Genotoxicity and effects of nanosilver contamination in drinking water disinfection

SUPPLEMENTARY MATERIAL

Water characterization

Dissolved organic carbon (DOC) concentrations, measured as NPOC (non-purgeable organic carbon), and total dissolved nitrogen (TN) concentrations were measured using a Shimadzu TOC-VP equipped with a TNM-1 total nitrogen measuring unit (Shimadzu Corporation, Japan).

Bromide, chloride, sulfate and phosphate were measured by ion chromatography using a Dionex ICS-3000 ion chromatograph equipped with an IonPac AS18 analytical column and conductivity detector (Dionex Corp., USA) according to method EN ISO 10304-1:1995.

Arsenic, iron and manganese were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) (Elan 9000, Perkin-Elmer, USA).

Disinfection treatment

Water samples were treated by adding an aliquot of either chlorine or chlorine dioxide stock solution sufficient to achieve the desired dose. All solutions were prepared by using ultrapure Milli-Q® water from Q-POD unit (Millipore, USA).

Stock solutions of chlorine were prepared by dissolving chlorine gas into Milli-Q® water and standardized spectrophotometrically by using a molar absorption coefficient of the triiodide ion (I$_3^-$), calculated to be 25,024 mol$^{-1}$ L cm$^{-1}$ at 351 nm. The concentrations of aqueous chlorine in treated samples were determined indirectly by UV-spectrophotometric measurement of triiodide generated by the reaction of residual chlorine with an excess of potassium iodide.

Chlorine dioxide stock solutions were prepared by adding diluted H$_2$SO$_4$ to a sodium chlorite solution according to Standard Method 4500-CIO$_2$ B (APHA et al. 1995) and standardized by direct photometric determination of the ClO$_2$ at 360 nm using a calculated absorption coefficient of 1,182 mol$^{-1}$ L cm$^{-1}$.

ClO$_2$ concentrations were determined according to USEPA Method 327.0, Revision 1.1 using lissamine green B and horseradish peroxidase (USEPA 2005).

All absorbance measurements were made using an HP 8453 UV-visible spectrophotometer (Hewlett Packard, USA).

Ozonization of water was achieved from dry pure oxygen by laboratory ozone generator Fischer 500M (Fischer Technology, Germany) with a maximum production capacity of 500 mg h$^{-1}$ O$_3$ following the procedure described elsewhere (Juretic et al. 2011).

In order to assess effect of silver nanoparticles on the genotoxicity and formation of disinfection by-products, selected water samples were additionally treated with 0.5 mg L$^{-1}$ of AgNP.

Analysis of trihalomethanes

All analyses were carried out using a Perkin-Elmer TurboMatrix HS-40 Trap coupled to a Clarus 500 GC–MS (Perkin-Elmer, USA). Elite-Volatiles capillary column (30 m $\times$ 250 $\mu$m i.d., 1.4 $\mu$m film thickness) from Perkin-Elmer was used for separation. The mass spectrometer was operated in electron impact ionization mode with ionization energy of 70 eV. Mass spectra were collected over the m/z range 35–300 with a scan time of 0.1 s and an inter-scan delay of 0.01 s. Quantification of trihalomethane (THMs) was performed in selected ion monitoring mode using internal standard calibration.

The total THM formation potential (TTHMFP) was measured by Standard Method 5710B (APHA et al. 1995). The samples subjected to the TTHMFP analysis were buffered at pH 7, chlorinated with an excess of free chlorine and stored at 25 °C for 7 days to allow the reaction to approach completion.
The temperature of water samples in all experiments were kept at 25 ± 1 °C.

**Determination of dicarbonyl compounds by LC-MS/MS method**

Mili-Q® water and HPLC-grade methanol (Merck, Germany) were used with analytical-grade formic acid (FA) (Acros Organics, Belgium) for mobile phase preparations.

LC-MS/MS (liquid chromatography-tandem mass spectrometry) analysis was run using an Agilent 1200 series HPLC system equipped with a binary pump, a vacuum membrane degasser, an automated autosampler and injector interfaced with 6410 triple quadrupole mass spectrometer (Agilent Technologies Inc., USA). Chromatographic separation was conducted using a Zorbax Eclipse XDB-C18 4.6 × 150 mm, 3.5 μm column (Agilent Technologies, USA). Solvents for the analysis were 0.1% FA in water (solvent A) and 0.1% FA in 90% methanol (solvent B). The gradient was applied as follows: 0 min 50% A (50% B); 0–8 min 50% A (50% B)–0% A (100% B); 8–14 min 0% A (100% B). Flow rate was 0.5 mL min⁻¹. Electrospray positive ionization-mass spectrometric multiple-reaction monitoring (ESI⁺/MRM) experiments were used for detection of glyoxals in water samples. The desolvation gas temperature was 350 °C with flow rate of 12.0 L min⁻¹. The capillary voltage was 4.0 kV. Nitrogen was used as a collision cell gas.

**REFERENCES**


USEPA 2005 Method 327.0—Revision 1.1, Determination of chlorine dioxide and chlorite ion in drinking water using lissamine green B and horseradish peroxidase with detection by visible spectrophotometry. Cincinnati, Ohio, Office of Ground Water and Drinking Water, US Environmental Protection Agency (EPA 815-R-05-008).