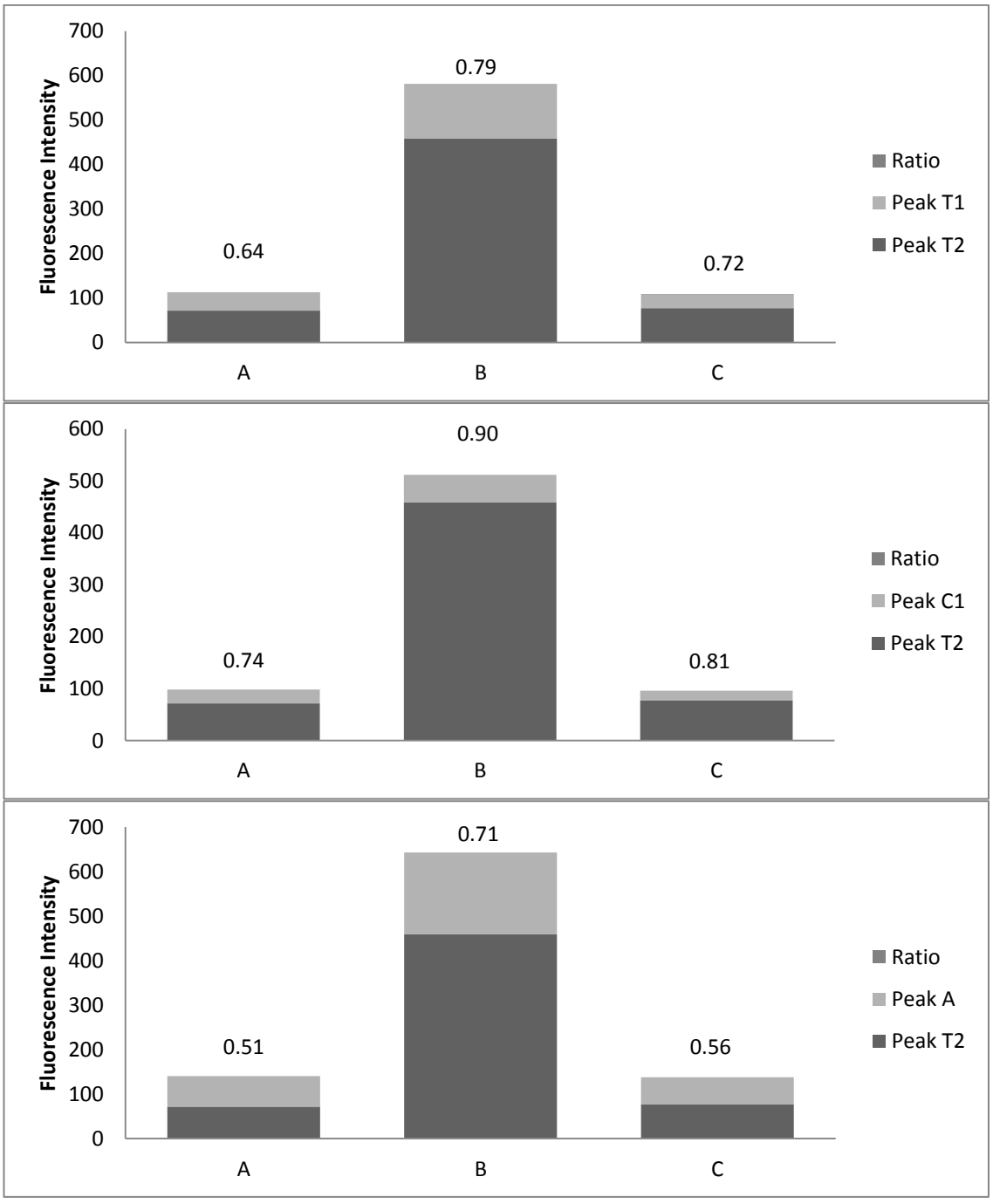


Figure 8.5 Comparison of ratios between peak T_2 to peaks T_1, C_1 and A

8.3.3 Correlations between fluorescence peaks and bather-derived DOM

Raw EEMs of swimming pool water samples with urine addition (Figure 8.6) show that the fluorescence spectrum is similar to the EEMs of swimming pool water (Figure 8.3) with higher fluorescence intensities occurring at the protein-like regions.

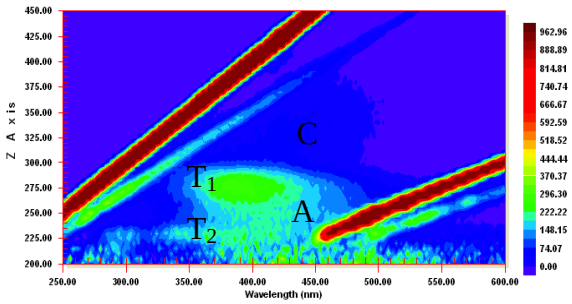
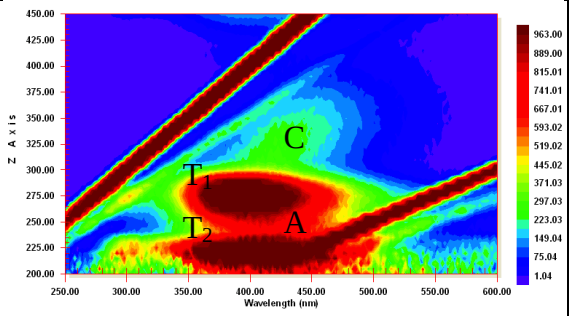
Pool water + 0.017% urine	Pool water + 0.033% urine
	

Figure 8.6 EEMs of swimming pool water samples with urine additions

The fluorescence EEMs show that the fluorescence intensity increases as the concentrations of urine increases in swimming pool water samples. The results from the laboratory experiments carried out to investigate the relationship between the various fluorescence peaks and human-derived body fluids (urine) are presented in Figure 8.7.

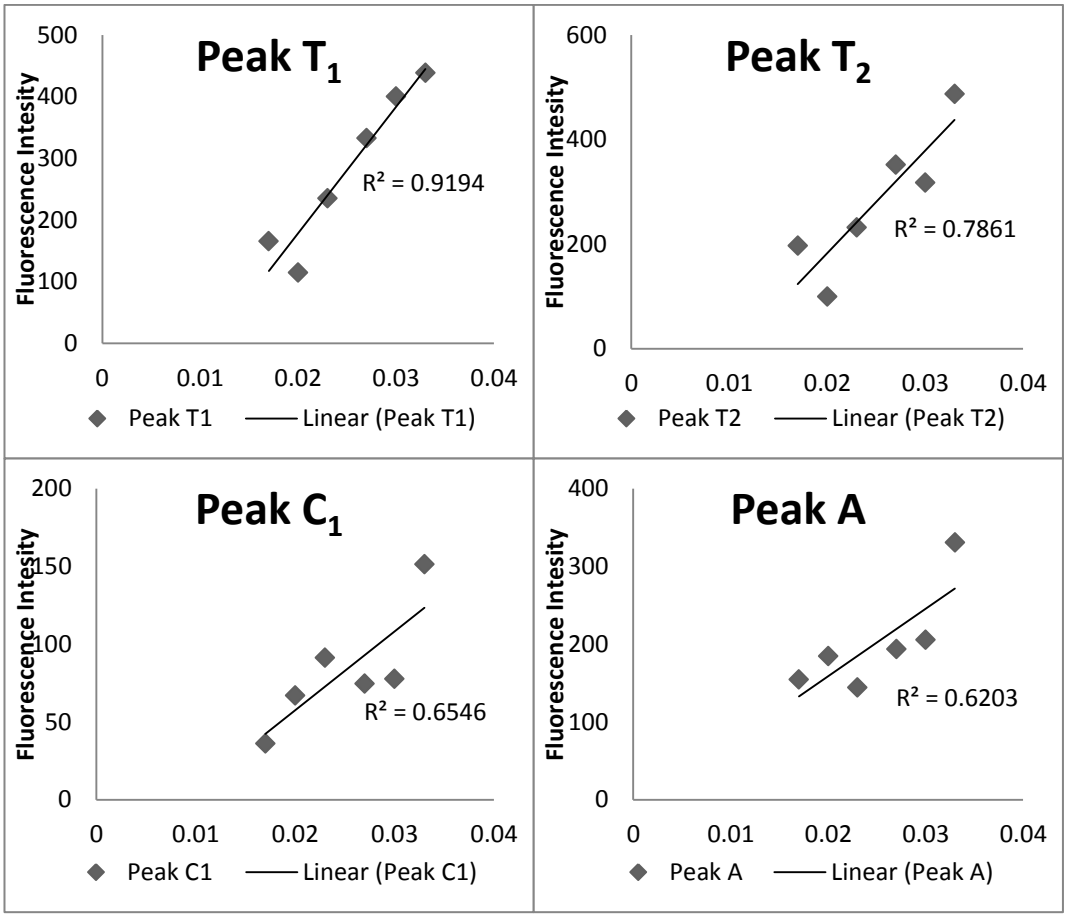


Figure 8.7 Correlation between fluorescence intensity and urine concentrations

It is observed that Peak T_1 showed the highest correlation (R^2 = 0.9194) between urine concentrations compared to peaks T_2, C_1 and A. Peak A had the lowest correlation with urine concentrations (R^2 = 0.6203). These results show that fluorescence monitoring of protein-like peaks is possible for monitoring the variability of bather-derived DOM in swimming pools. These measurements can potentially provide accurate indication of human-related contamination and the overall water quality in swimming pools.

8.4 Conclusions

The use of fluorescence provides a potentially fast, reliable and inexpensive method of monitoring the quality of swimming pool water. Protein-like peaks having an excitation and emission range of 225-285 nm and 300-350 nm respectively are likely the most suitable peaks for monitoring swimming pool water quality as they have been associated with anthropogenic DOM and microbial origins. As organic loadings from

swimmers are constantly introduced into the swimming pools, these peaks are likely the most relevant for monitoring. Furthermore, experiments have shown that the fluorescence intensities of protein-like peaks have a high correlation to anthropogenic organic loadings. From these results, it is apparent that the use of an online fluorimeter to monitor specific wavelengths focussing on the protein-like peaks has considerable potential to provide further insights to the possible use of online monitoring tools to measure water quality in swimming pools.

CHAPTER 9 RISK ASSESSMENT OF CHEMICALS IN SWIMMING POOLS

9.1 Introduction

In this chapter, a general risk assessment was undertaken using the Australian EnHealth Environmental Health Risk Assessment Guidelines (EnHealth, 2012b) to quantitatively assess risks to human health from exposure to chemical contaminants in swimming pools found in this study. According to EnHealth (2012b), 'a risk assessment provides a systematic approach for characterising the nature and magnitude of the risks associated with environmental health hazards'. The main aim of carrying out a quantitative risk assessment is to provide more information on possible risks which would enable suitable steps to be undertaken in order to minimise those risks.

The key components of a risk assessment provided by the EnHealth Council guidelines consist of issue identification, hazard assessment, exposure assessment, risk characterization and risk management. The relationship between each of these five components is shown in Figure 9.1.

The outcome of this risk assessment would help to indicate whether remedial measures are warranted to reduce the risk of exposure to chemical contaminants in swimming pools.

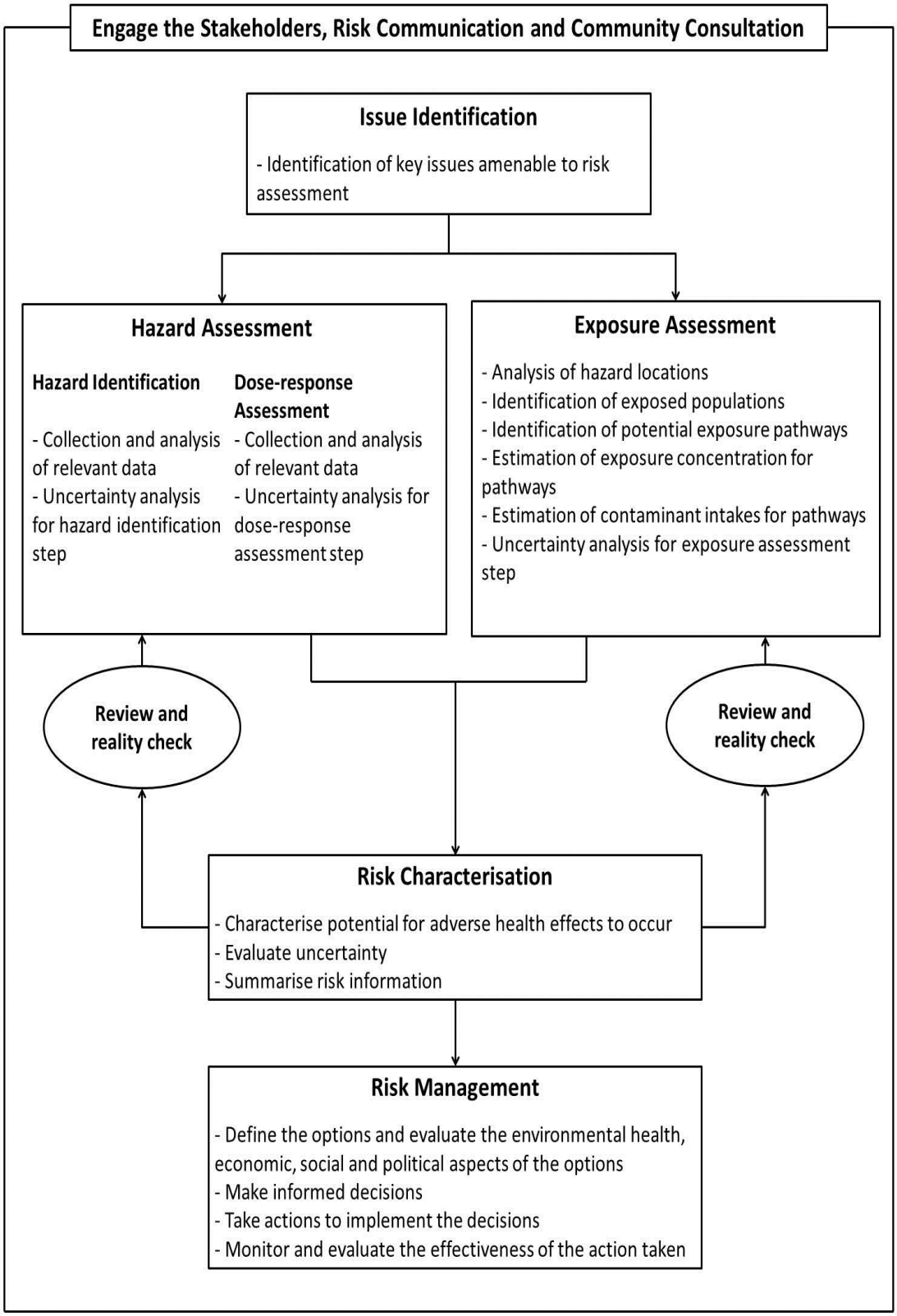


Figure 9.1 Risk assessment model (EnHealth, 2012b)

9.2 Issue identification

Various chemical contaminants have been identified in swimming pools including DBPs and personal care products (sunscreens and parabens) (Teo *et al.*, 2015). The constant recirculation and disinfection of swimming pool water may lead to the accumulation of unwanted chemicals in the pool. The input from bather-derived sources such as through bodily excretions and washing-off of cosmetics and lotions further contribute to the chemical contaminants occurring in swimming pools. The disinfection process of swimming pool waters may further produce by-products from these chemicals. Some of these chemicals may potentially have an adverse effect on human health.

Disinfection practices to maintain swimming pool water quality is mainly undertaken to prevent the transmission of diseases. Currently, there are no specific guidelines implemented to minimise the concentrations of chemical contaminants in swimming pools and control measures may need to be explored to manage swimming pool water quality from a chemical perspective. Although human health risks due to exposure to chemical contaminants in environmental waters have previously been addressed, not many have been conducted for swimming pool waters.

9.3 Hazard identification

The work in this thesis has led to the identification of a number of chemical contaminants occurring in swimming pools. The chemicals that have been identified in this study include of pharmaceuticals (caffeine and ibuprofen), PFRs (TNBP, TCEP, TCIPP, TDCIPP and TPHP) and *N*-nitrosamines (NDMA, NDEA and NMor). *N*-nitrosamines are known to be potentially carcinogenic (Lijinsky and Epstein, 1970). PFRs are also suspected carcinogens (Van der Veen and de Boer, 2012) and have been shown to exhibit endocrine disruption (Liu *et al.*, 2012a; Zhang *et al.*, 2014). It has been reported that TPHP and TDCIPP might be associated with altered thyroid levels and reduced semen quality in men (Meeker and Stapleton, 2010).

The pharmaceuticals and PFRs detected are most likely occurring within the swimming pools as they were not detected in fill water samples. Caffeine and ibuprofen are speculated to originate from bather-related sources (body fluid excretions via accidental

urinary excretions) while commonly used swimming equipment such as swimsuits were found to contribute to the concentrations of PFRs in swimming pools. *N*-nitrosamines, on the other hand, are most likely formed through the reactions of the disinfectants used and organic matter in the pool.

The quantitative risk assessment carried out in this chapter will evaluate the risk of exposure for all the chemicals that were analysed in this study and will only focus on the exposure assessment of accidental ingestion and dermal absorption as there is insufficient data on chemical volatilisation to account for inhalation exposure.

9.4 Dose-response assessment

A dose-response assessment was carried out to determine the relationships between adverse health effects of exposure. The non-cancer dose response was based on a reference dose (RfD) which is "an estimate of daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime" (EnHealth, 2012b). Exposure doses less than the RfD are generally considered to have no significant adverse health effect. RfDs used in this study are presented in Table 9.1.

Table 9.1 Reference dose (RfDs) for PFRs and PPCPs

Target compounds	Oral RfDs (mg/kg/d)	Reference
PFRs[a]		
TNBP	2.4	
TCEP	2.2	
TCIPP	8.0	
TDCIPP	1.5	
TPHP	7.0	
PPCPs		
Amitriptyline	N/A	
Atenolol	3.E-03	Snyder *et al.* (2008)
Caffeine	1.E-02	*
Carbamazepine	1.E-02	Snyder *et al.* (2008)
Clozapine	N/A	
DEET	7.E-02	*
Diazepam	1.E-03	Snyder *et al.* (2008)
Dilantin	N/A	
Enalapril	2.E-04	Snyder *et al.* (2008)
Fluoxetine	1.E-03	Snyder *et al.* (2008)

Hydroxyzine	N/A	
Ibuprofen	1.E-01	Schwab *et al.* (2005)
Meprobamate	8.E-03	Snyder *et al.* (2008)
Metformin	6.E-02	Schwab *et al.* (2005)
Methotrexate	N/A	
Omeprazole	N/A	
Paracetamol	5.E+00	*
Primidone	N/A	
Risperidone	1.E-05	Snyder (2008)
Sulfamethoxazole	1.E-01	Snyder (2008)
Triamterene	N/A	
Trimethoprim	1.E-01	Snyder (2008)
Verapamil	N/A	
Bisphenol A	5.E-02	US EPA (2012)
Diclofenac	7.E-02	Snyder *et al.* (2008)
Gemfibrozil	3.E-02	Snyder *et al.* (2008)
Ketoprofen	1.E-01	*
Naproxen	6.E-01	Snyder *et al.* (2008)
Nonylphenol	2.E+01	*
Propylparaben	N/A	
Simvastatin	5.E-04	Snyder (2008)
Simvastatin hydroxy acid	5.E-04	Snyder (2008)
Triclocarban	N/A	
Triclosan	1.E-02	Snyder (2008)

[a] RfDs for PFRs were taken from Van der Veen and de Boer (2012)
* RfDs were estimated based on guideline values provided by the Australian Guidelines for Water Recycling – Phase 2 (2008)
N/A not available

For carcinogenic chemicals, a cancer slope factor is used as a reference value to determine carcinogenic effect which is defined as "the plausible upper-bound estimate of the probability of a carcinogenic response per unit of intake over a lifetime" (EnHealth, 2012b). Cancer slope factors for *N*-nitrosamines are presented in Table 9.2.

Table 9.2 Slope factors for *N*-nitrosamines

N-nitrosamines:	Cancer slope factor[a] $(mg/kg/day)^{-1}$
NDMA	51
NMEA	22
NDEA	150
NDPA	7
NPyr	2.1
NDBuA	5.4

[a] Cancer slope factors were obtained from the IRIS database (US EPA, 2012)

9.5 Exposure assessment

The exposure assessment estimates the amount of intake of a chemical through specific exposure routes. Swimmers may be exposed to chemical contaminants in swimming pools via a variety of exposure routes including accidental ingestion of water, inhalation of volatile compounds and dermal absorption. The exposure to chemicals in swimming pools is dependent on body weight, body surface area, age and other specific variables depending on the route of exposure. A wide range of proposed default values for chemical exposure during swimming can be found from the EnHealth Guidelines and from the US EPA Exposure Factors Handbook. The default values used for exposure estimation in this study are presented in Table 9.3.

Table 9.3 Recommended default values for exposure estimation

Ingestion rate (L/hr)	IR	0.025	US EPA (2011)
Exposure frequency (d/yr)	EF	52	EnHealth (2012a)
Exposure duration (yr)	ED	70	US EPA (2011)
Exposure time (hr/d)	ET	1.5	EnHealth (2012b)
Event frequency (event/d)	EF	1	US EPA (2011)
Body weight (kg)	BW	70	US EPA (2011)
Skin surface area (cm^2)	SA	20,000	EnHealth (2012b)
Averaging time (d) (ED x 365 d/y)	AT	25,550	
Absorbed dose per event (mg/cm^2/event)	DA_{event}	chemical-specific	

The concentrations of chemicals used in this risk assessment study are presented in Table 9.4. The maximum concentrations of each compound measured in swimming pools were chosen as a representative value to evaluate for a worst-case scenario for the chemicals that were detected. The LOQs were used for the other chemicals that were analysed but were below the LOQs in swimming pools.

Table 9.4 Concentrations of chemicals used in risk assessment

Target compounds	Concentration (mg/L)
PFRs	
TNBP	3.E-05
TCEP	3.E-04
TCIPP	1.E-03
TDCIPP	7.E-04
TPHP	1.E-04
N-nitrosamines	
NDMA	9.E-06
NDEA	2.E-06
NMor	1.E-04
NMEA	<1.E-06
NDPA	<1.E-06
NPyr	<1.E-06
NDBuA	<1.E-06
PPCPs:	
Amitriptyline	<5.E-06
Atenolol	<5.E-06
Caffeine	2.E-03
Carbamazepine	<5.E-06
Clozapine	<5.E-06
Diazepam	<5.E-06
Dilantin	<5.E-06
Enalapril	<5.E-06
Fluoxetine	<5.E-06
Hydroxyzine	<5.E-06
Ibuprofen	8.E-05
Meprobamate	<5.E-06
Omeprazole	<5.E-06
Paracetamol	<5.E-06
Primidone	<5.E-06
Risperidone	<5.E-06
Sulfamethoxazole	<5.E-06
Triamterene	<5.E-06
Trimethoprim	<5.E-06

Verapamil	<5.E-06
Bisphenol A	<2.E-05
Gemfibrozil	<5.E-06
Ketoprofen	<1.E-05
Naproxen	<5.E-06
Nonylphenol	<1.E-05
Propylparaben	<1.E-05
Simvastatin	<5.E-06
Simvastatin hydroxy acid	<5.E-06
Triclocarban	<1.E-05
Triclosan	<5.E-06

Accidental ingestion:

The factors affecting the amount of water ingested include demographics, skills, experience and type of activity carried out in the pool. The accidental ingestion exposure can be calculated from Equation 9.1 as provided by the EnHealth Guidelines.

Equation 9.1 Calculation of exposure to chemicals through ingestion during swimming

$$I = \frac{C \times IR \times EF \times ED}{AT \times BW}$$

I = Intake of chemical (mg/kg/day)

C = Average of chemical concentration in swimming pools (mg/L)

IR = Ingestion rate (L/day)

EF = Exposure frequency (days/year)

ED = Exposure duration (years)

AT = Averaging time period over which the exposure is averaged (days)

BW = Body weight (kg)

Dermal absorption:

The dermal absorption exposure during swimming is dependent on several factors including the length of time in contact with water, water temperature and chemical concentration. For swimming scenarios, the US EPA recommends 100% exposure of

the skin surface. Thus, the total skin surface area available for contact during swimming is assumed to be 20,000 cm^2 for adults (EnHealth, 2012b). The dermal absorption of chemical contaminants in swimming pools can be estimated using Equation 9.2:

Equation 9.2 Calculation of dermal exposure to chemicals in swimming pools

$$I = \frac{DA_{event} \times SA \times EV \times EF \times ED}{AT \times BW}$$

I = Intake of chemical (mg/kg/day)

DA_{event} = dose absorbed per event (mg/cm^2/event)

SA = surface areas of skin exposed (cm^2)

EV = Event frequency (events/day)

EF = Exposure frequency (days/year)

ED = Exposure duration (years)

The calculations for dermal absorption were carried out using Microsoft Excel spreadsheets provided by the US EPA (2004). The DA_{event}, which is chemical specific, was estimated based on the dermal permeability (K*p*) for each compound (Table 9.5). The K*p* was predicted from the Log D and molecular weight of each compound which was presented in Table 3.1 (Chapter 3). Log D is the distribution constant which is a measure of the lipophilicity of a compound and is pH dependent. In this risk assessment, Log D was determined at pH 7, similar to the pH maintained in swimming pool waters.

Table 9.5 Dermal permeability (K*p*) of target compounds used in the risk assessment

Target compounds	**K*p* (cm/hr)**
PFRs	
TNBP	2.E-02
TCEP	4.E-04
TCIPP	1.E-03
TDCIPP	9.E-04
TPHP	3.E-02
N-nitrosamines	
NDMA	3.E-04
NDEA	9.E-04
NMor	1.E-04
NMEA	5.E-04
NDPA	3.E-03
NPyr	4.E-04
NDBuA	1.E-02
PPCPs:	
Amitriptyline	1.E-03
Atenolol	2.E-06
Caffeine	5.E-05
Carbamazepine	1.E-03
Clozapine	3.E-03
Diazepam	3.E-03
Dilantin	5.E-04
Enalapril	1.E-05
Fluoxetine	2.E-04
Hydroxyzine	3.E-04
Ibuprofen	5.E-04
Meprobamate	3.E-04
Omeprazole	7.E-04
Paracetamol	5.E-04
Primidone	3.E-04
Risperidone	8.E-05
Sulfamethoxazole	4.E-05
Triamterene	3.E-04
Trimethoprim	6.E-05
Verapamil	7.E-05
Bisphenol A	2.E-02
Gemfibrozil	1.E-03
Ketoprofen	8.E-05
Naproxen	2.E-04
Nonylphenol	1.E+00
Propylparaben	1.E-02

Simvastatin	1.E-02
Simvastatin hydroxy acid	9.E-05
Triclocarban	3.E-01
Triclosan	1.E-01

The oral and dermal exposure values during swimming for chemicals investigated in this study are summarised in Table 9.6. The results show that the predicted exposure to the target chemicals in swimming pools was significantly lower than the RfDs. This indicates that these chemicals present a generally low health risk to human health during swimming. The estimated exposure to chemicals in swimming pools was roughly similar between oral exposure and dermal absorption. Overall, caffeine and TCIPP presented the highest level of exposure with an estimated intake of 2.E-06 mg/kg/d.

Table 9.6 Oral and dermal exposure quantitation for chemicals analysed in swimming pools

Chemical compounds			**Oral exposure (mg/kg/d)**	**Dermal absorption (mg/kg/d)**	**Total exposure (mg/kg/d)**
Chemicals detected above the LOQs	PFRs	TNBP	3.E-08	5.E-08	8.E-08
		TCEP	4.E-07	1.E-08	4.E-07
		TCIPP	1.E-06	2.E-07	2.E-06
		TDCIPP	8.E-07	7.E-08	9.E-07
		TPHP	2.E-07	3.E-07	5.E-07
	N-nitrosamines	NDMA	1.E-08	4.E-10	1.E-08
		NDEA	2.E-09	4.E-10	3.E-09
		NMor	1.E-07	4.E-09	1.E-07
	PPCPs	Caffeine	2.E-06	9.E-09	2.E-06
		Ibuprofen	1.E-07	4.E-09	1.E-07
Chemicals below the LOQs	PPCPs	Amitriptyline	<6.E-09	<8.E-10	<7.E-09
		Atenolol	<6.E-09	<1.E-12	<6.E-09
		Carbamazepine	<6.E-09	<8.E-10	<7.E-09
		Clozapine	<6.E-09	<2.E-09	<8.E-09
		Diazepam	<6.E-09	<2.E-09	<8.E-09
		Dilantin	<6.E-09	<3.E-10	<6.E-09
		Enalapril	<6.E-09	<6.E-12	<6.E-09
		Fluoxetine	<6.E-09	<1.E-10	<6.E-09
		Hydroxyzine	<6.E-09	<2.E-10	<6.E-09
		Meprobamate	<6.E-09	<2.E-10	<6.E-09
		Omeprazole	<6.E-09	<4.E-10	<6.E-09
		Paracetamol	<6.E-09	<3.E-10	<6.E-09
		Primidone	<6.E-09	<2.E-10	<6.E-09
		Risperidone	<6.E-09	<5.E-11	<6.E-09
		Sulfamethoxazole	<6.E-09	<2.E-11	<6.E-09
		Triamterene	<6.E-09	<2.E-10	<6.E-09
		Trimethoprim	<6.E-09	<3.E-11	<6.E-09
		Verapamil	<6.E-09	<4.E-11	<6.E-09
		Bisphenol A	<2.E-08	<4.E-08	<7.E-08
		Gemfibrozil	<6.E-09	<9.E-10	<7.E-09
		Ketoprofen	<1.E-08	<9.E-11	<1.E-08
		Naproxen	<6.E-09	<1.E-10	<6.E-09
		Nonylphenol	<1.E-08	<2.E-07	<2.E-07
		Propylparaben	<1.E-08	<1.E-08	<3.E-08
		Simvastatin	<6.E-09	<5.E-09	<1.E-08
		Simvastatin hydroxy acid	<6.E-09	<5.E-11	<6.E-09
		Triclocarban	<1.E-08	<1.E-07	<1.E-07
		Triclosan	<6.E-09	<4.E-08	<5.E-08

	N-nitrosamines	NMEA	<1.E-09	<6.E-11	<1.E-09
		NDPA	<1.E-09	<8.E-10	<2.E-09
		NPyr	<1.E-09	<4.E-11	<1.E-09
		NDBuA	<1.E-09	<3.E-09	<4.E-09

9.6 Risk characterisation

Risk characterisation was carried out to quantify risks posed by the chemicals in swimming pools. For the non-cancer risk compounds, a hazard quotient (HQ) is calculated for the risk assessment. Risk assessment for carcinogenic compounds was calculated using the cancer slope factor to determine the increased cancer risk over a person's lifetime. The exposure doses used for calculating the hazard quotients and cancer risk were taken as the sum of exposure from the oral and dermal routes for each chemical compound. The risk assessment for non-cancer risks was determined by the calculation of a HQ using Equation 9.3.

Equation 9.3 Calculation of hazard quotients (HQ) for non-cancer risks

$$\text{Hazard Quotient (HQ)} = \frac{\text{Total exposure dose (mg/kg/day)}}{\text{RfD (mg/kg/day)}}$$

When the calculated HQ is less than 1, the exposure to the chemical compound is assumed to be at a safe level (US EPA, 2012).

The risk associated with exposure to carcinogens was determined using Equation 9.4.

Equation 9.4 Calculation of risk from exposure to carcinogenic chemicals

$$\text{Risk (R)} = \text{Cancer slope factor (mg/kg/day)}^{-1} \times \text{Exposure dose (mg/kg/day)}$$

The calculated risk is excess lifetime cancer risk. Generally, calculated risk values less than 1 in a million (10^{-6}) are considered acceptable as commonly used conservative international benchmark (US EPA, 2012).

The hazard quotients from PFRs and PPCPs measured in swimming pools are presented in Table 9.7.

Table 9.7 Hazard quotients (HQ) from PFRs and PPCPs in swimming pools

Target compounds	**HQ**
PFRs	
TNBP	3.E-08
TCEP	2.E-07
TCIPP	2.E-07
TDCIP	6.E-07
TPHP	7.E-08
PPCPs	
Amitriptyline	*
Atenolol	<2.E-06
Caffeine	2.E-04
Carbamazepine	<7.E-07
Clozapine	*
Diazepam	<8.E-06
Dilantin	*
Enalapril	<3.E-05
Fluoxetine	<6.E-06
Hydroxyzine	*
Ibuprofen	1.E-06
Meprobamate	<8.E-07
Omeprazole	*
Paracetamol	<1.E-09
Primidone	*
Risperidone	<4.E-04
Sulfamethoxazole	<5.E-08
Triamterene	*
Trimethoprim	<6.E-08
Verapamil	*
Bisphenol A	<1.E-06
Gemfibrozil	<2.E-07
Ketoprofen	<1.E-07
Naproxen	<1.E-08
Nonylphenol	<1.E-08
Propylparaben	*
Simvastatin	<2.E-05
Simvastatin hydroxy acid	<1.E-05
Triclocarban	*
Triclosan	<4.E-06

* RfDs were not available

Exposure to the chemicals analysed in this study were determined to have calculated hazard quotients of less than 1. This indicates that health risks from exposure to these chemicals are low.

The overall exposure to PFRs in swimming pools in this study were considerably lower (about four orders of magnitude lower) than the exposure to PFRs in indoor house dust as it was reported that the adult daily intake through ingestion was 5.E10-2 mg/kg/d for worst case scenarios (Van der Veen and de Boer, 2012). In another study, the HQ estimated for the ingestion of house dust was generally higher than the HQ estimated in this study with one PFR compound (TBOEP) exceeding the benchmark value of 1 (Mizouchi *et al.*, 2015). This indicates that swimming pools may not be a significant source of exposure to PFRs compared to other PFR sources. Furthermore, as PFRs have low Henry's Law constants (Table 3.1, Chapter 3), volatilization of these compounds from the water phase into air is negligible. However, as some PFRs have been detected in significant concentrations in the air especially TNBP, TCEP and TCIPP (Marklund *et al.*, 2005a; Reemtsma *et al.*, 2008), human exposure from air inhalation within a swimming pool facility from other sources may be of more significance and should be further investigated.

It has been reported that PFRs may have antiestrogenic and estrogenic effects at environmental matrix levels (Zhang *et al.*, 2014). Although the exposure levels for PFRs in swimming pools are shown to be at a safe level, these compounds could potentially cause endocrine-disrupting effects to swimmers. Future risk assessments may need to be conducted to assess the potential estrogenic effects of PFRs in swimming pools.

The estimated lifetime cancer risk from exposure to *N*-nitrosamines in swimming pools is presented in Table 9.8.

Table 9.8 Calculated risk from exposure to *N*-nitrosamines in swimming pools

***N*-nitrosamines**	**Risk**
NDMA	6.E-07
NDEA	4.E-07
NMor	1.E-06
NMEA	<3.E-08
NDPA	<1.E-08

NPyr	<3.E-09
NDBuA	<2.E-08

The calculated lifetime cancer risk for *N*-nitrosamines exposure from swimming was below the benchmark value (10^{-6}) of negligible risk defined by the US EPA. This indicates that the cancer risk to *N*-nitrosamines exposure in swimming pools was at an acceptable or 'tolerable' level. For some compounds, specifically NDMA, NDEA and NMor, the risk level was close or approaching the benchmark value thus, caution and further monitoring of these compounds in swimming pools may be appropriate. Furthermore, the contribution from the inhalation pathway is currently unknown. However, taking into account that the values used in this risk assessment were highly conservative, the health risk to these compounds is likely to be overestimated and are only indicative.

The cancer risks estimated for *N*-nitrosamines in this study were lower than the cancer risk estimated for other DBPs occurring in swimming pools with one study reporting cancer risk to THMs between 8.E10-4–1.E10-3 from inhalation exposure during swimming (Lee *et al.*, 2009). Another study reported cancer risk of swimmers to THM exposure between 8.E10-4–2.E10-3 (Panyakapo *et al.*, 2008). This suggests that other DBPs may be more of a significant cancer risk compared to *N*-nitrosamines. However, as *N*-nitrosamines are volatile compounds, the risk from exposure through the inhalation route may be of more significance and should be further investigated.

9.7 Conclusions

The results of this quantitative risk assessment revealed that exposure to PFRs, PPCPs and *N*-nitrosamines in swimming pools generally pose a very low health risk to swimmers and are below commonly applied health risk benchmarks. The risk assessment showed that the oral route of exposure to chemicals present a slightly higher level of exposure compared to the dermal route. It is important to note that some of the assumptions used in this risk assessment are for worst-case exposure scenarios and are highly conservative, thus the potential adverse human health effects are likely to be overestimated. Also, the concentration values used in this risk assessment were based on one swimming pool which may not be representative of the exposure of a wider

range of swimming pools. Inhalation exposure routes to chemicals in swimming pools may be of more significance to human health which may warrant further investigations.

The health risks from the exposure to chemical contaminants in swimming pools can be minimised by reducing the amount of contaminants entering the pool. This can be achieved by implementing control measures as discussed in Section 2.6 (Chapter 2). The risk of exposure to chemical contaminants in swimming pools can be further lowered through the use of fluorescence as an online monitoring tool to monitor chemicals in swimming pools. This will provide real-time data on the water quality of swimming pools. Corrective measures can then be undertaken immediately should the need arise to improve swimming pool water quality.

CHAPTER 10 CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

The aim of this research was to investigate the concentrations of chemical contaminants in swimming pools and to provide a simple risk assessment corresponding to the chemicals detected. This will provide important information regarding the quality of swimming pool waters.

Specific outcomes of this research are summarised below.

1. A comprehensive review of the literature showed that most of the existing research on chemical contaminants in swimming pools has focused on the occurrence of DBPs. The presence and concentrations of these chemical contaminants are dependent upon several factors including the types of pools, types of disinfectants used, disinfectant dosages, bather loads, temperature and pH of swimming pool waters. Chemical constituents of personal care products such as parabens and UV filters from sunscreens have also been reported. The by-products from reactions of these chemicals with disinfectants and UV irradiation have been reported and some may be more toxic than their parent compounds. Preventive measures can be implemented to remove not only DBPs but other chemicals occurring in swimming pools. Effective ways to reduce the concentrations of chemical contaminants could be by increasing public awareness and improving the hygiene of swimmers by implementing a shower rule prior to entering the pool. Alternative and emerging treatment methods such as activated carbon filtration and advanced oxidation processes may also improve swimming pool water quality.

2. A range of municipal wastewater analytical methods are adaptable for the analysis of swimming pool water. Swimming pool water was successfully analysed for a range of PPCPs using LC-MS/MS and *N*-nitrosamines using GC-MS/MS. Fluorescence EEMs analysis was also successfully adapted to analyse swimming pool water.

3. A rapid and reliable analytical method was developed for the analysis of five PFRs in various environmental waters using GC-MS/MS. The PFRs investigated were

TNBP, TCEP, TCIPP, TDCIPP and TPHP. The use of isotopically labelled compounds for each PFR ensured accurate quantification and accounted for analytical variabilities which may have been introduced during sample preparation and instrumental analysis. Method recoveries for all compounds were above 80% in all tested water samples. Method detection limits for all target analytes ranged from 0.3 – 24 ng/L in ultrapure water, tap water, seawater, surface water, secondary treated wastewater and swimming pool water. Validation of this method confirmed satisfactory method stability with less than 1% of coefficients of variation verifying that this approach produced good reproducibility for the analysis of environmental water samples.

4. From the investigation on the occurrence of PFRs in swimming pools, five PFRs namely TNBP, TCEP, TCIPP, TDCIPP and TPHP were detected in swimming pool waters with concentrations ranging from <5 – 27 ng/L (TNBP), <5 – 293 ng/L (TCEP), <50 – 1180 ng/L (TCIPP), <5 – 670 ng/L (TDCIPP) and <5 – 132 ng/L (TPHP). The concentrations of PFRs were generally higher in indoor swimming pools compared to outdoor swimming pools. In municipal water supplies used to fill the swimming pools, the five PFRs were all below the limit of quantifications eliminating this as the source. Potential leaching of PFRs from commonly used swimming equipment, including newly purchased kickboards and swimsuits was investigated. These experiments revealed that PFRs leached from swimsuits, indicating that swimming suits may be may be one of the many potential sources of PFR contamination in swimming pools.

5. Swimming pool water samples from chlorinated and seawater pools were analysed for 30 PPCPs. Results showed that all PPCPs were below the limits of quantification in the seawater pools. However, caffeine and ibuprofen were consistently detectable within the chlorinated swimming pools. Caffeine was detected in 12 chlorinated swimming pools at concentrations up to 1540 ng/L and ibuprofen was observed in 7 chlorinated pools at concentrations up to 83 ng/L. Caffeine and ibuprofen concentrations were below limits of quantification in all fill water samples, eliminating this as the source in swimming pools. The occurrence of these two compounds in swimming pools was attributed to swimmers' excretion of body

fluids such as accidental urinary excretion or sweat. High variations in caffeine concentrations monitored throughout the day roughly reflect bather loads in swimming pools and are likely to be markers of accidental urinary excretions during swimming.

6. From the analysis of *N*-nitrosamines by isotope dilution GC-MS/MS, three *N*-nitrosamines, NDMA, NDEA and NMor, were detected in indoor chlorinated swimming pools but were below the LOQs in fill water samples. *N*-nitrosamines were not detected in any of the outdoor pools and seawater pools which were attributed to UV degradation by sunlight. Furthermore, for seawater pools, the lack of disinfectants used and the high bromide content inhibited the formation of *N*-nitrosamines. UV disinfection used in swimming pools may be effective in reducing the concentrations of *N*-nitrosamines depending on the amount of precursors and applied UV dosage.

7. Fluorescence monitoring can be used as a quick and reliable method to monitor the water quality of swimming pool water. The monitoring of protein-like peaks in swimming pools provided an indication of the amount of anthropogenic organic loadings in the pools. The results obtained in this study indicated that the use of an online fluorimeter to monitor specific peaks in swimming pools has considerable potential to provide real-time data of pool water quality.

8. The quantitative risk assessment indicated that the health risk through oral and dermal exposure to chemicals detected in swimming pools in this study were generally low and below commonly applied health risk benchmarks. Swimming pools are not likely a significant source of exposure to PPCPs, PFRs and *N*-nitrosamines.

10.2 Recommendations for future research

From this research, the following recommendations for future investigations have been made:

1. During the development of the PFRs analytical method, it was observed that TCIPP contamination in procedural blanks occurred frequently. Further research is required to determine the source of this contamination. This could be done by eliminating the use of any plastic materials during sample preparation such as the use of SPE plastic cartridges. Experiments could also be carried out to investigate if the laboratory water purification system is a source of PFRs. Furthermore, as TCIPP is one of the more volatile PFRs, experiments could be carried out to investigate if the background concentrations in procedural blanks can be reduced by exposing the water samples to sunlight before analysis, thereby reducing background contamination.

2. This study has investigated the occurrence of TNBP, TCEP, TCIPP, TDCIPP and TPHP in swimming pools. A wider range of PFRs may be occurring in swimming pools such as trimethyl phosphate, tris(2-butoxyethyl) phosphate, tris(2,3-dibromopropyl) phosphate and tris(2-ethylhexyl) phosphate. Further research is needed to identify if other PFRs are present in pool water as they have been shown to be potential endocrine disrupting compounds and may therefore pose a risk to human health. Further investigations into the possible sources of PFRs occurring in swimming pools could also be undertaken for example other plastic or rubber swimming devices used during swimming such as goggles or flippers. The significance of PFRs in indoor air as a source to PFRs in swimming pool water could also be evaluated. Factors that may affect the leaching of PFRs in swimming pools from swimsuits under different conditions such as temperature could also be explored. This would help to determine if preventive measures could be undertaken to reduce PFR concentrations in swimming pools.

3. Swimmers' bodily excretion can potentially be indicated by either caffeine or ibuprofen as these chemicals are likely to be markers of accidental urinary excretions during swimming. However, as demographic variability may influence the occurrence of caffeine in swimming pools, the use of caffeine as surrogate indicators may be limited to monitor adult demographics. Measurement of these chemicals has the potential to provide quantitative indications of the quantities of human excreted substances in the pool. Further research aimed at translating

swimming pool concentrations to swimmer numbers could provide an estimate of the level of contamination based on the number of swimmers using the pool which would assist in the daily management and operations of swimming pools water quality.

4. The disinfectants used in swimming pools may react with the chemicals identified in this study to produce by-products which would contribute to the growing list of chemicals already identified in swimming pools. Reaction studies could give a better insight on the fate of these chemicals in swimming pool water. These findings may lead to the further identification of a wider range of chemicals.

5. Experiments have shown that fluorescence is a useful tool for monitoring the changes in swimming pool water quality. Field monitoring studies could be conducted to assess the potential application of an online fluorescence tool to measure anthropogenically-derived organic matter in swimming pools. In addition, chemical analyses of swimming pool water could be carried out simultaneously. Ultimately the potential relationships between chemical concentrations detected and protein-like fluorescence intensities could then be investigated. The use of fluorescence to monitor trace organic contaminants as indicators for anthropogenic sources could then be assessed which has potential as an alternative analytical method for real-time swimming pool water quality monitoring. However, it is important to note that there may be other sources of proteins in swimming pools (and other chemicals with overlapping fluorescence signals). Understanding the reliability of this measurement (and potential sources of interference) is now recommended for further research.

6. The quantitative risk assessment indicated that the risk from oral and dermal exposure to chemicals in swimming pools were generally low. The Henry's Law constants for PFRs and PPCPs are low and thus volatilisation of these compounds from swimming pool water into the air is negligible. However, the Henry's law constants for *N*-nitrosamines are higher compared to PFRs and PPCPs and some are of four orders of magnitude differences which suggest potential volatilisation of these compounds from water into air. The contribution from air inhalation to human

exposure for these *N*-nitrosamines compounds during swimming could be more significant (as compared to PFRs and PPCPs) and should be investigated.

7. Health risk assessments are generally chemical-specific. The potential human health significance are based on individual compounds and thus do not account for the mixture of chemicals occurring in the water matrix. A more comprehensive risk assessment to evaluate the overall exposure of these chemicals could be carried out to improve the assessment of potential health risk to chemicals in swimming pools.

8. Alternative operation and management processes for swimming pools to minimise chemical contaminants while maintaining microbial safety could also be explored to improve swimming pool water quality.

References

Adin A, Katzhendler J, Alkaslassy D, Rav-Acha C. (1991) Trihalomethane formation in chlorinated drinking water: A kinetic model. Water Research 25 (7):797-805.

Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail (ANSES) (2012) *Évaluation des risques sanitaires liés aux piscines.*

Aggazzotti G, Fantuzzi G, Righi E, Predieri G. (1995) Environmental and biological monitoring of chloroform in indoor swimming pools. Journal of Chromatography A 710 (1):181-190.

Aggazzotti G, Fantuzzi G, Righi E, Predieri G. (1998) Blood and breath analyses as biological indicators of exposure to trihalomethanes in indoor swimming pools. Science of the Total Environment 217 (1-2):155-163.

Aggazzotti G, Predieri G. (1986) Survey of volatile halogenated organics (VHO) in Italy: Levels of VHO in drinking waters, surface waters and swimming pools. Water Research 20 (8):959-963.

Alcudia-León MC, Lucena R, Cárdenas S, Valcárcel M. (2013) Determination of parabens in waters by magnetically confined hydrophobic nanoparticle microextraction coupled to gas chromatography/mass spectrometry. Microchemical Journal 110 (0):643-648.

Ali N, Dirtu AC, Van den Eede N, Goosey E, Harrad S, Neels H, Coakley J, Douwes J, Covaci A. (2012) Occurrence of alternative flame retardants in indoor dust from New Zealand: indoor sources and human exposure assessment. Chemosphere 88 (11):1276-1282.

Alvarez-Rivera G, Llompart M, Garcia-Jares C, Lores M. (2014) Identification of halogenated photoproducts generated after ultraviolet-irradiation of parabens and benzoates in water containing chlorine by solid-phase microextraction and gas chromatography–mass spectrometry. Journal of Chromatography A 1349:105-115.

Andresen J, Grundmann A, Bester K. (2004) Organophosphorus flame retardants and plasticisers in surface waters. Science of the Total Environment 332 (1):155-166.

Asami M, Oya M, Kosaka K. (2009) A nationwide survey of NDMA in raw and drinking water in Japan. Science of the Total Environment 407 (11):3540-3545.

Bacaloni A, Cavaliere C, Foglia P, Nazzari M, Samperi R, Laganà A. (2007) Liquid chromatography/tandem mass spectrometry determination of organophosphorus flame retardants and plasticizers in drinking and surface waters. Rapid Communications in Mass Spectrometry 21 (7):1123-1130.

Bacaloni A, Cucci F, Guarino C, Nazzari M, Samperi R, Laganà A. (2008) Occurrence of organophosphorus flame retardant and plasticizers in three volcanic lakes of central Italy. Environmental Science & Technology 42 (6):1898-1903.

Baker A. (2001) Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. Environmental Science & Technology 35 (5):948-953.

Baker A, Inverarity R. (2004) Protein-like fluorescence intensity as a possible tool for determining river water quality. Hydrological Processes 18 (15):2927-2945.

Balmer ME, Buser H-R, Müller MD, Poiger T. (2005) Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environmental Science & Technology 39 (4):953-962.

Barbot E, Moulin P. (2008) Swimming pool water treatment by ultrafiltration–adsorption process. Journal of Membrane Science 314 (1):50-57.

Beech JA, Diaz R, Ordaz C, Palomeque B. (1980) Nitrates, chlorates and trihalomethanes in swimming pool water. American Journal of Public Health 70 (1):79-82.
Benoit FM, Jackson R. (1987) Trihalomethane formation in whirlpool spas. Water Research 21 (3):353-357.
Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science & Technology 43 (3):597-603.
Berg M, Müller SR, Mühlemann J, Wiedmer A, Schwarzenbach RP. (2000) Concentrations and mass fluxes of chloroacetic acids and trifluoroacetic acid in rain and natural waters in Switzerland. Environmental Science & Technology 34 (13):2675-2683.
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.
Blatchley III ER, Cheng M. (2010) Reaction mechanism for chlorination of urea. Environmental Science & Technology 44 (22):8529-8534.
BŁędzka D, Gryglik D, Olak M, Gębicki JL, Miller JS. (2010) Degradation of *n*-butylparaben and 4-*tert*-octylphenol in H_2O_2/UV system. Radiation Physics and Chemistry 79 (4):409-416.
Bollmann UE, Möller A, Xie Z, Ebinghaus R, Einax JW. (2012) Occurrence and fate of organophosphorus flame retardants and plasticizers in coastal and marine surface waters. Water Research 46 (2):531-538.
Bottoni P, Bonadonna L, Chirico M, Caroli S, Záray G. (2014) Emerging issues on degradation by-products deriving from personal care products and pharmaceuticals during disinfection processes of water used in swimming pools. Microchemical Journal 112:13-16.
Brandsma SH, de Boer J, Leonards PE, Cofino WP, Covaci A. (2013) Organophosphorus flame-retardant and plasticizer analysis, including recommendations from the first worldwide interlaboratory study. TrAC Trends in Analytical Chemistry 43:217-228.
Brausch JM, Rand GM. (2011) A review of personal care products in the aquatic environment: environmental concentrations and toxicity. Chemosphere 82 (11):1518-1532.
Buerge IJ, Poiger T, Müller MD, Buser HR. (2003) Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environmental Science & Technology 37 (4):691-700.
Bull RJ, Reckhow DA, Li X, Humpage AR, Joll C, Hrudey SE. (2011) Potential carcinogenic hazards of non-regulated disinfection by-products: Haloquinones, halo-cyclopentene and cyclohexene derivatives, *N*-halamines, halonitriles, and heterocyclic amines. Toxicology 286 (1):1-19.
Butt S, Christensen T. (2000) Toxicity and phototoxicity of chemical sun filters. Radiation Protection Dosimetry 91 (1-3):283-286.
Caliman FA, Gavrilescu M. (2009) Pharmaceuticals, personal care products and endocrine disrupting agents in the environment–a review. CLEAN–Soil, Air, Water 37 (4-5):277-303.
Camarasa JG, Serra-Baldrich E. (1992) Allergic contact dermatitis from triphenyl phosphate. Contact dermatitis 26 (4):264-265.

Cammann K, Hübner K. (1993) False results in headspace—gas chromatographic analysis of trihalomethanes in swimming pool water due to elevated headspace temperatures. Journal of Chromatography A 648 (1):294-298.
Cammann K, Hübner K. (1995) Trihalomethane concentrations in swimmers' and bath attendants' blood and urine after swimming or working in indoor swimming pools. Archives of Environmental Health: An International Journal 50 (1):61-65.
Canosa P, Rodríguez I, Rubi E, Negreira N, Cela R. (2006) Formation of halogenated by-products of parabens in chlorinated water. Analytica Chimica Acta 575 (1):106-113.
Cardador M, Gallego M. (2010) Optimisation and comparison of several microextraction/methylation methods for determining haloacetic acids in water using gas chromatography. Analytical and Bioanalytical Chemistry 396 (3):1331-1343.
Cardador M, Gallego M. (2011) Haloacetic acids in swimming pools: swimmer and worker exposure. Environmental Science & Technology 45 (13):5783-5790.
Caro J, Gallego M. (2007) Assessment of exposure of workers and swimmers to trihalomethanes in an indoor swimming pool. Environmental Science & Technology 41 (13):4793-4798.
Cassan D, Mercier B, Castex F, Rambaud A. (2006) Effects of medium-pressure UV lamps radiation on water quality in a chlorinated indoor swimming pool. Chemosphere 62 (9):1507-1513.
Catto C, Sabrina S, Ginette CT, Manuel R, Robert T. (2012) Occurrence and spatial and temporal variations of disinfection by-products in the water and air of two indoor swimming pools. International Journal of Environmental Research and Public Health 9 (8):2562-2586.
Chambon P, Taveau M, Morin M, Chambon R, Vial J. (1983) Survey of trihalomethane levels in Rhône-Alps water supplies. Estimates on the formation of chloroform in wastewater treatment plants and swimming pools. Water Research 17 (1):65-69.
Chen D, Letcher RJ, Chu S. (2012) Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography–tandem quadrupole mass spectrometry. Journal of Chromatography A 1220:169-174.
Chen K-C, Wang Y-H. (2012) Control of disinfection by-product formation using ozone-based advanced oxidation processes. Environmental Technology 33 (4):487-495.
Chen W, Westerhoff P, Leenheer JA, Booksh K. (2003) Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environmental Science & Technology 37 (24):5701-5710.
Chen Z, Yang L, Zhai X, Zhao S, Li A, Shen J. (2010) *N*-nitrosamine formation during chlorination/chloramination of bromide-containing water. Water Science & Technology: Water Supply 10:462-471.
Choi KI, Lee SH, Osako M. (2009) Leaching of brominated flame retardants from TV housing plastics in the presence of dissolved humic matter. Chemosphere 74 (3):460-466.
Chowdhury S, Al-Hooshani K, Karanfil T. (2014) Disinfection byproducts in swimming pool: occurrences, implications and future needs. Water Research 52 (2014):68-109.

Chu H, Nieuwenhuijsen M. (2002) Distribution and determinants of trihalomethane concentrations in indoor swimming pools. Occupational & Environmental Medicine 59 (4):243-247.
Coble PG. (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry 51 (4):325-346.
Couri D, Abdel-Rahman MS, Bull RJ. (1982) Toxicological effects of chlorine dioxide, chlorite and chlorate. Environmental Health Perspectives 46:13-17.
Cristale J, Quintana J, Chaler R, Ventura F, Lacorte S. (2012) Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water. Journal of Chromatography A 1241:1-12.
Cuderman P, Heath E. (2007) Determination of UV filters and antimicrobial agents in environmental water samples. Analytical and Bioanalytical Chemistry 387 (4):1343-1350.
Daniel FB, DeAngelo AB, Stober JA, Olson GR, Page NP. (1992) Hepatocarcinogenicity of chioral hydrate, 2-chloroacetaldehyde, and dichioroacetic acid in the male B6C3F_1 mouse. Toxicological Sciences 19 (2):159-168.
Díaz-Cruz MS, Barceló D. (2009) Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. TrAC Trends in Analytical Chemistry 28 (6):708-717.
Dishaw LV, Powers CM, Ryde IT, Roberts SC, Seidler FJ, Slotkin TA, Stapleton HM. (2011) Is the PentaBDE replacement, tris (1,3-dichloro-2-propyl) phosphate (TDCPP), a developmental neurotoxicant? Studies in PC12 cells. Toxicology and Applied Pharmacology 256 (3):281-289.
EnHealth. (2012a) Australian Exposure Factor Guidance: Guidelines for assessing human health risks from environmental hazards. Department of Health and Ageing and enHealth Council, Canberra.
EnHealth. (2012b) Environmental Health Risk Assessment: Guidelines for assessing human health risks from environmental hazards. Department of Health and Ageing and enHealth Council, Canberra.
Erdinger L, Kirsch F, Sonntag HG. (1997) Potassium as an indicator of anthropogenic contamination of swimming pool water. Zentralblatt fur Hygiene und Umweltmedizin 200 (4):297-308.
Erdinger L, Kühn KP, Kirsch F, Feldhues R, Fröbel T, Nohynek B, Gabrio T. (2004) Pathways of trihalomethane uptake in swimming pools. International Journal of Hygiene and Environmental Health 207 (6):571-575.
Esplugas S, Bila DM, Krause LGT, Dezotti M. (2007) Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. Journal of Hazardous Materials 149 (3):631-642.
Esteban S, Gorga M, Petrovic M, González-Alonso S, Barceló D, Valcárcel Y. (2014) Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. Science of the Total Environment 466:939-951.
Fantuzzi G, Righi E, Predieri G, Ceppelli G, Gobba F, Aggazzotti G. (2001) Occupational exposure to trihalomethanes in indoor swimming pools. The Science of the Total Environment 264 (3):257-265.

Felix T, Hall BJ, Brodbelt JS. (1998) Determination of benzophenone-3 and metabolites in water and human urine by solid-phase microextraction and quadrupole ion trap GC–MS. Analytica Chimica Acta 371 (2):195-203.
Fishbein L. (1979) Overview of some aspects of occurrence, formation and analysis of nitrosamines. The Science of the Total Environment 13 (2):157 -188.
Florentin A, Hautemanière A, Hartemann P. (2011) Health effects of disinfection by-products in chlorinated swimming pools. International Journal of Hygiene and Environmental Health 214 (6):461-469.
Fono LJ, Sedlak DL. (2005) Use of the chiral pharmaceutical propranolol to identify sewage discharges into surface waters. Environmental Science & Technology 39 (23):9244-9252.
Font-Ribera L, Kogevinas M, Zock JP, Gómez FP, Barreiro E, Nieuwenhuijsen MJ, Fernandez P, Lourencetti C, Pérez-Olabarría M, Bustamante M. (2010) Short-term changes in respiratory biomarkers after swimming in a chlorinated pool. Environmental Health Perspectives 118 (11):1538-1544.
Fries E, Püttmann W. (2001) Occurrence of organophosphate esters in surface water and ground water in Germany. Journal of Environmental Monitoring 3 (6):621-626.
García-López M, Rodríguez I, Cela R. (2009) Pressurized liquid extraction of organophosphate triesters from sediment samples using aqueous solutions. Journal of Chromatography A 1216 (42):6986-6993.
García-López M, Rodríguez I, Cela R. (2010) Mixed-mode solid-phase extraction followed by liquid chromatography–tandem mass spectrometry for the determination of tri-and di-substituted organophosphorus species in water samples. Journal of Chromatography A 1217 (9):1476-1484.
Garcia-Villanova RJ, Oliveira Dantas Leite MV, Hernández Hierro JM, de Castro Alfageme S, García Hernández C. (2010) Occurrence of bromate, chlorite and chlorate in drinking waters disinfected with hypochlorite reagents. Tracing their origins. Science of the Total Environment 408 (12):2616-2620.
German Standard (2012) *DIN 19643 Treatment of water of swimming pools and baths* Beuth Verlag: Berlin.
Gibs J, Stackelberg PE, Furlong ET, Meyer M, Zaugg SD, Lippincott RL. (2007) Persistence of pharmaceuticals and other organic compounds in chlorinated drinking water as a function of time. Science of the Total Environment 373 (1):240-249.
Giokas DL, Sakkas VA, Albanis TA. (2004) Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography–photodiode array detection and gas chromatography–mass spectrometry. Journal of Chromatography A 1026 (1):289-293.
Giokas DL, Sakkas VA, Albanis TA, Lampropoulou DA. (2005) Determination of UV-filter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography–mass spectrometry after micelle mediated extraction-solvent back extraction. Journal of Chromatography A 1077 (1):19-27.
Giokas DL, Salvador A, Chisvert A. (2007) UV filters: From sunscreens to human body and the environment. TrAC Trends in Analytical Chemistry 26 (5):360-374.
Glassmeyer S, Shoemaker J. (2005) Effects of chlorination on the persistence of pharmaceuticals in the environment. Bulletin of Environmental Contamination and Toxicology 74 (1):24-31.
Glauner T, Frimmel F (2006) Advanced oxidation–A powerful tool for pool water treatment, In International Conference Ozone and UV, April 3rd 2006 Berlin

Glauner T, Kunz F, Zwiener C, Frimmel FH. (2005a) Elimination of swimming pool water disinfection by-products with advanced oxidation processes (AOPs). Acta Hydrochimica et Hydrobiologica 33 (6):585-594.
Glauner T, Waldmann P, Frimmel FH, Zwiener C. (2005b) Swimming pool water-fractionation and genotoxicological characterization of organic constituents. Water Research 39 (18):4494-4502.
Glezer V, Harris B, Tal N, Iosefzon B, Lev O. (1999) Hydrolysis of haloacetonitriles: linear free energy relationship, kinetics and products. Water Research 33 (8):1938-1948.
Goeres D, Palys T, Sandel B, Geiger J. (2004) Evaluation of disinfectant efficacy against biofilm and suspended bacteria in a laboratory swimming pool model. Water Research 38 (13):3103-3109.
Golden R, Gandy J, Vollmer G. (2005) A review of the endocrine activity of parabens and implications for potential risks to human health. CRC Critical Reviews in Toxicology 35 (5):435-458.
Golfinopoulos SK. (2000) Volatile halogenated organics in swimming pools. Toxicological & Environmental Chemistry 76 (3-4):219-228.
Gunkel K, Jessen HJ. (1988) On the problem of urea in baths waters. Zeitschrift fur die Gesamte Hygiene und Ihre Grenzgebiete 34 (4):248-250.
Hansen K, Willach S, Antoniou MG, Mosbæk H, Albrechtsen HJ, Andersen HR. (2012a) Effect of pH on the formation of disinfection byproducts in swimming pool water–Is less THM better? Water Research 46 (19):6399-6409.
Hansen K, Willach S, Mosbæk H, Andersen HR. (2012b) Particles in swimming pool filters–Does pH determine the DBP formation? Chemosphere 87 (3):241-247.
Hansen KM, Albrechtsen H-J, Andersen HR. (2013) Optimal pH in chlorinated swimming pools–balancing formation of by-products. Journal of Water and Health 11 (3):465-472.
Hansen KMS, Willach S, Mosbæk H, Albrechtsen HJ, Andersen HR (2011) Effect of selection of pH in swimming pools on formation of chlorination by-products, In 4th Swimming Pool & Spa International Conference Porto, Portugal
Hartmann PC, Bürgi D, Giger W. (2004) Organophosphate flame retardants and plasticizers in indoor air. Chemosphere 57 (8):781-787.
Haselkorn T, Whittemore AS, Udaltsova N, Friedman GD. (2006) Short-term chloral hydrate administration and cancer in humans. Drug Safety 29 (1):67-77.
Henderson R, Baker A, Murphy K, Hambly A, Stuetz R, Khan S. (2009) Fluorescence as a potential monitoring tool for recycled water systems: A review. Water Research 43 (4):863-881.
Hery M, Hecht G, Gerber J, Gender J, Hubert G, Rebuffaud J. (1995) Exposure to chloramines in the atmosphere of indoor swimming pools. Annals of Occupational Hygiene 39 (4):427-439.
Hsu H, Chen M, Lin C, Chou W, Chen J. (2009) Chloroform in indoor swimming-pool air: Monitoring and modeling coupled with the effects of environmental conditions and occupant activities. Water Research 43 (15):3693-3704.
Hudson N, Baker A, Reynolds D. (2007) Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters - a review. River Research and Applications 23 (6):631-649.
International Agency for Research on Cancer (IARC) (1999) *Monographs on the evaluation of carcinogenic risks to humans - Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances.* Vol. 73.

Jacobs J, Spaan S, Van Rooy G, Meliefste C, Zaat V, Rooyackers J, Heederik D. (2007) Exposure to trichloramine and respiratory symptoms in indoor swimming pool workers. European Respiratory Journal 29 (4):690-698.
Jonkers N, Sousa A, Galante-Oliveira S, Barroso CM, Kohler HPE, Giger W. (2010) Occurrence and sources of selected phenolic endocrine disruptors in Ria de Aveiro, Portugal. Environmental Science and Pollution Research 17 (4):834-843.
Judd S, Bullock G. (2003) The fate of chlorine and organic materials in swimming pools. Chemosphere 51 (9):869-879.
Jurado-Sánchez B, Ballesteros E, Gallego M. (2010) Screening of *N*-nitrosamines in tap and swimming pool waters using fast gas chromatography. Journal of Separation Science 33 (4-5):610-616.
Kanan A (2010) Occurrence and formation of disinfection by-products in indoor swimming pools water, A thesis presented to Clemson University.
Kanan A, Karanfil T. (2011) Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids. Water Research 45 (2):926-932.
Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Research 42 (13):3498-3518.
Kaydos-Daniels SC, Beach MJ, Shwe T, Magri J, Bixler D. (2008) Health effects associated with indoor swimming pools: a suspected toxic chloramine exposure. Public Health 122 (2):195-200.
Kelsall HL, Sim MR. (2001) Skin irritation in users of brominated pools. International Journal of Environmental Health Research 11 (1):29-40.
Keuten M, Peters M, Daanen H, de Kreuk M, Rietveld L, van Dijk J. (2014) Quantification of continual anthropogenic pollutants released in swimming pools. Water Research 53 (2014):259-270.
Keuten M, Schets F, Schijven J, Verberk J, van Dijk J. (2012) Definition and quantification of initial anthropogenic pollutant release in swimming pools. Water Research 46 (11):3682–3692.
Keuten M, Verberk J, Van Dijk J, van Loosdrecht M. (2009) In *Swimming Pool and Spa International Conference, London.*
Khan SJ, Wang L, Hashim NH, McDonald JA. (2014) Distinct enantiomeric signals of ibuprofen and naproxen in treated wastewater and sewer overflow. Chirality 26 (11):739-746.
Kim H, Han K. (2011) Swimmers contribute to additional formation of *N*-nitrosamines in chlorinated pool water. Toxicology and Environmental Health Sciences 3 (3):168-174.
Kim H, Shim J, Lee S. (2002) Formation of disinfection by-products in chlorinated swimming pool water. Chemosphere 46 (1):123-130.
Kim JW, Isobe T, Muto M, Tue NM, Katsura K, Malarvannan G, Sudaryanto A, Chang KH, Prudente M, Viet PH. (2014) Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. Chemosphere 116:91-97.
Kim S, Choi K. (2014) Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environment International 70:143-157.
Kim YJ, Osako M, Sakai S. (2006) Leaching characteristics of polybrominated diphenyl ethers (PBDEs) from flame-retardant plastics. Chemosphere 65 (3):506-513.

Koch J, Volker P. (1996) Formation of artefacts and its suppression during the determination of volatile halogenated hydrocarbons in water by means of headspace gas chromatography under the standard conditions of DIN 38407-F5. Part II: Increased trihalomethane formation in chlorinated swimming-pool water. Acta Hydrochimica et Hydrobiologica 24 (4):179-184.

Kockler J, Oelgemöller M, Robertson S, Glass BD. (2012) Photostability of sunscreens. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 13 (1):91-110.

Kogevinas M, Villanueva CM, Font-Ribera L, Liviac D, Bustamante M, Espinoza F, Nieuwenhuijsen MJ, Espinosa A, Fernandez P, DeMarini DM. (2010) Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environmental Health Perspectives 118 (11):1531-1537.

Kozłowska K, Polkowska Ż, Namieśnik J. (2006) Effect of treated swimming pool water on the levels of trihalomethanes in swimmer's urine. Toxicological & Environmental Chemistry 88 (2):259-272.

Kramer M, Hübner I, Rörden O, Schmidt C (2009) Haloacetonitriles-another important group of disinfection byproducts in swimming pool water, In Swimming Pool & Spa International Conference London

Kunz PY, Fent K. (2006) Multiple hormonal activities of UV filters and comparison of *in vivo* and *in vitro* estrogenic activity of ethyl-4-aminobenzoate in fish. Aquatic Toxicology 79 (4):305-324.

Kunz PY, Galicia HF, Fent K. (2006) Comparison of in vitro and in vivo estrogenic activity of UV filters in fish. Toxicological Sciences 90 (2):349-361.

Kupper T, Plagellat C, Brändli R, de Alencastro LF, Grandjean D, Tarradellas J. (2006) Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Research 40 (14):2603-2612.

Lahl U, Batjer K, Duszeln J, Gabel B, Stachel B, Thiemann W. (1981) Distribution and balance of volatile halogenated hydrocarbons in the water and air of covered swimming pools using chlorine for water disinfection. Water Research 15 (7):803-814.

Lambropoulou D, Giokas D, Sakkas V, Albanis T, Karayannis M. (2002) Gas chromatographic determination of 2-hydroxy-4-methoxybenzophenone and octyldimethyl-*p*-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. Journal of Chromatography A 967 (2):243-253.

Leakey JE, Seng JE, Latendresse JR, Hussain N, Allen LJ, Allaben WT. (2003) Dietary controlled carcinogenicity study of chloral hydrate in male $B6C3F_1$ mice. Toxicology and Applied Pharmacology 193 (2):266-280.

Lee J, Ha K-T, Zoh K-D. (2009) Characteristics of trihalomethane (THM) production and associated health risk assessment in swimming pool waters treated with different disinfection methods. Science of the Total Environment 407 (6):1990-1997.

Lee J, Jun MJ, Lee MH, Eom SW, Zoh KD. (2010) Production of various disinfection byproducts in indoor swimming pool waters treated with different disinfection methods. International Journal of Hygiene and Environmental Health 213 (6):465-474.

Legay C, Rodriguez MJ, Sérodes JB, Levallois P. (2010) Estimation of chlorination by-products presence in drinking water in epidemiological studies on adverse reproductive outcomes: A review. Science of The Total Environment 408 (3):456-472.

León-González Z, Ferreiro-Vera C, Priego-Capote F, de Castro MDL. (2011) Bioaccumulation assessment of the sunscreen agent 2-ethylhexyl 4-(*N*, *N*-dimethylamino) benzoate in human semen by automated online SPE-LC-MS/MS. Analytical and Bioanalytical Chemistry 401 (3):1003-1011.

Lévesque B, Ayotte P, Tardif R, Charest-Tardif G. (2000) Evaluation of the health risk associated with exposure to chloroform in indoor swimming pools. Journal of Toxicology and Environmental Health Part A 61 (4):225-243.

Lévesque B, Duchesne JF, Gingras S, Lavoie R, Prud'Homme D, Bernard E, Boulet LP, 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.

Li J, Blatchley III ER. (2007) Volatile disinfection byproduct formation resulting from chlorination of organic-nitrogen precursors in swimming pools. Environmental Science & Technology 41 (19):6732-6739.

Lijinsky W, Epstein S. (1970) Nitrosamines as environmental carcinogens. Nature 225:21-23.

Liu LY, Salamova A, He K, Hites RA. (2015) Analysis of polybrominated diphenyl ethers and emerging halogenated and organophosphate flame retardants in human hair and nails. Journal of Chromatography A 1406:251-257.

Liu W, Cheung L-M, Yang X, Shang C. (2006) THM, HAA and CNCl formation from UV irradiation and chlor(am)ination of selected organic waters. Water Research 40 (10):2033-2043.

Liu X, Ji K, Choi K. (2012a) Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquatic Toxicology 114:173-181.

Liu YS, Ying GG, Shareef A, Kookana RS. (2012b) Biodegradation of the ultraviolet filter benzophenone-3 under different redox conditions. Environmental Toxicology and Chemistry 31 (2):289-295.

Liviac D, Creus A, Marcos R. (2009) Genotoxicity analysis of two halonitromethanes, a novel group of disinfection by-products (DBPs), in human cells treated in vitro. Environmental Research 109 (3):232-238.

Liviac D, Wagner ED, Mitch WA, Altonji MJ, Plewa MJ. (2010) Genotoxicity of water concentrates from recreational pools after various disinfection methods. Environmental Science & Technology 44 (9):3527-3532.

Loos R, Barceló D. (2001) Determination of haloacetic acids in aqueous environments by solid-phase extraction followed by ion-pair liquid chromatography–electrospray ionization mass spectrometric detection. Journal of Chromatography A 938 (1):45-55.

López-Serna R, Jurado A, Vázquez-Suñé E, Carrera J, Petrović M, Barceló D. (2013) Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. Environmental Pollution 174:305-315.

Lourencetti C, Grimalt JO, Marco E, Fernandez P, Font-Ribera L, Villanueva CM, Kogevinas M. (2012) Trihalomethanes in chlorine and bromine disinfected swimming pools: Air-water distributions and human exposure. Environment International 45 (2012):59-67.

Ma R, Cotton B, Lichtensteiger W, Schlumpf M. (2003) UV filters with antagonistic action at androgen receptors in the MDA-kb2 cell transcriptional-activation assay. Toxicological Sciences 74 (1):43-50.

Maia R, Correia M, Pereira IMB, Beleza VM. (2014) Optimization of HS-SPME analytical conditions using factorial design for trihalomethanes determination in swimming pool water samples. Microchemical Journal 112 (2014):164-171.
Malliarou E, Collins C, Graham N, Nieuwenhuijsen M. (2005) Haloacetic acids in drinking water in the United Kingdom. Water Research 39 (12):2722-2730.
Marklund A (2005) Levels and sources of organophosphorus flame retardants and plasticizers in indoor and outdoor environments, A thesis presented to Umeå University.
Marklund A, Andersson B, Haglund P. (2003) Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere 53 (9):1137-1146.
Marklund A, Andersson B, Haglund P. (2005a) Organophosphorus flame retardants and plasticizers in air from various indoor environments. Journal of Environmental Monitoring 7 (8):814-819.
Marklund A, Andersson B, Haglund P. (2005b) Organophosphorus flame retardants and plasticizers in Swedish sewage treatment plants. Environmental Science & Technology 39 (19):7423-7429.
Marklund A, Andersson B, Haglund P. (2005c) Traffic as a source of organophosphorus flame retardants and plasticizers in snow. Environmental Science & Technology 39 (10):3555-3562.
Martínez-Carballo E, González-Barreiro C, Sitka A, Scharf S, Gans O. (2007) Determination of selected organophosphate esters in the aquatic environment of Austria. Science of the Total Environment 388 (1):290-299.
Martinez D, Borrull F, Calull M. (1999) Evaluation of different electrolyte systems and on-line preconcentrations for the analysis of haloacetic acids by capillary zone electrophoresis. Journal of Chromatography A 835 (1-2):187.
McDonald JA, Harden NB, Nghiem LD, Khan SJ. (2012) Analysis of *N*-nitrosamines in water by isotope dilution gas chromatography-electron ionisation tandem mass spectrometry. Talanta 99:146-154.
Meeker JD, Stapleton HM. (2010) House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environmental Health Perspectives 118 (3):318-323.
Meyer J, Bester K. (2004) Organophosphate flame retardants and plasticisers in wastewater treatment plants. Journal of Environmental Monitoring 6 (7):599-605.
Michalski R, Mathews B. (2007) Occurrence of chlorite, chlorate and bromate in disinfected swimming pool water. Polish Journal of Environmental Studies 16 (2):237-241.
Mills R, Adams S, Cliffe E, Dickinson W, Nicholson J. (1973) The metabolism of ibuprofen. Xenobiotica 3 (9):589-598.
Mizouchi S, Ichiba M, Takigami H, Kajiwara N, Takamuku T, Miyajima T, Kodama H, Someya T, Ueno D. (2015) Exposure assessment of organophosphorus and organobromine flame retardants via indoor dust from elementary schools and domestic houses. Chemosphere 123:17-25.
Montesinos I, Cardador M, Gallego M. (2011) Determination of halonitromethanes in treated water. Journal of Chromatography A 1218 (18):2497-2504.
Montesinos I, Gallego M. (2012) Headspace gas chromatography–mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water. Analytical and Bioanalytical Chemistry 402 (7):2315-2323.

Morohoshi K, Yamamoto H, Kamata R, Shiraishi F, Koda T, Morita M. (2005) Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. Toxicology in Vitro 19 (4):457-469.
Muellner MG, Wagner ED, McCalla K, Richardson SD, Woo YT, Plewa MJ. (2007) Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic? Environmental Science & Technology 41 (2):645-651.
Nakajima M, Kawakami T, Niino T, Takahashi Y, Onodera S. (2009) Aquatic fate of sunscreen agents octyl-4-methoxycinnamate and octyl-4-dimethylaminobenzoate in model swimming pools and the mutagenic assays of their chlorination byproducts. Journal of Health Science 55 (3):363-372.
Natural Resource Management Ministerial Council Environment Protection and Heritage Council and National Health and Medical Research Council (2008) *Autralian Guidelines for Water Recycling: Managing Health & Environmental Risks (Phase 2) - Augmentation of Drinking Water Supplies.* Canberra.
Nawrocki J, Andrzejewski P. (2011) Nitrosamines and water. Journal of Hazardous Materials 189 (1):1-18.
Negreira N, Canosa P, Rodríguez I, Ramil M, Rubi E, Cela R. (2008) Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography-mass spectrometry. Journal of Chromatography A 1178 (1-2):206-214.
Nikolaou AD, Golfinopoulos SK, Arhonditsis GB, Kolovoyiannis V, Lekkas TD. (2004) Modeling the formation of chlorination by-products in river waters with different quality. Chemosphere 55 (3):409-420.
Norin H, Renberg L. (1980) Determination of trihalomethanes (THM) in water using high efficiency solvent extraction. Water Research 14 (10):1397-1402.
Nsubuga H, Basheer C. (2013) Determination of haloacetic acids in swimming pool waters by membrane-protected micro-solid phase extraction. Journal of Chromatography A 1315 (2013):47-52.
Packer JL, Werner JJ, Latch DE, McNeill K, Arnold WA. (2003) Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. Aquatic Sciences 65 (4):342-351.
Padhye LP, Yao H, Kung'u FT, Huang C-H. (2014) Year-long evaluation on the occurrence and fate of pharmaceuticals, personal care products, and endocrine disrupting chemicals in an urban drinking water treatment plant. Water Research 51:266-276.
Panyakapo M, Soontornchai S, Paopuree P. (2008) Cancer risk assessment from exposure to trihalomethanes in tap water and swimming pool water. Journal of Environmental Sciences 20 (3):372-378.
Parinet J, Tabaries S, Coulomb B, Vassalo L, Boudenne JL. (2011) Exposure levels to brominated compounds in seawater swimming pools treated with chlorine. Water Research 46 (3):828-836.
Parrat J, Donzé G, Iseli C, Perret D, Tomicic C, Schenk O. (2012) Assessment of occupational and public exposure to trichloramine in Swiss indoor swimming pools: a proposal for an occupational exposure limit. Annals of Occupational Hygiene 56 (3):264-277.
Pasquarella C, Veronesi L, Napoli C, Castaldi S, Pasquarella ML, Saccani E, Colucci ME, Auxilia F, Gallè F, Di Onofrio V. (2014) What about behaviours in swimming pools? Results of an Italian multicentre study. Microchemical Journal 112:190-195.

Patterson J, Boateng J, Walker LT, Verghese M. (2012) Cytotoxic effects of multiple N-nitrosamines in human liver cell line Hep2G: Possible mechanisms of action. Journal of Pharmacology and Toxicology 7 (3):114-127.
Peck AM. (2006) Analytical methods for the determination of persistent ingredients of personal care products in environmental matrices. Analytical and Bioanalytical Chemistry 386 (4):907-939.
Peng X, Ou W, Wang C, Wang Z, Huang Q, Jin J, Tan J. (2014) Occurrence and ecological potential of pharmaceuticals and personal care products in groundwater and reservoirs in the vicinity of municipal landfills in China. Science of the Total Environment 490:889-898.
Peng X, Yu Y, Tang C, Tan J, Huang Q, Wang Z. (2008) Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. Science of the Total Environment 397 (1):158-166.
Pennsylvania Department of Health, Bureau of Community Health Systems (2008) *Public swimming & bathing places (Aquatic facilities): Operational & biological contamination protocol recommendations.* http://www.dsf.health.state.pa.us/health/lib/health/SOPRecommendations.pdf viewed 8 October 2014.
Plewa MJ, Wagner ED, Mitch WA. (2011) Comparative mammalian cell cytotoxicity of water concentrates from disinfected recreational pools. Environmental Science & Technology 45 (9):4159-4165.
Pozzi R, Bocchini P, Pinelli F, Galletti GC. (2011) Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry. Journal of Chromatography A 1218 (14):1808-1814.
Prieto-Blanco M, Alpendurada M, López-Mahía P, Muniategui-Lorenzo S, Prada-Rodríguez D, Machado S, Gonçalves C. (2012) Improving methodological aspects of the analysis of five regulated haloacetic acids in water samples by solid-phase extraction, ion-pair liquid chromatography and electrospray tandem mass spectrometry. Talanta 94 (2012):90-98.
Quintana JB, Rodil R, Reemtsma T, García-López M, Rodríguez I. (2008) Organophosphorus flame retardants and plasticizers in water and air II. Analytical methodology. TrAC Trends in Analytical Chemistry 27 (10):904-915.
Radomski J, Greenwald D, Hearn W, Block N, Woods F. (1978) Nitrosamine formation in bladder infections and its role in the etiology of bladder cancer. The Journal of Urology 120 (1):48-50.
Reemtsma T, Lingott J, Roegler S. (2011) Determination of 14 monoalkyl phosphates, dialkyl phosphates and dialkyl thiophosphates by LC-MS/MS in human urinary samples. Science of the Total Environment 409 (10):1990-1993.
Reemtsma T, Quintana JB, Rodil R. (2008) Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. TrAC Trends in Analytical Chemistry 27 (9):727-737.
Regnery J, Püttmann W. (2009) Organophosphorus flame retardants and plasticizers in rain and snow from Middle Germany. CLEAN–Soil, Air, Water 37 (4-5):334-342.
Regnery J, Püttmann W. (2010) Occurrence and fate of organophosphorus flame retardants and plasticizers in urban and remote surface waters in Germany. Water Research 44 (14):4097-4104.

Ribeiro IFF, Heitor AM, Nogueira J (2011) Occurrence of chlorite and chlorate in swimming pool water, In 4th International Conference swimming pool and spas Porto (Portugal)

Rice EW, Baird RB, Eaton AD, Clesceri LS (2012) *Standard methods for the examination of water & wastewater*. 22 ed. APHA, Washington DC, USA.

Richardson S. (2005) New disinfection by-product issues: emerging DBPs and alternative routes of exposure. Global Nest J 7 (1):43-60.

Richardson SD, DeMarini DM, Kogevinas M, Fernandez P, Marco E, Lourencetti C, Ballesté C, Heederik D, Meliefste K, McKague AB. (2010) What's in the pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. Environmental Health Perspectives 118 (11):1523-1530.

Richardson SD, Plewa MJ, Wagner ED, Schoeny R, DeMarini DM. (2007) Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutation Research/Reviews in Mutation Research 636 (1-3):178-242.

Righi E, Fantuzzi G, Predieri G, Aggazzotti G. (2014) Bromate, chlorite, chlorate, haloacetic acids, and trihalomethanes occurrence in indoor swimming pool waters in Italy. Microchemical Journal 113 (2014):23-29.

Rodil R, Moeder M, Altenburger R, Schmitt-Jansen M. (2009a) Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Analytical and Bioanalytical Chemistry 395 (5):1513-1524.

Rodil R, Quintana JB, López-Mahía P, Muniategui-Lorenzo S, Prada-Rodríguez D. (2009b) Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1216 (14):2958-2969.

Rodil R, Quintana JB, Reemtsma T. (2005) Liquid chromatography-tandem mass spectrometry determination of nonionic organophosphorus flame retardants and plasticizers in wastewater samples. Analytical Chemistry 77 (10):3083-3089.

Rook JJ. (1974) Formation of haloforms during chlorination of natural waters.

Rostkowska K, Zwierz K, Rozanski A, Moniuszko-Jakoniuk J, Roszczenko A. (1998) Formation and Metabolism of N-Nitrosamines. Polish Journal of Environmental Studies 7 (6):321-325.

Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. (1998) Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicology and Applied Pharmacology 153 (1):12-19.

Sá CSA, Boaventura RAR, Pereira IB. (2011) Analysis of trihalomethanes in water and air from indoor swimming pools using HS-SPME/GC/ECD. Journal of Environmental Science and Health Part A 46 (4):355-363.

Sá CSA, Boaventura RAR, Pereira IB. (2012) Analysis of haloacetic acids in water and air (aerosols) from indoor swimming pools using HS-SPME/GC/ECD. Journal of Environmental Science and Health, Part A 47 (2):176-183.

Sakkas V, Giokas D, Lambropoulou D, Albanis T. (2003) Aqueous photolysis of the sunscreen agent octyl-dimethyl-*p*-aminobenzoic acid: Formation of disinfection byproducts in chlorinated swimming pool water. Journal of Chromatography A 1016 (2):211-222.

Samples WR (1959) A study on the chlorination of urea, A thesis presented to Harvard University.

Sánchez-Martín J, Beltrán-Heredia J, Domínguez J. (2013) Advanced photochemical degradation of emerging pollutants: Methylparaben. Water, Air, & Soil Pollution 224 (5):1-12.
Santos AJM, Miranda MS, Esteves da Silva JCG. (2012) The degradation products of UV filters in aqueous and chlorinated aqueous solutions. Water Research 46 (10):3167-3176.
Sarrión M, Santos F, Galceran M. (2000) In situ derivatization/solid-phase microextraction for the determination of haloacetic acids in water. Analytical Chemistry 72 (20):4865-4873.
Sato N, Xie R, Yoneda T, Xing Y, Noro A, Robinson K, Villalobos R. (2014) Water quality improvement by combined UF, RO, and ozone/hydrogen peroxide system (HiPOx) in the water reclamation process. Ozone: Science & Engineering 36 (2):153-165.
Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W. (2001) *In vitro* and *in vivo* estrogenicity of UV screens. Environmental Health Perspectives 109 (3):239-244.
Schlumpf M, Kypke K, Wittassek M, Angerer J, Mascher H, Mascher D, Vökt C, Birchler M, Lichtensteiger W. (2010) Exposure patterns of UV filters, fragrances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. Chemosphere 81 (10):1171-1183.
Schlumpf M, Schmid P, Durrer S, Conscience M, Maerkel K, Henseler M, Gruetter M, Herzog I, Reolon S, Ceccatelli R. (2004) Endocrine activity and developmental toxicity of cosmetic UV filters—an update. Toxicology 205 (1):113-122.
Schwab BW, Hayes EP, Fiori JM, Mastrocco FJ, Roden NM, Cragin D, Meyerhoff RD, Vincent J, Anderson PD. (2005) Human pharmaceuticals in US surface waters: a human health risk assessment. Regulatory Toxicology and Pharmacology 42 (3):296-312.
Seiler RL, Zaugg SD, Thomas JM, Howcroft DL. (1999) Caffeine and pharmaceuticals as indicators of waste water contamination in wells. Groundwater 37 (3):405-410.
Seredyńska-Sobecka B, Stedmon CA, Boe-Hansen R, Waul CK, Arvin E. (2011) Monitoring organic loading to swimming pools by fluorescence excitation–emission matrix with parallel factor analysis (PARAFAC). Water Research 45 (6):2306-2314.
Serpone N, Salinaro A, Emeline AV, Horikoshi S, Hidaka H, Zhao J. (2002) An *in vitro* systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents. Photochemical & Photobiological Sciences 1 (12):970-981.
Shah AD, Mitch WA. (2011) Halonitroalkanes, halonitriles, haloamides, and N-nitrosamines: A critical review of nitrogenous disinfection byproduct formation pathways. Environmental Science & Technology 46 (1):119-131.
Shah M, Meija J, Cabovska B, Caruso JA. (2006) Determination of phosphoric acid triesters in human plasma using solid-phase microextraction and gas chromatography coupled to inductively coupled plasma mass spectrometry. Journal of Chromatography A 1103 (2):329-336.
Shen R, Andrews SA. (2011) Demonstration of 20 pharmaceuticals and personal care products (PPCPs) as nitrosamine precursors during chloramine disinfection. Water Research 45 (2):944-952.

Silva ZI, Rebelo MH, Silva MM, Alves AM, da Conceição Cabral M, Almeida AC, Aguiar FR, de Oliveira AL, Nogueira AC, Pinhal HR. (2012) Trihalomethanes in Lisbon indoor swimming pools: Occurrence, determining factors, and health risk classification. Journal of Toxicology and Environmental Health, Part A 75 (13-15):878-892.
Simard S, Tardif R, Rodriguez MJ. (2013) Variability of chlorination by-product occurrence in water of indoor and outdoor swimming pools. Water Research 47 (5):1763-1772.
Simazaki D, Fujiwara J, Manabe S, Matsuda M, Asami M, Kunikane S. (2008) Removal of selected pharmaceuticals by chlorination, coagulation-sedimentation and powdered activated carbon treatment. Water Science & Technology 58 (5):1129–1135.
Singer P. (1999) Humic substances as precursors for potentially harmful disinfection by-products. Water Science and Technology 40 (9):25-30.
Singer PC. (1994) Control of disinfection by-products in drinking water. Journal of Environmental Engineering 120 (4):727-744.
Singer PC, Zilli WB. (1975) Ozonation of ammonia in wastewater. Water Research 9 (2):127-134.
Snyder SA. (2008) Occurrence, treatment, and toxicological relevance of EDCs and pharmaceuticals in water. Ozone: Science and Engineering 30 (1):65-69.
Snyder SA, Adham S, Redding AM, Cannon FS, DeCarolis J, Oppenheimer J, Wert EC, Yoon Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202 (1):156-181.
Snyder SA, Bruce G, Hemming J, Pleus R, Snyder E, Trenholm R (2008), *Toxicological relevance of EDCs and pharmaceuticals in drinking water.* Awwa Research Foundation Denver, USA.
Soltermann F, Lee M, Canonica S, von Gunten U. (2013) Enhanced *N*-nitrosamine formation in pool water by UV irradiation of chlorinated secondary amines in the presence of monochloramine. Water Research 47 (1):79-90.
South Australian Health Commission (SAHC) (1991) *Standard for the Operation of Swimming Pools and Spa Pools in South Australia.* 2013 ed. Adelaide. ISBN 978-1-74243-514-5
Spiegelhalder B, Preussmann R. (1984) Contamination of toiletries and cosmetic products with volatile and nonvolatile *N*-nitroso carcinogens. Journal of cancer research and clinical oncology 108 (1):160-163.
Stackelberg PE, Gibs J, Furlong ET, Meyer MT, Zaugg SD, Lippincott RL. (2007) Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. Science of the Total Environment 377 (2):255-272.
Stapleton HM, Klosterhaus S, Eagle S, Fuh J, Meeker JD, Blum A, Webster TF. (2009) Detection of organophosphate flame retardants in furniture foam and US house dust. Environmental Science & Technology 43 (19):7490-7495.
Stapleton HM, Klosterhaus S, Keller A, Ferguson PL, van Bergen S, Cooper E, Webster TF, Blum A. (2011) Identification of flame retardants in polyurethane foam collected from baby products. Environmental Science & Technology 45 (12):5323-5331.
Stefan MI, Bolton JR. (2002) UV direct photolysis of *N*-nitrosodimethylamine (NDMA): Kinetic and product study. Helvetica Chimica Acta 85 (5):1416-1426.
Stokes R, Diffey B. (1999) The water resistance of sunscreen and day-care products. British Journal of Dermatology 140 (2):259-263.

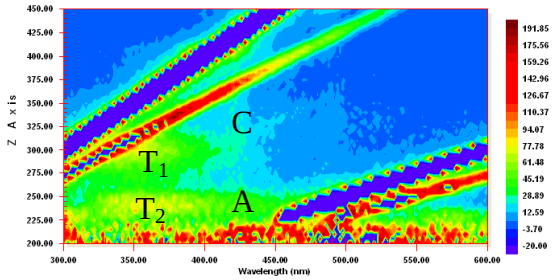
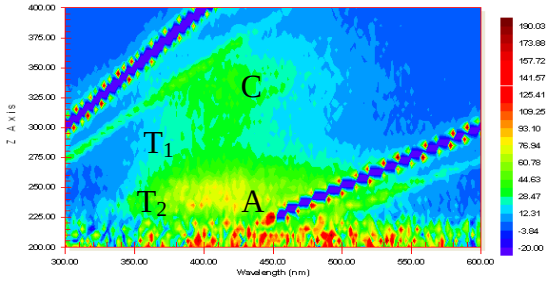
Sundkvist AM, Olofsson U, Haglund P. (2010) Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. Journal of Environmental Monitoring 12 (4):943-951.
Suppes LM, Abrell L, Dufour AP, Reynolds KA. (2013) Assessment of swimmer behaviors on pool water ingestion. Journal of Water & Health 12 (2):269–279.
Suzuki T, Kitamura S, Khota R, Sugihara K, Fujimoto N, Ohta S. (2005) Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. Toxicology and Applied Pharmacology 203 (1):9-17.
Takahashi Y, Onodera S, Morita M, Terao Y. (2003) A Problem in the Determination of Trihalomethane by Headspace-Gas Chromatography/Mass Spectrometry. Journal of Health Science 49 (1):1-7.
Tang-Liu D, Williams R, Riegelman S. (1983) Disposition of caffeine and its metabolites in man. Journal of Pharmacology and Experimental Therapeutics 224 (1):180-185.
Tanju Karanfil, Stuart W. Krasner, Paul Westerhoff, Xie Y (2008), *Disinfection by-products in drinking water - Occurrence, formation, health effects and control.* The American Chemical Society: Washington, DC.
Templeton M, Chen Z. (2010) NDMA and seven other nitrosamines in selected UK drinking water supply systems. Journal of Water Supply: Research and Technology—AQUA 59 (4):277-283.
Teo TLL, Coleman HM, Khan SJ. (2015) Chemical contaminants in swimming pools: Occurrence, implications and control. Environment International 76:16-31.
Terasaki M, Makino M. (2008) Determination of chlorinated by-products of parabens in swimming pool water. International Journal of Environmental and Analytical Chemistry 88 (13):911-922.
Terasaki M, Makino M, Tatarazako N. (2009) Acute toxicity of parabens and their chlorinated by-products with Daphnia magna and Vibrio fischeri bioassays. Journal of Applied Toxicology 29 (3):242-247.
Ternes TA, Joss A, Siegrist H. (2004) Peer reviewed: Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environmental Science & Technology 38 (20):392A-399A.
Thickett K, McCoach J, Gerber J, Sadhra S, Burge P. (2002) Occupational asthma caused by chloramines in indoor swimming-pool air. European Respiratory Journal 19 (5):827-832.
Tuazon EC, Carter WP, Atkinson R, Winer AM, Pitts JN. (1984) Atmospheric reactions of *N*-nitrosodimethylamine and dimethylnitramine. Environmental science & technology 18 (1):49-54.
Uhl W, Hartmann C. (2005) Disinfection by-products and microbial contamination in the treatment of pool water with granular activated carbon. Water Science & Technology 52 (8):71-76.
US EPA (2004) *Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment).* Office of Solid Waste & Emergency Response, Washington, DC. http://epa.gov/swerrims/riskassessment/ragse/.
US EPA (2011) *Exposure Factor Handbook: 2011.* Office of Research and Development, NCEA, Washington DC, USA.
US EPA (2012) *Integrated Risk Information System (IRIS).* United States Environmental Protection Agency http://www.epa.gov/iris/ viewed July 2015.

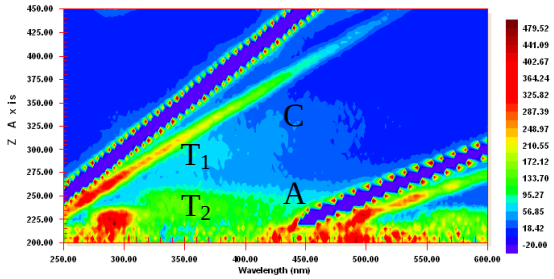
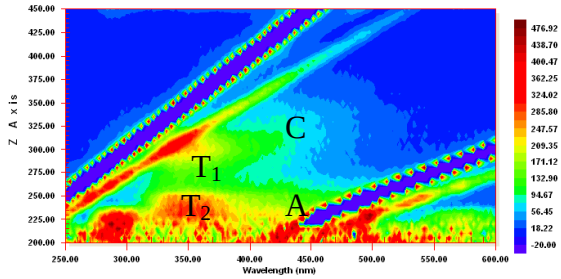
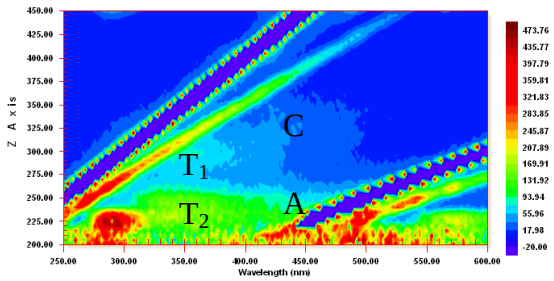
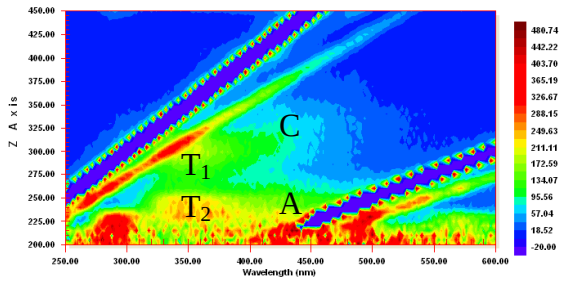
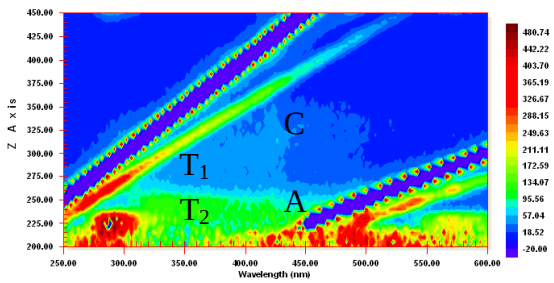
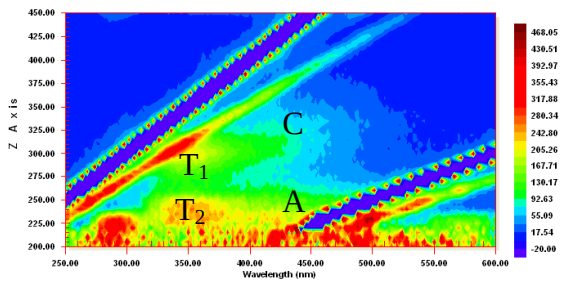
Van den Eede N, Dirtu AC, Neels H, Covaci A. (2011) Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environment international 37 (2):454-461.
Van den Eede N, Heffernan AL, Aylward LL, Hobson P, Neels H, Mueller JF, Covaci A. (2015) Age as a determinant of phosphate flame retardant exposure of the Australian population and identification of novel urinary PFR metabolites. Environment International 74:1-8.
Van der Veen I, de Boer J. (2012) Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88 (10):1119-1153.
Vanderford BJ, Snyder SA. (2006) Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. Environmental Science & Technology 40 (23):7312-7320.
Vasel J, Praet E. (2002) On the use of fluorescence measurements to characterize wastewater. Water Science & Technology 45 (4-5):109-116.
Vidal L, Chisvert A, Canals A, Salvador A. (2010) Ionic liquid-based single-drop microextraction followed by liquid chromatography-ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples. Talanta 81 (1-2):549-555.
Villanueva CM, Cantor KP, Grimalt JO, Malats N, Silverman D, Tardon A, Garcia-Closas R, Serra C, Carrato A, Castano-Vinyals G. (2007a) 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.
Villanueva CM, Gagniere B, Monfort C, Nieuwenhuijsen MJ, Cordier S. (2007b) Sources of variability in levels and exposure to trihalomethanes. Environmental Research 103 (2):211-220.
Virkutyte J, Al-Abed SR, Dionysiou DD. (2012) Depletion of the protective aluminum hydroxide coating in TiO_2-based sunscreens by swimming pool water ingredients. Chemical Engineering Journal 191 (2012):95-103.
Von Gunten U, Hoigne J. (1994) Bromate formation during ozonization of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. Environmental Science & Technology 28 (7):1234-1242.
Walse SS, Mitch WA. (2008) Nitrosamine carcinogens also swim in chlorinated pools. Environmental Science & Technology 42 (4):1032-1037.
Wang W, Qian Y, Boyd JM, Wu M, Hrudey SE, Li X-F. (2013) Halobenzoquinones in swimming pool waters and formation from personal care products. Environmental Science & Technology 47 (7):3275-3282.
Wang W, Qian Y, Li J, Moe B, Huang R, Zhang H, Hrudey SE, Li X-F. (2014a) Analytical and Toxicity Characterization of Halo-hydroxyl-benzoquinones as Stable Haloquinone Disinfection Byproducts in Treated Water. Analytical Chemistry 86 (10):4982–4988.
Wang X, Leal MG, Zhang X, Yang H, Xie Y. (2014b) Haloacetic acids in swimming pool and spa water in the United States and China. Frontiers of Environmental Science & Engineering 8 (6):820-824.
Wang X, Liu J, Yin Y. (2011) Development of an ultra-high-performance liquid chromatography–tandem mass spectrometry method for high throughput determination of organophosphorus flame retardants in environmental water. Journal of Chromatography A 1218 (38):6705-6711.

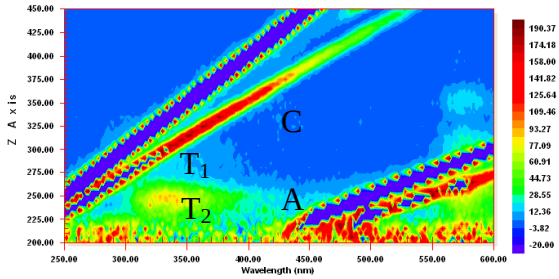
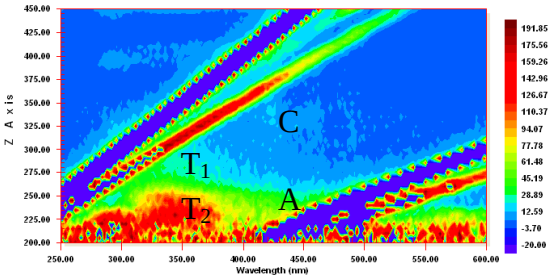
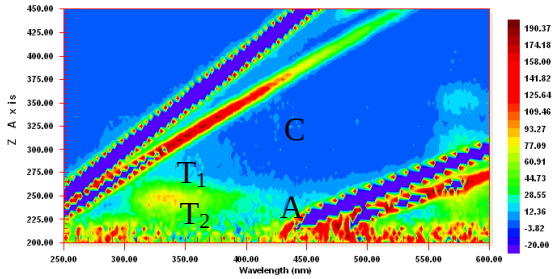
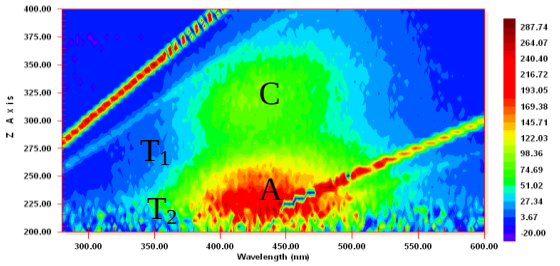
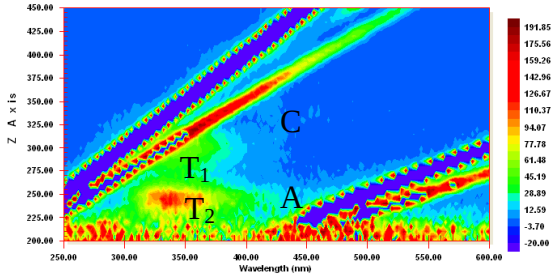
Watson K, Farré MJ, Birt J, McGree J, Knight N. (2014) Predictive models for water sources with high susceptibility for bromine-containing disinfection by-product formation: implications for water treatment. Environmental Science and Pollution Research:1-16.
Weaver WA, Li J, Wen Y, Johnston J, Blatchley MR, Blatchley ER. (2009) Volatile disinfection by-product analysis from chlorinated indoor swimming pools. Water Research 43 (13):3308-3318.
Weng S, Li J, Blatchley ER. (2012) Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. Water Research 46 (8):2674-2682.
Weng SC, Blatchley III ER. (2011) Disinfection by-product dynamics in a chlorinated, indoor swimming pool under conditions of heavy use: National swimming competition. Water Research 45 (16):5241-5248.
Weng SC, Sun P, Ben W, Huang CH, Lee LT, Blatchley ER. (2014) The Presence of Pharmaceuticals and Personal Care Products (PPCPs) in Swimming Pools. Environmental Science & Technology Letters.
Williams M, Kumar A, Ort C, Lawrence MG, Hambly A, Khan SJ, Kookana R. (2013) The use of multiple tracers for tracking wastewater discharges in freshwater systems. Environmental Monitoring and Assessment 185 (11):9321-9332.
World Health Organization (WHO) (2006) Guidelines for safe recreational water environments: Swimming pools and similar environments (Volume 2).
World Health Organization (WHO) (2008) Guidelines for drinking-water quality – 3rd ed. Chapter 12, 326-328.
Wright MW, Wright ST, Wagner RF. (2001) Mechanisms of sunscreen failure. Journal of the American Academy of Dermatology 44 (5):781-784.
Xue S, Zhao Q-L, Wei L-L, Jia T. (2008) Effect of bromide ion on isolated fractions of dissolved organic matter in secondary effluent during chlorination. Journal of Hazardous Materials 157 (1):25-33.
Yamamoto H, Tamura I, Hirata Y, Kato J, Kagota K, Katsuki S, Yamamoto A, Kagami Y, Tatarazako N. (2011) Aquatic toxicity and ecological risk assessment of seven parabens: Individual and additive approach. Science of the Total Environment 410-411 (2011):102-111.
Yeh RY, Farré MJ, Stalter D, Tang JY, Molendijk J, Escher BI. (2014) Bioanalytical and chemical evaluation of disinfection by-products in swimming pool water. Water Research 59 (2014):172-184.
Yoon Y, Ryu J, Oh J, Choi B-G, Snyder SA. (2010) Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Science of the Total Environment 408 (3):636-643.
Zhang DQ, Hua T, Gersberg RM, Zhu J, Ng WJ, Tan SK. (2013) Fate of caffeine in mesocosms wetland planted with *Scirpus validus*. Chemosphere 90 (4):1568-1572.
Zhang Q, Lu M, Dong X, Wang C, Zhang C, Liu W, Zhao M. (2014) Potential estrogenic effects of phosphorus-containing flame retardants. Environmental Science & Technology 48 (12):6995-7001.
Zhao Y, Qin F, Boyd JM, Anichina J, Li XF. (2010) Characterization and determination of chloro-and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water. Analytical Chemistry 82 (11):4599-4605.
Zwiener C, Richardson SD, De Marini DM, Grummt T, Glauner T, Frimmel FH. (2007) Drowning in disinfection byproducts? Assessing swimming pool water. Environmental Science & Technology 41 (2):363-372.

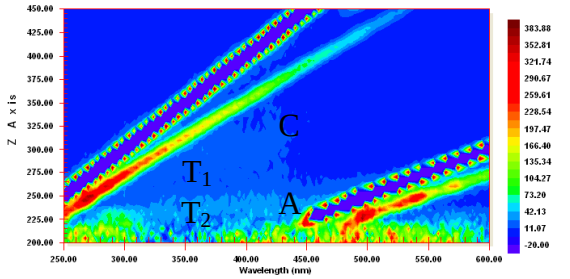
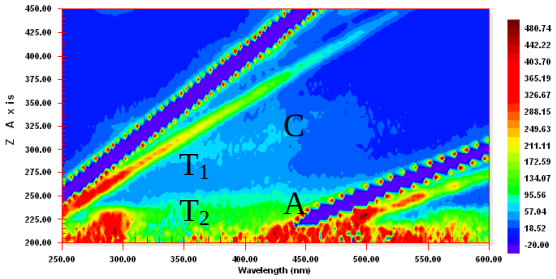
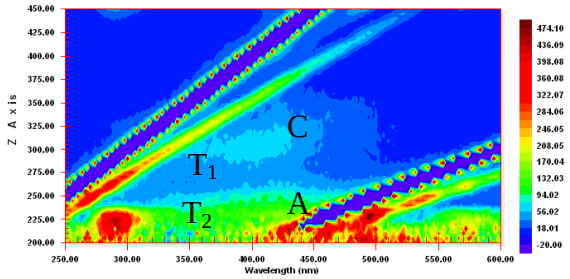
APPENDIX A

Raw fluorescence EEMs of various swimming pool waters consisting of indoor pools, outdoor pools and spa pools

Location A	
Indoor heated	Fill water
	

Location B	
Indoor main	Spa pool 1
	
Indoor wading	Spa pool 2
	
Indoor training	Spa pool 3
	

Location D	
Outdoor 22m	Waterslide
	
Outdoor 50m	Fill water
	
Toddlers pool (shaded)	
	

Location E	
Indoor 50m pool	Indoor training pool
	
Indoor wading/spa pool	
	

APPENDIX B