Supplemental Information

What's in the Pool? A Comprehensive Identification of Disinfection By-Products and Assessment of Mutagenicity of Chlorinated and Brominated Swimming Pool Water

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Materials and Methods

Preparation of water extracts and concentrates. Water samples were extracted immediately upon arrival using a previously published XAD resin process (Richardson et al. 2008). Briefly, water samples were acidified to pH <1 (using sulfuric acid) and concentrated on a column containing a combination of XAD-8 (40 mL) over XAD-2 (40 mL) resins. The resins were eluted with 350 mL ethyl acetate, and the ethyl acetate eluents were dried with sodium sulfate, rotoevaporated to approximately 5 mL, and further concentrated to 1.4 mL using a gentle stream of nitrogen. New XAD resins were pre-cleaned before use by Soxhlet extraction as described (Richardson et al. 1994).

Derivatization of extracts with diazomethane. Half of the 1.0-mL extract was derivatized with diazomethane (Ngan and Toofan 1991) to enable the identification of halo-acids (through their corresponding methyl esters); the other half was analyzed directly for other DBPs. Diazomethane was generated by dissolving 0.367 g Diazald (Sigma-Aldrich) in 1 mL of carbitol in the inner part of a diazomethane generator (Sigma-Aldrich), adding 2-3 mL of methyl *tert*-butyl ether (MTBE) in the outer part of the reactor, closing the vessel, and immersing it in ice. The reaction was initiated by adding 1.5 mL of KOH (37%) to the inner vessel and allowing the reaction to proceed for 1 h. The diazomethane formed collected in the MTBE, and 0.250 mL of this solution was added to the pool water extract (0.5 mL) and allowed to react for approx. 30 min, after which the reaction was quenched with approximately 50 mg of activated silica.

Synthesis of (E)- and (Z)-bromochlorobutenedioic acid. Bromine (16 g, 0.1 moles) was added over a few minutes to a stirred solution of chlorine (7.1 g, 0.1 moles) in dichloromethane (50 mL) with cooling in an ice bath. The solution was stirred for 20 min and then added to a stirred solution of dimethyl acetylene dicarboxylate (22.8 g, 0.16 moles) in dichloromethane (30

mL). The flask was stoppered and stirred overnight under illumination from a 200W light. After evaporation of the solvent from the decolorized reaction mixture, the product was fractionated by distillation through a 15-cm Vigreau column. Several additional fractionations through the column gave the following product: boiling point 136-140^{o}C at 25 mm, which contained 7% dichloro-, 84% bromochloro-, and 8% dibromobutenedioic acid dimethyl esters.

The dimethyl esters (1 g) were refluxed with stirring with 35% H_2SO_4 (5 mL) for 4 h to form the corresponding acids. The product was cooled, poured into H_2O (10 mL), and extracted with ether. The extracts were dried and evaporated to give the product (900 mg). Trituration with hexane gave a mixture of (*E*)- and (*Z*)-2-bromo-3-chlorobutenedioic acid as a beige product, mp. 103-108^{o}C.

Purification of (Z)-bromochlorobutenedioic acid. A mixture of the (*E*)- and (*Z*)-isomeric dimethyl esters obtained above (2.0 g), was fractionated on silica gel. Elution with hexane:ether, 4:1, initially gave the (*E*)-isomer, 93% purity followed by the (*Z*)-isomer, 86% purity. The (*Z*)-isomer (0.5 g) was hydrolyzed by stirring in 10% KOH in methanol:water, 9:1 (5 mL) at room temperature for 20 min. The product was diluted with water, acidified with 10% hydrochloric acid and extracted with ether. The solvent was evaporated and the product triturated with hexane to give (*Z*)-bromochlorobutenedioic acid as an off-white solid, mp. 123-125^{o}C. The structure was confirmed by gas chromatography (GC) before methylation (measurement of the anhydride) and after methylation (measurement of methyl ester).

References

Ngan F, Toofan M. 1991. Modification of preparation of diazomethane for methyl esterification of environmental-samples analysis by gas-chromatography. J Chromatogr Sci 29:8-10.

Richardson, S. D., Thruston, A. D., Jr., Collette, T. W., Patterson, K. S., and Lykins, B. W., Jr. 1994. Multispectral identification of chlorine dioxide disinfection byproducts in drinking water. *Environ. Sci. Technol*. 28: 592-599.

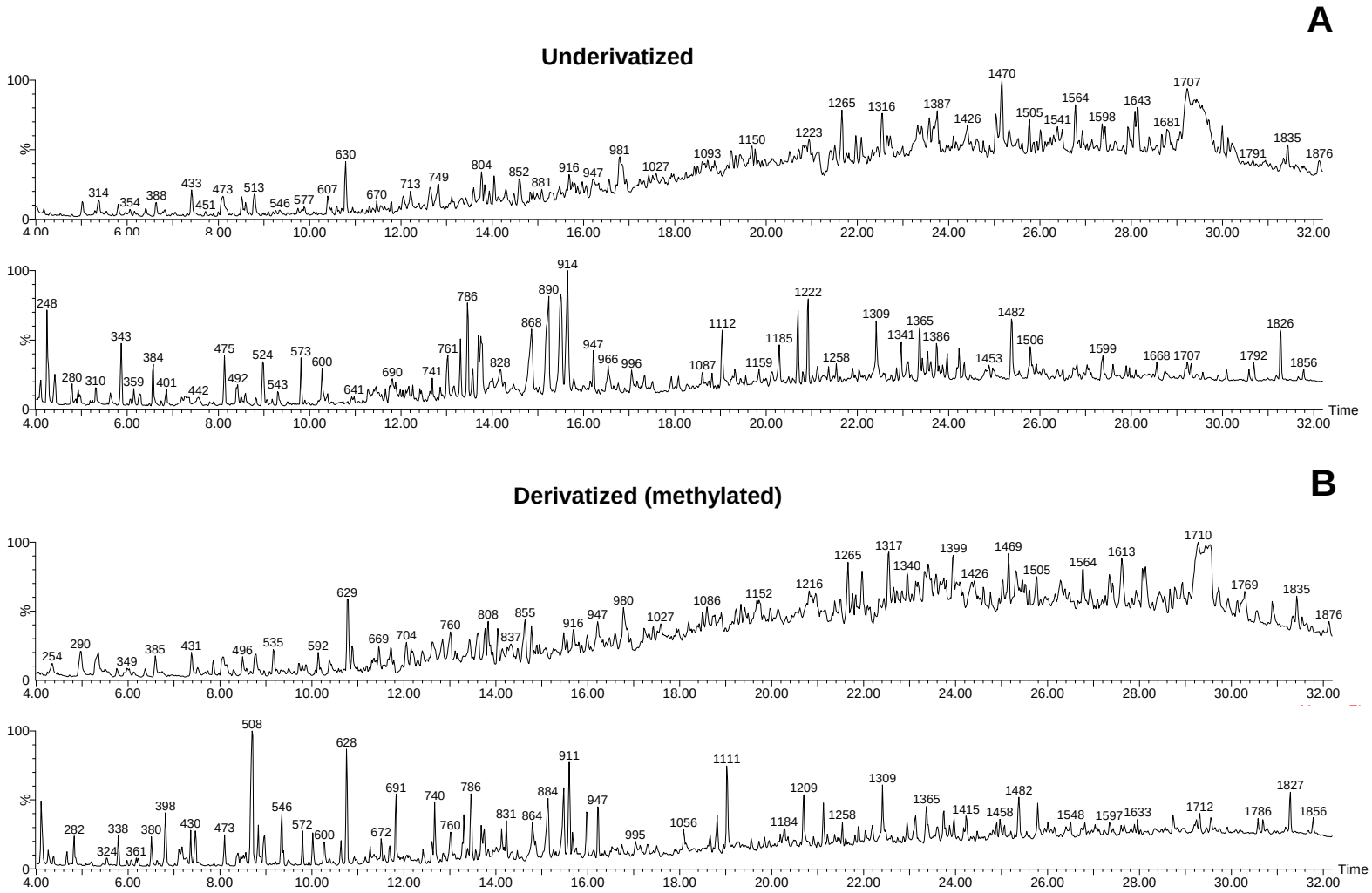

Figure S1. (A) GC/MS chromatograms showing underivatized extracts of chlorinated and brominated pool waters. (B) GC/MS chromatograms showing derivatized (methylated) extracts of chlorinated and brominated pool waters.

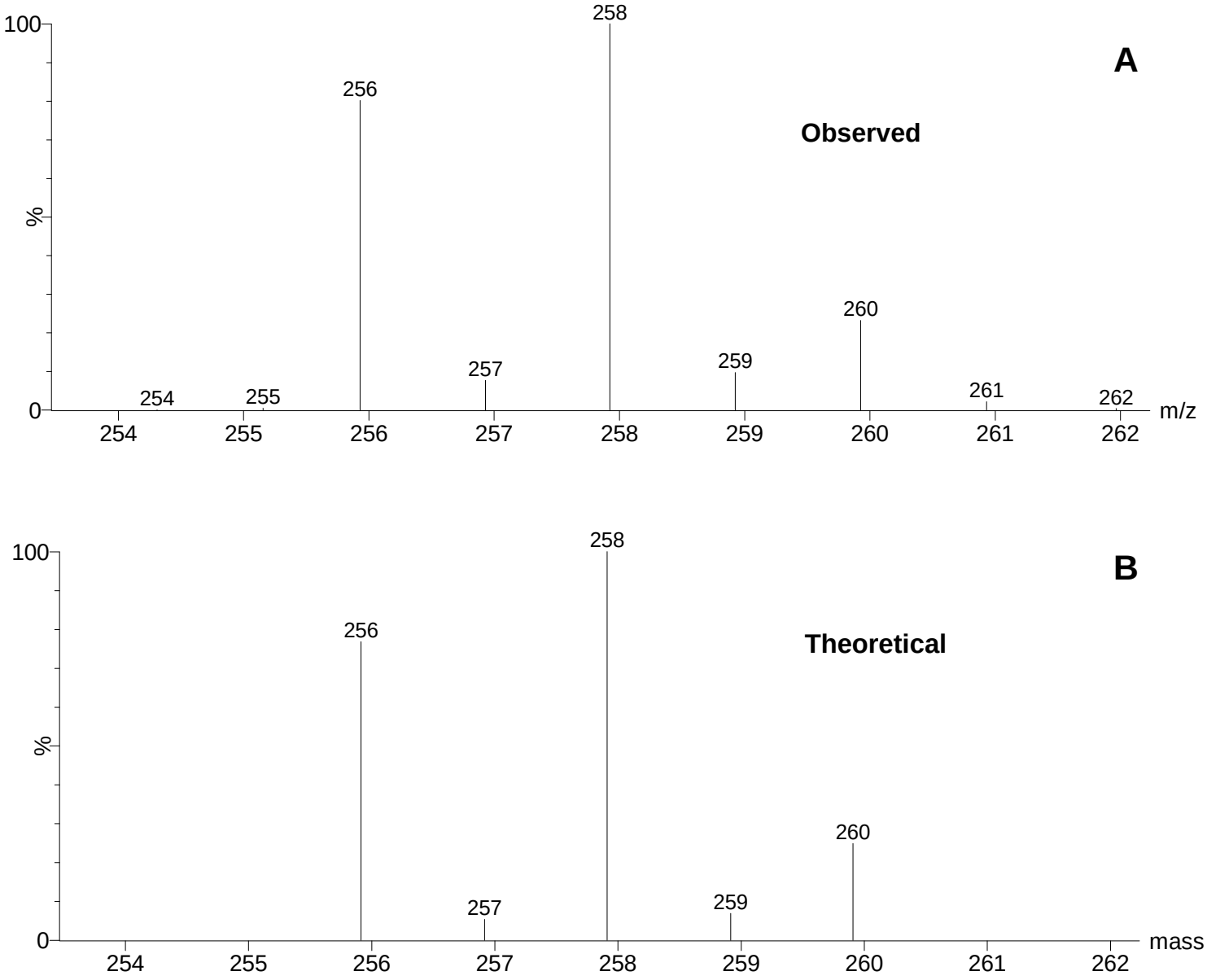

Figure S2. Experimentally observed (A) and theoretical isotopic distributions (B) molecular ion of (*Z*)-bromochlorobutenedioic acid dimethyl ester.