Supporting Information

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Manuscript Title:
Chemical and Biological Characterization of Newly Discovered Iodo-Acid Drinking Water Disinfection Byproducts

Drinking Water Sampling, Extraction, and Concentration. Upon receipt of samples at the U.S. EPA, water samples were acidified to pH 2, immediately concentrated using XAD-8 over XAD-2 resins, eluted with ethyl acetate, and concentrated to 1 mL using rotary evaporation followed by evaporation under nitrogen.

GC/MS Analysis. GC/MS analyses with electron ionization (EI) were performed on a Micromass Autospec II high resolution mass spectrometer equipped with an Agilent 6890 gas chromatograph. The high-resolution mass spectrometer was operated at an accelerating voltage of 8 kV and at resolutions of 1,000 and 10,000, for low and high resolution experiments, respectively. Perfluorokerosene (pfk) was used as the mass calibrant. Injections of 1–2 μL of the extracts (or standard solutions in ethyl acetate) were introduced via a split/splitless injector onto a J&W-Agilent Scientific DB-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The GC temperature program consisted of an initial temperature of 35°C for 4 min, followed by a rate increase of 9°C /min to 285°C, which was held for 30 min. A GC injection port temperature of 250°C and a GC/MS transfer line temperature of 285°C were used.

Chemicals and Reagents. General laboratory chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Pittsburgh, PA). Media supplies and fetal bovine serum (FBS) for the mammalian cells were purchased from HyClone Laboratories (Logan, UT).

***Salmonella* Microplate Cytotoxicity Assay.** The detailed procedures for the *Salmonella* microplate cytotoxicity assay were recently published (*21*). Briefly, frozen log-phase *S. typhimurium* TA100 cells were thawed, grown in Luria broth (LB) for 2 h and the cell titer was adjusted to an optical density (OD) of 0.030 at 595 nm. Each treatment vial contained 300 μL of titered cells, a known concentration of the DBP (<10 μL) and 100 mM potassium phosphate buffer, pH 7.4 in a total volume of 1 mL. The cells were treated for 1 h at 37°C while shaking at 200 rpm. Aliquots (100 μL) from each treatment vial were transferred into multiple wells of a 96-well microplate and 100 μL of 2× LB medium were added. The initial optical density of each well was measured using a Bio-Rad model 550 microplate reader at 595 nm which provided a blank reading for each microplate well. The microplate was placed in a plastic container and incubated at 37°C while shaking at 200 rpm for 210 min. The final OD of each well was determined at 595 nm with the microplate reader. For each well, the blank OD value (time 0 reading) was subtracted from the OD reading of that specific well after 210 min of incubation. A concurrent negative control of bacteria without DBP exposure was included with each microplate and the blank-corrected data for the negative control was set at 100%. The blank-corrected data for each DBP concentration was converted into a percentage of the negative control. In general, each DBP concentration was replicated 4 – 8 times per microplate and each experiment was repeated a minimum of 2 times.

***Salmonella* Preincubation Mutagenicity Assay.** *S. typhimurium* TA100 cells were grown from a single colony isolate in 100 mL LB medium supplemented with ampicillin (50 μg/mL) at 37°C with shaking (200 rpm) (*30*). The overnight culture was harvested by centrifugation, washed

twice with phosphate buffer and the titer was adjusted to 2×10^{10} cells/mL. Each reaction mixture was composed of a known DBP concentration, 2×10^{9} cells and phosphate buffer in a total volume of 1 mL. Each reaction vial was incubated for 1 h at 37°C while shaking. Triplicate 250 µl aliquots of the reaction vials (5×10^{8} cells) were added to 2 mL of molten Vogel Bonner (VB) top agar supplemented with histidine + biotin. Each top agar tube was poured onto a selective VB medium plate, the plates were incubated at 37°C for 72 h and the histidine revertant colonies were scored. The mutagenicity experiments were repeated 3 times.

Mammalian Cell Microplate Cytotoxicity Assay. A 96-well flat-bottomed microplate was prepared for a series of DBP concentrations. One column was the blank control consisting of 200 µl of F12 + 5% FBS medium only. The concurrent negative control column consisted of 3×10^{3} Chinese hamster ovary (CHO) cells plus F12 + FBS medium. The wells for the remaining columns contained 3×10^{3} CHO cells, F12 + FBS and a known DBP concentration in a total of 200 µL. The wells were covered with a sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) to prevent volatilization. The plate was placed on a rocking platform for 10 min at 37°C to ensure uniform distribution of the CHO cells and the cells were treated for 72 h at 37°C, in an atmosphere of 5% CO_2. The medium from each well was gently aspirated; the cells were fixed in 100% methanol for 20 min and stained for 30 min with a 1% crystal violet solution in 50% methanol. The wells were gently washed, drained and 50 µL of DMSO were added to each well and incubated at room temperature for 30 min. The microplate was analyzed at 595 nm with a microplate reader; the absorbancy of each well was automatically recorded and stored on an attached computer. The average absorbance of the blank wells (no cells) was subtracted from the

absorbance data from each well. The blank-corrected absorbancy value of the negative control (cells with medium only) was set at 100%. The absorbance for each treatment group well was converted into a percentage of the negative control. For each DBP concentration 8 replicate wells were analyzed per experiment and the experiments were repeated twice.

Single Cell Gel Electrophoresis (SCGE) Assay. The day before treatment, 4×10^4 CHO cells were added to microplate wells in 200 μL of F12 + 5% FBS and incubated overnight. The next day the cells were washed with Hank's balanced salt solution (HBSS) and treated with the DBP in F12 medium without FBS in a total volume of 25 μL for 4 h at 37°C, 5% CO_2. The wells were covered with sterile AlumnaSeal™. After the treatment the cells were washed twice with HBSS and harvested with 50 μL of 0.01% trypsin and 106 μM EDTA solution. The trypsin was inactivated with the addition of 70 μL of F12 + FBS. A 10 μL aliquot was removed to measure acute cytotoxicity using the trypan blue vital dye exclusion assay. We did not use the SCGE data if the acute cytotoxicity exceeded 30% (*33*). The remaining suspension from each well was mixed with 120 μL of molten 1% low melting point agarose (LMA) prepared with phosphate-buffered saline, and 90 μL of the suspension were spread on each of two SCGE microscope slides. The microscope slides had been previously coated with a layer of 1% normal melting point agarose prepared with deionized water and dried overnight. After the LMA solidified at 4°C for 5 min, a final layer of 0.5% LMA was applied to the slides. To remove the cell and nuclear membranes the slides were placed overnight at 4°C in freshly prepared lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% Triton X-100, and 10% DMSO). The slides then were placed in an alkaline buffer (1 mM Na_2EDTA, 300 mM

NaOH, pH >13.5) in an electrophoresis tank, and the DNA was allowed to denature for 20 min. The slides were electrophoresed at 0.72 V/cm (25 V, 300 mA) for 40 min at 4°C. The gels were neutralized with 700 mM Tris buffer, pH 7.5. The slides were rinsed in cold deionized water, dehydrated in cold (4°C) methanol for 20 min, dried for 20 min at 50°C and stored at room temperature in a covered slide box. For analysis the slides were hydrated in cold deionized water for 20 min, placed on a tray and stained with 65 μL of ethidium bromide (2 μg/mL) for 5 min. The slides were dipped in cold water to remove excess stain, a cover slip was placed over the gel, and the slides were analyzed with a Zeiss fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. A computerized image analysis system (Komet version 3.1, Kinetic Imaging Ltd., Liverpool, UK) was used to measure various SCGE parameters (i.e. % tail DNA and tail moment) of 25 randomly chosen nuclei per slide. The tail moment (integrated value of migrated DNA density multiplied by the migration distance) was used as the primary measure of DNA damage. The digitalized data were automatically transferred from the CCD camera to a computer-based spreadsheet for statistical analysis.