

# Drowning in Disinfection Byproducts? Assessing Swimming Pool Water

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Disinfection is mandatory for swimming pools: public pools are usually disinfected by gaseous chlorine or sodium hypochlorite and cartridge filters; home pools typically use stabilized chlorine. These methods produce a variety of disinfection byproducts (DBPs), such as trihalomethanes (THMs), which are regulated carcinogenic DBPs in drinking water that have been detected in the blood and breath of swimmers and of nonswimmers at indoor pools. Also produced are halogenated acetic acids (HAAs) and halo ketones, which irritate the eyes, skin, and mucous membranes; trichloramine, which is linked with swimming-pool-associated asthma; and halogenated derivatives of UV sun screens, some of which show endocrine effects. Precursors of DBPs include human body substances, chemicals used in cosmetics and sun screens, and natural organic matter. Analytical research has focused also on the identification of an additional portion of unknown DBPs using gas chromatography (GC)/mass spectrometry (MS) and liquid chromatography (LC)/MS/MS with derivatization. Children swimmers have an increased risk of developing asthma and infections of the respiratory tract and ear. A 1.6–2.0-fold increased risk for bladder cancer has been associated with swimming or showering/bathing with chlorinated water. Bladder cancer risk from THM exposure (all routes combined) was greatest among those with the *GSTT1-1* gene. This suggests a mechanism involving distribution of THMs to the bladder by dermal/inhalation exposure and activation there by *GSTT1-1* to mutagens. DBPs may be reduced by engineering and behavioral means, such as applying new oxidation and filtration methods, reducing bromide and iodide in the source water, increasing air circulation in indoor pools, and assuring the cleanliness of swimmers. The positive health effects gained by swimming can be increased by reducing the potential adverse health risks.

## Introduction

The development of treated water for swimming pools has made swimming a year-round activity, widely enjoyed for

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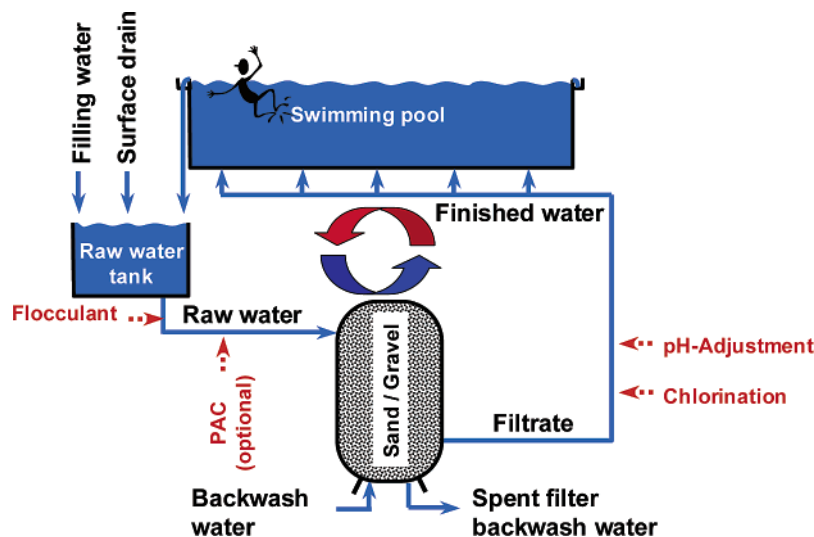
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leisure, as well as exercise. Swimming pools can be found in different kinds and sizes in public areas, hotels and spas, or at private homes. In the United States, more than 368 million people visit public swimming pools each year. In Germany ~250–300 million people visit pools each year, averaging 3 visits per capita. In the UK, one-third of the children and ~36% of adults (>15 years of age) visit swimming pools at least once a week; and 55% of children (5–9 years of age) use pools at least once a month. In the United States, there are ~250,000 public pools and ~10,000,000 residential pools. The highest numbers of existing in-ground and above-ground pools in Europe are found in France (773,000) and Germany (625,000), followed by the UK with 155,000, and Italy with 94,000 (1).

Swimming can provide health benefits and has some advantages over land-based activities for people of all ages and physical abilities. To conserve the positive aspects of aquatic activities, regulators and researchers have turned their attention to hygienic and chemical water quality. Thus, pool water and other recreational waters are increasingly regarded as a health priority around the world. The World Health Organization (2) has identified some of the potential hazards associated with recreational water use, which include infections caused by feces-associated microbes, such as viruses and bacteria, as well as protozoa, such as *Giardia* and *Cryptosporidium*, which are resistant to chlorine and other pool disinfectants. In the United States, the National Swimming Pool Foundation (NSPF) recently awarded the Centers for Disease Control and Prevention a grant to explore such health issues (3). “There is a compelling need to get some answers on the spread of recreational water illnesses,” says NSPF’s Chief Executive Officer, Thomas M. Lachocki (4). In the UK, technological developments, pool water quality, and governing standards were the topics of an International Conference held in 2002 in Cranfield (5). In Germany a recent collaborative research project focused on “Pool Water Chemistry and Health” with leading experts of university, industry, and the Federal Environmental Agency (Umweltbundesamt) (6).

Beyond the engineering aspects of the pool itself, sufficient disinfection with a minimum of disinfection byproducts (DBPs) is the other major issue in pool water treatment. The goal is to prevent illnesses associated with recreational water use (3) and to minimize any health impacts from excessive DBPs. Swimming pool water is a dynamic environment that changes with the climate, the number and behavior of people in the pool, activities of the swimmers, as well as environmental contaminants brought into the pool on the skin and



**FIGURE 1.** Scheme of conventional pool water treatment with flocculation – filtration – chlorination. PAC = powdered activated carbon (adapted from ref 15).

clothes (bather load). Pool water must achieve suitable disinfection, given this range of contamination, while also accommodating a wide range of people: young children, pregnant women, the elderly, people with compromised immune systems, Olympic athletes, etc. This is a challenging environment in which to achieve a suitable public health outcome.

### Treatment Characteristics of Swimming Pool Water

In this review, we draw a distinction between private (residential) and public (commercial) pools. Public pools have a more diverse group of users, a larger number of users, and a greater responsibility to meet standards for treatment techniques and water quality compared to private pools, which are under the exclusive responsibility of the private owner. Although the numbers of private pools and users of private pools might outnumber those of public pools, only public pools are monitored for infectious disease outbreaks or toxicological harm.

In the United States, the individual states regulate swimming pools, and responsibility is generally assigned to the department of health of each state (7). The codes in the U.S. are generally similar to those in Germany and typically describe requirements regarding construction, operation, and maintenance of the pool, as well as maintaining water quality. Requirements for pool water treatment were included in the German Federal Diseases Act (Bundesseuchengesetz) in 1980, and in 2000 these were incorporated in the succeeding Federal Infection Protection Law (8). Public pools in Germany rely primarily on the treatment scheme, operation, and surveillance described in the German Industrial Norm DIN 19643. Despite minor differences in threshold values, applied chemical concentrations, and the requirement for an initial flocculation step, the DIN 19643 can serve as a good example of pool water treatment that is similar to that in much of Western Europe, North America, and Australia. The different treatment options under the DIN are listed in Table 1.

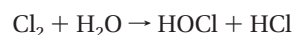
Flocculation and filtration are the first barriers in the DIN against microorganisms and chemical substances and may be accompanied by adsorption to activated carbon and/or oxidation by ozone to better control the levels of DBPs, especially THMs (Figure 1). Flocculation can considerably improve particle elimination and is, therefore, recommended for enhanced removal of *Cryptosporidium* oocysts and *Giardia* by conventional filter beds. However, this step has limited relevance to pool-water treatments outside of public

**TABLE 1.** Options for Pool Water Treatment According to DIN 19643.

DIN 19643	treatment combination
part 1	general requirements for construction, operation, and surveillance
part 2	adsorption – flocculation – filtration – chlorination
part 3	flocculation – filtration – ozonation – adsorption/ filtration – chlorination
part 4	flocculation – ozonation – mixed-bed filtration – chlorination
part 5	flocculation – filtration – adsorption on GAC – chlorination

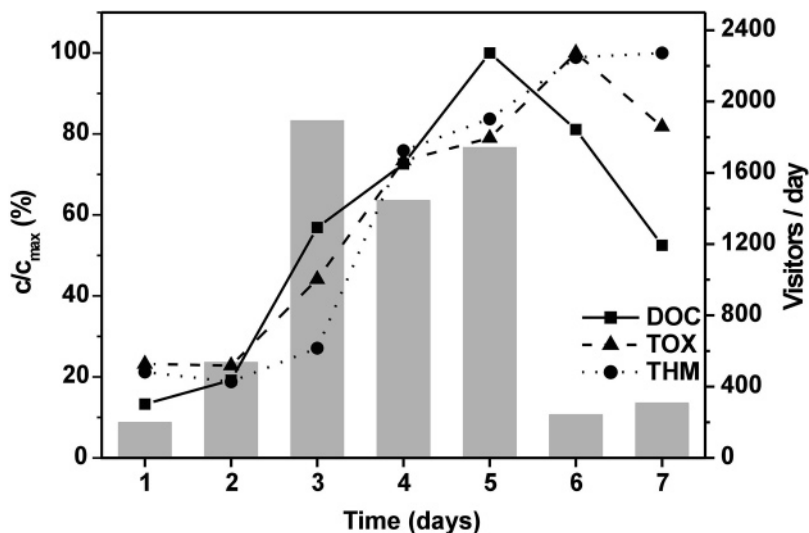
pools in Germany because flocculation is not generally used anywhere else in the world for treatment of swimming pool water.

Disinfection is the second barrier for microorganisms and is most important for poor quality water. According to DIN 19643, the disinfectant used must have the potency to remove the indicator microorganism, *Pseudomonas aeruginosa*, by 99.99% within 30 seconds (9). This is commonly achieved with chlorine-based chemicals. Gaseous chlorine or sodium hypochlorite is often used in large public swimming pools. Both chlorine and hypochlorite react in water to form hypochlorous acid.



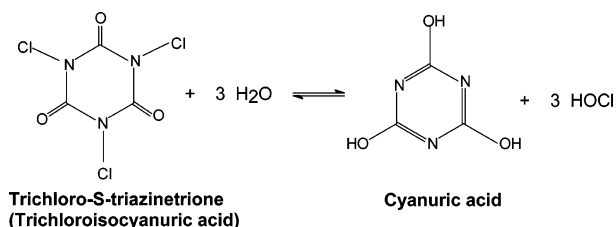
To achieve a sufficient disinfection capacity, in Germany the concentration of hypochlorous acid (so-called free chlorine) must be kept in the range of 0.3–0.6 mg/L in pool water and between 0.7 and 1.0 mg/L in spas (at a pH range between 6.5 and 7.6; DIN 19643). In the United States, the UK, and Australia, hypochlorous acid concentrations of 1–3 mg/L are recommended. Alternatives to chlorine-based disinfectants are not yet permitted in Germany. In the United States, alternative disinfectants must demonstrate a disinfection efficiency equivalent to that of hypochlorous acid (10).

As noted above, flocculation is generally not used other than in public pools in Germany. Instead, filtration is often done with cartridge filters, which provide good water quality, are easy to maintain, and are relatively cheap. Diatomaceous earth filters provide the best water quality, but they are the most expensive pool filters generally available. Stabilized



**FIGURE 2.** Profiles of DOC, TOX, and THMs in water from a public outdoor pool for a one-week period in the summer of 2001. The gray columns represent the visitors per day;  $c_{\max}(\text{DOC}) = 3.4 \text{ mg/L}$ ;  $c_{\max}(\text{TOX}) = 329 \text{ } \mu\text{g/L}$ ;  $c_{\max}(\text{total THMs}) = 125 \text{ } \mu\text{g/L}$  (adapted from (11)).

chlorine (typically trichloro-*S*-triazinetriene in stick or tablet form) is often used for disinfection of home swimming pools. One mole of trichloro-*S*-triazinetriene reacts in water to form one mole of cyanuric acid and 3 moles of hypochlorous acid (the active disinfectant). Alternative disinfectants include bromine, ozone, copper, and silver salts; however, these are not widely used and are not expected to replace chlorine-based chemicals in the next years.



Swimming pools generally involve constant recirculation of the water at elevated temperature and continuous loading of the water with organic carbon and nitrogen from swimmers, as well as the exposure of the pool water to sunlight. The bather load comprises a variety of substances such as body fluids, skin particles, hair, microorganisms, cosmetics, and other personal-care products that can get into the pool water. Consequently, substances that are not removed efficiently by the treatment process can accumulate in swimming pool water.

In general, the operation of the flocculation/filtration step for removing particles and microorganisms remains constant, independent of the number of swimmers, the degree of organic contamination, or other water-chemical parameters. However, this standardized treatment may not be adequate for pools with a highly dynamic bather load and the concomitant organic contamination found in open-air pools (see Table 2 in the Supporting Information).

### Bather Load and Treatment

Figure 2 shows an example of the accumulation of dissolved organic carbon (DOC) and the formation of total organic halide (TOX) and THMs in a public outdoor pool with flocculation and filtration treatment. Starting from a cold-weather period with low numbers of pool visitors, a hot-weather period with a large increase in visitors (gray columns) is mirrored by high values of DOC. At the same time, the increase of TOX and THMs occurs 24 and 48 h later,

respectively (11). These results can be interpreted in two ways. First, there is no efficient elimination of the organic bather load by the treatment process at high bather loads. Second, a swimming pool can be considered as a kind of "DBP reactor" where the continuous input of anthropogenic substances and the continuous dosing of reactive chlorine in each treatment cycle results in partial removal of DOC and accompanying formation of DBPs.

The reasons for the time-shift of TOX and THM formation are related more to the number of treatment cycles needed for efficient chlorination than to different formation kinetics. In general, organic matter in pool water is subjected to exhaustive chlorination, and after several treatment cycles, no further TOX formation can be measured in pool water (e.g., in the early morning) without freshly introduced contamination. In addition, part of the accumulated organic matter on a loaded sand filter may be another source for DBP formation (12).

Experiments with a pilot plant reported by Judd and Black (13) support the assumption that DOC removal is obtained by DOC oxidation by chlorine. They observed a steady state for the DOC concentration in a 2.2-m<sup>3</sup> capacity pilot plant with a continuous DOC load and chlorination. Mineralization and volatilization seem to be the only convincing explanations for DOC removal. This finding suggests that classical pool water treatment under constant operating conditions has to rely on a recovery period during which there is no input of bather load.

### New Treatment Technologies

New treatment technologies for swimming pool water should have features that permit them to address the constantly changing bather load and meet the following requirements: (1) removal of particles and microorganisms; (2) removal or minimization of organic matter and low-molecular-weight DBPs; and (3) efficient disinfection with minimal DBP formation.

Membrane filtration has emerged as a promising treatment method as an alternative to flocculation and traditional filtration with sand or diatomaceous earth because it can reduce DOC to a much greater extent and does not produce much waste compared to the suspension/filtration. Membranes designed with a low molecular weight cutoff (10 000–40 000 Da) can eliminate up to 60% of the DOC. Compared to sand filtration, ultrafiltration membranes also have a higher efficiency for removing particles and microorganisms. In a

recent study by Hagen (14), particle counts in the effluents of an ultrafiltration module and a multilayer filter were about 2/mL and 100/mL, respectively. Further, the application of a pilot-scale membrane filtration module in a public outdoor pool led to lower chlorine consumption compared to sand filtration.

However, efficient DOC and TOX removal requires filtration with a much lower molecular weight cutoff. TOX fractionation with membranes with a molecular weight cutoff of 1000 and 200 Da showed that only about 30% of TOX was found in the <200 Da fraction, whereas about 50% appeared in the 200–1000 Da fraction; a minor part (<10%) was present in the >1000 Da fraction (15 and Figure 1 in Supporting Information). Therefore, advanced oxidation processes like hydrogen peroxide/ozone in combination with nanofiltration were proposed for the removal of the low-molecular-weight fractions, which are also responsible for much of the irritation and health effects caused by DBPs in swimming pool water (16). Moreover, the nonvolatile, low-molecular-weight fraction was found to contain the majority of genotoxic compounds present in pool water (17). These and other new techniques require further study to determine if they are any better than conventional treatment methods.

### THMs and Other DBPs in Swimming Pool Water

Trihalomethanes (chlorinated and brominated THMs) are the best known and most intensively investigated class of DBPs since their discovery by Rook in 1974 (18, 19). Reports on THMs in swimming pool water first appeared in 1980. Weil et al. (20) determined THMs in pool water and in model experiments, and Beech et al. (21) identified nitrates, chlorates, and THMs in swimming pool water. Since then, THMs have been measured in swimming pool waters around the world, such as in Italy (22, 23), the United States (24), the UK (25), and Korea (26). Researchers also have studied the formation of THMs in pool model systems (27).

In 1993, THMs were also included as indicators for DBP formation in pool water in the German Industrial Norm DIN 19643. Similarly, THMs were also viewed as an indicator for other chlorination DBPs when they were regulated under the U.S. Safe Drinking Water Act in 1979 (28). However, controlling THM levels may not always control the levels of other DBPs. For example, low pH can reduce THM formation but can cause significant increases in the formation of haloacetic acids (HAAs) (29).

In addition to chlorinated DBPs, brominated and iodinated compounds are also of toxicological concern. Brominated HAAs and acetonitriles and only a few other targeted chlorinated DBPs, such as chloral hydrate, dichloroacetonitrile, and 1,1,1-trichloropropanone, have been identified previously in swimming pool water. No iodinated compounds, which can be important in spas with high iodide concentrations in water, have been identified in pool water so far.

Another emerging compound class of compounds of concern in pool water are the haloketones, which can irritate the eyes, skin, and mucous membranes (30), and also the chloramines, especially trichloramine, which is formed from urea and other nitrogen-containing compounds. Trichloramine is a highly volatile and irritating compound that has raised concern recently as an irritant to the respiratory tract as well as having a possible role in asthma (31, 32).

Unlike drinking water, where organics in the source water are the substrate for DBP formation, swimming pool water has additional sources of compounds that can serve as precursors to DBPs. These include chemicals used in sunscreens, human body substances (perspiration, urine, mucus, skin particles, hair, etc.), leaves from surrounding trees (in the case of outdoor pools), algae, and other biota, as well as any natural organic matter already present in the

source water. This bather load adds an additional complication to the disinfection and toxicological safety of swimming pool water.

### New DBPs Identified in Drinking Water and Swimming Pool Water

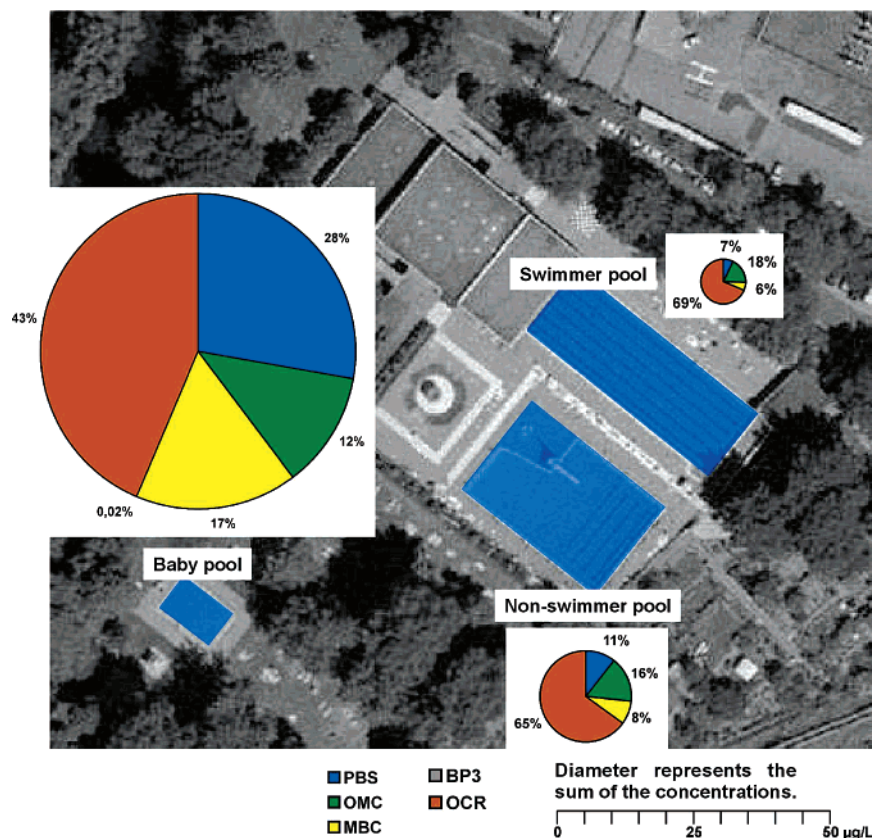
Previous studies of swimming pool water (and chlorinated drinking water) have focused largely on target compounds such as THMs. New DBP research on drinking water is taking a different, more comprehensive approach to include a much broader range of compounds, from low-molecular-weight and volatile ones to those that are more polar and have high molecular weights. This research is targeted to identify an additional portion of unknown DBPs as well as other compound classes of toxicological concern, such as carbonyls (ketones and aldehydes). The results of such studies may have relevance to swimming pool water; however, the toxicological significance of new compound classes, as well as the high-molecular-weight compounds, is unknown at the start of the analytical work. Nonetheless, identification is the first step to improving our knowledge of the chemical composition and toxicological properties of swimming pool water. In this regard, gas chromatography (GC)/mass spectrometry (MS), liquid chromatography (LC)/MS, and LC tandem mass spectrometry (MS/MS) have been useful in compound identification, sometimes in combination with derivatization reactions of different functional groups (33, 34).

Zwiener et al. (35–37) used LC/MS/MS with electrospray ionization (ESI) to analyze carbonyl compounds after derivatization with 2,4-dinitrophenylhydrazine (DNPH). DNPH derivatization is a well-known method for carbonyl measurements in air (38–40) and was used recently to determine DBPs of ozonated drinking water by Richardson et al. (41). The method was optimized for LC/MS/MS applications by Zwiener et al. (42). DNPH derivatization can be used as a type of labeling to measure carbonyl compounds selectively by tandem mass spectrometry (35). DNPH derivatives show common fragmentation patterns, including the occurrence of abundant ions at  $m/z$  152,  $m/z$  163, and  $m/z$  182, representing carbonyls in general, aldehydes, and dicarbonyls, respectively.

In treated drinking water samples, several homologues of the compound classes of oxoacids, hydroxydicarbonyls, and dicarbonyls could be tentatively identified (see Table 3 in the Supporting Information). Side reactions of DNPH, such as the substitution of chlorine atoms in halogenated carbonyls, however, limit the application. Derivatization reagents with hydroxylamine as the reactive functional group are potential alternatives for the identification of halogenated carbonyls (35). Thus, a promising target for further research is the application of derivatization labeling and selective LC/MS/MS measurements for different functional groups.

New work by Zwiener et al. (43) has also identified several active ingredients of sunscreens and their halogenated reaction products. LC/MS/MS with ESI was used to identify five compounds from UV screens in outdoor pools: 2-hydroxy-4-methoxybenzophenone (BP3), ethylhexyl methoxycinnamate (OMC), 2-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate (OCR), 2-phenyl-1H-benzimidazole-5-sulfonic acid (PBS), and 4-methylbenzylidene camphor (MBC). The compounds comprised derivatives of benzophenone, camphor, cinnamic acid, dibenzoylmethane, or *p*-aminobenzoic acid, the most common compound classes found in UV screens (Figure 3).

Compound patterns and concentrations of sunscreens in pool waters differed due to different sunscreen products and bather load. Consequently, the highest concentrations (40  $\mu\text{g/L}$ ) of the five sunscreen components were detected



**FIGURE 3.** Active ingredients of sunscreens found in outdoor pools. The diameters of the graphs represent the sum of the concentrations of PBS (2-phenyl-1H-benzimidazole-5-sulfonic acid), OMC (2-ethylhexyl-4-methoxycinnamate), MBC (4-methylbenzylidene camphor), BP3 (2-hydroxy-4-methoxybenzophenone), and OCR (2'-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate).

in baby pools with high bather load. Ten times lower concentrations were measured in adult pools with comparable lower bather loads. Some of the sunscreen compounds like BP3 can be chlorinated efficiently and, therefore, can contribute considerably to TOX and THM formation (see Figure 2 in Supporting Information). Interestingly, some of the sunscreens (as well as their chlorinated byproducts) show endocrine effects (44).

Richardson et al. (45 and Supporting Information) used GC/MS to comprehensively identify DBPs in chlorinated swimming pool water in two private outdoor pools in the United States. Several DBPs were identified in these pools, including haloacetic acids, other haloacids (3-carbon acids, 5-carbon oxoacids, and 4-carbon diacids), haloketones, a halosulfone, and nonhalogenated byproducts, including cyanuric acid, which was the major byproduct formed due to its release from the stabilized chlorine (Table 2). THMs were not detected in these outdoor pools, likely due to volatilization of DBPs during the hot summer month sampled (August). DBPs confirmed by the match of mass spectra and retention times to chemical standards are noted in italics. Although the others have been characterized by low- and high-resolution MS, and some have excellent library matches, the lack of matching standards requires that their identification be considered tentative. To our knowledge, this represents the first identification for most of these DBPs in swimming pool water.

An example of a DBP not present in the mass spectral library databases was the compound identified tentatively as 5,5,5-trichloro-4-oxopentanoic acid (identified in its methyl ester form; Figure 3 in Supporting Information). This halo-oxo-acid and all of the other DBPs identified have been found previously in chlorinated drinking water (46). Only one DBP (dibromoacetic acid) contained bromine in its structure; the

**TABLE 2.** DBPs Identified in Outdoor Chlorinated Swimming Pools<sup>a</sup>

haloacetic acids
<i>dichloroacetic acid*</i>
<i>dibromoacetic acid*</i>
<i>trichloroacetic acid*</i>
other haloacids
2-chloro-2-methylpropanoic acid*
<i>2,2-dichloropropanoic acid*</i>
5,5-dichloro-4-oxo-pentanoic acid
5,5,5-trichloro-4-oxo-pentanoic acid*
isomer of 5,5,5-trichloro-4-oxo-pentanoic acid*
2-chlorobutanedioic acid*
2,3-dichlorobutanedioic acid*
2,2-dichlorobutanedioic acid*
haloketones
1,3-dichloropropanone*
<i>1,1,1-trichloropropanone*</i>
1,1,3-trichloropropanone*
<i>1,1,3,3-tetrachloropropanone*</i>
1,1,1,3-tetrachloropropanone
<i>1,1,1,3,3-pentachloropropanone</i>
other halogenated compounds
dichloromethyl methyl sulfone
nonhalogenated compounds
cyanuric acid

<sup>a</sup> DBPs found in both swimming pools are marked with an asterisk; DBPs not marked with an asterisk were found in only one swimming pool, which used groundwater as source water. DBP identifications confirmed by analysis of an authentic standard are noted in italics; all other identifications should be considered tentative. Total organic halide (TOX) concentrations were 673 and 272 µg/L for the two pools, respectively.

other halogenated DBPs contained chlorine only. The predominance of chlorine-containing byproducts was not unexpected because the source waters treated in these

swimming pools were low in natural bromide, which can react with hypochlorous acid to form hypobromous acid, the active brominating species that gives rise to bromine-containing DBPs.

### Exposure Routes

Human exposure studies have shown that THMs can be found in the blood, plasma, and exhaled breath of swimmers and even of nonswimmers within an indoor pool setting (22, 23, 47–50). Inhalation and dermal exposures are likely to be important routes of human exposure to volatile DBPs in swimming pools, with ingestion from accidental swallowing of water being a minor route (51, 52). Swimming in chlorinated pools and the use pattern of drinking water are the main determinants of blood levels of THMs. Whitaker et al. (52) found that mothers who swam regularly received greater doses of chloroform than nonswimmers, revealing that THM blood levels were determined largely by pool attendance, which was influenced by frequency, activity, and THM levels in the pool water.

Because THMs and  $\text{NCl}_3$  readily volatilize from water, inhalation is an important route of exposure for these classes of DBPs, especially for swimmers (and lifeguards and other nonswimmers present) in indoor pools and to a lesser extent for those in outdoor pools (50, 53). In addition, the THMs and the more nonpolar haloketones can permeate the skin (54), making dermal exposures important. In contrast, the more polar HAAs do not readily permeate the skin (54). Because the HAAs are also not volatile, the only route of exposure for this class of DBP via swimming pool water is by accidental ingestion, resulting in a much lower uptake of HAAs than THMs.

### Epidemiological Studies

The strongest epidemiological evidence for adverse health effects from swimming in chlorinated pool water has come from studies on respiratory function and asthma. Swimming in chlorinated pool water has been associated with an increase in lung epithelium permeability (55), risk of developing asthma (56), and respiratory complaints (57) among children. Lagerkvist et al. (58) found that swimming also had adverse effects on Clara cell function in children. Nystad et al. (59) found an association between baby swimming and recurrent respiratory tract and ear infections. Thickett et al. (53) found that nitrogen trichloride (trichloramine) at 100–570  $\text{mg}/\text{m}^3$  in the air was a possible cause of occupational asthma among two lifeguards and a swimming teacher. Nitrogen trichloride exposure from swimming has also been linked to various adverse biomarker changes in the lung, in addition to asthma (32, 60). Nitrogen trichloride has also been implicated as a cause of eye and upper respiratory tract irritation reported by life guards and swimmers (61). Chlorinated whirlpool baths have been shown to increase airway reactivity in patients with mild asthma (62).

In contrast to respiratory effects, very few other health endpoints have been investigated for swimming pool water. However, a new study by Villanueva et al. (63) shows a significant increased risk of bladder cancer for swimmers vs nonswimmers. Bladder cancer has also been the primary adverse health endpoint associated with drinking water DBPs (64). In swimming pool studies that investigated reproductive endpoints, however, no adverse effects were observed (65, 66). In contrast, several studies of drinking water have found adverse reproductive effects, including low birth weight, preterm delivery, spontaneous abortions, stillbirth, and birth defects (67–72). Grummt (73) used cytogenetic endpoints as biomarkers and found no genotoxic effects in swimmers.

In a case-control bladder cancer study of the effects of route of exposure to THMs from chlorinated drinking water,

Villanueva et al. (63) found an overall 2-fold risk for bladder cancer among men without considering whether the men drank chlorinated or bottled water. Genotyping showed that most of the risk was among people who had the *GSTT1-1* gene (74). As described below, these results are consistent with laboratory studies indicating that dermal/inhalation exposure would permit the activation of selected THMs by *GSTT1-1* in target organs, such as the bladder, whereas oral exposure (i.e., drinking the water) would result in the inactivation of these THMs by enzymes in the liver. Thus, the emerging epidemiology on drinking water and route of exposure and genotyping provide some basis for additional studies of the potential carcinogenic effects of swimming pool water.

### Toxicity Studies of DBPs

The toxicity of the THMs, HAAs, and other DBPs in drinking water, such as the mutagen MX, have been reviewed extensively (75, 76) and are not covered here. However, we note that many of the DBPs in drinking water, including some of the THMs that have been identified in swimming pool water and in swimmers (see above), are mutagenic and carcinogenic. As noted earlier, exposure to DBPs via swimming pool water occurs almost exclusively by inhalation and dermal routes, and as the studies of Villanueva et al. (63) and Cantor et al. (74) suggest, these routes of exposure may result in a different risk for health effects than might result from oral exposure.

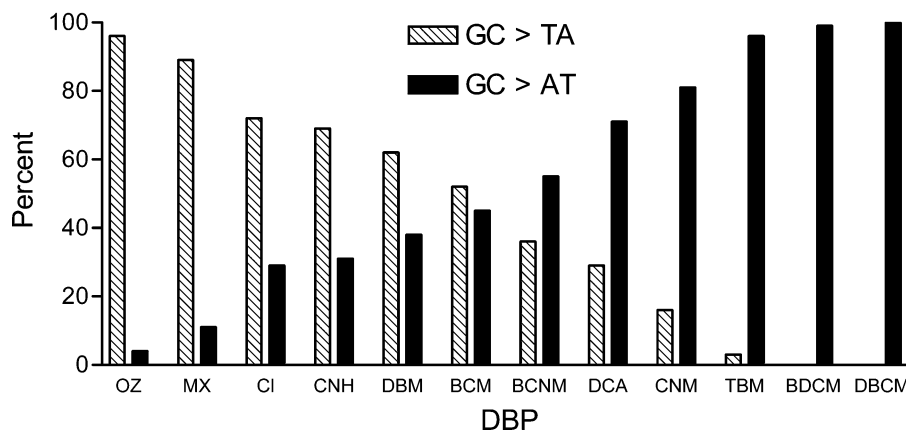
Studies nearly a decade ago showed that the THMs other than chloroform are activated to mutagens by the enzyme glutathione-S-transferase-theta (*GSTT1-1*) in a transgenic strain of *Salmonella* bacteria containing the rat *GSTT1-1* gene (77, 78). A recent study in rats (79) found that the levels of *GSTT1-1* enzyme available to activate one of these THMs, bromodichloromethane (BDCM), relative to the levels of the cytochrome enzyme CYP2E1 to inactivate BDCM were greater in the kidney and large intestine than in the liver. Studies in humans have found higher levels of THMs in the blood when people are exposed to chlorinated water via showering (dermal/inhalation) than when exposed orally (80).

These studies have led to the proposal (79) that oral exposure to the THMs result in the inactivation and elimination of the THMs by first-pass metabolism in the liver before the THMs can reach the systemic circulation. In contrast, dermal or inhalation exposure would result in the THMs entering the blood stream directly (bypassing the liver), and being distributed to various organs throughout the body. In those organs in which cancer has been observed, such as the bladder, the THMs other than chloroform might then be activated to mutagens by the *GSTT1-1* enzyme, initiating the production of cancer.

Such a mechanism provides support for the recent observations of Villanueva et al. (63) and Cantor et al. (74) showing that people who showered or bathed with chlorinated water had a significant risk for bladder cancer. Those who had the *GSTT1-1* gene were at greatest risk based on total THM exposure (all routes combined). Swimming was also associated with an increased risk of bladder cancer (63). If borne out with additional studies both in humans and animal models, these results could have important implications for the long-term risks of swimmers for bladder cancer. Populations of swimmers might also display variable risk because the *GSTT1-1* gene is present in ~80% of Caucasians but in only ~20% of Asians (81).

New studies and survey data on alternative methods to disinfect drinking water indicate that disinfection of water containing high levels of bromide results in high levels of bromine-containing DBPs, which are generally more cytotoxic and mutagenic than chlorine-containing DBPs (82–87). Thus, it would be interesting to know if disinfection of

## Summary of Mutation Spectra of Disinfection By-products in TA100



**FIGURE 4.** Two classes of mutations are detected by strain TA100 of *Salmonella*, and the various DBPs and organic extracts of drinking water prepared by different treatment methods produce the full range of these mutations—all the way from agents inducing primarily guanine–cytosine (GC) to thymine–adenine (TA) base substitutions to those producing exclusively GC to AT base substitutions. The agents from left to right are OZ (organic extract of ozonated water), MX (the chlorinated furanone that is a primarily mutagen in drinking water), CI (organic extract of chlorinated water), CNH (organic extract of chloraminated water), DBM (dibromomethane), BCM (bromochloromethane), BCNM (bromochloronitromethane), DCA (dichloroacetic acid), and CNM (chloronitromethane). The remaining three DBPs were tested in a homologue of strain TA100 that contains the rat *GSTT1-1* gene; these are TBM (tribromomethane), BDCM (bromodichloromethane), and DBCM (dibromochloromethane). Adapted from refs 77, 83, 89, 90.

high-bromide water by methods other than chlorination would produce water that was more mutagenic than that produced using low-bromide water—and what the implications of this might be for alternative methods of treating swimming pool water.

A newly discovered class of DBPs in drinking water, the halonitromethanes, whose formation is increased by pre-ozonation, appears to be more cytotoxic and genotoxic than their halomethane homologues in *Salmonella* bacteria (83) as well as in mammalian cells in culture (82). Some of these halonitromethanes are also present in drinking water at levels similar to those of some halomethanes (87, 88); however, whether the halonitromethanes are present in swimming pool water is currently unknown.

Figure 4 compares the mutation spectra (i.e., the types of mutations in DNA) induced by some of the halonitromethanes in the *Salmonella* (Ames) bacterial mutagenicity assay to the mutations produced by other DBPs in this assay. The results show that DBPs produce a range of DNA base-pair substitutions, with some, such as the mutagen MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) and organic extracts of disinfected water, producing primarily guanine–cytosine (GC) to thymine–adenine (TA) base-pair substitutions in DNA. Some of the brominated halomethanes and halonitromethanes produced ~50% GC to TA and ~50% GC to AT base-pair substitutions in DNA, whereas the brominated halomethanes activated by the enzyme GSTT1-1 produced exclusively GC to AT base-pair substitutions. Thus, even the limited number of drinking water extracts and DBPs shown in Figure 4 induce a wide range of different types of mutations that likely result from a variety of mutational mechanisms. Thus, one cannot assume a single mechanism or genetic effect for most DBPs or even for all of those within the same chemical class. For example, all of the trihalomethanes except chloroform are activated to mutagens by the enzyme GSTT1-1 (77, 78).

Finally, the emerging epidemiological and experimental evidence for adverse reproductive effects of chlorinated drinking water and selected DBPs (71) may warrant the initiation of studies to determine whether DBPs induce germ-cell mutations or act as endocrine disruptors. The examples above illustrate how mechanistic toxicity research can provide

guidance for understanding the potential health effects associated with exposure to DBPs. Although most of these toxicological studies have been performed with regard to drinking water, the results, as noted for dermal/inhalation exposure to THMs, may have relevance to exposure to DBPs via swimming pool water.

### Measures to Control DBPs in Swimming Pool Water

Measures to control the formation of DBPs in pool water should include reducing the input of DBP precursors (natural organic matter and bather load inputs) and improving treatment technology. The bather load can be reduced considerably by the behavior of swimmers before and during swimming. For example, showering and using toilet facilities, washing off sunscreen lotions, and applying water-tight diapers can reduce the bather load and help to reduce the potential for DBP formation.

From a technological point of view, the treatment efficiency to reduce DBP precursors and already-formed DBPs can be improved by more efficient filtration and oxidation. This includes membrane filtration and advanced oxidation. Removal of the low-molecular-weight fraction would be advised because it contains a large part of the toxicity. Toxicity data also suggest keeping bromide and iodide concentrations in pool water low and not using bromine because brominated and iodinated DBPs have been found to be even more toxic than chlorinated DBPs (82–84). It may also be beneficial to increase air circulation in indoor pool settings to reduce the levels of volatile DBPs.

In the end, it will be important to maintain microbial disinfection while minimizing potentially harmful DBPs. The goal would be to maintain the positive health effects of swimming through exercise while reducing other potential adverse health risks.

### Acknowledgments

The German Ministry of Research and Education (BMBF) is acknowledged for financial support of the Joint Project “Pool Water Chemistry and Health”. We thank E. Roskamp and D. Eichelsdörfer for their referee work during the project and all research partners for the fruitful cooperation. We thank

Richard Miltner of EPA's National Risk Management Research Laboratory for some of the TOX analyses, and we thank Jeffrey Ross and Russell Owen for their helpful comments on this manuscript. This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA.

### Note Added after ASAP Publication

No abstract was present in the version of this Critical Review published on the web November 17, 2006. An abstract was added to the version published December 14, 2006. Reference 74 was revised and the manuscript received dates were adjusted in the version published December 14, 2006.

### Supporting Information Available

DBP identification, determination of UV screens, table of LC and MS operating parameters, treatment characteristics of swimming pool water, table of dynamics of visitor numbers and organic pollutants in an outdoor swimming pool, new treatment technologies, molecular size fractions of TOX and DOC received by membrane fractionation, new DBP research, table of carbonyl compounds tentatively identified in treated waters, chlorination reaction, electron ionization mass spectrum of tentatively identified DBP, toxicity studies, mutagenicity of organic extracts of drinking water, and references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Received for review March 2, 2006. Revised manuscript received September 27, 2006. Accepted October 5, 2006.

ES062367V