**Supporting Information**

**C. Zwiener et al. "Drowning in disinfection by-products? - Assessing Swimming Pool Water"**

## Experimental Section

### DBP Identification

Forty liters of pool water were collected from two outdoor swimming pools treated with stabilized chlorine. Stabilized chlorine was added in doses to achieve a chlorine residual of approximately 1 ppm. Samples were acidified to pH 2 and extracted using XAD resins (XAD-8 over XAD-2). The XAD columns were eluted with ethyl acetate, residual water was removed using separatory funnels and sodium sulfate, and the extract was concentrated to 1 mL using rotary evaporation and a gentle stream of nitrogen. XAD resins were cleaned by Soxhlet extraction prior to use according to a published procedure (*1*). An ethyl acetate resin blank and pool source waters (groundwater and chlorinated tap water, respectively) were also analyzed as controls. $BF_3$/methanol was used to methylate a portion of the ethyl acetate extracts to aid in the identification of halo-acids (*2*). The total organic halide (TOX) was measured at the US EPA's National Risk Management Research Laboratory using the adsorption-pyrolysis-titrimetric method (*3*).

Gas chromatography (GC) with low- and high-resolution mass spectrometry (MS) was used to identify the by-products. Because several of the DBPs were not present in the mass spectral library databases (NIST or Wiley), extensive interpretation of the mass spectra was necessary. Low- and high-resolution GC/electron ionization (EI)–MS analyses were performed on a Micromass Autospec high-resolution magnetic sector mass spectrometer

(Waters Inc.) equipped with an Agilent 6890 GC. The high-resolution mass spectrometer was operated at an accelerating voltage of 8 kV. Low-resolution analyses were carried out at 1000 resolution and high-resolution analyses at 10,000 resolution. Injections of 1–2 µL of the extract were introduced via a split/splitless injector onto a GC column (DB-5, 30-m × 0.25-mm ID, 0.25-µm film thickness, J&W Scientific/Agilent). The GC temperature program consisted of an initial temperature of 35ºC, which was held for 4 min, followed by an increase at a rate of 9ºC/min to 285ºC, which was held for 30 min. Transfer lines were held at 280ºC, and the injection port was controlled at 250ºC. 2,2'-Difluorobiphenyl was used as an internal standard.

**Determination of UV screens**

Pool water samples were collected in 1-L brown glass flasks directly from the pool and subsequently quenched with 0.3 mL of a solution of sodium thiosulfate (0.05 M). The samples were refrigerated and stored in the dark. Solid-phase extraction of 500-mL samples on Oasis HLB cartridges (Waters, Milford, MA) was done within 24 h. Prior to pre-concentration, the cartridges were pre-washed and conditioned with 3 mL ethyl acetate, 2 x 3 mL methanol, and 2 x 4.5 mL water. Samples were acidified to pH 2 with HCl (1 M) and extracted on the cartridges at a flow rate of 5 mL/min. After washing with 3 mL water, the cartridges were dried in a stream of nitrogen and eluted with 5 mL methanol and 5 mL ethyl acetate. The combined organic extracts were evaporated and the residue re-dissolved in 0.5 mL methanol/water (50:50, v/v), to which the internal standard was added previously (0.1 µmol/L fluorohydroxybenzophenone, FHBP). The UV screens were measured by LC-ESI-MS-MS on an API 3000 instrument (Applied Biosystems, Toronto) at the following operating parameters (Table 1).

Table 1. LC and MS operating parameters for the determination of UV screens in pool waters.

| | | |
|---|---|---|
| HPLC | Column: | Purospher STAR RP-18e, 125 x 2 mm, $d_P$ = 5 µm (Merck, Darmstadt) |
| | Eluents: | A: water with 0.1 % (v) acetic acid |
| | | B: methanol with 0.1 % (v) acetic acid |
| | Flow rate: | 0.3 mL/min |
| | Injection volume: | 50 µL |
| | Gradient: | in 10 min from 50 % B to 90 % B |
| | | 8 min isocratic at 90 % B |
| | | in 4 min from 90 % B to 100 % B |
| | | 5 min isocratic at 100 % B |
| ESI | Ionization voltage: | + 5000 V and – 5000 V |
| | Spray gas flow rate: | 1.48 L/min |
| | Dry gas flow rate: | 7 L/min |
| | Dry gas temperature: | 450 °C |
| MS/MS | Curtain gas flow rate: | 1.73 L/min |
| | Collision gas curtain : | $2.19 \cdot 10^{17}$ molecules/cm$^2$ |
| | Period 1: | First 10 min in negative ionization mode |
| | Period 2: | Second 20 min in positive ionization mode |
| | Mass transitions: | FHBP (IS) $[M-H]^-$ m/z 215.0 → 92.1; 95.0; 187.2 |

| | |
|---|---|
| PBS | $[M-H]^-$ m/z 273.0 → 193.2; 80.2; 115.1 |
| OMC | $[M+H]^+$ m/z 291.3 → 161.2; 179.2; 133.2 |
| BP3 | $[M+H]^+$ m/z 229.3 → 151.0; 77.1; 105.0 |
| BMDBM | $[M+H]^+$ m/z 311.3 → 135.2; 161.3; 77.0 |
| MBC | $[M+H]^+$ m/z 255.4 → 105.1; 171.3; 90.8 |
| OCR | $[M+H]^+$ m/z 362.4 → 250.2; 232.2; 105.1 |
| ODPABA | $[M+H]^+$ m/z 278.3 → 151.1; 166.2; 134.2 |

Further voltages of electrical lenses and ion guide were optimized for each mass transition separately.

## Results

### Treatment characteristics of swimming pool water

Table 2 shows the highly dynamic bather load and the concomitant organic contamination found in open-air pools.

**Table 2.** Dynamics of visitor numbers and organic pollutants in an outdoor swimming pool in the years 2000–2003 in Germany.

| Number of visitors | DOC (mg/L) | TOX (µg/L) | THM (µg/L) | Combined chlorine (mg/L) |
|---|---|---|---|---|

| | | | | | (mg/L) |
|---|---|---|---|---|---|
| Mean value (n = 60) | 2244 | 1.3 | 200 | 39 | 0.1 |
| Median | 793 | 1.1 | 196 | 34 | 0.1 |
| Minimum | 0 | 0.3 | 45 | 5 | 0.0 |
| Maximum | 7902 | 4.6 | 451 | 125 | 0.3 |

**New treatment technologies**

TOX fractionation with membranes with a molecular weight cut-off of 1000 Da 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 (Figure 1).

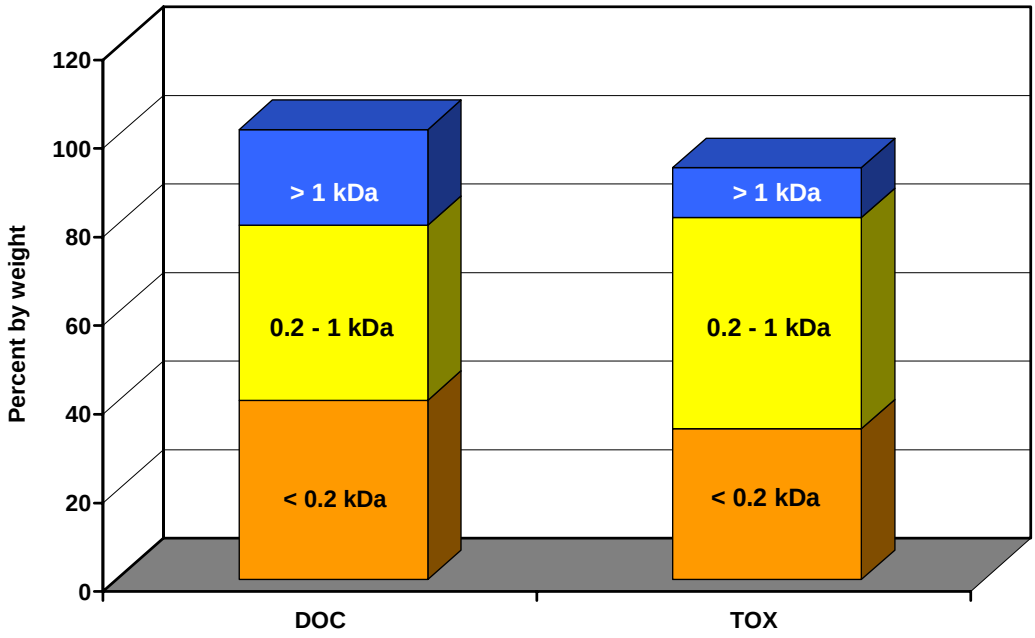


**Figure 1.** Molecular size fractions of TOX and DOC received by membrane fractionation with a molecular weight cut-off of 200 Da and of 1000 Da.

**Source:** Adapted from *(4,5)*.

**New DBP research**

In treated water samples, several homologues of the compound classes of oxoacids, hydroxydicarbonyls, and dicarbonyls could be tentatively identified (*4* and Table 3).

**Table 3.** Carbonyl compounds tentatively identified in treated waters.

**Source:** Adapted from *(6)*.

| $[M-H]^-$ ion of DNPH derivative | Carbon atoms |
|---|---|
| Oxoacids | |
| 253.4 | 2 |
| 267.3 | 3 |
| 281.4 | 4 |
| 295.4 | 5 |
| 309.3 | 6 |
| Hydroxy dicarbonyls | |
| 461.5 | 4 |
| 489.6 | 6 |
| 503.4 | 7 |
| Dicarbonyls | |
| 445.5 | 4 |
| 459.5 | 5 |

Some of the sunscreen compounds like BP3 can be efficiently chlorinated and, therefore, can contribute considerably to TOX and THM formation (Figure 2).

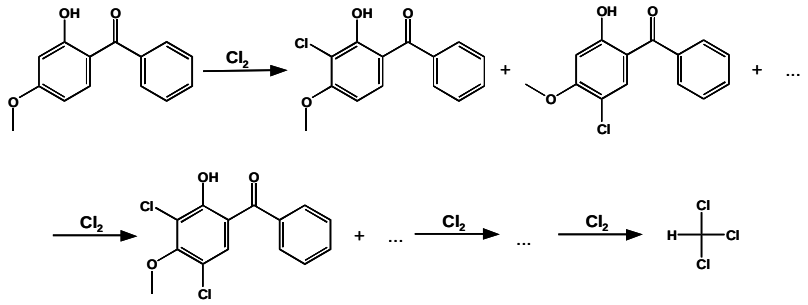


**Figure 2.** Chlorination reaction of the benzophenone sunscreen BP3.

An example of a DBP not present in the mass spectral library databases was the compound tentatively identified as 5,5,5-trichloro-4-oxopentanoic acid (identified in its methyl ester form, see Figure 3). This halo-oxo-acid has been found previously as a DBP in chlorinated drinking water.

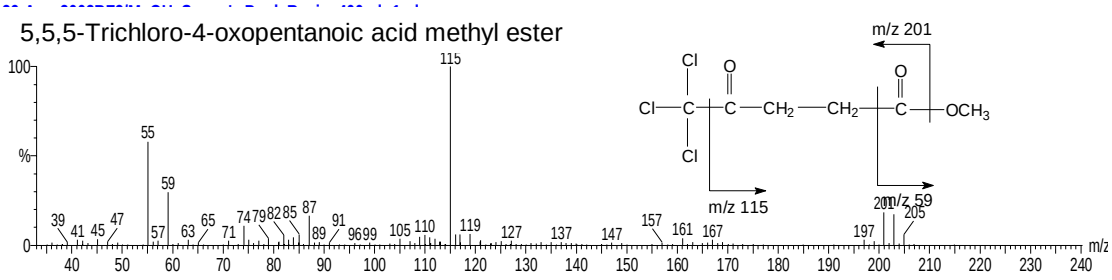


**Figure 3.** Electron ionization mass spectrum of tentatively identified DBP.

**Toxicity studies**

Although alternative methods of drinking water disinfection, such as ozonation and chloramination, have accomplished the intended reduction in the levels of regulated THMs and HAAs, they have also produced higher levels of other DBPs and new classes of DBPs, some of which appear to be more toxic than those currently regulated (7-*10*). Nonetheless, early studies (*11-13*) of the mutagenicity of extracts of drinking water prepared by various disinfection methods showed that all the organic extracts of water prepared by alternative disinfection methods produced organic extracts that were less mutagenic in the *Salmonella* bacterial mutagenicity assay than were extracts from water disinfected by chlorine (Figure 4). These studies also showed that the extracted organic mixture induced primarily base-substitution mutations because the organic extracts were more mutagenic in the base-substitution strain TA100 than in the frameshift strain TA98.

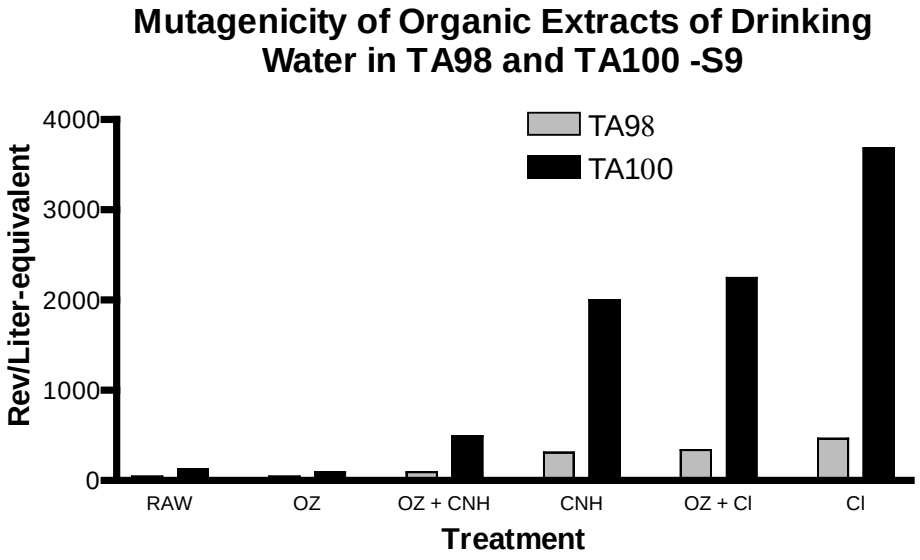


**Figure 4.** Mutagenic potencies in the *Salmonella* mutagenicity assay of XAD/ethyl acetate extracts of drinking water prepared by different treatment methods. Strain TA98 permits the recovery of frameshift mutations, whereas strain TA100 permits the recovery of base-substitution mutations. Raw, untreated river water; OZ, ozonated; OZ + CNH, ozonated

followed by chloramine; CNH, chloramine; OZ + Cl, ozone followed by chlorine; Cl, chlorine.

**Source:** Adapted from *(11)*.

**References for supporting information**

(1) Richardson, S. D.; Thruston, A. D., Jr.; Collette, T. W.; Patterson, K. S.; Lykins, B. W., Jr.; Majetich, G.; Zhang, Y. *Environ. Sci. Technol.* **1994**, *28*, 592.

(2) Kanniganti, R.; Johnson, J. D.; Ball, L. M.; Charles, M. J. *Environ. Sci. Technol.* **1992**, *26*, 1998.

(3) *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association, 1998.

(4) Glauner, T.; Frimmel, F. H.; Zwiener, C. Swimming pool water – the required quality and what can be done technologically. *GWF Wasser Abwasser* **2004,** *145*, 706-713 (in German).

(5) Glauner, T., Zwiener, C., Waldmann, P., Frimmel, F.H. Swimming pool water - fractionation and genotoxicological characterization of organic constituents. *Water Res*. **2005**, 39, 4494-4502.

(6) Zwiener, C.; Glauner, T.; Frimmel, F. H. Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry and Derivatization for the Identification of Polar Carbonyl Disinfection By-Products. In: I. Ferrer, E.M. Thurman (Eds.), Liquid Chromatography/Mass Spectrometry, MS/MS and Time-of-Flight MS – Analysis of Emerging Contaminants. ACS Symp. Ser., Vol. 850, Ch. 21, pp 356-375, Am. Chem. Soc. Oxford Univ. Press, Washington, 2003.

(7) Plewa, M. J.; Wagner, E. D.; Jazwierska, P.; Richardson, S. D.; Chen, P. H.; Mckague, A. B. Halonitromethane Drinking Water Disinfection Byproducts: Chemical Characterization and Mammalian Cell Cytotoxicity and Genotoxicity. *Environ. Sci. Technol.* **2004**, *38*, 62-68.

(8) Plewa, M. J.; Wagner, E. D.; Richardson, S. D.; Thruston, A. D., Jr.; Woo, Y.-T.; McKague, A. B. Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts. *Environ. Sci. Technol*. **2004**, *38* (18), 4713-4722.

(9) Kundu, B.; Richardson, S. D.; Granville, C. A.; Shaughnessy, D. T.; Hanley, N. M.; Swartz, P. D.; Richard, A. M.; DeMarini, D. M. Comparative mutagenicity of halomethanes and halonitromethanes in Salmonella TA100: structure-activity analysis and mutation spectra. *Mutat. Res*. **2004,** *554*, 335-350.

(10) Kundu, B.; Richardson, S. D.; Swartz, P. D.; Matthews, P. P.; Richard, A. M.; DeMarini, D. M. Mutagenicity in Salmonella of halonitromethanes: a recently recognized class of disinfection by-products in drinking water. *Mutat. Res.* **2004,** *562*, 39-65.

(11) DeMarini, D. M.; Abu-Shakra, A.; Felton, C. F.; Patterson, K. S.; Shelton, M. L. Mutation spectra in Salmonella of chlorinated, chloraminated, or ozonated drinking water extracts: comparison to MX. *Environ. Mol. Mutagen.* **1995**, *26*, 270-285.

(12) Noot, D. K.; Anderson, W. B.; Daignault, S. A.; Williams, D. T.; Huck, P. M. Evaluating treatment processes with the Ames mutagenicity assay. *J. Am. Water Works Assoc*. **1989**, *81*, 87-102.

(13) Patterson, K. S., Lykins, B. W. Jr., Richardson, S.D. Mutagenicity of Drinking Water Following Disinfection. *Aqua* **1995**, *44*, 1-9.