CHEMICALS OF CONCERN IN RECREATIONAL WATERS

OCCURRENCE AND ASSESSMENT OF POTENTIAL HUMAN HEALTH RISKS OF CHEMICALS IN PUBLIC SWIMMING POOLS

Report to the
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by

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EXECUTIVE SUMMARY

BACKGROUND

During the summer months, thousands of South Africans head to municipal and holiday resort swimming pools to cool off. By far, drowning is the most common risk associated with swimming, but it is also the most preventable by installed pool safety fences and covers limiting unsupervised pool access. Apart from dangers associated with drowning, swimming pool water, if not well managed, may act as a medium for exposure to harmful pathogens and chemicals. The World Health Organisation (WHO) Guidelines for safe recreational water environments (Volume 2: Swimming pools and similar environments) (WHO, 2006) provide guidance on the management of risks related to swimming pools, as well as broad perspective on water quality health risks. In South Africa, the quality of public swimming pool water has to meet the South African National Standards (SANS) 241 for drinking water of 2015 as was stipulated by Notice 943 of 2013 (Government Gazette, 2013). However, current practice shows that public swimming pools are largely managed only to protect the bather from pathogens that might have been introduced by bathers themselves. The focus of this study was to investigate the presence of selected chemical compounds of concern in public swimming pools and evaluate the potential risks due to exposure during swimming.

The quality of the water depends on the quality of the management of the pool water. The biggest concern of most managers is to make sure that the microbial parameters are met, with very little concern for the chemical constituents of the pool water. Numerous research reports have shown that pool water may contain a wide variety of chemicals of concern that may originate from the source of the water, i.e. borehole or tap water provided by the municipality (treated river water), and those that can be formed due to addition of treatment chemicals into the pool water. For example, while the addition of disinfecting chemicals (e.g. chlorine) may inhibit the presence and proliferation of pathogens, they may also contribute to the formation of potentially harmful by-products. And a third source of chemicals introduced to the pool water is the bathers themselves. Apart from the personal care products that wash off their bodies, the bodily excretions such as urine and sweat can contribute to the presence of toxic organic compounds, as well as pharmaceuticals and their partially metabolised versions thereof. The human health risks associated with exposure to such chemicals is well documented, with a fair amount of information also available on the possible disinfectant by-products that may develop as well as their detrimental effects on human health. However, one factor that has not been investigated extensively is the endocrine disruption effect of swimming pool water, especially due to the presence of mixtures of chemicals of concern such as metals, disinfectant by-products and other organic compounds of concern such as pharmaceuticals and personal care products. The latter two classes of compounds are known for their unintended ability to cause interferences in the hormonal processes of vertebrate bodies. This study, serves as a baseline for establishing the health implications due to exposure of bathers to chemicals of concern in public swimming pools.

AIMS

The following were the aims of the project:

1. Conduct a review of literature on public swimming pool water quality, human health risks and regulation, and highlight best practices.
2. Conduct initial screening to determine the presence of human health priority substances in public swimming pool water, at the start and end of each day during peak swimming season.
3. Compare the endocrine disrupting effects of open pools to covered pools that receive the same treatment and source water.
4. Determine the levels of selected human priority substances (such as disinfectant by-products, and metals) and determine toxicity effects, including endocrine disrupting effects.

5. Formulate recommendations for public swimming pools monitoring and develop an advisory document for water quality management for public swimming pools.

METHOD

The study was conducted in collaboration with managers from public swimming pools in municipalities and holiday resorts. At each site, samples were collected from the source water (i.e. municipal inlet, reservoir, borehole), in the pool and also the backwash water of each pool. Water was sampled at different times of the day: early in the morning before the bathers arrive (am) and at the end of the day after most of them have left (pm). From the samples collected, instrumental analysis was done to determine the levels of selected metals, disinfectant by-products and organic compounds. Water quality parameters (e.g. pH, temperature, NO_3^-, PO_4^{3-}, etc.) were also measured for each of the swimming pools under investigation. These levels were compared to drinking water quality guideline levels of South Africa as well as to other relevant international water quality guidelines as there is no public swimming pool water quality standards or guidelines in South Africa. A limited human health risk assessment was done investigating both cancerous and non-cancerous risks due to ingestion and dermal exposure. This could only be done for the four trihalomethanes (THMs): chloroform, bromoform, dibromochloroform and bromodichloroform as well as the metals. *In vitro* screening of the pool water was done to determine endocrine disrupting effects. Reporter gene-bioassays were used to determine the endocrine disruption ability of the water. These assays all measure the ability of the compounds to bind to hormonal receptors through which the endogenous hormone affects its function. Both activation of the receptor as well as inhibition were investigated. The “reporter” in the assays is the firefly luciferase gene that had been encoded into the mammalian cells’ genome. In the presence of the endogenous hormone/hormone mimic, light is emitted by the cells and this luminescence is quantified. Sample extracts were also investigated for their cytotoxicity and to this end, viability assays were run in parallel to the luminescence assays.

RESULTS AND DISCUSSION

For most of the instrumentally determined values, most of the guideline levels were not exceeded and if there was an exceedance, it was for the backwash water and the pond into which discarded water was dumped. None of the trihalomethanes (THMs), for instance, was greater than their respective guideline levels, and the total THMs in only one sample exceeded the guideline levels. However, nitrites almost invariably exceeded the South African drinking water quality standard. Most of the physical, aesthetic and chemical determinands were within the drinking water guideline levels. The most common pharmaceuticals and personal care products that washed off in the pool water included fluconazole, articaine, efavirenz and methylene blue which were only detected at the municipality pools. Zoloperone, detanosal, euprocin, dihyprylone, ampyrimine, indeloxazine and pyridinolcarbamate were unique to the holiday resort pools.

In terms of the toxicity assessments, only the backwash samples were cytotoxic. There was evidence of androgen and oestrogen activity, of both the agonistic and antagonistic type, but none of these responses exceeded any internationally available guidelines. The South African guideline for oestrogen-like responses was exceeded only once and that was for a backwash sample. The South African guideline is more sensitive than the one derived for international use. The human health risk assessment revealed that for both hazard quotient (HQ) and cancer risk (CR), backwash water is the most harmful and if the water is returned to natural sources aquatic biota might be harmed. Exposure through the skin was greater than through the ingestion of swimming pool water and it was the metals that were responsible for the highest HQ and CR compared to the THMs. Chloroform was one of the THMs that contributed to health risk. The children were more at risk than the adults to develop detrimental health effects due to these compounds and elements that occur in public swimming pools.

CONCLUSIONS

- There was evidence of disinfectant by-products but these were not exceeding drinking water standards except in one instance.
- There were quantifiable levels of metals in the pool water but mostly not exceeding drinking water quality standards.
- Nitrites were without exception too high, when compared with the drinking water quality guideline, most likely due to chloramination.
- Dermal exposure to THM and metals may lead to greater human health risk than the ingestion exposure pathway and children are more at risk than the adults.
- The elements in the source water contributed more than the THMs to the human health risk. Two THMs contributed to a lesser extent.
- Pharmaceuticals and personal care products were detected in the pool water.
- There were quantifiable levels of androgen as well as oestrogen activity in the swimming pool water, but none of the available guideline levels were exceeded.
- Most of the time, the samples that did show bio-assay responses were those collected at the end of the day, i.e. after many people spent the day in the pool, or backwashed water, that is supposed to contain the highest concentration of toxicants.

RECOMMENDATIONS

Recommendations regarding the regulation of public swimming pools:

- Results from the current study highlight the need for regular monitoring of public swimming pool water and monitoring for more than the limited list of compounds on the SANS 241 document.
- Instrumental analysis for every chemical known (and not yet known) to man is not feasible. A solution that is currently being investigated for application in drinking water quality, is to apply *in vitro* biological assays, to screen for selected biological endpoints, the so-called effects-based approach, to determine the safety of the water for human consumption. This approach is a solution that may be extended to include public swimming pool water (recreational water) as well as other water uses, i.e. ecological, agricultural, and industry. A variety of biological endpoints can be tested for using biological assays, including cytotoxicity, genotoxicity, mutagenic, oxidative stress, and endocrine disruptive.
- Bathers should be informed about the role pharmaceuticals and personal care products may play in contaminating swimming pool water with endocrine disruptors and that making use of the showers (often available at public swimming pools) before entering the pool might help to curb overloading the pool with contaminants.
- Bathers should also be made aware of the detrimental health effects they may cause other bathers when relieving themselves in the water, not only because of hygienic reasons, but also because partially metabolised pharmaceuticals entering the swimming pool in this manner. These pharmaceuticals may contribute to the load of endocrine disruptive compounds. The public should be made aware of the possible risks they expose themselves to when using public swimming pools, especially at more sensitive life stages.
- Although UV and ozone may contribute to the creation of new compounds from existing ones, consider using UV and/or ozone as an alternative to chemical disinfecting products.
- If chlorine and bromine based disinfectants are used, ensure that they are applied at levels indicated by the WHO (2006).
- Care should be taken NOT to return backwashed water into the pool since it contains the highest levels of contaminants.
- Backwash water should be treated before being disposed of into the environment.

Recommendations to improve the research side of the project:

- A larger sample size of swimming pools should be analysed on a regular basis over a longer time period to confirm the endocrine disruptive effects observed.
- The pharmaceuticals and personal care products identified should be quantified as well if analytical standards are available and affordable.
- The effect of temperature, and whether a pool is enclosed or not, may be included in the research, provided that all other variables are controlled for. These variables include the size of the pool and the source water.
- Although the main biological endpoint of this project was endocrine disruption, other biological endpoints might be screened for using the *in vitro* bioassays, such as oxidative stress and genotoxicity.
- The pond that received the backwash water from the three swimming pools on the resort should be monitored closely. It contains aquatic life, and some of the fish should be sampled and an in-depth investigation into their health will reveal if the toxicants in the backwash water are at levels that are detrimental to wildlife.

GENERAL OVERVIEW OF THE STUDY

While the aims had been achieved in this research, like in most research, limitations were experienced and gaps identified. From the results it is clear that there are disinfectant by-products present as well as elements probably coming from the source water in the public swimming pool water, but long term monitoring of a greater number of pools will give a better estimation as to what the mean variability of these levels are throughout the four seasons. Personal care products and pharmaceuticals have been discovered in the pool water, but the sample size was limited and although the study proved their presence, little else could be determined, i.e. which are the most common ones and how it varies from province to province, for instance, are not known at this stage. The small sample size also hampers the validity of the human health risk assessment although certain trends were observed: metals seemed to have contributed more to the risk to human health than the THMs for instance and the children are more at risk than adults.

Clear evidence of the endocrine disruptive effects of compounds from the pool water had also been established in this study, however, a greater number of pools sampled regularly over a longer period will give a better idea of the variability of the endocrine disruptive effects and whether it ever reaches a level at which we should be concerned.

Comparison between warm and cold water swimming pools and the differences between indoor and outdoor pools can only be investigated under very controlled environments where the exact same bathers enter the pool, spending the same amount of time and using the same PPCPs. Although this was an initial aim, such controlled environments were not available during this study. Learning the effect of a covered swimming pool on the creation of DBPs for instance would require temperature control of both pools of the same size filled with the same source water and treated with disinfectants at the exact same time and rate. Literature has shown that temperature and UV availability influences the formation of DBPs.

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PIETERS, R., HORN, S., JOUBERT, S., TSHIAMO, S., GERBER, E. Hormone receptor binding by compounds from public swimming pool water. 2019. 2nd African Conference on health effects of endocrine disruptors, Pretoria, South Africa, 4-7 Nov 2019. Presented by Pieters	

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ACRONYMS & ABBREVIATIONS

AhR	aryl hydrocarbon receptor
AR	androgen receptor
BCDMH	bromochlorodimethylhydantoin
CDI	Chronic daily intake
cdtFBS	charcoal dextran-treated foetal bovine serum
CHO	Chinese hamster ovary (cells)
CR	Cancer risk
DBP	disinfectant by-product
DEq	dexamethasone equivalents
EBT	effect-based trigger value
EDC	endocrine disrupting compound/chemical
EEq	oestradiol equivalents
ER	oestrogen receptor
FBS	foetal bovine serum
FC	fold change
GR	glucocorticoid receptor
HAAs	haloacetic acids
HBQ	halobenzoquinone
HHRA	Human health risk assessment
HI	Hazard index
HQ	Hazard quotient
HRA	Health risk assessment
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD	optical density
PPCPs	pharmaceuticals and personal care products
REP	relative potency
RLU	relative light units
SANS	South African National Standards
TCC	triclocarban
TCS	triclosan
TEq	testosterone equivalent
THM	trihalomethane
US EPA	United States Environment Protection Agency
VC	vehicle control
WHO	World Health Organization

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1 BACKGROUND

1.1 INTRODUCTION

The quality of public swimming pool water in South Africa has to meet the South African National Standards (SANS) 241 for drinking water of 2015 as was stipulated by Notice 943 of 2013 (Government Gazette, 2013). Various municipal by-laws contain limited information regarding swimming pool water quality management but they are all aligned with SANS 241 and the World Health Organisation (WHO) guidelines for recreational water environments (WHO, 2006), which states that parameters such as pH, turbidity, residual disinfectant, and microbiological must be frequently monitored. The WHO guidelines state that public swimming pool water should be managed in such a manner that it would ensure the safety of the bathers: turbidity for instance must be such that the body of a small child is visible at the bottom of the pool from the vantage point of the lifesaver; there should be no threat from contracting a microbial or protozoan-carried pathogen; and no harm should come from the pH or chemicals added to treat the water.

In this report the focus is on the presence and risks of chemical of concern in swimming pool water, specifically those that are contributed to by the bathers, and thus include compounds that cannot necessarily be controlled by regulation or swimming pool operators. These compounds include bodily excretions such as sweat, urine and small amounts of faecal matter, as well as personal care products such as sunscreens, that wash off the bodies. Included in the small amounts of urine there could be pharmaceuticals in the various stages of metabolism. Bathers would also be exposed to compounds that originate from the source water with which the swimming pools are filled and this could include metals. The disinfectants added to the water to prevent the spread of microbial or protozoan diseases react to the organic matter in the swimming pool water to form the so-called disinfectant by-products (DBPs) and these compounds may have detrimental health effects, especially on the respiratory system.

Numerous research reports have shown that the presence of trace amounts of these chemical contaminants in swimming pool water may have a negative influence on the health of the bathers. *In vitro* tests using Chinese Hamster Ovary (CHO) cells showed that swimming pool water was more toxic than tap water and according to the Ames test, swimming pool water were similarly mutagenic as chlorinated potable water (Tao et al., 2015). In another study, it seemed as if brominated disinfectants caused the swimming pool water to be the most genotoxic. In fact, when the disinfectant is bromochlorodimethylhydantoin (BCDMH) the pool water is four times as genotoxic compared with chlorine-based and UV disinfectants (Tao et al., 2015). Another DBP is chloramine which might be the source of respiratory symptoms and eye irritations in prolonged exposures. Outdoor pool water is likely five times less genotoxic than indoor pools and a possible explanation is the potentially increased volatilisation of chemicals in the open air.

1.2 PROJECT AIMS

The following were the aims of the project:

1. Conduct a review of literature on public swimming pool water quality, human health risks and regulation, and highlight best practices
2. Conduct initial screening to determine presence of human health priority substances in public swimming pool water, at the start and end of each day during peak swimming season
3. Compare the endocrine disrupting effects of open pools to covered pools that receive the same treatment and source water

4. Determine the levels of selected human priority substances (e.g. disinfectant by-products, metals, etc.) and determine toxicity effects, including endocrine disrupting effects.
5. Formulate recommendations for public swimming pool monitoring and develop an advisory document for water quality management for public swimming pools.

1.3 SCOPE AND LIMITATIONS

The nature of what is described as a public swimming pool is wide and varied and include swimming pools operated by gymnasiums, holiday resorts, sport facilities of universities, and municipalities, just to name a few. The source water can be fresh or marine. In this project the focus was on municipal swimming pools of a town in the North-West province, South Africa, as well as a holiday resort known for its good maintenance of pool water because the pools are its main tourist attraction. We investigated therefore only freshwater swimming pools. The focus was on swimming pools only and did not include spa baths or jacuzzis. Although a broad spectrum of elements were detected, only four of the most common disinfectant by-products were quantified as well as “total trihalomethanes”. The elements were quantified on the campus of the North-West University (NWU), but the quantification of the DBPs had to be outsourced and because of the limited number of samples that could be accommodated by this laboratory, beyond that which they regularly analyse for their own purposes, and the time constraints, not all of the samples were analysed for these compounds. The laboratory can only accommodate 10 samples which must be delivered to them as soon as possible and the analysis should be done without delay. Water samples should not be stored for long when the target compounds to be analysed include trihalomethanes. This study was based on grab samples collected during the duration the study and a small sample size, but enough information was gathered to make recommendations.

2 LITERATURE REVIEW

2.1 INTRODUCTION

Public swimming pools in South Africa are often managed only to protect the bather from microbial pathogens that might have been introduced by bathers themselves. Perhaps the most important guidance for the quality of public swimming pool water in South Africa is that the potable water supply serving the swimming pool should comply with the drinking water quality standards (SANS 241) and thus was used as the overall yardstick for water sampled in this study. This standard provides levels for either acute or chronic health effects. However, as the project progressed and results were obtained, it became clear that SANS 241 is perhaps not the ideal document to refer to for public swimming pool water quality. Because there is no other more applicable guideline currently available to guide public swimming pool water quality in South Africa, it is still referred to in this report.

In light of the fact that people are not expected to be drinking large quantities of swimming pool water, the focus of this study is on chronic exposures rather than the levels capable of acute toxicity. And less attention is given to volatile compounds because these would quickly dissipate from the large surface area of swimming pools. Volatile compounds may play a role if the swimming pool is enclosed, and the ventilation is poor. However, the focus of this report is on water quality, and not air quality. Since the main focus of this report is on the effects of chemical compounds found in public swimming pool water, and not on the microbial safety, all of the literature review is on the chemical compounds, with special reference to those which are not addressed by any water quality guideline, but may influence human health. For this reason, brief mention is made to metal pollutants, but only in reference to their potential harmful effects to human health, and not with regards to the harm they may have on the wear and tear of the swimming pool seal and appearance and pumps.

The bathers themselves may contribute compounds to swimming pool water that may be harmful to human health. These include parabens and UV-filters from sunscreens and these not yet regulated. The mixture of disinfectants and the personal care products (PCPs) together with UV irradiation have been reported to create by-products that may be more toxic than their parent compounds (Teo et al., 2015). Immunocompromised individuals may be at higher risk from microbial or chemical hazards (WHO, 2006). The degree of water contact would influence the amount of exposure to whatever is dissolved or suspended in the water. Bathers are also exposed to aerosols that might harbour contaminants.

2.2 MANAGING WATER QUALITY IN PUBLIC SWIMMING POOLS

This section contains information found in literature, both in science databases but also guidelines regarding public pool maintenance published by countries from all over the world. The latter often offer extensive information regarding the physical requirements of a swimming pool which include references to underwater benches and ledges, and where the first aid kit should be kept. However, the focus of this report was on chemical water quality and therefore it is the focus of this section as well. Because microbial activity in swimming pools is controlled by chemicals, microbial activity is briefly referred to as well.

2.2.1 Diseases transmitted in swimming pools and current guidelines

Serious diseases may be contracted in swimming pool water due to pathogens of faecal origin, such as bacteria, viruses, fungi, enteric and free living protozoans and other parasites. These usually originate from

humans using the water but sometimes they may come from pumps, filters, water piping, external wet surfaces, heating, ventilation, and air conditioning, to name a few (Bottoni et al., 2014). Poorly managed swimming pools have caused illnesses such as enteritis, lung infections, sore throats, dermal infections such as rash, and fungal infections of hair, skin, and nails. Viral otitis, conjunctivitis and infections of the urinary tract also have been documented (WHO, 2006; Brandi et al., 2007). According to the Norms and Standards for Environmental Health published in the South African Government Gazette Notice 943 (South Africa, 2013), the water should be regularly monitored for heterotrophic plate count bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Legionella*-species. The total viable bacteriological count may not exceed 100 organisms per mℓ of water and *E. coli* type 1 may not be present in any 100 mℓ of swimming pool water.

This same guideline could be found in municipal by-laws of South Africa. These can be summarised from the public health by-law for the city of Johannesburg (Gauteng Provincial Gazette no 179, 2004) which states that "the total viable bacteriological count of any sample submitted for analysis, must not exceed 100 organisms per mℓ of water; and *Escherichia coli* type 1 bacteria must not be present in any 100 mℓ of water"

2.2.2 Chemical treatment options available

A variety of treatment approaches are available to create and maintain public swimming pool water fit for human use: multiple disinfection options coupled with chemicals for pH control, algal control, stabilisers, and flocculating agents (Bottoni et al., 2007). Chlorination is a disinfectant that had been introduced for water treatment in 1902, but other chemicals are also used: chloramine, chlorine dioxide, ozone and UV irradiation (Chowdhury et al., 2014.). Chlorine disinfectants include sodium hypochlorite (which is liquid bleach), calcium hypochlorite, or chlorine gas for indoor pools and examples of stabilised chlorine products are stabilised chlorine granules, chlorinated isocyanurates and chlorine tablets. The use of the chlorine product creates a relatively high dose of free residual chlorine (FRC). This, together with the usually higher temperatures of swimming pool water (colder than ambient temperature of piped water for drinking), constant organic load input by bathers (sweat, urine, skin particles, cosmetics and other personal care products) and contact with air at the water surface, form disinfectant by-products (DBPs) (Chowdhury et al., 2014).

The version of chlorine that is responsible for its disinfecting properties is referred to as FRC or free available chlorine or free chlorine. This refers to the concentration of hypochlorous acid and the hypochlorite ion in equilibrium concentration in the pool water. It is good practice to attain breakpoint before the first chlorine measurements are taken each day. Breakpoint chlorination means that all of the chlorine is available as free chlorine and is achieved by adding sufficient chlorine to burn out all the combined chlorine, so that the free chlorine equals total chlorine. In South Africa the municipal by-laws prescribe that where chlorine based disinfectants are used, it must be maintained between a minimum of 0.5 mg/ℓ, with a maximum of 3 mg/ℓ free available chlorine residual. And if a disinfectant other than chlorine is used, the residual level must be equivalent in effect to the requirements for chlorine (Gauteng Provincial Gazette no 179, 2004).

Some physical and chemical water characteristics such as the turbidity and pH are also mentioned by the Norms and Standards document. The role of pH is important because an alkaline or acidic pH decreases the disinfection power of free chlorine (Queensland Health, 2004). The pH should be kept between 7.2 and 8.0 depending on the nature of the disinfectant used—chlorine or bromine. The municipal by-laws echo this: The pH value of the water must be not less than 7 and not greater than 8. The pool water must also be free from floating, suspended or settled debris or swimming organisms and the walls, floor, access ladders or steps and gutters, must be free from slime and algae (Gauteng Provincial Gazette no 179, 2004).

2.2.3 Monitoring pool water quality

In South Africa the public pool water maintained by municipalities, must be sent for "chemical and bacteriological examination" (Gauteng Provincial Gazette no 179, 2004). This must be done by the environmental health officer and the frequency of the testing intervals are described as "…at intervals which he or she considers appropriate…". These samples must be submitted to an analyst authorised in terms of section 12 of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 to do the analysis. This section 12 describes that the analyst should use methods according to regulation (Government Gazette, 1972). The by-laws mention that the free chlorine, pH and bacteriological levels should be tested but have no clear specifications for the frequency of this testing. This is in contrast to the degree of detail given in other countries' guidelines levels. An example was found in the Australian state of Queensland. Their swimming pools with high use frequency "should be monitored five times a day for free chlorine, total chlorine, other forms of disinfectant and pH" and water should be sampled once a month for microbial analysis. There is also a description of the method to be followed when the sampling is done (Queensland Health, 2004). According to the WHO guidelines, only the parameters that are easy and inexpensive to measure reliably and of immediate operational health relevance, such as turbidity, residual disinfectant and pH) should be monitored frequently. Microbial monitoring is definitely a requirement for public swimming pools (WHO, 2006).

2.3 HUMAN EXPOSURE TO CHEMICAL COMPOUNDS IN SWIMMING POOL WATER

There are a variety of factors that influence the level of human exposure to the chemical compounds in the pool water. Some of these factors belong to the physico-chemical nature of the compounds themselves, i.e. their volatility and solubility in water, but other factors belong to the human bathers and their behaviours: surface area of the skin, and duration of being submerged in the water. And yet another category of factors includes the physical environment of the swimming pool: is it covered or open, and the frequency of treating the water with disinfectants, and period to renew the entire volume of the pool. When predicting human health risk using approaches adopted by the WHO and United States Environmental Protection Agency (USEPA), many of these variables are taken into consideration. Here attention is given to the three main routes by which chemical compounds might enter the human body: ingestion, inhalation, and dermal contact.

2.3.1 Ingestion

Human behaviour will greatly influence the amount of water that will be ingested. Children may spend long periods in the swimming pool, and are more prone than adults to accidentally swallow water (WHO, 2006). According to Suppes et al. (2013) adults ingest 4 mℓ/h and children 26 mℓ/h swimming pool water and this happens when being exposed to surface splashing and not when submerged. Previously, Evans et al. (2001) reported that children, on average, ingest 37 mℓ and adults 16 mℓ of swimming pool water however, these authors did not reflect the period of exposure. According to the same authors, there was also a gender difference: men and boys ingested more water (on average 22 mℓ and 45 mℓ respectively) but women and girls ingested less (12 mℓ and 30 mℓ respectively). In a more recent study in Ohio, USA, by Dufour et al. (2017) the study population consisted of 549 participants from nine public swimming pools, did they found that adults ingest on average 32 mℓ/h and children swallowed four times more, i.e. > 120 mℓ/h. In their paper, these authors summarised previous assumptions regarding quantities of ingested swimming pool water to range between 10 mℓ and 50 mℓ per event.

2.3.2 Inhalation

Bathers would inhale from the air just above the water's surface and any of the volatilised compounds, such as many of the DBPs. According to Chowdhury et al. (2014) the major routes of uptakes of DBPs from

swimming pools are inhalation and dermal contact, rather than from ingestion. The volume of air inhaled depends on the intensity of the physical activity and the period thereof (WHO, 2006). Whether a swimming pool is covered or not might also influence the level of THMs and other DBPs in the air above the water, however, the results are variable, according to a study by Simard et al. (2013). Comparing 15 indoor pools in Quebec, Canada to 39 outdoor pools fed with the same source water showed that the ratio between indoor and outdoor was 2.6-0.6 for THMs indicating that the THMs can be lower in the outdoor pools than in the indoor pools. But the HAAs were always higher in the outdoor pools than the indoor pools. Although HAAs are also formed as a result of chlorinated and brominated treatment of pool water, these were not investigated in this study. There are also no guideline levels for these in SANS 241 pertaining to HAAs.

2.3.3 Dermal contact

The skin is probably the area of the human body that is the most exposed to swimming pool water and depending on the physico-chemical nature of the chemical compound, there are two different pathways for lipophilic and hydrophilic compounds to cross the outer layer of the skin. Lipophilic compounds are transported via the lipid portion of the cell membranes and the hydrophilic compounds via the protein component. Lipid content of the stratum corneum (outer layer of the skin) varies greatly in lipid content between humans. The higher the lipid content, the greater the transport of lipophilic compounds across the membranes (Raykar et al., 1988). The extent of uptake through the skin is furthermore influenced by the duration of exposure, the temperature of the water and the concentration of the chemical (WHO, 2006).

2.4 WATER QUALITY CHEMICAL DETERMINANDS

The chemicals selected to be discussed here, are those listed in the SANS 241 (2015) drinking water standards for South Africa, because of the lack of any other swimming pool guideline level. The reason for this is that the by-laws all stipulate that the public swimming pool maintained by municipalities (Gauteng Provincial Gazette no 179, 2004), should be filled with municipal water, which by default, should meet drinking water quality standards. From the literature, it is evident that a limited chemical analysis is required for pool water quality, both nationally and internationally. The standards for drinking water are stricter and contain allowable levels for more compounds. There are health risks associated by amongst others, the DBPs, but none of the national and international guidelines for public swimming pool health included any of these DBPs.

2.4.1 Disinfectant by-products

It has been known for some time that the disinfectants added to swimming pool water to prevent microbial infections (Teo et al., 2015), form harmful by-products that are regulated (SANS 241:2015). The presence of trihalomethanes (THMs) in swimming pools was reported for the first time by Weil et al. (1980) and Beech et al. (1980). Over 600 DBPs have been identified in chlorinated waters Richardson et al. reported in 2007 and by 2015 Teo et al. reported a number greater than 700 and many of them are mutagenic and carcinogenic. And since swimming pool water is also mostly treated with chlorine, these compounds are also present in pool water. Various factors such as high free residual chlorine, high temperatures, organic precursors, constant organic loads, exposure routes, contact of water surface with air as well as water recirculation could affect the formation of DBPs compared with drinking water DBPs (Hang et al., 2016). Swimming pool water has been shown to be genotoxic, mutagenic and cytotoxic because of its large variety of chemical contaminants that had been added to disinfect the water, but also that washed off of bathers' skin (Teo et al., 2015). The organic compounds coming from the bathers, such as skin lipids, may be the main contributor to the production of carbonaceous DBPs (Keuten et al., 2014).

Trihalomethanes are generated from the complex reaction between chlorine and organic matter. They consist of one carbon atom and at least one (often two) halogens with the fourth bond on the carbon filled with a hydrogen atom. Parameters, other than the presence of organic matter that influence the formation of THMs, are chlorine concentration, contact time, water pH, temperature, and bromide ion concentration. When pH increases, the THM concentration increases. In the presence of bromides, brominated THMs are formed preferentially, most likely because the bromination reaction is quicker than chlorination (Florentin et al., 2011). Although most countries do not have a regulatory limit for DBPs, Germany set a guideline level of 20 µg/ℓ for total THMs and the French have a maximum limit of 100 µg/ℓ. THMs include chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$) (Teo et al., 2015). South Africa has chronic guideline levels for the four THMs listed here and that is why they have been included in the current study. Because of their lipid solubility, THMs are easily absorbed by biota, but they have a relatively short half-life in the biota (DWAF, 1996).

Haloacetic acids (HAAs) are another DBP class that may occur in pool water but usually not at the same levels as THMs. The most common congeners are monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloracetic acid (TCAA), monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA). They are less volatile than THMs. Halobenzoquinones (HBQs) were found in pools treated with chlorine or a combination of chlorine and UV disinfectants. The body creams and sunscreens from bathers' bodies are possible sources for HBQ precursors and may increase the formation of HBQs in pool water (Teo et al., 2015). Trichloramine (NCl_3), chloral hydrate, chlorate, chlorite and bromate all have been detected in swimming pool water. For an extensive review on all of the possible DBP that have been identified up to date, the reader is referred to Teo et al. (2015).

Although there is a long list of DBPs that may form in a swimming pool, in this section, on their potential hazard to human bathers, the focus is on those four that were instrumentally analysed for this study: chloroform, bromoform, bromodichloroform and dibromochloroform.

Chloroform, bromoform and bromodichloroform are classified as Group B2 probable human carcinogens by the United States Environment Protection Agency (US EPA) whereas dibromochloromethane is regarded as a Group 3 carcinogen which means that although there is some evidence in literature that it might be cancer forming in animal models, it had not been confirmed in humans; i.e. a probable carcinogen. Bromoform is also labelled a mutagen. Non-cancer effects of chloroform and bromoform on animals were observed when exposure was through inhalation as well as ingestion. Organs that were affected include kidney, liver, the nasal epithelium (chloroform only), developmental epithelium (chloroform only) and colon (bromoform only) (OEHHA, 2019). Non-cancer hazard had been proven for both bromodichloroform and dibromochloroform in laboratory animals and it caused liver, kidney, and reproductive toxicity. The brominated and nitrogenous DBPs are more cytotoxic than the chlorinated DBPs (Hang et al., 2016).

Exposure to these compounds in swimming pool water is not only via the inopportune ingestion of small quantities of water, but also through dermal absorption and inhalation. The latter happens because these THMs are volatile organic compounds (McKone, 1987). Of the four under consideration chloroform is the most volatile, then bromodichloroform and then dibromochloroform (Kerger et al., 2005). Another route of getting these organic compounds into gaseous phase, is the diffusion taking place between the surface of the water and the air (OEHHA, 2019). The absorption of the THM takes place not only through the intact skin when swimming, but is absorbed by the lung epithelium when inhaled and absorbed via the inside of the mouth. It is also rapidly absorbed by the gastrointestinal track after ingestion. In fact, the majority of ingested THMs are absorbed (Mathews et al., 1990) and 90% of it in the mouth (OEHHA, 2019). According to Panyakapo et al. (2008) skin and gastrointestinal exposure while swimming is the main intake route of THM in comparison to other exposure routes. In a study by Xu et al. (2002), the permeability coefficients of these THMs ranged from 0.16 to 0.21 cm/hour when tested *in vitro* using human skin. Bromoform had the highest permeability coefficient value, and chloroform was the least permeable through the skin.

Once inside the body the distribution of the THMs are determined by the logarithm of their octanol/water partition coefficient ($\log K_{OW}$) values which indicate that they are between 93 (chloroform) and 250 (bromoform) times more soluble in octanol than in water, which means that they will partition to lipids where it will be stored. The four THMs are removed from the body through urine and faeces, but most of it is eliminated via exhalation without metabolic change. A portion of the metabolised THM is respired as carbon dioxide (OEHHA, 2019).

Chloroform is also considered a developmental toxicant because it decreases birth mass in humans and animals (OEHHA, 2019) and in inhalation studies in rats and mice there was increase in the percentage embryo implantations that were resorbed (Schwetz et al., 1974). There were also foetal malformations depending on the dose inhaled by the pregnant dams.

2.4.2 Metals, arsenic and cyanide

There are no guideline levels in South Africa for metals in public swimming pool water apart from the drinking water standards to which is alluded in the Norms and Standards for Environmental Health published in the South African Government Gazette Notice 943 (South Africa, 2013). And the South African National Standard for drinking water quality (SANS 241-1:2015a) lists a number of important elements that should be regulated in drinking water and the levels are set to prevent chronic health risks. Chronic health risks may develop after consuming 2 ℓ water every day for 70 years by a person weighing 60 kg. Guideline levels determined by this consideration also have safety factors included in the estimation. The metals for which chronic health standards exist in South Africa include: antimony (Sb), barium (Ba), boron (B), cadmium (Cd), total chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), and uranium (U). The metal aluminium (Al) also has a guideline level, but the reason this was included is because of operational, and not so much health related reasons (SANS 241-1, 2015a). There is a guideline for arsenic (As) included in this list of micro-determinands as well as cyanide (CN^-).

It is well-known that metals affect organisms on cellular level as well as on systemic level. Various cellular structures such as the cell membrane, endoplasmic reticulum, mitochondria, lysosome, and nuclei are influenced. On biochemical level, enzymes important to metabolism, detoxification, and damage repair are often affected and metal ions are known to cause DNA damage leading to cell cycle changes, controlled cell death and carcinogenesis. Among these metals, five have been identified as being very toxic and they are cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and arsenic (As) (not strictly a metal). These metallic elements may cause multiple organ damage and are classified as human carcinogens. But their toxicity depends on various variables such as dose, route of exposure, and the oxidation state of the metal. More factors that also influence their effect on human health are age, gender, and baseline health of the individual (Tchounwou et al., 2012). Cadmium may cause kidney damage, but also skeletal damage (Järup, 2003). Reversible effects of chronic inorganic Hg poisoning are characterised by neurological and psychological symptoms, which include tremors, changes in personality, restlessness and many more. The main symptom of methyl Hg poisoning is nervous system damage (Järup, 2003). Prolonged exposure to Pb may lead to memory deterioration, slowed reaction time and difficulty in understanding. It may also lead to anaemia. Low-level Pb exposure in children may be the cause of diminished intellectual capacity (Järup, 2003). Populations that are exposed to 100 µg/ℓ arsenic through their drinking water have higher risk of lung, bladder, and kidney cancer (Järup, 2003; WHO, 2018).

Cyanide (CN^-) inhibits the enzyme cytochrome c oxidase in the mitochondrial respiratory chain. This prevents tissues, especially tissues with high oxygen consumption like the brain and heart, to use oxygen. This reduction in oxygen is enough to cause cell death. Other enzymes that may also be inhibited by CN^-, are catalase, peroxidase, phosphatase, ascorbic acid oxidase, to name a few (ATSDR, 2006; CDC, 2018). But the guideline level for CN^- in South Africa's drinking water quality is for acute toxicity and no further attention is given to this micro-determinand.

Treatment chemicals can contribute to human health risk and therefore aluminium, iron, disinfectant residuals and ammonia are examples of compounds that may occur in the swimming pool water because of operational processes (SANS 241, 2015b).

2.4.3 Pharmaceuticals and personal care products

Many personal care products have been detected in swimming pools some of which are parabens and ultraviolet filters found in sunscreen lotions, as well as insect repellent such as N, N-diethyl-meta-toluamide (DEET), caffeine and tris(2-carboxyethyl) phosphine (TCEP) (Alcudia-León et al., 2013; Vidal et al., 2010; Weng et al., 2014). More examples of personal care products that are common are briefly mentioned below. The compounds mentioned were selected based on literature evidence that they are endocrine disruptive. The discussion is by no means exhaustive. However, studies showed that different chemical reactions between these compounds and disinfectants may transform them into products more harmful than the original PPCP (Bottoni et al., 2014).

Ultraviolet (UV) filters from sunscreen lotions, lipsticks, and shampoos, and parabens are found in a variety of body products. These compounds have endocrine disrupting capabilities (Teo et al., 2015). Children's pools seem to have a higher concentration of sunscreens than pools for adults. And it is possibly true that there would be more sunscreen in the pool during summer time than in winter time. Some sunscreens undergo photo-degradation (Rodil et al., 2009) and react with chlorine (Díaz-Cruz & Barceló, 2009). These processes may produce by-products which may be more harmful (Díaz-Cruz & Barceló, 2009) than the compound originally released into the pool.

Parabens are added to personal care products because it is an antimicrobial preservative (Terasaki et al., 2009). These compounds have been found to be oestrogenic, actively binding to both the oestrogen receptors, ERα and ERβ (Routledge et al., 1998; Watanabe et al., 2013); binding to the androgen receptor (AR) (Watanabe et al., 2013) as well as binding to the glucocorticoid receptor (GR) (Klopčič et al., 2015.)

Phthalates may be utilised as solvents for perfumes, are used in aerosols, and as additives to prevent the hardening of nail products. It is also found in body lotions, hair spray and deodorants (Witorsch & Thomas, 2010). It is usually regarded as antiandrogens (Erkekoglu & Kocer-Gumusel, 2016), is showed to have a role in the development of obesity and glucose metabolism disorders (De Toni et al., 2017), and inhibits testosterone production in the foetal testes (Witorsch & Thomas, 2010).

Synthetic polycyclic musks are a source of fragrances in cosmetics, soaps, and perfume (Witorsch & Thomas, 2010) and some of them had been found to be antagonistic toward the ERβ, the AR and the progestin receptor (PR). Antimicrobials such as triclocarban (TCC) and triclosan (TCS), that act as antibacterial and/or antifungal agents in personal care products such as liquid and bar soaps, toothpastes, mouthwashes, and cosmetics. Both TCC and TCS have aryl hydrocarbon receptor (AhR) antagonistic effects but of the two, only TCS has agonistic effects on the AhR (Witorsch & Thomas, 2010). (For notes on the endocrine disrupting role of the AhR refer to section 1.6.1. Triclosan is inhibitive of the ER and does not cause activation of the ER. Although TCC is not a ligand for the AR, it seems to enhance the action of androgen hormones through mechanisms other than binding to the AR (Witorch & Thomas, 2010).

Apart from what can be washed off of bathers' bodies, pharmaceuticals consumed by the bathers may be excreted through urine and/or sweat. Non-steroidal anti-inflammatory drugs such as diclofenac and ibuprofen have been shown to cause elevated plasma oestradiol levels and induction of the female vitellogenin protein in male fish (Hong et al., 2007; Han et al., 2010). Ibuprofen also modulates the thyroid endocrine system of amphibians (Veldhoen et al., 2014). Carbamazepine influenced the reproduction of male fish (Galus et al., 2013a). And a mixture of acetaminophen, carbamazepine, gemfibrozil, and venlafaxine significantly changed reproductive endpoints of female zebrafish (Galus et al., 2013b). Although the examples of the aforementioned

pharmaceuticals all are of effects in aquatic vertebrates, they are useful examples because if it can happen to fish, it may happen to a human, also a vertebrate. Steroidal pharmaceutical substances that might also be present in public swimming pool water include the natural 17β-oestradiol, oestrone, and the artificial oestrogen, 17α-ethinyloestradiol; progestogens such as norenthindrone and progesterone; the oestrogen antagonist, tamoxifen, and androgens and glucocorticoids such as testosterone, beclometasone and hydrocortisone (Tijani et al., 2013).

2.5 ENDOCRINE DISRUPTING CHEMICALS

2.5.1 Overview

The biggest motivation for this study was to learn the endocrine disruptive effects of swimming pool water, because this is an effect not often considered in monitoring regimes. And the biggest contributor of endocrine disrupting effects by swimming pool water is likely to be pharmaceuticals and personal care products (PPCPs). The term “endocrine disruptor chemical/compound” (EDC) is a collective name for all of the compounds, organic and inorganic, that have the ability to interfere with the functioning of the endocrine system in an animal body. The United States Environment Protection Agency (USEPA) defines an EDC as “exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for maintenance of homeostasis, reproduction, development, and/or behaviour” (Kavlock et al., 1996). Endocrine disruption can occur via: 1) agonists/antagonists of hormonal receptors, 2) selective modulators in coactivator/corepressor recruitment or 3) cross-talk between different receptor types (Yeung et al., 2011). EDCs can interfere with the hormones naturally produced in the body: from altering the amount of hormone synthesised and transported by the circulatory system, to the amount that reaches the target organs and how potently the hormone can activate its receptor (Gore et al., 2014). EDCs thus have the ability to interfere with the fundamentals of hormone signalling.

Because of the broad and interwoven role that hormones play in the body, disrupting their functioning has wide implications, from reproduction and fertility, to nervous and immune system related effects (Connolly et al., 2011). Detrimental effects include the development of cancers (Diamanti-Kandarakis et al., 2009), hepatic and cardiovascular diseases, reproductive disorders, learning and behavioural problems, as well as immune related health problems (WHO & UNEP, 2012).

2.5.2 In vitro measurement of endocrine disruptive effects

Research on the effect of EDCs on the endocrine system mainly focused on their direct effect on reproductive processes mediated by the family of nuclear receptors (NRs). The NRs are ligand-regulated transcription factors and are responsible for reproduction, homeostasis and metabolism, as well as responding to xenobiotics (Swedenborg et al., 2009). Members of the NR family include the ER and the AR. Generally, chemicals with oestrogenic-, androgenic- and thyroid-like activity are mediated through the respective nuclear receptors, but other modes of actions are also possible such as the aryl hydrocarbon receptor (AhR) (Swedenborg et al., 2009).

In vitro bioassays are used to determine if compounds can act as agonists or antagonists of these receptors. Reporter gene assays make use of genetically manipulated eukaryotic cells that produce a specific gene product, such as luciferase, in response to stimuli (New et al., 2003). The enzymatic activity of these cells can be monitored easily and thus ‘reports’ on the stimulus by producing light. The amount of light emitted is directly proportional to the enzymatic activity (New et al., 2003). A sample’s response is expressed in terms of the amount of light emitted by that of a known concentration of the reference compounds (See also Chapter 4).

The H4IIE-*luc* bioassay is the best characterised reporter gene assay model for determining AhR-mediated responses to ligands (Janošek et al., 2006). The H4IIE-*luc* cells are rat hepatoma cells that had been stably transfected with a luciferase reporter gene under the control of the dioxin responsive element (DRE) (Vrzal et al., 2005, Sanderson et al., 1996). This cell line expresses the CYP1A1 enzyme (Jacobs et al., 2008). The CYP1A1 enzyme is the most studied xenobiotic-metabolising enzyme, as it is the most consistently and commonly expressed isoform of the cytochrome P450 (CYP) family (Denison & Nagy, 2003, Shanle & Xu, 2010). Cross-talk can occur between the AhR and the signalling pathways of NRs such as the ER (Janošek et al., 2006). Activation of the AhR has been shown to exert ER independent anti-oestrogenic effects through the interference with ER signalling pathways (Janošek et al., 2006), resulting in reduced levels of oestrogens (Göttel et al., 2014).

The MDA-kb2 cells are human breast cancer cells that express the AR and the GR (Wilson et al., 2002). These cells were stably transfected with a mouse mammary tumour virus (MMTV) luciferase-neo reporter gene construct. The bioassay is used to screen for AR agonists and antagonists as well as GR agonists (Wilson et al., 2002). Glucocorticoid hormones are secreted by the adrenal glands and are responsible for maintaining homeostasis. They are necessary for growth, reproduction, intermediary metabolism, immune and inflammatory reactions as well as in the functioning of the central nervous and cardiovascular system (Klopčič et al., 2015).

2.5.3 The role of in vitro assays in effect-based approach of water quality monitoring

In the light of ever increasing occurrence of organic pollutants in the environment, more of them are bound to find their way into drinking water (which is what is filling specifically municipal swimming pools). There is a global drive towards effect-based analysis of water to ensure water quality, especially from Europe (Escher et al., 2018; Brack et al., 2019). Targeted instrument analysis of water samples can be limiting because only a selection of compounds is targeted for quantification. Non-target screening for monitoring remains challenging because it is dependent on the comprehensiveness of the database against which the screening is done (Brunner et al., 2020). In a country such as South Africa, routine instrumental analysis for a wide variety of chemicals in treated potable water is not realistic, let alone the regular testing of swimming pool water. A solution might be the use of a battery of *in vitro* bioassays by which swimming pool water can be screened regularly.

Because different chemicals cause different effects, water quality monitoring will require a relevant and efficient set of bioassays, based on health effects of water relevant chemicals (Escher et al., 2014). Cell models, tissues or small organisms have been developed to measure effects of chemicals on generic to specific biological processes (Brunner et al., 2020). A decided advantage of bioassays monitoring water quality is that they can measure the combined effects of low-level mixtures of chemicals (Brack et al., 2019).

It is prudent to remember that not every positive response in a bioassay is associated with a potential risk to human health. Therefore, individual effect levels at which potential risks cannot be excluded, so called effect-based trigger values (EBTs) need to be derived for each bioassay and for potential risk on human (and environmental health) (Brand et al., 2013; Escher et al., 2015; Van der Oost et al., 2017).

3 METHODS FOR SAMPLE COLLECTION AND ANALYSIS

3.1 INTRODUCTION

Swimming is a popular recreational activity in South Africa, especially during the hot summer months to cool-off from the high temperatures that may vary from 25-40°C. Municipal and/or borehole water is used to fill public swimming pools of towns and holiday resorts. Swimming pool water is treated with various substances to kill off microbes that may be pathogenic/spread diseases. As a result of these treatment processes DBPs form in the water. The source water may also introduce other contaminants for example metals.

The levels of four DBPs and a host of metals were determined for the municipality swimming pools as well as the holiday resort. The concentrations were determined only once for each of the pools in most instances to form an idea of the approximate values expected to be found. The main aim was to establish if there would be a difference between the water early in the morning before the swimmers entered the pool and at the end of the day. The concentrations were also determined for the source and the backwash water. Apart from the determination of concentrations of certain compounds, the identification of PPCPs was also done to learn the nature of the types of PPCPs that could find its way into public swimming pools.

Limited human health risk assessment was done for those compounds that were quantified (Chapter 3), i.e. some of the metals and the THMs, but not for the PPCPs because these were not quantified. It is however interesting to learn to which compounds swimmers inadvertently expose themselves due to that is rinsed off and excreted from other bathers. When going to a public swimming pool one is usually aware of the presence of disinfecting chemicals in the water as well as all of the PPCPs on and inside your own body, but one seldom thinks of those PPCPs coming from other bathers to which you are also exposed to.

3.2 MATERIALS AND METHODS

3.2.1 Site selection

Swimming pools from two different categories were chosen as sampling sites. Samples were collected at a public holiday resort (BP sites) that falls into the semi-public category and a municipal swimming pools (PD & PT sites) (public category), all of these were located in the North West province. The BP sites are the semi-public pools of the holiday resort with source water from boreholes and a water utility. The latter is only used when the borehole water is not enough to fill the swimming pools. The public pools from the town are designated PD and PT to distinguish between the two pools and their source water is municipal tap water. The two public swimming pools were in a town in the North West. Both uncovered and not heated except by the sun.

3.2.2 Water sampling

Water from three swimming pools (two outdoor heated swimming pools, BP pool A and BP pool C, and one indoor heated swimming pool, BP pool B) were sampled, as well as water from the reservoir, borehole, and backwash of each pool. Water was also sampled from the tap supplying the resort's utility water. Water was sampled at different times of the day: early in the morning before the bathers arrive (am) and at the end of the day after most of them have left (pm). In this way "clean" water could be compared to water now containing washed-off personal care products and body excretions like sweat and urine. Only surface water was sampled because that is the region in the swimming pool with the highest likelihood to also find UV filters

(Poiger et al., 2004). Water was sampled in 1 ℓ Schott glass bottles pre-cleaned with acid and kept 4°C (De Jager et al., 2011).

3.2.3 Water sample analysis

Immediately after sampling, the pH, temperature, dissolved oxygen (DO_2) and total dissolved solids (TDS) were measured using the Lovibond® Water Testing probe (Lasec). Nutrients were measured using a HACH spectrophotometer (CTE Water Tech) and these included: nitrates (NO_3-N), nitrites (NO_2), free chlorine (Cl_2), sulphates (SO_4^{2-}), phosphorous (PO_4^{3-}), nitrogen ammonia (NH_3-N) and sulphide (S^{2-}). The limit of quantification (LOQ) was defined as the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy under the stated conditions of a test (Shrivastava & Gupta, 2011). The LOQs for the various nutrients were: 0.3 mg/ℓ for nitrates (NO_3-N); 2 mg/ℓ for nitrites (NO_2); 0.02 mg/ℓ for free chlorine (Cl_2); 2 mg/ℓ for sulphates (SO_4^{2-}); 0.02 mg/ℓ for phosphorous (PO_4^{3-}); 0.01 mg/ℓ for nitrogen ammonia (NH_3-N) and 5 mg/ℓ for sulphide (S^{2-}). Alkalinity was measured using a commercially available HTH kit.

3.2.4 Determination of concentrations of selected chemicals of concern

3.2.4.1 Disinfection by-products

Analysis of disinfection by-products was performed by Rand Water (SANAS accredited laboratory) using a headspace gas chromatograph with an electron capture detector.

3.2.4.2 Metals

The water sampled for the metal analysis was collected in 50 mℓ centrifuge tubes and acidified with diluted HNO_3. Metal analysis were analysed at an in-house facility (Eco-Analytica) located on the NWU Potchefstroom campus with an inductively coupled plasma-mass spectrometer (ICP-MS) (PerkinElmer, Elan 6000) equipped with an auto-sampler system (PerkinElmer, AS-90). Water samples were diluted in a ratio 1:2 with a 1% nitric acid dilution (analytical grade, Merck). Internal standards yttrium and Tm were added at concentration 10 µg/ℓ. The operational parameters of the ICP-MS were: 1 000 W plasma power, 14 ℓ/min plasma gas flow, 0.95 ℓ/min nebulizer gas flow and a sample flow-rate of 1 mℓ/min which was regulated by a peristaltic pump. Calibration of the ICP-MS was done using a series of 11 dilutions of a multi-element standard solution. With this calibration, the concentrations of the analytes were calculated in the samples using corresponding regression lines with a correlation factor of >0.999 (Erasmus et al., 2020).

3.2.5 Screening of pharmaceuticals and personal care products

3.2.5.1 Automated solid-phase extraction of water samples

Swimming pool water was extracted using the SPE-DEX® automated solid-phase extraction system (Horizon Technology, Salem, NH, USA). The method targeted endocrine disrupting hormones developed by Ebitson (2015). Hydrophilic-lipophilic balance-low (HLB-L) disks (47 mm, Oasis, Horizon Technology) were used for the extraction. The method is briefly summarized as conditioning disks with methanol twice followed by another conditioning with deionised water (18 MΩ·cm) twice. One litre of the swimming pool water was passed through the disk, followed by a three-minute air dry period. The target compounds were eluted with four methyl tertiary-butyl ether (MTBE) cycles. The eluent was concentrated to near dryness using nitrogen gas (Ebitson, 2015).

The samples were constituted in 500 µℓ ethanol. This means that a water sample was concentrated 2 000 times.

3.2.5.2 Instrumentation

An ultra-high-performance liquid chromatography (UHPLC) system was used and consisted of an Agilent 1290 Infinity binary pump (G4220A); 1290 Infinity autosampler (G4226A) and 1290 Infinity thermostatted column Compartment (G1316C). The UHPLC was coupled to an Agilent 6540 Accurate mass quadrupole time-of-flight (Q-TOF) / mass spectrometer (MS) (G6540A) (Agilent Technologies, Santa Clara, CA, USA). The desolvation and ionisation of the sample extracts were achieved by positive and negative electrospray ionisation (ESI) that were enhanced with Agilent Jet Stream (AJS) technology. The Q-TOF was set to scan from 50 to 950 m/z and the instrument was set to extended dynamic range (2 GHz). The software used was MassHunter data acquisition (version B.05.00) and MassHunter qualitative analysis (version B.05.00). The mass axis of the Q-TOF was calibrated for positive and negative ionisation with tuning mixes (G1969-85000, Agilent). During each run a solution with masses of 121.050873 [M+H] and 922.009798 [M+H] were constantly infused as accurate mass references.

3.2.5.3 UHPLC and Q-TOF parameters

The swimming pool samples were extracted to target EDCs specifically. These extracts were subjected to untargeted chemical analysis to screen for hormones as well as pharmaceuticals with different chromatographic methods (Table 3-1 and Tabe 3-2).

Table 3-1 Chromatographic method and Q-TOF parameters used for pharmaceutical screening

Parameters	**Positive ionisation**	
Injection volume	1 µℓ	
Column	Poroshell 120 Bonus-RP column (Agilent, 2.1 mm x 100 mm, 2.7 µm)	
Column temperature	25˚C	
Flow rate	0.6 mℓ/min	
Mobile phase A	Water + 0.05% formic acid	
Mobile phase B	ACN + 0.05% formic acid	
Gradient (min)	A (%)	B (%)
0	90	10
8.5	90	10
8.6	50	50
13	50	50
13.3	0	100
14.3	0	100
15	90	10
Post run-time	min	
Total run-time	17 min	
Drying gas temperature	275°C	
Drying gas flow	10 ℓ/min	
Nebuliser pressure	310.26 kPa	
Drying gas temperature	275°C	
Sheath gas temperature	400°C	
Sheath gas flow	10 ℓ/min	
VCap	3 000 V	
Nozzle voltage	0 V	
Fragmentor	130 V	
Skimmer	48 V	

Parameters	Positive ionisation
Injection volume	1 µℓ
Column	Poroshell 120 Bonus-RP column (Agilent, 2.1 mm x 100 mm, 2.7 µm)
Column temperature	25˚C
Flow rate	0.6 mℓ/min
Mobile phase A	Water + 0.05% formic acid
Mobile phase B	ACN + 0.05% formic acid
OCT RF peak-to-peak voltage (Vpp)	750 V

Table 3-2 Chromatographic method and Q-TOF parameters used for hormone screening

Parameters	Positive ionisation	Negative ionisation
Injection volume	5 µℓ	
Column	Poroshell 120 Phenyl-Hexyl (Agilent 2.1 mm x 100 mm 1.9 µm)	
Column temperature	45˚C	
Flow rate	0.35 mℓ/min	
Mobile phase A	Water + 1% formic acid + 0.154 g/ℓ ammonium acetate	Water + 0.036 g/ℓ ammonium fluoride
Mobile phase B	Methanol + 1% formic acid + 0.154 g/ℓ ammonium acetate	Methanol + 0.036 g/ℓ ammonium fluoride
Gradient (min)	A (%)	B (%)
0	50	50
5	30	70
9	10	90
10	50	50
Total run-time	10 min	
Drying gas temperature	300°C	
Drying gas flow	8 ℓ/min	
Nebuliser pressure	310.26 kPa	
Sheath gas temperature	400°C	
Sheath gas flow	11 ℓ/min	
VCap	2 500 V	
Nozzle voltage	0 V	
Fragmentor	130 V	
Skimmer	75 V	
OCT RF Vpp	750 V	

3.2.5.4 Screening of PPCPs

The data obtained after the chromatographic analysis were utilised to screen for and identify some of the compounds present in the extracts. Compound possibilities were generated based on molecular features and subjected to the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL). The library includes 9 200 compounds including: human doping drugs, designer drugs, veterinary drugs, pesticides, mycotoxins, cannabinoids, hallucinogens, stimulants, benzodiazepines, hypnotics, neuroleptics, barbiturates, antidepressants, cardiovascular medicine, anti-epileptics, opioids, anabolic agents, pharmaceuticals and personal care products and hormones. This PCDL combined with the accurate mass capabilities of the Q-TOF instrument confirm the presence of compounds based on accurate monoisotopic mass, isotope patterns, fragment confirmations and retention time.

3.3 RESULTS

Results of the physical and chemical determinands are presented based on the groupings in the SANS 241 (2015) drinking water guideline document (Table 3-3 to Table 3-5). The levels were compared to the guideline for each sampling site from the various swimming pools. Table 3-6 and Table 3-7 contains metal concentrations (chemical determinands) for which there are no SANS 241 numerical limits.

3.3.1 Physical and aesthetic determinands

The water temperatures ranged from 21.5-36.0˚C (Table 3-3). The indoor, heated pool: BP Pool B had the highest temperature for the am and pm sampling 33.4-36.0˚C. The temperatures of all samples taken after people spent the day in the swimming pool water (denoted as pm) were higher when compared to samples taken before people entered the pool (denoted as am) with the exception of BP pond which was sampled late afternoon (day 1) and early morning (day 3) (Table 3-3). The pH ranged from 5.1-6.7 (Table 3-3). The dissolved oxygen (DO_2) ranged from 3.9-8.4 mg/ℓ. Generally, samples taken in the "am" had higher DO_2 than those taken in the "pm" except for BP pond and PD pool where there were no differences between am and pm samples with regards to DO_2 (Table 3-3). The total dissolved solids (TDS) ranged from 98-996 mg/ℓ with BP utility source water and BP borehole being outliers with low levels TDS, when compared to the rest of the samples. The alkalinity ranged from 50-180 mg/ ℓ (Table 3-3).

Table 3-3 Physical and aesthetic determinands

Sample ID	Temperature °C	pH	Dissolved O_2 %	Dissolved O_2 mg/ℓ	TDS mg/ℓ	Alkalinity mg/ℓ
(SANS 241:2015)		≥5 to ≤9.7	--	--	≤ 1 200	--
BP utility source	24.5	6.5	21.2	7.6	130	60
BP borehole	22.0	6.2	21.5	7.7	98	60
BP reservoir	23.8	5.9	11.4	3.9	458	110
BP pool A am	31.4	6.1	18.9	5.9	934	100
BP pool A pm	34.4	6.2	16.8	5.1	996	110
BP pool A backwash	33.6	6.4	16.7	5.1	991	110
BP pool B am	33.4	6.1	18.0	5.7	670	90
BP pool B pm	36.0	6.5	16.0	4.8	671	100
BP pool B backwash	34.6	6.4	19.7	6.0	683	100
BP pool C am	28.7	6.2	19.0	6.5	670	70
BP pool C pm	32.5	6.3	19.1	6.1	690	100
BP pool C backwash	29.2	6.4	21.4	6.7	659	100
BP pond day 1	23.8	5.7	21.6	7.5	356	80
BP pond day 3	21.5	5.1	19.8	7.5	354	60
PD source water	23.3	6.7	20.5	8.3	480	160
PD pool am	24.0	6.2	20.9	8.3	513	80
PD pool pm	24.3	6.0	20.7	8.3	569	80
PD backwash	22.7	6.5	20.7	8.4	590	80
PT source water	22.1	6.3	16.3	6.5	473	180
PT pool am	22.9	6.0	20.7	8.4	552	60
PT pool pm	23.7	5.8	21.7	7.4	549	50
PT backwash	25.4	5.7	21.6	7.3	555	50

-- no guideline available, TDS = total dissolved solids

3.3.2 Chemical determinands: macro-determinands

The macro determinands measured for each site are presented in Table 3-4. Nitrates (NO_3-N) ranged from <LOQ-5.8 mg/ℓ, nitrites (NO_2) from <LOQ-13 mg/ℓ, free chlorine (Cl_2) from <LOQ-8.3 mg/ℓ, sulphates (SO_4^{2-}) from 2-120 mg/ℓ, phosphorous (PO_4^{3-}) from <LOQ-1.5 mg/ℓ, nitrogen ammonia (NH_3-N) from <LOQ-0.4 mg/ℓ and sulphide (S^{2-}) from <LOQ-47 mg/ℓ. Chlorine ranged from nd-305.2 mg/ℓ, Na from nd-3349 mg/ℓ and Zn nd-13.5 mg/ℓ. The free chlorine for BP pool C am exceeded the SANS 241 limits. This could because of the recent dosing of the pool water with chlorine. It is expected that free chlorine would be higher in public swimming pools than drinking water because of the greater application of chlorine disinfectants (Teo et al., 2015). The nitrate levels were below the stipulated levels for drinking water in South Africa, but the nitrite levels were, apart from the pond water, exceeding the standards.

3.3.3 Chemical determinands: micro-determinands

The metals for which limits are available are summarised in Table 3-5. Copper (Cu) was the most detected metal with 100% frequency among samples with barium (Ba) the second most. Aluminium (Al) was the least detected with a frequency of 23.5%. The highest concentrations were those of iron (Fe) and none of the levels in the swimming pools exceeded the chronic health limits of SANS 241. In three instances the aesthetic guideline for Fe was exceeded: the utility water that acts as a substitute source of water on the holiday resort in the very rare occasion that the borehole, their preferred water source, becomes unavailable; the backwash water of pool C; and the pond water. The Fe levels were never higher than the levels for chronic health. The water from the borehole exceeded the drinking water levels for Pb, and so did the backwash water of pool C (Table 3-5). Fortunately, the reservoir into which the borehole feeds had contradictorily, a much lower level compared with the borehole.

A number of other metals were analysed and are presented in Table 3-6 and Table 3-7. There are no numerical limits for these metals in the drinking water standards (SANS 241) in South Africa. The data for BP pool A pm (Table 3-5 through Table 3-7) is suspect because it is the sample with most non-detects and compared to its “am” sample, is outside of what was expected. These samples were taken on the same day and it is not normal that there should be this big difference between samples taken from the same pool. Magnesium (Mg), palladium (Pd), rubidium (Rb), antimony (Sb), selenium (Se), thallium (Tl) and uranium (U) were detected in all of the samples (even in that of “BP pool A pm”), followed by gold (Au), calcium (Ca), potassium (K), strontium (Sr) and vanadium (V) with 94% frequency. Interestingly, nickel (Ni) was detected in the municipal pool (Table 3-6) and only in the backwash water of one holiday resort pool. Platinum (Pt) was also not detected in any of the pool water except for the backwash water of pool C of the holiday resort (Table 3-7).

Table 3-4 Chemical determinands: Macro-determinands

Sample ID	Free chlorine	Nitrates	Nitrites	Sulphates	Phosphorus	Nitrogen ammonia	Sulfide	Cl	Na	Zn
	mg/ℓ Cl_2	mg/ℓ NO_3-N	mg/ℓ NO_2	mg/ℓ SO_4^{2-}	mg/ℓ PO_4^{3-}	mg/ℓ NH_3-N	ug/ℓ S^{2-}	mg/ℓ	mg/ℓ	mg/ℓ
SANS 241:2015	≤ 5^	≤ 11*	≤ 0.9*	≤ 250# ≤ 500*	--	≤ 1.5#	--	≤ 300#	≤ 200#	≤ 5#
BP utility source	<LOQ	1.5	4.0	58	<LOQ	0.4	0.0	11.6	7.8	0.343
BP borehole	<LOQ	0.9	4.0	2	0.6	<LOQ	<LOQ	nd	11.4	0.030
BP reservoir	0.2	2.1	6.0	20	0.6	<LOQ	<LOQ	100.2	9.1	0.026
BP pool A am	0.6	3.1	<LOQ	24	1.4	<LOQ	<LOQ	305.2	13.2	0.011
BP pool A pm	0.02	3.1	5	8	1.62	0.03	<LOQ	195.6	1.8	0.004
BP pool A backwash	<LOQ	2.5	2.0	60	1.5	<LOQ	6.0	281.9	193.8	0.097
BP pool B am	1.2	3.2	3.0	22	1.0	<LOQ	<LOQ	153.7	78.5	0.047
BP pool B pm	0.5	3.0	2.0	28	1.1	<LOQ	<LOQ	162.2	79.6	0.030
BP pool B backwash	0.3	2.6	4.0	28	1.2	0.1	15.0	211.8	75.6	0.089
BP pool C am	8.3	5.8	13.0	20	0.6	<LOQ	<LOQ	169.2	138.3	0.046
BP pool C pm	<LOQ	2.4	4.0	46	0.7	<LOQ	<LOQ	117.5	141.6	0.043
BP pool C backwash	0.4	3.4	4.0	42	0.9	<LOQ	27.0	166.8	52.3	0.348
BP pond day 1	0.2	0.6	1.0	8	0.3	0.1	13.0	70.7	27.0	0.020
BP pond day 3	0.3	0.5	1.0	8	0.4	0.1	22.0	82.7	-	-
PD source water	2.1	1.5	<LOQ	122	0.5	<LOQ	<LOQ	91.0	31.7	0.347
PD pool am	0.1	0.8	6.0	110	0.3	<LOQ	<LOQ	123.5	33.9	0.008
PD pool pm	<LOQ	0.9	3.0	114	0.1	<LOQ	<LOQ	84.7	37.3	0.009
PD backwash	<LOQ	<LOQ	8.0	116	0.7	0.1	35.0	107.0	38.5	0.033
PT source water	1.8	1.4	5	112	0.13	<LOQ	1.0	23.1	-	-
PT pool am	3.9	2.4	3.0	96	0.2	<LOQ	<LOQ	125.5	-	-
PT pool pm	0.7	0.8	5.0	120	0.3	<LOQ	<LOQ	148.0	-	-
PT backwash	<LOQ	<LOQ	4.0	106	0.5	0.2	47.0	106.1	-	-

-- no guideline available, - no value determined; # aesthetic; * acute health; ^ chronic health; <LOQ below limit of quantification; nd non-detect

Table 3-5 Chemical determinands: micro-determinands

Sample ID	Ba µg/ℓ	Cd µg/ℓ	Cr µg/ℓ	Cu µg/ℓ	Fe µg/ℓ	Pb µg/ℓ	Mn µg/ℓ	Al µg/ℓ
SANS 241:2015	≤ 700^	≤ 3^	≤ 50^	≤ 2 000^	≤ 300# ≤ 2 000^	≤ 10^	≤ 100# ≤ 400^	≤ 300~
BP utility source	39.63	0.02	nd	14.51	676.90	5.57	32.37	nd
BP borehole	26.65	0.01	7.75	18.93	12.45	10.54	0.35	nd
BP reservoir	28.64	0.17	0.62	15.97	17.35	0.54	0.98	nd
BP pool A am	2.37	nd	1.51	11.40	nd	nd	nd	nd
BP pool A pm	Nd	nd	0.84	8.17	nd	nd	nd	nd
BP pool A backwash	74.43	0.08	20.19	36.23	114.90	3.22	20.85	465
BP pool B am	58.20	0.02	11.87	54.37	13.05	2.21	0.91	nd
BP pool B pm	59.03	nd	13.42	10.94	10.72	1.57	0.54	nd
BP pool B backwash	66.31	0.03	16.05	22.06	81.13	3.27	15.54	1010
BP pool C am	57.87	0.02	14.23	17.16	18.22	1.39	0.57	nd
BP pool C pm	58.37	nd	16.35	27.40	13.24	2.10	0.88	nd
BP pool C backwash	104.90	0.06	27.71	228.40	1047.00	45.93	229.30	1449
BP pond day 1	51.83	nd	11.14	24.23	465.40	1.51	490.20	nd
PD source water	9.99	0.02	nd	105.20	94.93	1.11	4.09	nd
PD pool am	10.27	0.02	0.13	2.31	55.15	0.23	2.93	178
PD pool pm	9.95	0.01	0.04	3.39	36.57	0.37	1.24	127
PD backwash	13.79	0.06	0.15	8.32	64.16	6.76	26.31	1117

nd non-detect; - no value determined; # aesthetic; * acute health; ^ chronic health; ~ operational health

Table 3-6 Chemical determinands: micro-determinands. There are no guideline levels for these metals in SANS 241 (2015)

Sample ID	Ag	As	Au	B	Be	Bi	Ca	Co	Hg	K	Mg	Mo	Ni
	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ
BP utility source	0.187	0.405	1.264	nd	0.012	0.1125	15 970	0.278	6.211	2 727	5 726	0.671	nd
BP borehole	0.524	nd	0.807	nd	nd	0.2384	19 640	0.090	3.538	565	48 640	0.141	nd
BP reservoir	0.656	0.563	3.948	91.8	0.182	1.4300	18 430	0.267	22.320	598	33 800	2.371	nd
BP pool A am	0.522	nd	0.592	1290.0	nd	0.0063	2 139	0.008	2.239	1 324	2 230	nd	nd
BP pool A pm	0.188	nd	0.469	nd	nd	nd	nd	0.009	1.491	nd	450	nd	nd
BP pool A backwash	1.775	0.285	0.224	8873.0	0.015	nd	59 180	0.654	1.154	7 333	48 950	nd	nd
BP pool B am	0.823	0.124	0.351	6.3	nd	nd	33 860	0.146	2.129	3 926	45 140	0.120	nd
BP pool B pm	0.721	0.060	0.341	nd	nd	nd	33 780	0.196	2.123	3 274	45 110	0.105	nd
BP pool B backwash	1.777	0.139	0.202	176.7	0.019	0.0042	33 780	0.428	1.525	3 371	44 400	0.193	nd
BP pool C am	0.208	nd	0.314	nd	nd	nd	29 010	0.077	1.430	2 707	44 350	0.120	nd
BP pool C pm	0.293	0.109	0.233	nd	nd	nd	29 370	0.061	1.515	2 606	45 420	0.171	nd
BP pool C backwash	2.179	0.570	0.183	25.3	0.103	nd	41 640	6.547	0.941	1 918	44 730	nd	7.717
BP pond day 1	0.633	nd	0.151	999.4	nd	nd	12 970	0.513	1.383	2 409	11 650	nd	nd
PD source water	nd	0.265	0.008	3.9	nd	0.0002	54 390	1.987	0.025	1 744	46 550	0.144	0.623
PD pool am	nd	0.247	0.017	3.3	nd	0.0011	56 000	1.746	0.026	2 179	43 590	0.195	0.678
PD pool pm	nd	0.267	0.017	4.1	nd	0.0028	59 550	1.778	0.065	2 403	47 880	0.199	0.490
PD backwash	0.003	0.288	nd	4.4	0.013	nd	73 330	4.691	0.056	2 474	49 940	0.075	1.669

nd non-detect

Table 3-7 Chemical determinands: More micro-determinands. There are no guideline levels for these metals in SANS 241 (2015)

Sample ID	P	Pd	Pt	Rb	Sb	Se	Sr	Th	Ti	Tl	U	V
	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ
BP utility source	0.2	0.2	nd	0.8	0.1	1.3	60	1.2	0.2	0.1	0.2	3.7
BP borehole	nd	0.2	nd	2.5	0.1	0.7	68	0.8	4.3	0.1	0.0	11.5
BP reservoir	nd	0.7	nd	1.2	0.2	4.8	64	1.9	2.3	0.8	0.5	7.9
BP pool A am	24.0	0.1	nd	0.6	0.4	1.4	4	0.7	nd	0.0	0.1	0.8
BP pool A pm	nd	0.0	nd	0.1	0.0	0.7	nd	0.5	nd	0.0	0.1	nd
BP pool A backwash	835.9	0.2	nd	7.3	0.3	2.0	160	0.5	5.6	0.0	0.2	11.0
BP pool B am	348.7	0.1	nd	3.9	0.1	1.9	118	0.7	3.8	0.0	0.2	10.1
BP pool B pm	355.3	0.1	nd	3.4	0.1	1.7	117	0.6	3.7	0.0	0.2	9.5
BP pool B backwash	415.8	0.1	nd	3.3	0.2	2.5	117	0.4	4.8	0.0	0.2	10.6
BP pool C am	247.8	0.1	nd	2.9	0.1	1.7	106	0.6	3.7	0.0	0.2	10.4
BP pool C pm	250.5	0.1	nd	2.8	0.1	1.2	106	0.6	3.9	0.0	0.1	10.4
BP pool C backwash	347.7	0.2	0.1	2.4	0.3	2.9	155	0.4	11.0	0.2	0.7	15.6
BP pond day 1	22.5	0.0	nd	4.7	0.1	0.6	42	0.3	1.0	0.0	0.1	2.0
PD source water	nd	0.0	nd	4.1	0.0	1.0	48	nd	0.7	0.0	1.3	0.3
PD pool am	nd	0.0	nd	4.8	0.1	0.4	52	0.0	1.5	0.0	1.8	0.4
PD pool pm	0.3	0.0	nd	4.9	0.1	0.6	52	nd	0.8	0.0	1.8	0.4
PD backwash	10.4	0.0	nd	5.0	0.1	0.5	58	nd	0.8	0.0	0.8	0.1

nd non-detect

3.3.4 Chemical determinands: organic

Four compounds that are part of disinfectant by-products were determined in each sample and the total THMs were determined by using a ratio calculation. Chloroform was the compound with the highest concentration in all the samples that were analysed for DBPs, except for BP pool A am (Table 3-8) where bromodichloroform was the highest. Even so, it did not exceed the drinking quality limits. The total THM level for drinking water was exceeded in one sample, BP pool C pm (Table 3-8) and very close to the guideline limit in three other samples: BP pool A backwash, BP pool C am, and BP pool C backwash.

Table 3-8 Chemical determinands: organic determinands

Sample ID	Bromo-dichloroform	Bromoform	Chloroform	Dibromo-chloroform	Total THM	TOC
	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	mg/ℓ C
SANS 241:2015	≤ 60^	≤ 100^	≤ 300^	≤ 100^	≤ 1^	≤ 10^
BP utility source	19	1.3	25	13	0.54	3.5
BP borehole	<0.5	<0.5	<0.5	<0.5	0.02	<2
BP reservoir	<0.5	7.9	24	<0.5	0.17	<2
BP pool A am	30	<0.5	9.7	9.8	0.64	2.6
BP pool A pm	33	<0.5	91	12	0.98	2.9
BP pool A backwash	30	0.56	85	11	0.9	2.6
BP pool B am	27	4.4	41	18	0.81	5
BP pool B pm	28	4.7	44	19	0.85	3.6
BP pool B backwash	24	2	44	12	0.69	4.1
BP pool C am	28	11	45	26	0.99	3.4
BP pool C pm	36	11	46	32	1.18	4.6
BP pool C backwash	29	5.3	50	21	0.91	3.4
BP pond day 1	2.8	<0.5	12	<0.5	0.10	4.1
BP pond day 3	5.3	<0.5	17	1.4	0.16	3
PD source water	26	2.2	33	18	0.75	-
PD pool am	8.5	<0.5	46	2.9	0.33	-
PD pool pm	6.9	<0.5	39	2.2	0.27	-
PD backwash	8.7	<0.5	73	2	0.41	-
PT source water	-	-	-	-	-	-
PT pool am	-	-	-	-	-	-
PT pool pm	-	-	-	-	-	-
PT backwash	-	-	-	-	-	2

- no value determined; ^ chronic health; total THM: calculated by value determined divided by the limit, use sum of ratios per compound to compare

3.3.5 Comparing the THMs values: 2018 vs 2019

One of the municipality's swimming pools was sampled again in 2019 for their THM load (Table 3-9. The pool that is the better maintained of the two previously used, was selected. This was done because of its regular use for competitions (swim gala and underwater hockey) and training by local swim clubs. For both sampling events the individual as well as the total THMs were within the drinking water standards.

Table 3-9 THM comparison for same municipal swimming pool

Sample ID	Bromo-Dichloroform	Bromoform	Chloroform	Dibromo-chloroform	Total THM
	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ
SANS 241:2015	≤ 60^	≤ 100^	≤ 300^	≤ 100^	≤ 1^
		2018			
PD source water	26	2.2	33	18	0.75
PD pool am	8.5	<0.5	46	2.9	0.33
PD pool pm	6.9	<0.5	39	2.2	0.27
PD backwash	8.7	<0.5	73	2	0.41
		2019			
PD source water	23	<10	23	19	0.75
PD pool am	<10	<10	29	<10	0.46
PD pool pm	<10	<10	25	<10	0.45
PD backwash	<10	<10	28	<10	0.46

3.3.6 Screening of PPCPS

The calibration of the analytical instrument was successful with reference compounds detected during the runs. The screening results revealed the presence of various compounds detected in the swimming pool water extracted specifically for EDCs (Table 3-10 to Table 3-12). A shortened list for each sample is presented in this section (full list is available upon request). This list comprises of the 26 compounds that were detected with the highest abundance and a score greater than 75% in each of the samples.

Table 3-10 Screening results: Shortened list of compounds detected in the swimming pool water of the public holiday resort (BP) (holiday resort)

BP utility source	BP reservoir	BP borehole	BP pond day 1	BP pond day 3
Piquizil	Loxanast	Octodrine	Loxanast	Androstane-3,17-dione
6-Acetylmorphin-D3	Bumadizone	Sulfadimidine	Androstane-3,17-dione	Empenthrin
Adenosine N-ethyl-carboxamide	Lauroylsarcosine	Dinoprost	Empenthrin	Bumadizone
Simarubidin	Butoxycaine	Alantolactone	Renanolone	Ketocainol
Sorbic acid	Ethyldibunate	Octodrine	Bumadizone	Piquizil
Bumadizone	Netilmicin	Pentagestrone acetate	Ketocainol	6-Acetylmorphin-D3
Gibberellic acid	Pirimicarb	Nandrolone phenpropionate	Ibuverine	Androstane-3,17-dione
Zoloperone	Azimexone	Falipamil	11-Ketotestosterone	Empenthrin
Metolachlor	Trimethylolmelamine	Irbesartan	Trimethobenzglycine	Renanolone
Propazine	Cicloxilic acid	Nandrolone phenpropionate	Estradiol diacetate	Ibuverine
Trimethylolmelamine	Falipamil	Pirimicarb	Renanolone	Adenosine N-ethylcarboxamide
Carsalam	Amylpenicillin	Trimethylolmelamine	Heptyl heptanoate	Trimethobenzglycine
Atrazine	Pentagestrone acetate	Azimexone	4-Nitrophenol	Simarubidin
Trospium	Citronellal hydrate	Leptacline	Enisoprost	Heptyl heptanoate
MNFA	Fluocinolone acetonide	Narasin	Gloxazon	Netilmicin
Isothipendyl	Congocidin	Azithromycin	p-Nonylphenol	Sorbic acid
Meglumine	Azithromycin	Benzophenone	1-(2,3-dihydroxypropyl)-Theobromine	Drinidene
Hydrazinopyridazin-methylaminopropanol	Minaxolone	Orphenadrine	Dihyprylone	Oestradiol diacetate
Atraton	Tetroquinone	Nabumetone	4-Aminosalicylic acid	Renanolone
Simarubidin	4-Stilbazole	Asparagine	Nicotinaldehyde	Zoloperone
Triciribine	Flumoxonide	Valdetamide	Ethinylcyclohexanol	Gibberellic acid
Valine	Valine	Valine	Ketocainol	Piquizil
4-Nitrophenol	Narasin	Tolmetin	Guaiol	4-Nitrophenol
Atrazine-desisopropyl	Asparagine	Glutamine	17 a-Oestradiol	Ethyldibunate
	Heptolamide	Diphenylmethoxyacetic acid	Androst-4-ene-3,17-dione	Betamethasone

BP utility source	BP reservoir	BP borehole	BP pond day 1	BP pond day 3
Acetiamine	Piribedil	Karbutilate	Cinflumide	Desocriptine

Table 3-11 Screening results (cont): Shortened list of compounds detected in the swimming pool water of the public holiday resort (BP) (holiday resort)

BP pool A am	BP pool A pm	BP backwash A	BP pool B am	BP pool B pm	BP backwash B	BP pool C am	BP pool C pm	BP backwash C
Bumadizone	Bumadizone	Bumadizone	Bumadizone	Bumadizone	Bumadizone	Detanosal	Bumadizone	Bumadizone
Detanosal	Piquizil	Netilmicin	Citronellal hydrate	Detanosal	Detanosal	Dihyprylone	Detanosal	Detanosal
Euprocin	6-Acetylmorphin-D3	Dihyprylone	Dihyprylone	Dihyprylone	Barban	Bumadizone	Dihyprylone	Barban
Dihyprylone	Amifloverine	Desocriptine	Alantolactone	Euprocin	Euprocin	Ampyrimine	Terfluranol	Dihyprylone
Netilmicin	Dihyprylone	Euprocin	Detanosal	Moxisylyt	Dihyprylone	Indeloxazine	Exiproben	Citronellal hydrate
Ethyldibunate	Citronellal hydrate	Despropionylbezitramide	Dodecanal	Netilmicin	Citronellal hydrate	Pyridinolcarbamate	Netilmicin	Ampyrimine
Betamethasone	Adenosine N-ethylcarboxamide	Fluorandrenolone	Netilmicin	2-Phenylbutyramide	Desocriptine	Pirimicarb	Ampyrimine	Indeloxazine
Ampyrimine	Netilmicin	Terfluranol	Kessyl alcohol	Citronellal hydrate	Indeloxazine	Citronellal hydrate	Indeloxazine	Pyridinolcarbamate
Indeloxazine	Ethyldibunate	Citronellal hydrate	Loxanast	Ampyrimine	Ampyrimine	Tridihexethyl	Pyridinolcarbamate	Terfluranol
Pyridinolcarbamate	Exiproben	Exiproben	Dicyclopentadiene	Indeloxazine	Pyridinolcarbamate	Ethyldibunate	Pirimicarb	Euprocin
Detanosal	Euprocin	Hymechromon	Hydroprene	Moxisylyt	Despropionylbezitramide	Netilmicin	Citronellal hydrate	Piquizil
Despropionylbezitramide	Picrotoxinin	Apiol	Butylscopolaminium	Betamethasone	Fluorandrenolone	Betamethasone	Tridihexethyl	Netilmicin
Fluorandrenolone	Sorbic acid	Uridin	Heptyl heptanoate	Ethyldibunate	6-Acetylmorphin-D3	Ruscogenin	Despropionylbezitramide	Exiproben
Congocidin	Dicyclopentadiene	Conessin	Trethocanic acid	Despropionylbezitramide	Piquizil	Terfluranol	Fluorandrenolone	Alantolactone
Furonazide	Piquizil	Picrotoxinin	Tolpiprazole	Fluorandrenolone	Mexrenoate	Conessin	Desocriptine	Uridin
Fluocinolone acetonide	Zoloperone	Ampyrimine	Valtrate	Alantolactone	Iproheptin	Dinoprost	Betamethasone	Adenosine N-ethylcarboxamide
Citronellal hydrate	Congocidin	Indeloxazine	2-Phenylbutyramide	Dodecanal	Hymechromon	Congocidin	Dicyclopentadiene	Apiol
Tridihexethyl	Fluocinolone acetonide	Pyridinolcarbamate	Iproniazid	Congocidin	Uridin	Desocriptine	Fluocinolone acetonide	Hymechromon
Flumoxonide	Gibberellic acid	Dicyclopentadiene	Pentagestrone acetate	Fluocinolone acetonide	Dimantine	Fluorandrenolone	Flumoxonide	Dicyclopentadiene
2-Phenylbutyramide	Alphameprodine	Fluocinolone acetonide	Karbutilate	Hymechromon	Betamethasone benzoate	Trimethylolmelamine	Congocidin	Fluorandrenolone
Apiol	Despropionylbezitramide	Flumoxonide	Valproic acid	Apiol	Flumoxonide	Dicyclopentadiene	Adenosine N-ethylcarboxamide	Betamethasone

BP pool A am	BP pool A pm	BP backwash A	BP pool B am	BP pool B pm	BP backwash B	BP pool C am	BP pool C pm	BP backwash C
Adenosine N-ethylcar-boxamide	Fluorandrenolone	Betamethasone	Ethyl 2-acetyl-3-oxo-tetradecanoate	Cotoin	Dimoxaprost	Carsalam	Euprocin	Ethyldibunate
Cotoin	6-Acetylmorphin-D3	Dodecanal	Tolpiprazole	Uridin	Dotarizin	Butylscopolaminium	Valtrate	Desocriptine
Uridin	Desocriptine	Helvolic acid	Draquinolol	Picrotoxinin	Congocidin	Alantolactone	Ethiofencarb	Despropionylbezitramide
Hymechromon	Imuracetam	Iproheptin	Citronellol	Dicyclopentadiene	Fluocinolone aceto-nide	Morphin-D3	Atropine methyl	Somantadine
Bepridil	Betamethasone ben-zoate	Trimethylolmelamine	Promoxolane	Flumoxonide	Dodecanal	Pentagestrone ace-tate	Bromochlorochinolinol	Morphin-D3

Table 3-12 Screening results: Shortened list of compounds detected in the municipal swimming pool water (PT and PD) (municipality)

PD source water	PD pool am	PD pool pm	PD backwash	PT source water	PT pool am	PT pool pm	PT backwash
Pirimicarb	Asparagine	Asparagine	Losulazine	Pirimicarb	Ethyldibunate	Pirimicarb	Citronellal hydrate
Glutamine	Glutamine	Glutamine	Piribedil	Bumadizone	Betamethasone	Adenosine N-ethylcar-boxamide	Bumadizone
Fluconazole	Fluconazole	Netilmicin	Netilmicin	Carsalam	Netilmicin	Bumadizone	Azimexone
Methylene blue	6-Acetylmorphin-D3	Fluconazole	Mycophenolic acid	Trimethylolmelamine	Fluorandrenolone	Adenosine N-ethylcar-boxamide	Pirimicarb
4'-Methyl-alpha-pyrroli-dinopropiophenone	Netilmicin	Ethyldibunate	Desocriptine	Piribedil	Despropionylbezitramide	Morphin-D3	Trimethylolmelamine
Betamethasone	Ethyldibunate	Betamethasone	Triciribine	Heptolamide	Congocidin	Carsalam	Undecenamide 10-
Fepitrizole	Desocriptine	Pirimicarb	Morphin-D3	Morphin-D3	Fluocinolone acetonide	Heptolamide	Leptacline
Trimethylolmelamine	Methylene blue	Despropionylbezitramide	Taloximine	Phenamidine	Desocriptine	Azimexone	Dimethylacetal-anisal-dehyde
Articaine	Gabapentin	Fluorandrenolone	Trimethylolmelamine	Valine	Bepridil	Trimethylolmelamine	Eplerenone
Methylene blue	Bumadizone	Methylene blue	Methylene blue	Pentagestrone acetate	Betamethasone benzoate	Rocuronium	Trospium
Aprindine	Trimethylolmelamine	8-Hydroxychinolin	Ethyldibunate	Flupirtine	Aprindine	Trimethoxybenzene	Octodrine
Phenamidine	Trospium	Congocidin	Cubebin	Hexyl dodecanoate	Flumoxonide	Valine	Loxanast
Carsalam	Methylene blue	Betamethasone benzo-ate	Flumoxonide	Azimexone	Pirimicarb	Citronellal hydrate	Azelaic acid
Efavirenz	Losulazine	Flumoxonide	Betamethasone	Asparagine	Picrotoxinin	Pentagestrone acetate	Anisohydrocinnamol
Bumadizone	Articaine	Citronellal hydrate	Despropionylbezitramide	Midamaline	Trimethylolmelamine	Tolonium	Valine
Phenylpropanol	Fluocinolone acetonide	Bumadizone	Helvolic acid	Tiapride	Glutamine	Tolonium	Dimethyl-4-aminophe-nol

PD source water	PD pool am	PD pool pm	PD backwash	PT source water	PT pool am	PT pool pm	PT backwash
Paraldehyde	Efavirenz	Fluocinolone acetonide	Fluorandrenolone	Tetroquinone	Benafentrine	Cyclarbamate	Carsalam
Piribedil	Sorbic acid	Articaine	Cyprodenate	Hydrocodon-D3	Asparagine	Menbutone	Mupirocin
Citronellal hydrate	Carsalam	Methylene blue	Phenamidine	Glutamine	Citronellal hydrate	Piribedil	Heptolamide
Acetanilide	Losulazine	Bepridil	Tenocyclidine	Anhalonidine	4'-Methyl-alpha-pyrrolidinopropiophenone	Flupirtine	Piribedil
Pentagestrone acetate	Citronellal hydrate	3,4,5,6-Tetrahydro-phthalic acid anhydride	Mifentidine	Piribedil	Pentagestrone acetate	Formoterol	Triciribine
Fepitrizole	6-Acetylmorphin-D3	Umbelliferon	Amifloxacin	Valdetamide	Morphin-D3	p-Methoxyetilampheta-mine	Benzoic acid
Tetroquinone	2,4-Dimethylphenol (2,4-Xylenol)	Helvolic acid	Triflusulfuron-methyl	Mesulergine	4-Cyclohexylphenol	Hexyl dodecanoate	Tetroquinone
Desocriptine	Eplerenone	Efavirenz	WY-3654	Triciribine	Hexyl hydroxybenzoate	Hydrocodon-D3	Floverine
Undecenamide 10-	Flumoxonide	Triflusulfuron-methyl	Valine	Octodrine	Piribedil	Minaxolone	Mesulergine
Valine	Piquizil	Piribedil	Cinfenoac	3,5-Dibromosalicylic acid	Valine	Mesulergine	Alantolactone

3.4 SUMMARY

3.4.1 General findings on the quality of the water in the selected pools

None of the samples' pH values or TDS levels exceeded the South African standards for drinking water (Table 3-3) (SANS 241:2015). It was however slightly acidic and therefore not completely conducive for the disinfection power of free chlorine. Currently, there are standards prescribed in South Africa for temperature, dissolved oxygen and alkalinity. However, according to Association of Pool and Spa Professionals (APSP) (2014), the alkalinity should be 60-180 mg/ℓ $CaCO_3$, thus only two samples (PT pool pm and PT backwash) were below this guideline (Table 3-3). One sample, BP Pool C am, with a value of 8.3 mg/ℓ Cl_2 exceeded the standards for free chlorine (Table 3-4). This high value may be due to the addition of chlorine prior to sampling. Chlorine is known to be the most common disinfectant used in swimming pools as it rapidly kills viruses and bacteria (Spiliotopoulou et al., 2015).

None of the samples exceeded the drinking water levels of the following nutrient parameters: nitrates, sulphates, and nitrogen ammonia (Table 3-4). In contrast to this, most of the samples exceeded the drinking water limits for nitrites. The nitrite levels ranged between 0-13 mg/ℓ. Only two samples' (BP pool A am and PD source water) values were below the guideline level with regards to nitrites. There are a variety of factors that might have contributed to this. The most likely is the addition of an algaecide didecyl dimethyl ammonium chloride, which is a form of chloramination and known to form nitrites (WHO, 2011). Didecyl dimethyl ammonium chloride is definitely used by the holiday resort (personal communication: R Herrington). However, nitrates in stagnant water can be transformed to nitrites by the bacterium *Nitrosomonas* in the presence of galvanised pipes (and tanks) (WHO, 2011). And a small percentage (5%) of dietary nitrates are converted to nitrites by nitrate reductase in saliva (WHO, 2011).

No drinking water quality limits were found for phosphorus and sulphide. One sample, BP pool A am, exceeded the SANS 241 limit for Cl. Backwash water from pool A at the holiday resort almost reached the stated level for Zn (Table 3-4) but fortunately no one swims in the backwash water. This gets pumped to a pond on the premises. None of the samples exceeded the Na guideline level.

None of the metals for which there are SANS 241 chronic health risks exceeded these levels in any of the swimming pools (Table 3-5). The water from the borehole exceeded the limits for Pb (Table 3-5), but the reservoir into which the borehole feeds had contradictorily, a much lower level. This phenomenon is worth investigating further in a follow-up study because the borehole is the main water source for the holiday resort. One of the backwash samples also had a very high Pb concentration, but the bathers would not be exposed to that, unless the water is returned to the pool, which was not the practice at the resort during the site visit. However, the biota in the pond into which the water is discarded might be impacted negatively over prolonged exposure to similar high levels. The aqueous phase in the pond had a comparatively low 1.51 µg/ℓ Pb, but the metal might be partitioning to the sediment where it may accumulate and enter the food web via benthic organisms (Newman, 2015).

The only exceedance of SANS 241 limits was manganese (Mn) in the pond that was 490.2 µg/ℓ compared with the drinking water quality limit of 400 µg/ℓ. However, no swimming is allowed in this pond and humans are not exposed to these levels. The only exceedance for Fe was the aesthetic limits which would not be cause for concern regarding human health. The Fe levels were never higher than the guideline level for chronic health. The exceedance of the Al levels is also not of any health concerns, because the purpose for this value is for safe up-keep of the infrastructure in and around the swimming pool.

None of the samples exceeded the SANS 241 drinking water standards for the organic determinands: THMs and total organic content (Table 3-6) except for BP Pool C pm, which exceeded the total THM level. However, it is the long-term exposure to high concentrations of THMs that have a greater health concern than short-term periods of exposure, such as those experienced at a swimming pool. In the South African Water Quality Guideline for domestic use (DWAF, 1996), the risk criterion is a one in 100 000 chance of developing cancer over and above the background risk if there is a lifetime exposure to levels greater than those provided by the SANS 241 document (see Table 3-8). And for not one of the samples these individual guidelines were exceeded. However, when the total THM is considered, a number of samples were close to the 1 µg/mℓ or exceeding it. Since the backwash water is not the type of water people would be swimming in, these exceedances are not of concern. But, two pools, A and C sampled in the morning, were very close to the total THM guideline level. And pool C showed the same situation at the end of the day (Table 3-8). This is not likely to be hazardous because the major effect of these compounds is caused through long-term exposure and this would then increase cancer risk (DWAF, 1996).

3.4.2 General findings on the presence of PPCPs

The swimming pool samples were extracted to specifically target the steroid hormones. Compounds that co-eluted, but which are not steroid hormones *per se*, were also detected. A summary of the most frequently detected compounds was compiled to learn if their presence might explain the endocrine disruptive effects detected by the luminescence bio-assays. Some compounds were detected exclusively at either the municipal pools or the resort pools: fluconazole, articaine, efavirenz and methylene blue were only detected at the municipality pools (Table 3-10; Table 3-11). Zoloperone, detanosal, euprocin, dihyprylone, ampyrimine, indeloxazine and pyridinolcarbamate were only detected in the resort pools (Table 3-10; Table 3-11). Apart from efavirenz, all of the preceding compounds also appear in Table 3-13 and belong to the most frequently detected compounds list.

Table 3-13 The most frequently detected compounds across all the sites

*% Frequency	Compound name	Compound description/uses
86	Bumadizone	Non-steroidal anti-inflammatory drug
68	Citronellal hydrate	Terpenoid chemical that give citronella oil its distinctive lemon scent
68	Netilmicin	Semisynthetic antibiotic (action similar to gentamicin)
64	Betamethasone	Synthetic corticosteroid used to treat inflammation
55	Trimethylolmelamine	Fire retardant additive in paints, paper & plastics
50	Desocriptine	α- and β-Adrenoceptor antagonist
50	Ethyldibunate	Cough suppressant
45	Pirimicarb	Carbamate insecticide used to control aphids on vegetable, cereal and orchard crops
45	Flumoxonide	Synthetic glucocorticoid corticosteroid which was never marketed
45	Fluocinolone acetonide	Corticosteroid used in dermatology
45	Dihyprylone	Sedative drug
45	Despropionylbezitramide	Pain relief (anaesthesia)
41	Congocidin	Polyamide with antibiotic and antiviral activity.
41	Valine	Amino acid
36	Piribedil	Piperazine dopamine agonist
36	Fluorandrenolone	Topical corticosteroid
36	Pentagestrone acetate	Synthetic topical corticosteroid
32	Carsalam	nonsteroidal anti-inflammatory drug
32	Adenosine N-ethylcarboxamide	Long-lasting adenosine derivative with vaso-activity (increase or decrease of blood pressure)
32	Euprocin	An anaesthetic
32	Ampyrimine	Diuretic drug

*% Frequency	Compound name	Compound description/uses
32	~Indeloxazine	Antidepressant
32	~Detanosal	Analgesic anti-inflammatory
27	Morphin-D3	Pain medication of the opiate family
27	Glutamine	Amino acid
27	Piquizil	Bronchodilator
27	Asparagine	Amino acid
27	Alantolactone	Chemotherapeutic agent
27	~Pyridinolcarbamate	Treatment of atherosclerosis and other vascular disorders
23	6-Acetylmorphin-D3	Heroin metabolite
18	#Methylene blue	Medication to treat met-haemoglobin anaemia and a dye
18	~Zoloperone	Anxiolytic drug
14	#Fluconazole	Antifungal
14	#Articaine	Dental anaesthetic

*% samples in which the compound was detected; #compounds exclusive to the municipal pools; ~compounds exclusive to the resort pools

The endocrine side-effects of some of the compounds listed in Table 3-13 have been discovered with a literature search: Betamethasone, which is a steroidal medication that was detected in 64% of the samples may cause endocrine menstrual irregularities, be responsible for growth retardation in children and the cause of secondary adrenocortical and pituitary unresponsiveness. These side effects were reported by patients exposed to clinical doses. It also decreased carbohydrate tolerance and increased need of insulin in diabetics (Drugs.com, 2019a). Fluocinolone is category C pregnancy drug which means that there is evidence of its ability to adversely affect animal foetuses, but its influence on the human foetus is unknown (Drugs.com, 2019b). Congenital malformations have been reported in women treated with fluconazole (Nørgaard et al., 2008) and it is known to exhibit some degree of drug-drug interaction due to its metabolism by the CYP450 system. Fluconazole is a substrate of CYP3A4 and inhibitor of CYP2C9 and 2C19 (Neofytos et al., 2010). Fluorandrenolone has been reported to cause, amongst others, hypothalamic-pituitary-adrenal axis suppression in children, and adrenal suppression that include linear growth retardation (Aqua Pharmaceuticals, 2006). One compound with definite activity via the ER is alantolactone that is used as an anticancer treatment effect on the human breast cancer cell line MCF7 (Liu et al., 2018).

4 HUMAN HEALTH RISK ASSESSMENT

4.1 INTRODUCTION

The risk assessment process includes four steps: 1) hazard identification; 2) hazard characterisation; 3) exposure assessment and 4) risk characterisation (WHO, 2010). The hazard identification step identifies the ability of compounds/metals to cause various health effects. Hazard characterisation includes a qualitative and quantitative description of the properties of an agent having the potential to cause adverse health effects. The exposure assessment evaluates the concentration of a particular agent that reaches a target population. The final step is risk characterisation that summarises the finding to use for advice in decision-making. Much of the hazard identification and characterisation of the THMs and metals were addressed in the literature review (Chapter 2). What follows here is a description of the process of exposure assessment. The risk characterisation is completed once the results have been obtained.

4.2 MATERIAL AND METHODS

The probability to develop health effects, both harmful non-cancerous effects (hazard quotient: HQ) and cancer risk (CR) were determined using the concentrations determined for the THMs and the metals. Because the determination of the concentrations of the THMs and the elements were done only once, and we are not reporting on a long term monitoring of a large sample size, and because these concentrations will vary over time and between different sites, the health risk assessment done for this project is not representative for all public swimming pools of South Africa. It only gives a prediction of the possible risks adults and children are exposed to at these very specific concentrations that were determined only once.

We focused on only two exposure routes, dermal and ingestion, because the concentrations of the compounds were determined for the water phase only, and not the vapour phase above the swimming pool water. Also, according to Panyakapo et al. (2008) and Abbasnia et al. (2019), skin and gastrointestinal exposures are the main intake route of THM that mainly pose cancer risk in comparison to other exposure routes.

The hazard index (HI) was calculated for each sample and for each compound the necessary constants were available. The total of the HI per sample is referred to as the hazard quotient (HQ). Some values used in the calculations were selected arbitrarily since these were not based on any measurements. These include the number of times South Africans would visit a public swimming pool per year (EF in Table 4-1), and the mean duration spent in the pool (ET in Table 4-1).

The following equations were used:

$$CDI_{ing} = \frac{C_w \times IR \times EF \times ED}{BM \times AT}$$

and

$$CDI_{der} = \frac{C_w \times SA \times PC \times ET \times EF \times ED \times F}{BM \times AT}$$

where CDI_{ing} is the chronic daily intake (mg/kg-day) via ingestion and CDI_{der} the chronic daily intake via dermal absorption (mg/kg-day) (IRIS, 2019). The meaning of the abbreviations and their respective units and values are summarised in Table 4-1. Cancer risk (CR) and hazard index (HI) were calculated using the following formula:

$$CR = \sum_{i=1}^{m} CDIij \times SFij$$

and

$$HI = \sum_{i=1}^{m} \left(\frac{CDIi}{RfDi} \right)$$

where i = refers to chloroform, bromodichloroform, dibromochloroform, and bromoform, which can be varied from 1 to m; j represents the two routes of exposure that could be various from 1 to n. For the compound specific permeability coefficients (PF), slope factors (SF) and reference doses (RfD), refer to Table 4-2. The same SF was used for both ingestion and dermal exposure (Abbasnia et al., 2019; Mishra et al., 2014). The SF shows the 95-percentile upper-bound lifetime cancer risk from exposure to the carcinogen and the RfD is the safe dose that may be ingested with no adverse effect (IRIS, 2019).

Table 4-1 Meaning of the symbols and where applicable, the values used for the coeffiients used in the formulae for hazard and cancer risk

Input parameters	Units	Value	Reference
Cw: concentration	mg/ℓ	as quantified in this study	
BM: body mass	kg	adult: 66 kg	Pheiffer et al. (2018)
		child: 35 kg	
IR: ingestion rate	ℓ/day	adult: 32 mℓ/h x *2.5 h/day = 0.08	Dufour et al. (2017)
		child: 4 x adults' = 0.32	Dufour et al. (2017)
ET: duration of swimming	h/event	*2.5 h	
EF: swimming frequency	events/year	*6	
ED: exposure duration	year	adult: 70 y child: 12 y	
F: conversion factor	%	skin fraction in contact with water = 100	
AT: average time	days	*6 x ED	
SA: skin surface area	m^2	Using Mostellar formula with mean SA adult mass and mean length of SA adult: 1.73 m^2 child: 1.33 m^2	Global RPh (2020) NCD-RisC (2016) Puoane et al. (2002)
PC: permeability coefficient	cm/h	chloroform: 0.00683 bromodichloroform: 0.00402 dibromochloroform: 0.00289 bromoform: 0.00235	Mishra et al. (2014)
SF: slope factor (dermal and ingestion)	$(mg/kg/d)^{-1}$	chloroform: 0.0061 bromodichloroform: 0.062 dibromochloroform: 0.084 bromoform: 0.0079	Mishra et al. (2014)
RfD: reference dose	mg/kg/d	chloroform: 0.01 bromodichloroform: 0.02 dibromochloroform: 0.02 bromoform: 0.02	Mishra et al. (2014)

*Arbitrarily selected for this study

Table 4-2 The compound and exposure pathway specific permeability coefficient (PC), reference dose (RfD), and slope factor (SF) used in the health risk assessment.

Compound/ metal	Dermal permeability coefficient (PC) (cm/h)	Reference dose oral (RfD_{oral}) (mg/kg/day)	Reference dose dermal (RfD_{dermal}) (mg/kg/day)	Cancer slope factor (SF) $(mg/kg\text{-}day)^{-1}$
chloroform	0.00683*	0.0061*		0.01*
bromodichloroform	0.00402*	0.062*		0.02*
dibromochloroform	0.00289*	0.084*		0.02*
bromoform	0.00235*	0.0079*		0.02*
Cr	0.002#▲	0.003#	0.000015#	0.05▲
Cu	0.001#▲	0.04#	0.012#	-
Fe	0.001#▲	0.7°	0.045#	-
Pb	0.004#▲	0.0014#	0.00042#	8.5▲
Mn	0.001#▲	0.02#	0.0008#	-
Ni	0.0002#▲	0.02#	0.0054#	-
Cd	0.001#▲	-	-	0.006▲

-no data available; *Mishra et al. (2014); #Karim et al. (2011); ▲Hashmi et al. (2014);°USEPA, 2006

4.3 RESULTS

The results presented here are the CRs and HQ for the four THMs and those metals for which SFs and RfDs (both dermal and ingestion) are available. The SF for ingestion was used also to calculate CR for dermal exposure due to lack of availability of a dermal SF. This approach was also followed by Abbasnia et al. (2019) and Mishra et al. (2014). The HQs per compound per sample (and subsequent hazard index (HI) for all compounds in the sample) are summarised in tables Table 4-3 to Table 4-6. The HI_{total} in Table 4-4 and Table 4-6 represents the total HI per site through dermal and ingestion exposure routes. A HI < 0.1 indicates no hazard; 0.1 < HI < 1 indicates a low hazard risk; 1.1 < HI < 10 shows moderate risk, and HI > 10 points to a high hazardous effect (Lemly, 1996). A cancer risk calculated for ingestion (Table 4-7 and Table 4-9), which is less than 1×10^{-4} or 1 in 10 000 is considered acceptable risk. Similarly, a risk less than 1×10^{-6} or 1 in 1 000 000 is considered negligible for dermal exposures (Table 4-8 and Table 4-10). Any CR values greater than these acceptable risks, means that cancer may develop due to exposures of the target compounds evaluated in the risk assessment under the assumptions made.

4.3.1 Hazard quotient and hazard index

The HI was the highest for dermal exposure in both age groups compared with ingestion (Table 4-3 to Table 4-4 and Table 4-5 to Table 4-6) and when the HI was determined for each sample all of them had a HI greater than 10 indicating a high risk to non-cancerous risk. The backwash samples of the resort pools always had the highest HI. Considering those samples that represented proper pool samples (not backwash, source water or pond water) BP pool C pm had the highest HI. Overall, there are more exceedances of an acceptable hazard risk for children than for adults (compare Table 4-3 and Table 4-4 to Table 4-5 and Table 4-6). The compound class with the highest probability of being a non-cancerous hazard are the metals, especially Cr, followed by $CHCl_3$ (Table 4-4 and Table 4-6).

4.3.2 Cancer risk

Similar to the HI, the CR was also the highest for the dermal exposure. Compare Table 4-7 to Table 4-8 and Table 4-9 to Table 4-10. All of the compounds except for Cd had the possibility of causing cancer in all of the samples. And as for the HI, the pool sample that has the highest CR is pool C. The compound with the highest probability of causing cancer through dermal and ingestion exposure is Pb followed by $CHBrCl_2$.

Table 4-3 Hazard quotient (HQ) (per compound) and hazard index (HI) (entire sample) for adults when ingesting swimming pool compounds

HQ	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Fe	Pb	Mn	Cr	Cu	Ni	HI
BP utility source	1.9E-05	1.3E-05	5.1E-05	1.3E-05	1.9E-05	7.9E-05	3.2E-05	–	7.2E-06	–	2.4E-04
BP borehole	2.6E-07	2.6E-07	5.1E-07	2.6E-07	3.5E-07	1.5E-04	3.5E-07	5.1E-05	9.4E-06	–	2.1E-04
BP reservoir	2.6E-07	8.1E-06	4.9E-05	2.6E-07	4.9E-07	7.7E-06	9.8E-07	4.1E-06	8.0E-06	–	7.9E-05
BP pool A am	3.1E-05	2.6E-07	2.0E-05	1.0E-05	–	–	–	1.0E-05	5.7E-06	–	7.6E-05
BP pool A pm	3.4E-05	2.6E-07	1.9E-04	1.2E-05	–	–	–	5.6E-06	4.1E-06	–	2.4E-04
BP pool A backwash	3.1E-05	5.7E-07	1.7E-04	1.1E-05	3.3E-06	4.6E-05	2.1E-05	1.3E-04	1.8E-05	–	4.4E-04
BP pool B am	2.8E-05	4.5E-06	8.4E-05	1.8E-05	3.7E-07	3.1E-05	9.1E-07	7.9E-05	2.7E-05	–	2.7E-04
BP pool B pm	2.9E-05	4.8E-06	9.0E-05	1.9E-05	3.1E-07	2.2E-05	5.4E-07	8.9E-05	5.4E-06	–	2.6E-04
BP pool B backwash	2.5E-05	2.0E-06	9.0E-05	1.2E-05	2.3E-06	4.7E-05	1.5E-05	1.1E-04	1.1E-05	–	3.1E-04
BP pool C am	2.9E-05	1.1E-05	9.2E-05	2.7E-05	5.2E-07	2.0E-05	5.7E-07	9.5E-05	8.5E-06	–	2.8E-04
BP pool C pm	3.7E-05	1.1E-05	9.4E-05	3.3E-05	3.8E-07	3.0E-05	8.8E-07	1.1E-04	1.4E-05	–	3.3E-04
BP pool C backwash	3.0E-05	5.4E-06	1.0E-04	2.1E-05	3.0E-05	6.5E-04	2.3E-04	1.8E-04	1.1E-04	7.7E-06	1.4E-03
BP pond day 1	2.9E-06	2.6E-07	2.5E-05	2.6E-07	1.3E-05	2.1E-05	4.9E-04	7.4E-05	1.2E-05	–	6.4E-04
PD source water	2.7E-05	2.2E-06	6.7E-05	1.8E-05	2.7E-06	1.6E-05	4.1E-06	–	5.2E-05	6.0E-07	1.9E-04
PD pool am	8.7E-06	2.6E-07	9.4E-05	3.0E-06	1.6E-06	3.3E-06	2.9E-06	8.6E-07	1.2E-06	7.0E-07	1.2E-04
PD pool pm	7.0E-06	2.6E-07	8.0E-05	2.2E-06	1.0E-06	5.3E-06	1.2E-06	2.7E-07	1.7E-06	5.0E-07	9.9E-05
PD backwash	8.9E-06	2.6E-07	1.5E-04	2.0E-06	1.8E-06	9.6E-05	2.6E-05	1.0E-06	4.1E-06	1.7E-06	2.9E-04

-not calculated due to unavailable values necessary for the calculation

Table 4-4 Hazard quotient (HQ) (per compound) and hazard index (HI) (entire sample) for adults via dermal exposure to swimming pool compounds

HQ	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Fe	Pb	Mn	Cr	Cu	Ni	HI	*HI_{total}
BP utility source	2.5E+02	1.0E+02	1.1E+03	1.2E+02	9.7E+02	3.4E+02	2.6E+03	–	7.8E+01	–	5.6E+03	5.6E+03
BP borehole	3.3E+00	1.9E+00	1.1E+01	2.4E+00	1.8E+01	6.5E+02	2.8E+01	6.7E+04	1.0E+02	–	6.8E+04	6.8E+04
BP reservoir	3.3E+00	6.2E+01	1.1E+03	2.4E+00	2.5E+01	3.3E+01	7.9E+01	5.3E+03	8.6E+01	–	6.7E+03	6.7E+03
BP pool A am	4.0E+02	1.9E+00	4.4E+02	9.4E+01	–	–	–	1.3E+04	6.1E+01	–	1.4E+04	1.4E+04
BP pool A pm	4.4E+02	1.9E+00	4.1E+03	1.1E+02	–	–	–	7.2E+03	4.4E+01	–	1.2E+04	1.2E+04
BP pool A backwash	4.0E+02	4.4E+00	3.8E+03	1.1E+02	1.7E+02	2.0E+02	1.7E+03	1.7E+05	2.0E+02	–	1.8E+05	1.8E+05
BP pool B am	3.6E+02	3.4E+01	1.9E+03	1.7E+02	1.9E+01	1.4E+02	7.4E+01	1.0E+05	2.9E+02	–	1.1E+05	1.1E+05
BP pool B pm	3.7E+02	3.7E+01	2.0E+03	1.8E+02	1.5E+01	9.7E+01	4.4E+01	1.2E+05	5.9E+01	–	1.2E+05	1.2E+05
BP pool B backwash	3.2E+02	1.6E+01	2.0E+03	1.1E+02	1.2E+02	2.0E+02	1.3E+03	1.4E+05	1.2E+02	–	1.4E+05	1.4E+05
BP pool C am	3.7E+02	8.6E+01	2.0E+03	2.5E+02	2.6E+01	8.6E+01	4.6E+01	1.2E+05	9.2E+01	–	1.3E+05	1.3E+05
BP pool C pm	4.8E+02	8.6E+01	2.1E+03	3.1E+02	1.9E+01	1.3E+02	7.1E+01	1.4E+05	1.5E+02	–	1.4E+05	1.4E+05
BP pool C backwash	3.9E+02	4.1E+01	2.3E+03	2.0E+02	1.5E+03	2.8E+03	1.9E+04	2.4E+05	1.2E+03	1.8E+01	2.7E+05	2.7E+05
BP pond day 1	3.7E+01	1.9E+00	5.4E+02	2.4E+00	6.7E+02	9.3E+01	4.0E+04	9.6E+04	1.3E+02	–	1.4E+05	1.4E+05
PD source water	3.5E+02	1.7E+01	1.5E+03	1.7E+02	1.4E+02	6.8E+01	3.3E+02	–	5.7E+02	1.4E+00	3.1E+03	3.1E+03
PD pool am	1.1E+02	1.9E+00	2.1E+03	2.8E+01	7.9E+01	1.4E+01	2.4E+02	1.1E+03	1.2E+01	1.7E+00	3.7E+03	3.7E+03
PD pool pm	9.2E+01	1.9E+00	1.8E+03	2.1E+01	5.3E+01	2.3E+01	1.0E+02	3.4E+02	1.8E+01	1.2E+00	2.4E+03	2.4E+03
PD backwash	1.2E+02	1.9E+00	3.3E+03	1.9E+01	9.2E+01	4.2E+02	2.1E+03	1.3E+03	4.5E+01	4.1E+00	7.4E+03	7.4E+03
Key	0.1 < HI/HQ < 1			1.1 < HI/HQ < 10				HI/HQ > 10				

-not calculated due to unavailable values necessary for the calculation; *HI_{total} represents the total HI per site through both dermal and ingestion exposure routes

Table 4-5 Hazard quotient (HQ) (per compound) and hazard index (HI) (entire sample) for children when ingesting swimming pool compounds

HQ	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Fe	Pb	Mn	Cr	Cu	Ni	HI
BP utility source	1.4E-04	9.8E-05	3.8E-04	9.8E-05	1.4E-04	5.6E-04	2.3E-04	–	5.1E-05	–	1.7E-03
BP borehole	1.9E-06	1.9E-06	3.8E-06	1.9E-06	2.5E-06	1.1E-03	2.5E-06	3.7E-04	6.7E-05	–	1.5E-03
BP reservoir	1.9E-06	5.9E-05	3.6E-04	1.9E-06	3.5E-06	5.5E-05	7.0E-06	2.9E-05	5.7E-05	–	5.7E-04
BP pool A am	2.3E-04	1.9E-06	1.5E-04	7.4E-05	–	–	–	7.1E-05	4.0E-05	–	5.6E-04
BP pool A pm	2.5E-04	1.9E-06	1.4E-03	9.0E-05	–	–	–	4.0E-05	2.9E-05	–	1.8E-03
BP pool A backwash	2.3E-04	4.2E-06	1.3E-03	8.3E-05	2.3E-05	3.3E-04	1.5E-04	9.5E-04	1.3E-04	–	3.2E-03
BP pool B am	2.0E-04	3.3E-05	6.2E-04	1.4E-04	2.6E-06	2.2E-04	6.5E-06	5.6E-04	1.9E-04	–	2.0E-03
BP pool B pm	2.1E-04	3.5E-05	6.6E-04	1.4E-04	2.2E-06	1.6E-04	3.8E-06	6.3E-04	3.9E-05	–	1.9E-03
BP pool B backwash	1.8E-04	1.5E-05	6.6E-04	9.0E-05	1.6E-05	3.3E-04	1.1E-04	7.6E-04	7.8E-05	–	2.2E-03
BP pool C am	2.1E-04	8.3E-05	6.8E-04	2.0E-04	3.7E-06	1.4E-04	4.0E-06	6.7E-04	6.1E-05	–	2.0E-03
BP pool C pm	2.7E-04	8.3E-05	6.9E-04	2.4E-04	2.7E-06	2.1E-04	6.2E-06	7.7E-04	9.7E-05	–	2.4E-03
BP pool C backwash	2.2E-04	4.0E-05	7.5E-04	1.6E-04	2.1E-04	4.7E-03	1.6E-03	1.3E-03	8.1E-04	5.5E-05	9.8E-03
BP pond day 1	2.1E-05	1.9E-06	1.8E-04	1.9E-06	9.4E-05	1.5E-04	3.5E-03	5.3E-04	8.6E-05	–	4.5E-03
PD source water	2.0E-04	1.7E-05	5.0E-04	1.4E-04	1.9E-05	1.1E-04	2.9E-05	–	3.7E-04	4.3E-06	1.4E-03
PD pool am	6.4E-05	1.9E-06	6.9E-04	2.2E-05	1.1E-05	2.3E-05	2.1E-05	6.1E-06	8.2E-06	5.0E-06	8.5E-04
PD pool pm	5.2E-05	1.9E-06	5.9E-04	1.7E-05	7.4E-06	3.7E-05	8.8E-06	1.9E-06	1.2E-05	3.5E-06	7.3E-04
PD backwash	6.5E-05	1.9E-06	1.1E-03	1.5E-05	1.3E-05	6.8E-04	1.9E-04	7.1E-06	3.0E-05	1.2E-05	2.1E-03

-not calculated due to unavailable values necessary for the calculation

Table 4-6 Hazard quotient (HQ) (per compound) and hazard index (HI) (entire sample) for children via dermal exposure to swimming pool compounds

HQ	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Fe	Pb	Mn	Cr	Cu	Ni	HI	$*HI_{total}$
BP utility source	3.6E+02	2.4E+02	9.4E+02	2.4E+02	1.4E+03	5.0E+02	3.8E+03	–	1.1E+02	–	7.6E+03	7.60E+03
BP borehole	4.7E+00	4.7E+00	9.4E+00	4.7E+00	2.6E+01	9.4E+02	4.1E+01	9.7E+04	1.5E+02	–	9.8E+04	9.80E+04
BP reservoir	4.7E+00	1.5E+02	9.0E+02	4.7E+00	3.6E+01	4.8E+01	1.1E+02	7.7E+03	1.2E+02	–	9.1E+03	9.13E+03
BP pool A am	5.7E+02	4.7E+00	3.7E+02	1.8E+02	–	–	–	1.9E+04	8.9E+01	–	2.0E+04	2.01E+04
BP pool A pm	6.2E+02	4.7E+00	3.4E+03	2.3E+02	–	–	–	1.0E+04	6.4E+01	–	1.5E+04	1.48E+04
BP pool A backwash	5.7E+02	1.1E+01	3.2E+03	2.1E+02	2.4E+02	2.9E+02	2.4E+03	2.5E+05	2.8E+02	–	2.6E+05	2.59E+05
BP pool B am	5.1E+02	8.3E+01	1.5E+03	3.4E+02	2.7E+01	2.0E+02	1.1E+02	1.5E+05	4.2E+02	–	1.5E+05	1.52E+05
BP pool B pm	5.3E+02	8.9E+01	1.7E+03	3.6E+02	2.2E+01	1.4E+02	6.3E+01	1.7E+05	8.5E+01	–	1.7E+05	1.71E+05
BP pool B backwash	4.5E+02	3.8E+01	1.7E+03	2.3E+02	1.7E+02	2.9E+02	1.8E+03	2.0E+05	1.7E+02	–	2.1E+05	2.05E+05
BP pool C am	5.3E+02	2.1E+02	1.7E+03	4.9E+02	3.8E+01	1.2E+02	6.7E+01	1.8E+05	1.3E+02	–	1.8E+05	1.81E+05
BP pool C pm	6.8E+02	2.1E+02	1.7E+03	6.0E+02	2.8E+01	1.9E+02	1.0E+02	2.0E+05	2.1E+02	–	2.1E+05	2.08E+05
BP pool C backwash	5.5E+02	1.0E+02	1.9E+03	4.0E+02	2.2E+03	4.1E+03	2.7E+04	3.5E+05	1.8E+03	2.7E+01	3.8E+05	3.84E+05
BP pond day 1	5.3E+01	4.7E+00	4.5E+02	4.7E+00	9.7E+02	1.3E+02	5.7E+04	1.4E+05	1.9E+02	–	2.0E+05	1.98E+05
PD source water	4.9E+02	4.1E+01	1.2E+03	3.4E+02	2.0E+02	9.9E+01	4.8E+02	–	8.2E+02	2.1E+00	3.7E+03	3.71E+03
PD pool am	1.6E+02	4.7E+00	1.7E+03	5.5E+01	1.1E+02	2.1E+01	3.4E+02	1.6E+03	1.8E+01	2.4E+00	4.1E+03	4.08E+03
PD pool pm	1.3E+02	4.7E+00	1.5E+03	4.1E+01	7.6E+01	3.3E+01	1.5E+02	5.0E+02	2.6E+01	1.7E+00	2.4E+03	2.43E+03
PD backwash	1.6E+02	4.7E+00	2.7E+03	3.8E+01	1.3E+02	6.0E+02	3.1E+03	1.9E+03	6.5E+01	5.9E+00	8.7E+03	8.72E+03
Key	0.1 < HI/HQ < 1				1.1 < HI/HQ < 10			HI/HQ > 10				

-not calculated due to unavailable values necessary for the calculation; $*HI_{total}$ represents the total HI per site through both dermal and ingestion exposure routes

Table 4-7 Cancer risk for adults via ingestion of swimming pool compounds. Red scale = CR > 10^{-4} = unacceptable risk

	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Pb	Cd	Cr	SUM
BP utility source	2.4E-08	2.1E-09	3.1E-09	2.2E-08	1.3E-08	6.6E-08	–	1.3E-07
BP borehole	3.2E-10	4.0E-11	3.1E-11	4.3E-10	2.5E-08	3.3E-08	5.1E-05	5.2E-05
BP reservoir	3.2E-10	1.3E-09	3.0E-09	4.3E-10	1.3E-09	5.6E-07	4.1E-06	4.7E-06
BP pool A am	3.8E-08	4.0E-11	1.2E-09	1.7E-08	–	–	1.0E-05	1.0E-05
BP pool A pm	4.2E-08	4.0E-11	1.1E-08	2.1E-08	–	–	5.6E-06	5.7E-06
BP pool A backwash	3.8E-08	9.0E-11	1.1E-08	1.9E-08	7.5E-09	2.7E-07	1.3E-04	1.3E-04
BP pool B am	3.4E-08	7.1E-10	5.1E-09	3.1E-08	5.2E-09	6.6E-08	7.9E-05	7.9E-05
BP pool B pm	3.5E-08	7.6E-10	5.5E-09	3.3E-08	3.7E-09	–	8.9E-05	8.9E-05
BP pool B backwash	3.0E-08	3.2E-10	5.5E-09	2.1E-08	7.7E-09	1.0E-07	1.1E-04	1.1E-04
BP pool C am	3.5E-08	1.8E-09	5.6E-09	4.5E-08	3.3E-09	6.6E-08	9.5E-05	9.5E-05
BP pool C pm	4.6E-08	1.8E-09	5.7E-09	5.5E-08	4.9E-09	–	1.1E-04	1.1E-04
BP pool C backwash	3.7E-08	8.6E-10	6.2E-09	3.6E-08	1.1E-07	2.0E-07	1.8E-04	1.8E-04
BP pond day 1	3.5E-09	4.0E-11	1.5E-09	4.3E-10	3.5E-09	–	7.4E-05	7.4E-05
PD source water	3.3E-08	3.6E-10	4.1E-09	3.1E-08	2.6E-09	6.6E-08	–	1.4E-07
PD pool am	1.1E-08	4.0E-11	5.7E-09	5.0E-09	5.4E-10	6.6E-08	8.6E-07	9.5E-07
PD pool pm	8.7E-09	4.0E-11	4.9E-09	3.8E-09	8.7E-10	3.3E-08	2.7E-07	3.2E-07
PD backwash	1.1E-08	4.0E-11	9.1E-09	3.4E-09	1.6E-08	2.0E-07	1.0E-06	1.2E-06

-not calculated due to unavailable values necessary for the calculation

Table 4-8 Cancer risk for adults via dermal exposure of swimming pool compounds. Red scale = CR > 10^{-6} = unacceptable risk

	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Pb	Cd	Cr	SUM
BP utility source	3.1E-01	1.6E-02	6.9E-02	2.1E-01	1.2E+00	7.8E-06	–	1.8E+00
BP borehole	4.1E-03	3.1E-04	6.9E-04	4.0E-03	2.3E+00	3.9E-06	5.0E-02	2.4E+00
BP reservoir	4.1E-03	9.7E-03	6.6E-02	4.0E-03	1.2E-01	6.6E-05	4.0E-03	2.1E-01
BP pool A am	5.0E-01	3.1E-04	2.7E-02	1.6E-01	–	–	9.8E-03	6.9E-01
BP pool A pm	5.5E-01	3.1E-04	2.5E-01	1.9E-01	–	–	5.4E-03	1.0E+00
BP pool A backwash	5.0E-01	6.9E-04	2.3E-01	1.8E-01	7.1E-01	3.1E-05	1.3E-01	1.7E+00
BP pool B am	4.5E-01	5.4E-03	1.1E-01	2.9E-01	4.9E-01	7.8E-06	7.7E-02	1.4E+00
BP pool B pm	4.6E-01	5.8E-03	1.2E-01	3.1E-01	3.5E-01	–	8.7E-02	1.3E+00
BP pool B backwash	4.0E-01	2.5E-03	1.2E-01	1.9E-01	7.2E-01	1.2E-05	1.0E-01	1.5E+00
BP pool C am	4.6E-01	1.4E-02	1.2E-01	4.2E-01	3.1E-01	7.8E-06	9.2E-02	1.4E+00
BP pool C pm	5.9E-01	1.4E-02	1.3E-01	5.1E-01	4.6E-01	–	1.1E-01	1.8E+00
BP pool C backwash	4.8E-01	6.5E-03	1.4E-01	3.4E-01	1.0E+01	2.3E-05	1.8E-01	1.1E+01
BP pond day 1	4.6E-02	3.1E-04	3.3E-02	4.0E-03	3.3E-01	–	7.2E-02	4.9E-01
PD source water	4.3E-01	2.7E-03	9.1E-02	2.9E-01	2.4E-01	7.8E-06	–	1.1E+00
PD pool am	1.4E-01	3.1E-04	1.3E-01	4.7E-02	5.1E-02	7.8E-06	8.4E-04	3.7E-01
PD pool pm	1.1E-01	3.1E-04	1.1E-01	3.5E-02	8.1E-02	3.9E-06	2.6E-04	3.4E-01
PD backwash	1.4E-01	3.1E-04	2.0E-01	3.2E-02	1.5E+00	2.3E-05	9.7E-04	1.9E+00

-not calculated due to unavailable values necessary for the calculation

Table 4-9 Cancer risk for children via ingestion of swimming pool compounds. Red scale = CR > 10^{-4} = unacceptable risk

	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Pb	Cd	Cr	SUM
BP utility source	1.8E-07	1.5E-08	2.3E-08	1.6E-07	9.3E-08	4.7E-07	–	9.5E-07
BP borehole	2.3E-09	3.0E-10	2.3E-10	3.2E-09	1.8E-07	2.4E-07	2.2E-05	2.2E-05
BP reservoir	2.3E-09	9.4E-09	2.2E-08	3.2E-09	9.0E-09	4.0E-06	1.8E-06	5.8E-06
BP pool A am	2.8E-07	3.0E-10	8.9E-09	1.2E-07	–	–	4.3E-06	4.7E-06
BP pool A pm	3.1E-07	3.0E-10	8.3E-08	1.5E-07	–	–	2.4E-06	2.9E-06
BP pool A backwash	2.8E-07	6.6E-10	7.8E-08	1.4E-07	5.4E-08	1.9E-06	5.7E-05	6.0E-05
BP pool B am	2.5E-07	5.2E-09	3.8E-08	2.3E-07	3.7E-08	4.7E-07	3.4E-05	3.5E-05
BP pool B pm	2.6E-07	5.6E-09	4.0E-08	2.4E-07	2.6E-08	–	3.8E-05	3.9E-05
BP pool B backwash	2.2E-07	2.4E-09	4.0E-08	1.5E-07	5.5E-08	7.1E-07	4.6E-05	4.7E-05
BP pool C am	2.6E-07	1.3E-08	4.1E-08	3.3E-07	2.3E-08	4.7E-07	4.0E-05	4.2E-05
BP pool C pm	3.4E-07	1.3E-08	4.2E-08	4.0E-07	3.5E-08	–	4.6E-05	4.7E-05
BP pool C backwash	2.7E-07	6.3E-09	4.6E-08	2.7E-07	7.7E-07	1.4E-06	7.9E-05	8.1E-05
BP pond day 1	2.6E-08	3.0E-10	1.1E-08	3.2E-09	2.5E-08	–	3.2E-05	3.2E-05
PD source water	2.4E-07	2.6E-09	3.0E-08	2.3E-07	1.9E-08	4.7E-07	–	9.9E-07
PD pool am	7.9E-08	3.0E-10	4.2E-08	3.7E-08	3.8E-09	4.7E-07	3.7E-07	1.0E-06
PD pool pm	6.4E-08	3.0E-10	3.6E-08	2.8E-08	6.2E-09	2.4E-07	1.1E-07	4.8E-07
PD backwash	8.1E-08	3.0E-10	6.7E-08	2.5E-08	1.1E-07	1.4E-06	4.3E-07	2.1E-06

-not calculated due to unavailable values necessary for the calculation

Table 4-10 Cancer risk for children via dermal exposure of swimming pool compounds. Red scale = CR > 10^{-6} = unacceptable risk

	$CHBrCl_2$	$CHBr_3$	$CHCl_3$	$CHBr_2Cl$	Pb	Cd	Cr	SUM
BP utility source	4.4E-01	3.9E-02	5.7E-02	4.1E-01	1.8E+00	1.1E-05	–	2.7E+00
BP borehole	5.8E-03	7.4E-04	5.7E-04	7.9E-03	3.4E+00	5.6E-06	7.3E-02	3.4E+00
BP reservoir	5.8E-03	2.4E-02	5.5E-02	7.9E-03	1.7E-01	9.6E-05	5.8E-03	2.7E-01
BP pool A am	7.0E-01	7.4E-04	2.2E-02	3.1E-01	–	–	1.4E-02	1.0E+00
BP pool A pm	7.7E-01	7.4E-04	2.1E-01	3.8E-01	–	–	7.9E-03	1.4E+00
BP pool A backwash	7.0E-01	1.7E-03	2.0E-01	3.5E-01	1.0E+00	4.5E-05	1.9E-01	2.5E+00
BP pool B am	6.3E-01	1.3E-02	9.4E-02	5.7E-01	7.0E-01	1.1E-05	1.1E-01	2.1E+00
BP pool B pm	6.5E-01	1.4E-02	1.0E-01	6.0E-01	5.0E-01	–	1.3E-01	2.0E+00
BP pool B backwash	5.6E-01	6.0E-03	1.0E-01	3.8E-01	1.0E+00	1.7E-05	1.5E-01	2.2E+00
BP pool C am	6.5E-01	3.3E-02	1.0E-01	8.2E-01	4.4E-01	1.1E-05	1.3E-01	2.2E+00
BP pool C pm	8.4E-01	3.3E-02	1.1E-01	1.0E+00	6.7E-01	–	1.5E-01	2.8E+00
BP pool C backwash	6.8E-01	1.6E-02	1.1E-01	6.6E-01	1.5E+01	3.4E-05	2.6E-01	1.6E+01
BP pond day 1	6.5E-02	7.4E-04	2.8E-02	7.9E-03	4.8E-01	–	1.0E-01	6.9E-01
PD source water	6.1E-01	6.5E-03	7.6E-02	5.7E-01	3.5E-01	1.1E-05	–	1.6E+00
PD pool am	2.0E-01	7.4E-04	1.1E-01	9.2E-02	7.3E-02	1.1E-05	1.2E-03	4.7E-01
PD pool pm	1.6E-01	7.4E-04	9.0E-02	7.0E-02	1.2E-01	5.6E-06	3.7E-04	4.4E-01
PD backwash	2.0E-01	7.4E-04	1.7E-01	6.3E-02	2.2E+00	3.4E-05	1.4E-03	2.6E+00

-not calculated due to unavailable values necessary for the calculation

4.4 SUMMARY

The main findings of the human health risk assessment are that under the assumptions made, children are more at risk than adults, and there is more risk through dermal exposure than ingestion. This latter phenomenon is corroborated by a study of Abbasnia et al. (2019). These authors focussed on THMs only.

The health risk assessment done based on dermal and ingestion exposure in this study should be interpreted with caution for a number of reasons: concentrations used were based on a small sample size during a once off event and the samples were quantified once. The numbers for some of the variables used in the calculations of hazard quotient and cancer risk, like the number of exposure events and average time spent in a swimming pool were selected randomly and were not based on any literature or project related sampling. Despite these limitations the meaningfulness of the health risk assessment (HRA) is based on its comparative value.

Comparing between samples and screening for tendencies: the backwash water samples of the resort had the highest HI and CR in for all three pools. This is not directly of concern to human health but at this resort the water is pumped to a pond that feeds into a river close by. Aquatic biota might be affected negatively. Another observation is that the exposure through absorption through the skin is greater than through the ingestion of swimming pool water. One of the reasons for this is likely that the entire skin, which is the biggest organ of the human body, is usually submerged. At least, this is what was assumed in the calculations, i.e. that a 100% of the body surface is submerged. This is an over estimation because the head is not always submerged, but in the HRA the worst case scenario was investigated. A third observation that emerged is the fact that it was the metals that were responsible for the highest HI and CR. This was specifically the case at the moment the pools were sampled and it might not always be the situation that it is the components of most likely the source water and not the THMs that is cause for concern. A fourth and very alarming observation is that the children were the most at risk to develop detrimental health effects due to these compounds and elements that occur in public swimming pools.

5 EFFECT BASED ANALYSIS OF POOL WATER QUALITY

5.1 INTRODUCTION

The levels of DBPs, metals and water parameters can be determined and compared to the drinking water standards, but they cannot predict any biological effects. Of particular concern in this study is the possible endocrine activity of these mixtures. Many natural and synthetic compounds have been identified as endocrine disrupting chemicals (EDCs), that may interfere with endogenous hormone homeostasis (Sikka & Wang, 2008). *In vitro* assays are widely used to screen compounds and mixtures from environmental matrices for endocrine disrupting effects. A suite of bioassays was applied to assess the endocrine disruptive potential of swimming pool water extracts.

5.2 MATERIALS AND METHODS

The method of sampling water for the biological assays, as well as the extraction process followed to concentrate the compounds are the same as for the screening of the samples by the LC/MS-QTOF.

5.2.1 (Anti)oestrogenic activity

The United States Environmental Protection Agency (USEPA) developed a stable cell line to measure oestrogen related effects. The T47D human breast cancer cells with oestrogen receptors (ERα and ERβ) were genetically modified to modulate oestrogen-dependent gene transcription (Wilson et al., 2004). T47D-KBluc cells were obtained from the American Type Culture Collection (ATCC, USA, catalogue no. CRL-2865).

5.2.1.1 Maintenance of cells

T47DKBluc cells were cultured in RPMI 1640 growth medium (Sigma-Aldrich, USA) supplemented with 2.5 g/ℓ glucose (Merck, Germany), 10 mM HEPES (Sigma-Aldrich, USA), 1 mM sodium pyruvate (Sigma-Aldrich, USA), 1.5 g/ℓ sodium carbonate (Sigma-Aldrich, USA), 10% foetal bovine serum (FBS) (Biowest). They were incubated at 37˚C in a 5% CO_2 atmosphere. One week before the assay, the cells' growth medium containing 10% FBS was replaced with 10% dextran-charcoal treated foetal bovine serum (cdt FBS) (Hyclone, Separations SA) (which is free of hormones).

5.2.1.2 Experimental procedure

The cells were seeded at 5×10^4 cells per well in a 96-well plate (100 µℓ per well) with growth media containing 5% cdt FBS. After 24 h incubation, the plates received sample extracts and reference compounds. These dosing solution were prepared using 5% cdt FBS growth media and the vehicle control (VC), ethanol, may not exceed 0.2% (v/v). The quality control for each plate received the VC as negative control; an ER agonist, β-oestradiol (E_2) as positive control (0.1 nM; 30 pM; 10 pM; 3 pM; 1 pM; 0.3 pM; 0.1 pM); ICI (0.1 µM) as the antagonist negative/background control; E_2 and ICI as the antagonist control. Each sample extract was tested alone and co-incubated with 0.1 nM E_2 or ICI. The plates were incubated for the 24 h exposure time.

After incubation, the plates were washed with phosphate buffer saline (PBS). The cells were lysed with 25 µℓ lysis buffer (Promega) and frozen at -80°C overnight. Luciferase activity was measured using a Berthold TriStar LB 941 plate reader (Germany) and recorded as relative light units (RLUs). The reaction buffer consisted of

1 M glycylglycine, 0.1 M ATP, 0.1 mg/mℓ bovine serum albumin (BSA) solution, 1 M $MgCl_2$ of which 25 µℓ were added to each well, followed by 25 µℓ 1 mM D-luciferin 5 seconds later. The RLUs were transformed to create a fold induction greater than the VC.

Oestrogenic activity was determined by fitting the E_2 standard curve with the sigmoidal, variable slope function using Graphpad Prism (version 7) to calculate the EC_{50} values. The samples responses were interpolated from the oestradiol standard curve to oestradiol equivalents (EEqs). Both controls (VC and E2) were also included as they competed with 0.1 µM ICI, an ER antagonist, to assess ER-specific responsiveness and background.

5.2.2 (Anti) androgenic activity

MDA-MB-453 human breast cancer cells, were stably transformed with murine mammalian tumour virus (MMTV)-luciferase neo reporter construct into the MDA-kb2 cell line (Wilson et al., 2002). This assay can detect both the (anti-)activation of the androgen receptor (AR) and the activation of the glucocorticoid receptor (GR) (Aït-Aïssa et al., 2010). The cells were a gift from the University of Saskatchewan, Canada.

5.2.2.1 Maintenance of cells

The MDA-kb2 cells were grown in Leibovitz L-15 media supplemented with 10% FBS and incubated at 37˚C without CO_2.The assays performed using L-15 media containing 10% cdtFBS

5.2.2.2 Experimental procedure

MDAkb-2 cells were seeded at 12 × 10^4 cells per well in 250 µℓ assay medium and allowed to attach for 48 h. Sample extracts and standards were dosed in triplicate. The vehicle control was ethanol. The AR agonist, testosterone was the positive control in the AR activation assays and given to the cells at 0.007; 0.03; 0.18; 0.92; 4.6; 23 µg/mℓ. A slight modification to the method was done to test for AR inhibition and GR activation. Exposure to 0.032, 0.16, 0.8, 4, 20 and 100 µg/mℓ flutamide was included as an AR antagonist in the AR inhibition assay. The compounds' ability to inhibit the AR, was tested by co-exposing the cells to 23 µg/mℓ testosterone throughout the assay. Dexamethasone was used as GR agonist and the standard curve consisted of 0.12; 0.6; 3; 15; 75 and 375 ng/mℓ. Glucocorticoid receptor activation could be monitored by co-incubating the cells with 0.2 µg/mℓ flutamide to block the AR. Exposure time for all three types of assays were 48 h.

At the end of the assays the plates were washed with PBS containing Mg^{2+} and Ca^{2+}. The cells were lysed by adding 25 µL lysis buffer (Promega) and frozen at -80°C. A Berthold multimode microplate reader (model LB941) was used to measure luminescence. The luciferase assay reagent (LAR) consisted of 20 mM tricine (Sigma-Aldrich), 1.07 mM $Mg(CO_3)_2Mg(OH)_2{\cdot}5H_2O$ (Sigma-Aldrich), 2.67 mM $MgSO_4$ $7H_2O$ (Sigma-Aldrich), 0.1 mM EDTA (ethylene-diamine-tetra-acetic acid)-disodium salt (Sigma-Aldrich), 33.3 mM dithiothreitol (DTT) (Sigma-Aldrich), 270 µM coenzyme A (Sigma-Aldrich), 530 µM ATP (Sigma-Aldrich) and 470 µM beetle luciferin (Melford) (Villeneuve et al., 1999). Luminescence were recorded as RLUs and the samples' responses were expressed in terms of the standards' dose-response curves and expressed as the percentage maximum (%T max, %D max and % F max). A minimum of 3 data points on the linear part of the curves were used to calculate the slope as well as the y-intercept in the straight line equation (y = mx + c). Using these calculations, effective concentrations (ECs) which elicited a 20%, 50% and 80% response were calculated. For each sample, relative potency values (REPs) were calculated by dividing the reference testosterone EC_{20}–EC_{80} by the EC_{20}–EC_{80} of the sample (Villeneuve et al., 2000). The final REP_{20}–REP_{80} values for each sample were expressed as bioassay equivalents (TEq and DEq) derived by back calculation based on the concentration factor of the extracts.

During the AR inhibition assay, testosterone, an AR agonist, were added to the cells to slightly activate the AR. The vehicle control wells only receive ethanol, and no other compounds to inhibit the AR. The ability of the samples to inhibit the existing cellular binding was determined by comparing their RLUs to the VC's RLUs. A fold change (FC) below 1 indicate inhibition and a FC above 1 show possible activation of the AR. The significance in differences were determined by using a non-parametric test, Mann-Whitney. IBM SPSS Statistics software (version 25) was used in this comparison and inhibition effects were regarded as statistically significant when $p < 0.05$.

5.2.3 Aryl hydrocarbon receptor activation

The H4IIE-luc cells are rat hepatoma cells that have been genetically modified and transfected with a firefly luciferase reporter gene (Aarts et al., 1995). The H4IIE-*luc* assay has the ability to screen for aryl hydrocarbon receptor (AhR) activity and indirectly measure cytochrome P450 induction, which is an endpoint in the AhR mediated response (Denison et al., 2004). The cells were a gift from John Giesy (University of Saskatchewan, Canada).

5.2.3.1 Maintenance of cells

This cell-line was maintained in Dulbecco's Modified Eagle's Medium (DMEM) and incubated at 37˚C in a 5% CO_2 atmosphere. The assay was conducted using DMEM supplemented with 10% cdtFBS (HyClone, Separations).

5.2.3.2 Experimental procedure

The cells were seeded at 8 x 10^4 per well in 250 µℓ and incubated for 24 h. After attachment, the cells were dosed with sample extracts and the positive control 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) in concentrations of 0.05, 0.19, 0.75, 3, 12, 48 ng/mℓ. The exposure time was 72 h (Chan et al., 2013). The same protocol was used for the end of this assay as discussed before. The samples' responses were expressed in terms of the TCDD standard curve and TCDD-eq were calculated by using regression of the TCDD and back calculation based on the concentration factor of the extracts.

5.2.4 Viability assay (mtt)

The cytotoxicity of the sample extracts was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mossman, 1983). The MTT assays were performed simultaneously to the reporter-gene assays. These results were used to validate the reporter gene bio-assay data by preventing false negative responses since low responses (i.e. no luminescence) do not necessarily indicate the presence of antagonists or absence of agonists, but possible cytotoxicity. The principle of the assay is based on the enzyme reaction in which yellow tetrazolium salt (MTT) is metabolised to a purple crystalline, insoluble formazan product. The formazan salts are dissolved and can then be quantified spectrophotometrically (Mossman, 1983).

Absorbance was measured at 560 nm in a multimode microplate reader (Berthold TriStar LB 941, Germany). The optical density (OD) of the solution is proportional to the living. The mean of the OD values from the negative control (methanol-killed cells) were subtracted from exposed cells. This value was expressed in terms of the solvent control (100% viable cells) and percentage viability was calculated. A viability percentage lower than 80% was subjected to non-parametric tests to determine if these samples were statistically significant cytotoxic that may affect reporter-gene response interpretations.

5.3 RESULTS

The swimming pool water extracts were assayed using the H4IIE-*luc,* T47Dkb*luc*, and MDA-kb2 reporter-gene assays. The sample responses were compared to the receptor specific reference compounds' responses. Sample responses below 20% were considered as <LOQ. The sample responses were converted to REP 20 values. Samples were regarded as cytotoxic if cell viability was lower than 70%. It was only the extracts from the backwash water that were cytotoxic (Table 5-1). This was to be expected because these samples would contain the highest concentration of the toxicants.

5.3.1 AhR activation

The %TCDDmax values of all the samples except for the BP utility source water, were below 20% and the REP 20 values could not be calculated as the samples responses were below the limit of quantification (Table 5-1). Most of the samples were not cytotoxic to the cells, except for the BP pool A, B, C and PT backwash samples which had a cell viability <70%.

Table 5-1 TCDD-eqs (AhR activation) and the percentage viability of the cells during the exposure

Sample ID	TCDDeq µg/ℓ	Viability %
BP utility source REP 20	0.34 ± 0.05	93 ± 0.07
BP borehole	<LOQ	101 ± 0.09
BP reservoir	<LOQ	114 ± 0.04
BP pool A am	<LOQ	92 ± 0.13
BP pool A pm	<LOQ	87 ± 0.06
BP pool A backwash	<LOQ	*23 ± 0.08
BP pool B am	<LOQ	100 ± 0.06
BP pool B pm	<LOQ	100 ± 0.12
BP pool B backwash	<LOQ	*0 ± 0
BP pool C am	<LOQ	100 ± 0.25
BP pool C pm	<LOQ	74 ± 0.71
BP pool C backwash	<LOQ	*0 ± 0
BP pond day 1	<LOQ	74 ± 0.11
BP pond day 3	<LOQ	99 ± 0.04
PD source water	<LOQ	99 ± 0.08
PD pool am	<LOQ	108 ± 0.07
PD pool pm	<LOQ	117 ± 0.04
PD backwash	<LOQ	84 ± 0.16
PT source water	<LOQ	101 ± 0.13
PT pool am	<LOQ	99 ± 0.09
PT pool pm	<LOQ	81 ± 0.07
PT backwash	<LOQ	*0 ± 0

<LOQ – below limit of quantification; REP – relative effect potencies; am – before swimming activity; pm – after swimming activity; * cytotoxicity; TCDDeq TCDD equivalents; AhR aryl hydrocarbon receptor.

5.3.2 (Anti-)oestrogen activity

The swimming pool water samples were screened for (anti-)oestrogenic activity. Six of the extracts were regarded as cytotoxic and no inhibition was present for these particular samples (Table 5-2). Thus the cytotoxicity had no effect on the inhibition assay and therefore, where inhibition was quantified, it was due to ligands blocking the ER and not because of a decline in cell viability. The activation bio-assay was done in triplicate, and the mean concentration and standard deviation are summarised as EEq values (Table 5-2). Due to time constraints, the inhibition assay could only be done once. The results therefore are only indicative of possible inhibition and should these assays be repeated to confirm these initial results. The total oestrogenic activity in the swimming pool water samples was estimated by comparing the values with the activity of the natural oestrogen, 17-*β*-estradiol (E_2) and expressed as oestradiol equivalents (EEq). Inhibitory effects were expressed in terms of the known concentration of the reference compound for inhibition, ICIeqs. In the case where the samples were cytotoxic at high concentrations of the extract, no response from the ER activation luminescence assay could be observed. But, if there are enough ER agonists present at the lower concentrations that enabled the cells to survive, it would be still possible to calculate an EEq.

Table 5-2 EEq and ICI-eqs (ER activation and inhibition) and the percentage viability of the cells during the exposure.

Sample ID	EEq	ICIEq	Viability
	ng/ℓ	µg/ℓ	%
Trigger value (Escher et al., 2018)	3.8		
BP utility source	<LOQ	0.35	85 ± 0.11
BP borehole	0.142 ± 0.109	<LOQ	*62 ± 0.1
BP reservoir	<LOQ	<LOQ	94 ± 0.25
BP pool A am	<LOQ	<LOQ	102 ± 0.19
BP pool A pm	<LOQ	<LOQ	*47 ± 0.19
BP pool A backwash	0.024 ± 0.014	<LOQ	80 ± 0.17
BP pool B am	<LOQ	<LOQ	*43 ± 0.17
BP pool B pm	<LOQ	<LOQ	*62 ± 0.15
BP pool B backwash	1.013 ± 0.205	<LOQ	*0 ± 0
BP pool C am	<LOQ	0.03	97 ± 0.04
BP pool C pm	0.045 ± 0.005	0.21	102 ± 0.04
BP pool C backwash	<LOQ	<LOQ	*0 ± 0
BP pond day 1	0.458 ± 0.431	0.306	95 ± 0.06
BP pond day 3	0.29 ± 0.286	0.44	128 ± 0.04
PD source water	0.186 ± 0.034	<LOQ	75 ± 0.09
PD pool am	0.426 ± 0.138	1.07	232 ± 0.56
PD pool pm	0.338 ± 0.127	0.81	253 ± 0.11
PD backwash	0.116 ± 0.087	0.85	212 ± 0.18
PT source water	<LOQ	0.73	98 ± 0.29
PT pool am	<LOQ	<LOQ	76 ± 0.13
PT pool pm	0.041 ± 0.002	<LOQ	84 ± 0.06
PT backwash	<LOQ	<LOQ	88 ± 0.19

<LOQ – below limit of quantification; am – before swimming activity; pm – after swimming activity; * cytotoxicity; EEq oestradiol equivalents; ICIeq ICI equivalents; ER oestrogen receptor

Estrogenic activity was detected in 11 of the 22 swimming pool samples, ranging from 0.02 ng EEq/ℓ (BP pool A backwash) to 1.01 ng EEq/ℓ (BP pool B backwash). Eight samples showed no (anti-)oestrogenic activity (<LOQ). Some of these samples were also cytotoxic, like BP pool A pm, BP pool B am, BP pool B pm and BP pool C backwash, and it was therefore impossible to tell if these samples contained any ER ligands. Evidence of inhibition was found in nine samples ranging from 0.03 µg/ℓ ICIeq (BP pool C am) to 1.07 µg/ℓ ICIeq (PD pool am). All the samples that showed inhibiting action had a high percentage cell viability (Table 5-2). This indicates that there was no cytotoxicity present and the inhibition of the cell receptors is due to compounds present in the water samples, and not cytotoxicity of the cells. Both controls (vehicle and E_2) were also compared with 0.1 mM ICI, an ER antagonist, to assess ER-specific responsiveness and background. High background is always a potential problem when working with oestrogen-responsive cells; therefore, a control that could be used to assess background was included on each plate.

4.3.3 (Anti-)androgen activity

The %Tmax values of all the samples except for one sample (BP pool B pm) were below 20% and the REP 20 values could not be calculated as the samples responses were below the limit of quantification (Table 5-3). Androgen receptor inhibition was quantified as fold change by dividing the response of the samples by the untreated cells (solvent control). A fold-change greater than 1 indicated activation and a fold change less than 1 indicated inhibition. There were two samples (BP pool B am and pm), from the same pool taken at the holiday resort that had fold changes greater than 1 but only the one sample collected at the end of the day (BP pool pm) had the most significant response (Table 5-3). This particular sample extract (BP pool B pm) was the only one out of all the samples that displayed a quantifiable agonistic activity with a %Tmax of 38% and a REP 20 value of 0.02 ng TEq/ℓ (Table 5-3). In order to determine which receptor was activated, a further GR activation test was done. Here, dexamethasone was used as a positive control and BP pool B had a %Dmax of 10.5% and the REP 20 value was not reported as the response of the sample was below the limit of quantification. With this in mind, BP pool B pm showed clear indication that the response from this particular sample was due to AR activation and not GR activation.

Because most of the fold changes were less than 1, it seemed to indicate inhibition of the AR. It is important, when interpreting the inhibition effects, that the viability results are considered simultaneously. Both samples from BP pool B were cytotoxic as their viability was less than 70%. Even so, both these sample extracts caused excitation of the androgen receptor with one sample being quantifiable, despite this decline in cell viability. If there was no cytotoxicity, the AR activation may have been higher.

Inhibition can occur if the cells are dead or dying and therefore no light is emitted by them, or, despite good viability, there were no binding to the receptor. The samples BP utility source, BP borehole and BP reservoir all showed statistically significant inhibition (FC < 1; Table 5-3). Of these three, BP utility source and BP reservoir were not cytotoxic, and therefore the observed inhibition is a valid observation. However, because BP borehole caused the cells to die (viability < 70%; Table 5-3), it is impossible to say if there was also an inhibitory effect caused by the extract. This cytotoxicity may indicate that the total response of the AR to the samples is not measured because some samples killed the cells even though AR responses are seen.

Inhibition effects caused by BP backwash A, BP pond day 1 and day 3, PD pool am and pm, PD backwash, as well as PT source water and PT pool am was confirmed as the samples were not cytotoxic (Table 5-3). No androgenic activity could be obtained when the cells were dead for instance BP backwash B and BP backwash C (Table 5-3). When the inhibition assays were run, the observation of the inhibition caused by the samples, could not be confirmed.

Table 5-3 Testosterone (TEq), dexamethasone (DEq) equivalents (AR and GR activation), the fold change (AR inhibition) and the percentage viability of the cells during the exposure

Sample ID	TEq (ng/ℓ)	DEq (µg/ℓ)	Fold change (AR inhibition)	Viability%
Trigger value (Escher et al., 2018)	14	21		
BP utility source	<LOQ	<LOQ	**0.48**	85 ± 0.11
BP borehole	<LOQ	<LOQ	**0.81**	*62 ± 0.1
BP reservoir	<LOQ	<LOQ	**0.79**	94 ± 0.25
BP pool A am	<LOQ	<LOQ	0.49	102 ± 0.19
BP pool A pm	<LOQ	<LOQ	0.95	*47 ± 0.19
BP pool A backwash	<LOQ	<LOQ	**0.14**	80 ± 0.17
BP pool B am	<LOQ	<LOQ	1.87	*43 ± 0.17
BP pool B pm REP 20	0.02 ± 0.002	<LOQ	4.09	*62 ± 0.15
BP pool B pm REP 50	0.004 ± 0.001	<LOQ	-	*62 ± 0.15
BP pool B backwash	<LOQ	<LOQ	**0.01**	*0 ± 0
BP pool C am	<LOQ	<LOQ	0.79	97 ± 0.04
BP pool C pm	<LOQ	<LOQ	0.92	102 ± 0.04
BP pool C backwash	<LOQ	<LOQ	**0.01**	*0 ± 0
BP pond day 1	<LOQ	<LOQ	**0.57**	95 ± 0.06
BP pond day 3	<LOQ	<LOQ	**0.44**	128 ± 0.04
PD source water	<LOQ	<LOQ	0.79	75 ± 0.09
PD pool am	<LOQ	<LOQ	**0.82**	232 ± 0.56
PD pool pm	<LOQ	<LOQ	**0.68**	253 ± 0.11
PD backwash	<LOQ	<LOQ	**0.72**	212 ± 0.18
PT source water	<LOQ	<LOQ	**0.82**	98 ± 0.29
PT pool am	<LOQ	<LOQ	**0.81**	76 ± 0.13
PT pool pm	<LOQ	<LOQ	0.96	84 ± 0.06
PT backwash	<LOQ	<LOQ	0.78	88 ± 0.19

- no value determined; <LOQ – below limit of quantification; REP – relative effect potencies; am – before swimming activity; pm – after swimming activity; *cytotoxicity; bold indicates statistical significant differences when compared to the control; Teq – testosterone equivalents; Deq – dexamethasone equivalents; AR – androgen receptor; GR – glucocorticoid receptor

5.4 SUMMARY

Guideline levels based on concentrations only, do not take the effects of the mixture of compounds into consideration. Multiple compounds mediating a similar mode of action might occur together in the environment, but instrumental analysis of environmental matrices cannot quantify them all, because the chemist is unaware of their presence. Because of this limitation, bio-assays were developed that would detect and quantify a similar mode of action caused by the mixture of compounds. The reporter gene assays employed in the current study may serve as an example of this. The result is that we are able to evaluate a health risk without identifying (all) the compounds responsible for it. Effect-based trigger (EBT) values were developed for endocrine disruption effects through exposure to the respective endocrine associated reference compounds, to which samples can be compared to in a suite of *in vitro* assays (Kase et al., 2018; Escher et al., 2018).

These trigger values were derived based on acceptable daily intake (ADI) values for some well-known endocrine reference compounds, combined with pharmacokinetic factors representing the adsorption, distribution, metabolism, excretion as well as exposure assumptions. There are some considerations when interpreting the exceedance of the EBT values:

- children are more at risk because of their smaller body mass;
- volumes of water consumed may influence exposure;
- susceptibility to EDCs are different at specific stages of development (Vandenberg et al., 2012).

5.4.1 AhR ACTIVATION

One sample, BP utility source, elicited a quantifiable response of REP 20/50 0.34 and 0.86 µg/ℓ TCDD eq. This sample had excess nitrites, Zn, Cd Fe and Mn that exceeded the drinking water quality guideline. That there was any sample causing a response from the AhR was a surprise because the physico-chemical nature of the compounds that were extracted using the described extraction method are not typical for the ligands binding to the AhR. It was even more of a surprise since the response came from a sample taken from the pipe supplying water to the holiday resort from the water utilities company. This particular source of water is only used by the resort if their borehole is not sufficient enough. And this was not necessary in the recent past. It is therefore possible that we inadvertently sampled stale and stagnant water, even though we had the tap run for at least 15 minutes.

5.4.2 (Anti-)oestrogenic activity

Although there were a few samples that caused quantifiable activation of the ER, none of these responses exceeded the international drinking water bio-assay derived EBT value of 3.8 ng/ℓ EEq (Escher et al., 2018). Only one of the samples exceeded the South African guideline level of 0.7 ng/ℓ (Genthe et al., 2010) and that was a backwash sample (Table 5-2). Backwash samples would contain the highest concentration of toxicants. Fortunately, bathers are not exposed to backwash water per se. As far as we know, there is no ICIEq value yet to which our results can be compared.

5.4.3 (Anti-)androgenic activity

The response quantified from activation of the AR of one sample did not exceed the drinking water EBT value of 14 ng/ℓ TEqq (Escher et al., 2018).

5.4.4 Covered pools vs open pools

There does not seem to be any significant difference between covered (BP pool B vs BP pools A and C) and uncovered pools in any of the measured endpoints. This is however, an almost impossible assessment to make, because although the presence of UV radiation may influence the breakdown of the compounds, there are too many other variables that might have played a role in the levels and effects observed: temperature differences and number of people in the pools, are but two of these variables. Not only the number of people but also the nature of the people in the pools could have influenced the data: the type of PPCP in and on their bodies, how much time have they spent in the water and there would be a difference between the PPCPs from adults and that of children

6 CONCLUSIONS & RECOMMENDATIONS

6.1 CONCLUSIONS

It was confirmed that most of the public swimming pools that were investigated in this study contained the expected list of compounds: DBPs such as chloroform, bromoform, dibromochloroform and bromodichloroform as well as a list of elements. The DBPs originate from the chlorine and bromine disinfecting products added to a public swimming pool to prevent microbial infections. The disinfecting compounds interact with the natural organic matter in source water as well as the organic compounds coming off the swimmers' bodies such as sweat, urine, hair, skin particles, and the personal care products (Kristensen et al., 2010). The presence of all three of these classes of compounds have been confirmed and the concentrations of some have been determined. The fact that humans are exposed to the cocktail of compounds is alarming since there might synergistic effects on human health that might be far more detrimental than the predicted single compound effect.

The concentrations of THMs and a number of elements, such as Cr, Pb, Cu and Cd occurred at levels able to cause non-cancerous risk as well as cancer under certain assumed conditions: 6 swimming events per year, 2.5 h per day for South Africans living to 70 years or children of age 12 y. Backwash water was usually the most harmful and generally contained the compounds at the highest concentrations.

Nitrites were without exception too high, when compared with the drinking water quality values, most likely due to chloramination.

The screening for PPCPs confirmed their presence and a long list have been identified. Some of these were unique to the municipality pools and others to the resort pools.

There were definite endocrine disrupting effects, both activating and inhibiting the oestrogen and androgen receptors. These effects were evident in samples collected at the end of the day, i.e. after many people spent the day in the pool.

Another, and important conclusion to this project, is the observation that there should be guidelines made available specifically for public swimming pool water focussed on the allowable levels of chemical determinands found in public swimming pool water. The values stipulated in SANS 241 for safe drinking water, are not addressing all of the compounds found in public swimming pools. Examples of these are the myriad pharmaceuticals and personal care product constituents that might find their way into the pool water. Another issue that needs addressed is the combinatorial effect of all these compounds. The bathers are exposed to the mixture of compounds simultaneously present in the pool. These compounds might cause synergistic effects, enhancing effects seen in single exposures or because of competing for the same cellular receptors, might inhibit each other's effect, lessening the expected harmful expect. The guidelines for the various uses of water in South Africa, do not address mixture effects at all. And worldwide this lack in addressing mixture effects are recognised as a serious gap. The main problem is that instrumental analytical quantification cannot predict toxic effects, even if it were possible to quantify every toxicant known to man. In a country like South Africa where there is limited capacity to quantify concentrations on a regular basis for known contaminants, it is not a feasible solution to have only maximum concentration levels to protect human health listed in a regulatory document.

5.2 RECOMMENDATIONS

Recommendations regarding regulation of public swimming pool:

- Results from the current study highlight the need for regular monitoring of public swimming pool water and monitoring for more than the limited list of compounds on the SANS 241 document.
- Instrumental analysis for every chemical known (and not yet known) to man is not feasible. A solution that is currently being investigated for application in drinking water quality, is to apply *in vitro* biological assays, to screen for selected biological endpoints, the so-called effects-based approach, to determine safety of the water for human consumption. This approach is a solution that may be extended to include public swimming pool water (recreational water) as well as other water uses, i.e. ecological, agricultural, and industry. A variety of biological endpoints can be tested for using biological assays, including cytotoxicity, genotoxicity, mutagenic, oxidative stress, and endocrine disruptive.
- Bathers should be informed about the role pharmaceuticals and personal care products may play in contaminating swimming pool water with endocrine disruptors and that making use of the showers (often available at public swimming pools) before entering the pool might help to curb overloading the pool with contaminants.
- Bathers should also be made aware of the detrimental health effects they may cause other bathers when relieving themselves in the water, not only because of hygienic reasons, but also because partially metabolised pharmaceuticals entering the swimming pool in this manner. These pharmaceuticals may contribute to load of endocrine disruptive compounds. Public should be made aware of the possible risks they expose themselves to when using public swimming pools, especially the more sensitive life stages.
- Although UV and ozone may contribute to the creation of new compounds from existing ones, consider using UV and/ozone as an alternative to chemical disinfecting products.
- If chlorine and bromine based disinfectants are used, ensure that they are applied at levels indicated by the WHO (2006).
- Care should be taken NOT to return backwashed water into the pool since it contains the highest levels of contaminants.
- Backwashed water should be treated before being disposed of into the environment.

Recommendations to improve the research side of the project

- A larger sample size of swimming pools should be analysed on a regular basis over a longer time period to confirm the endocrine disruptive effects observed.
- Where possible the chemicals other than DBPs and source elements should be identified should be quantified as well if analytical standards are available and affordable.
- The effect of temperature, and whether a pool is enclosed or not, may be included in the research, provided that all other variables are controlled for. These variables include the size of the pool and the source water.
- Although the main biological endpoint of this project was endocrine disruption, other biological endpoints might be screened for using the *in vitro* bioassays, such as oxidative stress and genotoxicity.
- The pond that received the backwash water from the three swimming pools on the resort should be monitored closely. It contains aquatic life, and some of the fish should be sampled and in-depth investigation into their health will reveal if the toxicants in the backwash water are at levels that are detrimental to wildlife.

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ADDENDUM: AN ADVISORY FOR MANAGING THE CHEMICAL WATER QUALITY WATER FOR PUBLIC SWIMMING POOLS

BACKGROUND

Currently, there is no separate document in South Africa that regulates or guides the chemical water quality parameters for public swimming pools. There is brief mention of it in the Norms and Standards for Environmental Health published in the South African Government Gazette Notice 943 (South Africa, 2013) and the South African National Standard for drinking water quality (SANS 241-1:2015a): the water should meet drinking water quality for all determinands and physical water parameters as well as the microbial specifications.

Meeting these criteria is not necessarily addressing all the issues of human health when bathers make use of public swimming pools:

1 Often, the emphasis of a holiday resort or local municipality is to prevent the spread of microbes that can cause all sorts of illnesses and in order to do so, disinfecting products are added to the water, more than what is required. Even under circumstances where the dosing of the disinfectants is precisely regulated, disinfectant by-products form (DBPs). Overdosing of the disinfectants would exacerbate the production of DBPs. These by-products are known for their genotoxicity and mutagenicity (Teo et al., 2015). More than 700 DBPs had been described but the guideline levels of only four occur in the SANS 241 guideline. They are the trihalomethanes (THMs): chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$).

   In South Africa there are not enough analytical capacity to quantify these four most common THMs, let alone quantifying 700 more that might be present.

2 Another shortcoming of applying SANS 241 on swimming pool water, is that the limits for THMs for instance are geared towards the ingestion pathway of exposure, and to a lesser extent bathing and showering. At least, when doing the daily ablutions, the body is submerged by water for a relatively shorter time—in comparison to swimming. Literature has shown that exposure through the skin creates a much greater risk to human health than the ingestion pathway (Abbasnia et al., 2019). The results of this study support these findings. The longer the bather stays in the water the greater the absorption through the skin will be, and the greater the risk, both cancerous and non-cancerous.

3 A factor that is unique to public swimming pool water, that is not shared with drinking water, and therefore not considered in the drinking water standards, is the presence of pharmaceuticals and personal care products (PPCPs) that wash off the bathers' bodies and are excreted by the same bodies. Non-metabolised pharmaceuticals and partially metabolised pharmaceuticals are excreted through sweat and urine and enters the swimming pool water in small quantities. Personal care products such as sunscreen lotions and skin care lotions contain parabens. It has been shown that PPCPs have, among others, endocrine disruptive effects. This means that they have the ability to interfere with the normal functioning of the hormone control functions in the body. This may disrupt normal development of foetuses, cause cancer and immune responses to name a few consequences. Exposure to endocrine disruptive compounds (EDCs) occur throughout the entire day to a variety of other chemicals, from pesticides to vapours from carpet glue. In a swimming pool the nature of the EDCs is likely more bioactive because they were created to be so, especially in the case of pharmaceuticals.

4 Guideline levels independent of their specific target audience, give maximum levels for individual compounds, and these should not be exceeded, but no guideline level take into consideration that the

body is exposed, through various pathways, to the mixture of the compounds, and therefore would be suffering from combinatorial effects, not regulated by allowable maximum levels for single compounds.

RECOMMENDATIONS AND CONSIDERATIONS

The statements made here are by no means to be exhaustive, but will shed some light on which issues should be addressed in guidelines developed to regulate public swimming pool water regarding the chemical compounds in the water. To this end applicable operational guidelines for public swimming pools found in literature is added here because they are sound advice to maintain good quality water.

1 Source water used to keep the water levels in the pool filled, should be of high quality. The source water may already contain disinfectants (e.g. free residual chlorine) when the water comes from a water utility or municipality, as well as precursors that react with the disinfectants. This would contribute to the addition of the disinfectants added by the maintenance personnel to ensure that no microbial infections are transmitted to the bathers. If the source water could be replaced with ground water, then the DBPs would be lesser to start with. The quality of the source water (ground water) should be known and therefore an annual assessment for natural occurring elements such as arsenic, lead and mercury would be advisable. These compounds would contribute to the combinatorial effects bathers would be exposed to in addition to the cocktail of compounds introduced into the pool after filling it. In some instances, the natural occurring heavy metals could be contributing even more to human health risk than the DBPs or the PPCPs.

2 Personnel responsible for pool water treatment should be made aware of the possible harmful effects of DBPs and that they should take care not to over-dose the treatment. This often happens when the pools are used intensively by many people and the water is treated more than regularly.

The following is useful information regarding chlorine and bromine disinfectants found in literature: The version of chlorine that is responsible for its disinfecting properties is referred to as free residual chlorine (FRC) or free available chlorine or free chlorine. This refers to the concentration of hypochlorous acid and the hypochlorite ion in equilibrium concentration in the pool water. It is good practice to attain breakpoint before the first chlorine measurements are taken each day. Breakpoint chlorination means that all of the chlorine is available as free chlorine and is achieved by adding sufficient chlorine to burn out all the combined chlorine, so that the free chlorine equals total chlorine. The role of pH is important because an alkaline or acidic pH decreases the disinfection power of free chlorine (Queensland Health, 2004).

Bromine is a weaker disinfectant than chlorine, and needs to be between 50 and 60% higher than chlorine, but should never be used together with ozone because carcinogens may be formed (Queensland Health, 2004).

3 It is advisable to replace the water of a pool daily with untreated water, especially in seasons when the pool is frequented intensively, as a precautionary measure. This would prevent a gradual accumulation of harmful compounds, keeping them at a low level. All public pools should be equipped with an effective water circulations system, a filtration system, and have a continuous disinfectant dosing control system. The volume turnover period should be between 5 to 6 hours. (Turnover period refers to the time taken for the total pool water volume to pass through the filters and treatment plant and return to the pool (Queensland Health, 2004).

4 It would be useful to include some form of biological affect-based screening tests to investigate the combinatorial effect of all of the compounds simultaneously on an organism. Although these biological assays are not commonly used yet, they have the benefit to indicate biological effect without even

knowing the identity of the compounds causing it. This is one of the advantages that biological assays have, above instrumental analysis: it gives a biological response, indicating possible toxicity, of the mixture of compounds. The instrumental analysis can only provide the concentration of the compounds targeted for analysis. They could all be within the limits prescribed by a guideline, but their sum total might be harmful. Or, unidentified toxicants might be present and it would be unknown to the maintenance personnel. Biological response would include those of the unknowns too. Guideline levels had been in the process of validation for some of these *in vitro* assays. Ideally, a response from the bio-assay should be followed with an instrumental analytical investigation to determine which compounds were responsible for the biological response. This would direct mitigation steps. However, risk to bathers will be known even if the identity of the compounds is unknown.

Examples of these suggested biological assays include the tissue culture based assays where mammalian cells in Petri dishes are used and can detect genotoxicity, cytotoxicity, endocrine disruption, oxidative stress and even possible neurotoxicity.

5 The public in general, and bathers in particular, should be made aware of the risk they put themselves in when using public swimming pools. The risk referred to here is specifically related to chemical exposure to the compounds in the source water, those introduced as disinfectants, the DBPs as well as the PPCPs that are introduced by the bathers themselves.

6 Where possible, bathers should be motivated to make use of the showers often available at public swimming pools before every entry into the water. This would mitigate the constant addition of freshly applied personal care products that could possibly have endocrine disruptive and other biological effects. Showering before swimming is common in some countries and is advised by the WHO (2006). The advice in this document is to frequently shower during the visit to the swimming pool. Bathers should also be dissuaded to urinate in the water.

7 All personnel involved with maintenance of pool water quality should be made aware of the harm backwash water could do to the environment if released untreated. This water contains a higher concentration of the toxicants found in the pool and all attempts should be made to remove these from the water before releasing it into the environment.

For a complete overview of managing public swimming pools, where guidance to even the bather load and the nature and type of filters that should be installed is made, the reader is referred to chapter five of the “Guidelines for safe recreational water environments Vol 2 Swimming pools and similar environments” of the WHO that was published in 2004.

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