Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

# Comparative mammalian cell cytotoxicity of wastewater with elevated bromide and iodide after chlorination, chloramination, or ozonation

*Shengkun Dong*[1,3,*], *Thanh H. Nguyen*[1,3], *Michael J. Plewa*[2,3]

1. *Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
2. *Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
3. *Safe Global Water Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

ARTICLE INFO

*Article history:*
Received 2 February 2017
Revised 11 March 2017
Accepted 16 March 2017
Available online 29 March 2017

*Keywords:*
Cytotoxicity
Ozonation
Chlorination
Chloramination
Wastewater
Bromide
Iodide
Disinfection

ABSTRACT

Recycling wastewater is becoming more common as communities around the world try to better control their water resources against an increased frequency of either prolonged droughts or intense flooding. For communities in coastal areas, wastewaters may contain elevated levels of bromide ($Br^-$) and iodide ($I^-$) from seawater intrusion or high mineral content of source waters. Disinfection of such wastewater is mandatory to prevent the spread of pathogens, however little is known about the toxicity of wastewater after disinfection in the presence of $Br^-$ and $I^-$. In this study we compared the induction of chronic cytotoxicity in mammalian cells in samples of municipal secondary wastewater effluent amended with elevated levels of $Br^-/I^-$ after disinfection by chlorine, chloramines or ozone to identify which disinfection process generated wastewater with the lowest level of adverse biological response. Chlorination increased mammalian cell cytotoxicity by 5 times as compared to non-disinfected controls. Chloramination produced disinfected wastewater that expressed 6.3 times more cytotoxicity than the non-disinfected controls and was 1.3 times more cytotoxic than the chlorinated samples. Ozonation produced wastewater with cytotoxicity comparable to the non-disinfected controls and was at least 4 times less cytotoxic than the chlorine disinfected wastewaters. These results indicate that compared to chlorination and chloramination, ozonation of wastewater with high $Br^-/I^-$ levels yielded the lowest mammalian cell cytotoxicity, suggesting its potential as a more favorable method to disinfect wastewater with minimizing the biological toxicity in mind.


## Introduction

Wastewater reuse was identified as an important means to alleviate pressure on freshwater resources and is attracting attention in the United States (National Research Council Committee, 2012). Reuse requires that the recycled wastewater is disinfected to prevent the spread of pathogens. Due to its efficacy and affordability, chlorine-based disinfection is the most widely adopted technology for wastewater disinfection (Metcalf & Eddy Inc., 2013). Chlorine-based disinfection includes free chlorine (referred to as chlorination hereinafter) or chloramines (referred to as chloramination hereinafter). The latter occurs when the ammonia concentration of the wastewater is sufficiently high. However, chlorine is not only ineffective at low

* *Corresponding author*. E-mail: sdong6@illinois.edu (Shengkun Dong).

http://dx.doi.org/10.1016/j.jes.2017.03.030
1001-0742/

concentrations against certain pathogens, such as *Cryptosporidium parvum* (Korich et al., 1990), but also generates disinfection byproducts (DBPs) (Crittenden et al., 2012; Reckhow et al., 1990). Compared to chlorination, chloramination produces lower concentrations of regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs), and therefore many drinking water utilities are considering switching from chlorination to chloramination (Seidel et al., 2005). In coastal areas and regions with high halogen content in source waters, $Br^-$ and $I^-$, two common halide anions, are elevated (Crittenden et al., 2012; Ludzack and Noran, 1965). Chlorination and chloramination can oxidize $Br^-$ to hypobromous acid and $I^-$ to hypoiodous acid (Qi et al., 2004; Sun et al., 2009). In the presence of wastewater organic matter (WOM) the slow decay kinetics of the hypobromous and hypoiodous acids may encourage the formation of brominated DBPs (Br-DBPs) and iodinated DBPs (I-DBPs) from reactions between hypobromous (Qi et al., 2004; Sun et al., 2009) and hypoiodous acids with organic precursors (Bichsel and Von Gunten, 2000).

In addition to chlorine-based disinfection, ozonation is being increasingly used for wastewater disinfection (Gottschalk et al., 2010). However, more studies of pathogen disinfection, DBP formation, and cytotoxicity related to ozonation were conducted in drinking water rather than in wastewater. Under drinking water conditions, ozone has been shown to inactivate a number of pathogens such as the Norwalk virus (Shin and Sobsey, 2003), poliovirus (Majumdar et al., 1973), and even chlorine-resistant pathogens, such as *C. parvum* (Cho and Yoon, 2007; Corona-Vasquez et al., 2002; Kim et al., 2007, 2004; Tang et al., 2005). However, enhanced $Br^-/I^-$ levels found in source waters in coastal areas could pose a potential problem, because ozonation has been shown to generate Br- and I-DBPs (Bichsel and Von Gunten, 1999, 2000; Zeng et al., 2016). Compared to the chlorinated DBPs (Cl-DBPs), Br-DBPs and I-DBPs are more cytotoxic and genotoxic to mammalian cells (Plewa and Wagner, 2009; Richardson et al., 2008), and therefore are of potential public health concerns. Because ozonation of wastewater for reuse has been shown to form a broad range of DBPs due to the high concentration of organic and inorganic constituents (Wert et al., 2007), safe practice of wastewater reuse requires systematic studies focusing on toxicity of ozonated wastewater.

The cytotoxicity and genotoxicity of disinfected drinking water were found to highly correlate with total organic bromine (TOBr) and total organic iodine (TOI) and weakly and inversely correlate with total organic chlorine (TOCl) (Yang et al., 2014). Thus, the generated Br- and I-DBPs rather than the Cl-DBPs were proposed to be the forcing agents for cytotoxicity and genotoxicity in drinking water containing high level of $Br^-/I^-$ (Yang et al., 2014). However, lowered genotoxicity in the presence of $Br^-$ after chlorination of a municipal secondary effluent was also reported (Wu et al., 2010). These partially contradictory results may be attributed to the complex chemical composition of the wastewater, suggesting the need to comparatively quantify the cytotoxicity of wastewater for reuse after different disinfection technologies. The objective of this study was, therefore, to identify which disinfection technology would generate disinfected wastewater effluents with the lowest mammalian cell cytotoxicity when enhanced $Br^-/I^-$ levels were present in a secondary effluent wastewater. The use of a single wastewater source allowed the comparison to be conducted without the complication of different WOM. The findings will shed light on selecting the disinfection technology that minimizes the potential biological toxicity.

## 1. Materials and methods

### 1.1. Water sampling, processing, and characterization

Samples were collected from the Northeast Wastewater Treatment Plant (NEP) in Urbana, Illinois. At the NEP, the raw sewage flows through a series of preliminary, primary, secondary, and tertiary treatments to remove the majority of the solids, organic matter, and ammonia, before it is disinfected and discharged. The samples were taken from a secondary clarifier after the activated sludge treatment but before the nitrification tower. To eliminate the interference of suspended solids, these samples were filtered through 1.6 μm glass fiber filters and stored in the dark at 4°C until used (within a week of collection). The total organic carbon (TOC) was measured by a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD) to be 7.3 mg C/L. The absorbance at 254 nm was measured by a Beckman UV–vis spectrophotometer (Beckman Coulter Life Sciences, Indianapolis, IN) to be 0.144 $cm^{-1}$. Specific UV Absorbance at 254 nm ($SUVA_{254}$) was, therefore, calculated to be 1.97 $m^{-1}mg^{-1}L$. Background total bromine and iodine was determined by ICP-MS previously to be both below the detection limit of 10 μg/L (Dong, 2016). Ammonia nitrogen (7.5 mg/L), free and total chlorine were measured using Hach kits (Loveland, CO). Combined chlorine was calculated as the difference between the total and free chlorine. The measured pH of the filtered wastewater was 7.5 at room temperature.

### 1.2. Disinfection experiments

Thirteen liters of the secondary effluent samples was treated with free chlorine (75 mg/L as $Cl_2$, $Cl_2$ to $NH_3$-N mass ratio of 10), combined chlorine (17.3 mg/L as total $Cl_2$, $Cl_2$ to $NH_3$-N mass ratio of 2.3), or ozone (3 mg/L) in the presence of $Br^-$ (500 μg/L) and $I^-$ (100 μg/L). All disinfectant concentrations were of engineering relevance. The concentrations of $Br^-$ and $I^-$ were taken from previous studies to represent waters that were impacted by high levels of $Br^-$ and $I^-$, such as desalinated seawater (Yang et al., 2014; Agus et al., 2009). For chlorine-based disinfection experiments, all reactions were carried out in amber glass bottles with Teflon-lined caps wrapped in aluminum foil. Ozonation took place in clear round bottom flasks sealed with caps. To achieve breakpoint chlorination, previous studies reported $Cl_2$ to $NH_3$-N mass ratio of between 7.6 to 10 (Mitch, 2009) and 15 (Stover et al., 1986). We conducted preliminary experiments to ensure that when operated at a $Cl_2$ to $NH_3$-N mass ratio of 10, more than 97% of available chlorine was free chlorine after rapid mixing. Similarly, for chloramination, a preliminary experiment was conducted to ensure that more than 94% of available chlorine was combined chlorine after rapid mixing. For both free and combined chlorine, the residual total chlorine at the end of the 30 min contact time was measured and the reactions were stopped using sodium bisulfite ($NaHSO_3$ to $Cl_2$ mass ratio of 1.63)

(Metcalf and Eddy Inc., 2013). For ozonation, 24 h were passed before sample concentration to guarantee the absence of residual ozone.

### 1.3. Sample concentration for cytotoxicity assays

The organic compounds from the wastewater samples were concentrated by adsorption onto Soxhlet-cleaned XAD resins. A volume of 55 mL each of XAD-2 (Amberlite XAD 2, Sigma Aldrich, MO) and XAD-8 (Supelite DAX 8, Sigma Aldrich, MO) resins was packed above a plug of glass wool in a glass chromatography column. The maximum ratio of water to resins was 770:1 to minimize breakthrough and maximize the adsorption of organics (Ringhand et al., 1987; Schenck et al., 1990). The wastewater samples were acidified to pH < 2 by sulfuric acid prior to being added into the column for extraction, to ensure protonation of carboxylic organics. The resins were eluted with 400 mL of optima grade ethyl acetate (Fisher Scientific, PA) to elute the organic compounds (Kronberg et al., 1988). The residual water in the ethyl acetate eluent was removed using a separatory funnel, followed by passing the hydrophobic fraction through a column of anhydrous sodium sulfate. The ethyl acetate extract was then reduced to 1–1.5 mL by a rotary evaporator at 50–60°C and further blown down to a sludge using a gentle stream of nitrogen gas. For each sample, 130 μL of dimethyl sulfoxide (DMSO) was used to dissolve the organic extract, resulting in a $10^5$-fold concentrated organic compounds derived from the original wastewater samples.

### 1.4. Biological and chemical reagents, Chinese hamster ovary cells

Chinese hamster ovary (CHO) K1 cell line AS52, clone 11–4–8 was used for all of the cytotoxicity experiments (Wagner et al., 1998). The CHO cells were maintained in Hams F12 medium containing 5% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotics (100 μg/mL streptomycin sulfate, 100 units/mL sodium penicillin G, and 0.25 μg/mL amphotericin B in 0.85% saline) at 37°C in a humidified incubator containing 5% $CO_2$.

### 1.5. CHO cell chronic cytotoxicity assay

The CHO cell chronic cytotoxicity assay measures the reduction in cell density as a function of the concentration of the test samples over 72 h (Wagner and Plewa, 2017; Plewa et al., 2002; Wagner et al., 1998). Details of the cytotoxicity assay can be found elsewhere (Dong et al., 2016; Plewa et al., 2002; Plewa and Wagner, 2009). Briefly, the test samples in DMSO were diluted in F12 plus FBS cell culture medium, and the corresponding concentrations were calculated and expressed as concentration factors of the organics derived from the original volume of the wastewater samples. After incubation with the cells for 72 h, the cell density expressed as the percentage of the concurrent negative control was recorded. These data were used to construct concentration-response curves.

### 1.6. Statistical analysis

To determine if a statistically significant difference existed between treatments, $LC_{50}$ values (the concentration of the organic extract that induced a cell density 50% of the negative control) were determined through regression analyses for each concentration–response curve (Fig. 1). These values were converted into cytotoxicity index values (CTI) = $(LC_{50}^{-1})(10^3)$ to allow for ANOVA statistical tests, which enabled us to rank the samples that were disinfected using different techniques, from the most to the least cytotoxic (Fig. 2). One-way ANOVA tests were conducted to determine the lowest concentration factor that induced a statistically significant level of cell death as

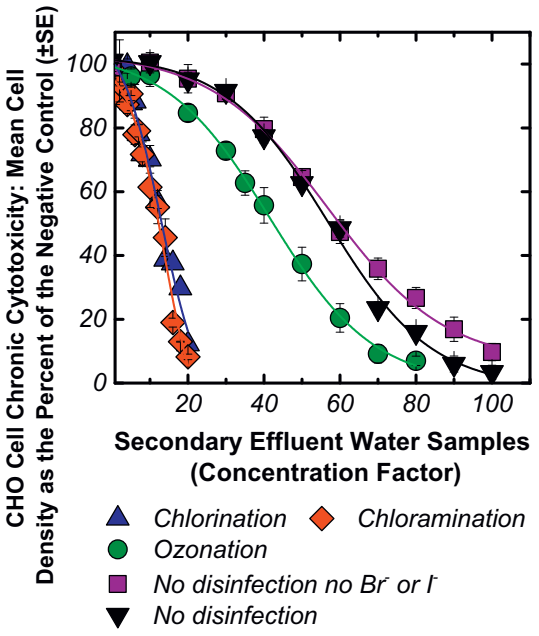


**Fig. 1 – The cytotoxicity concentration-response data for the secondary effluent samples under different disinfection treatment conditions. All treatments contained 500 and 100 μg/L as $Br^-$ and $I^-$, respectively, unless specified otherwise. Error bars correspond to the standard error of five to eight replicates.**

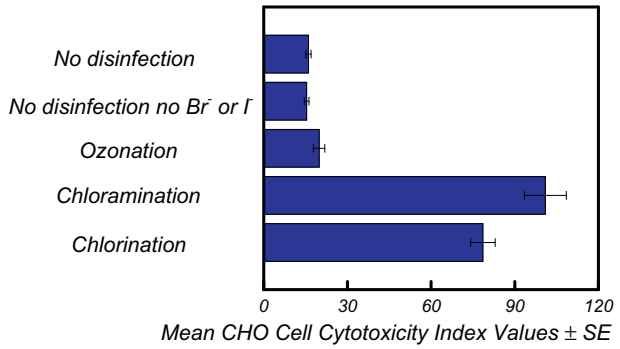


**Fig. 2 – The mean cytotoxicity index values for the secondary effluent samples under different disinfection treatment conditions. All treatments contained 500 and 100 μg/L as $Br^-$ and $I^-$, respectively, unless specified otherwise. Cytotoxicity ranking: chloramination > chlorination > ozonation ≈ No disinfection no added $Br^-/I^-$ ≈ No disinfection. Error bars correspond to the standard error of five to eight replicates.**

**Table 1 – Induction of chronic cytotoxicity in CHO cells by the secondary effluent in the presence of 500 and 100 μg/L as $Br^-$ and $I^-$, respectively.**

| Wastewater sample | Lowest cytotoxic conc. factor [a] | $r^2$ [b] | $LC_{50}$ (conc. factor) [c] | ANOVA test statistics [d] |
|---|---|---|---|---|
| Chlorination | 7× | 0.97 | 12.9× | $F_{10,41} = 26.8$; $p \leq 0.001$ |
| Chloramination | 6× | 0.98 | 10.2× | $F_{10,49} = 18.5$; $p \leq 0.001$ |
| Ozonation | 30× | 0.99 | 54.5× | $F_{10,77} = 98.1$; $p \leq 0.001$ |
| No disinfection no $Br^-/I^-$ | 40× | 0.99 | 66.6× | $F_{10,77} = 120.1$; $p \leq 0.001$ |
| No disinfection | 40× | 0.99 | 64.2× | $F_{10,77} = 350.1$; $p \leq 0.001$ |

CHO: Chinese hamster ovary.
[a] The lowest cytotoxic concentration factor was the lowest concentration factor of the corresponding sample that produced a statistically significant reduction in cell density as compared to the negative control.
[b] $r^2$ is the coefficient of determination for the regression analysis that derived the $LC_{50}$ value.
[c] The $LC_{50}$ value is the sample concentration factor that induced a cell density that was 50% of the negative controls.
[d] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test results and the resulting probability value.

compared to the negative control ($p \leq 0.05$) (Table 1). The power of the test was maintained at ≥0.8 at $\alpha = 0.05$.

## 2. Results and discussion

The goal for this research was to identify the lowest level of mammalian cell cytotoxicity induced by a secondary effluent wastewater with enhanced levels of $Br^-$ and $I^-$ after disinfection using breakpoint chlorination, chloramination, or ozonation. First, we compared the cytotoxicity of wastewater before and after chlorine-based disinfection methods, *i.e.*, chlorination and chloramination, in the presence of elevated $Br^-/I^-$. To achieve breakpoint chlorination in the tested wastewater with an ammonium nitrogen concentration of 7.5 mg/L, a high sodium hypochlorite dose (75 mg/L as $Cl_2$) was required (Stover et al., 1986). The high chlorine concentration produced a finished wastewater 5 times more cytotoxic than the controls (Figs. 1 and 2). Chloramination disinfection was conducted at a $Cl_2$ to $NH_3$-N mass ratio of 2.3 to generate monochloramine, following previous studies (Mitch, 2009; Stover et al., 1986). Despite the much lower chlorine dose (17.3 mg/L as $Cl_2$) than chlorination, chloramination of the $Br^-/I^-$ amended wastewater induced a significantly higher cytotoxicity than chlorination ($p < 0.05$), and was more than 6 times more cytotoxic than the negative controls (Figs. 1 and 2). This is an important finding because both chlorination and chloramination of wastewaters are common. The disinfection by chlorine-based chemicals (chlorine or chloramines) has been shown to generate a wide range of DBPs by reactions between the organics in the source water and the disinfectants (Crittenden et al., 2012; Reckhow et al., 1990). The observed lower cytotoxicity after chlorination compared to chloramination in the presence of $Br^-/I^-$ could be explained in part by the fast reaction between free chlorine with hypoiodous acid to iodate (Bichsel and Von Gunten, 1999, 2000). This fast reaction with free chlorine compared to those with chloramines might have prevented the formation of I-DBPs, which are a much more cytotoxic group of DBPs compared to Cl-DBPs (Plewa and Wagner, 2009; Richardson et al., 2008).

Ozonation produced finished wastewater significantly less cytotoxic than either chlorination (75% less toxic) or chloramination (80% less toxic) (Figs. 1 and 2). These findings are consistent with the results from a previous study (Dong et al., 2016). In addition, the observed cytotoxicity between ozonated and non-disinfected controls was similar ($p > 0.05$).

During ozonation, $I^-$ was oxidized primarily to iodate, and $Br^-$ was oxidized to both bromate and hypobromous acid, the latter of which can react with WOM to form a number of Br-DBPs (Haag and Hoigne, 1983; Von Gunten, 2003). The formation of Br-DBPs would be exacerbated during ozonation of wastewaters due to the high concentration of wastewater organics compared to the source water for drinking water (Metcalf & Eddy Inc., 2013; Wert et al., 2007). It is important to note that bromate is a regulated DBP (US Environmental Protection Agency, 2006). The data from the present study demonstrate the impact of elevated $Br^-/I^-$ in municipal secondary effluent after alternative disinfection processes. Ozonation produced disinfected wastewater with the lowest mammalian cell cytotoxicity, as compared to chlorination or chloramination disinfection. Although more regulated DBPs may have been produced by chlorination, lower overall cytotoxicity was observed with chlorination than chloramination. However, for secondary effluent wastewaters with high ammonia content, such as the one used in this study, very high chlorine concentrations would be needed to reach breakpoint and may not be feasible for utilities.

These data suggest that a higher potential to produce regulated DBPs may not suggest higher overall adverse biological effects of the disinfected wastewater. These data also demonstrate the importance of the chemical composition of the wastewater effluent (*e.g.*, concentration of $Br^-/I^-$) and the disinfection method employed. The findings are particularly of interest to municipal wastewater effluents from regions with source waters contaminated with high $Br^-/I^-$ levels, such as in coastal regions. The use of ozone to disinfect municipal wastewater effluents may lower the adverse biological impact of these effluents and protect the public health.

## Acknowledgment

This project was partially supported by grant RD83582201-0 from the U.S. Environmental Protection Agency (EPA) and a USDA grant 2017-68007-26307. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the EPA. We acknowledge Professor J. Gary Eden and EP Purification Inc. for providing the microchannel plasma ozone generator. The EPA does not endorse the

purchase of any commercial products or services mentioned in the publication.

## REFERENCES


Agus, E., Voutchkov, N., Sedlak, D.L., 2009. Disinfection by-products and their potential impact on the quality of water produced by desalination systems: a literature review. Desalination 237, 214–237.

Bichsel, Y., Von Gunten, U., 1999. Oxidation of iodide and hypoiodous acid in the disinfection of natural waters. Environ. Sci. Technol. 33, 4040–4045.

Bichsel, Y., Von Gunten, U., 2000. Formation of iodo-trihalomethanes during disinfection and oxidation of iodide-containing waters. Environ. Sci. Technol. 34, 2784–2791.

Cho, M., Yoon, J., 2007. Quantitative evaluation and application of *Cryptosporidium parvum* inactivation with ozone treatment. Water Sci. Technol. 55, 241–250.

Corona-Vasquez, B., Samuelson, A., Rennecker, J.L., Mariñas, B.J., 2002. Inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. Water Res. 36, 4053–4063.

Crittenden, J.C., Trussell, R.R., Hand, D.W., Howe, K.J., Tchobanoglous, G., 2012. MWH's Water Treatment: Principles and Design. third ed. John Wiley & Sons.

Dong, S., 2016. Disinfection of Wastewater for Reuse Using Microchannel Plasma Ozone Generator. Doctoral Dissertation, Department of Civil and Environmental Engineering. University of Illinois Urbana–Champaign, Urbana.

Dong, S., Lu, J., Plewa, M.J., Nguyen, T.H., 2016. Comparative mammalian cell cytotoxicity of wastewaters for agricultural reuse after ozonation. Environ. Sci. Technol. 50, 11752–11759.

Gottschalk, C., Libra, J.A., Saupe, A., 2010. Ozonation of Water and Waste Water: A Practical Guide to Understanding Ozone and Its Applications. second ed. John Wiley & Sons.

Haag, W.R., Hoigne, J., 1983. Ozonation of bromide-containing waters: kinetics of formation of hypobromous acid and bromate. Environ. Sci. Technol. 17, 261–267.

Kim, J.-H., von Gunten, U., Mariñas, B.J., 2004. Simultaneous prediction of *Cryptosporidium parvum* oocyst inactivation and bromate formation during ozonation of synthetic waters. Environ. Sci. Technol. 38, 2232–2241.

Kim, J.-H., Elovitz, M.S., Von Gunten, U., Shukairy, H.M., Mariñas, B.J., 2007. Modeling *Cryptosporidium parvum* oocyst inactivation and bromate in a flow-through ozone contactor treating natural water. Water Res. 41, 467–475.

Korich, D., Mead, J., Madore, M., Sinclair, N., Sterling, C.R., 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. Appl. Environ. Microbiol. 56, 1423–1428.

Kronberg, L., Holmbom, B., Reunanen, M., Tikkanen, L., 1988. Identification and quantification of the Ames mutagenic compound 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone and of its geometric isomer (E)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid in chlorine-treated humic water and drinking water extracts. Environ. Sci. Technol. 22, 1097–1103.

Ludzack, F., Noran, D., 1965. Tolerance of high salinities by conventional wastewater treatment processes. J. Water Pollut. Control Fed. 37, 1404–1416.

Majumdar, S.B., Ceckler, W.H., Sproul, O.J., 1973. Inactivation of poliovirus in water by ozonation. J. Water Pollut. Control Fed. 2433–2443.

Metcalf & Eddy Inc., 2013. Wastewater Engineering: Treatment and Resource Recovery. fifth ed. New York, McGraw-Hill Education.

Mitch, W.A., 2009. Occurrence and Formation of Nitrogenous Disinfection by-Products. Water Research Foundation.

National Research Council Committee, 2012. Water Reuse: Potential for Expanding the Nation's Water Supply through Reuse of Municipal Wastewater. National Academies Press.

Plewa, M.J., Wagner, E.D., 2009. Mammalian Cell Cytotoxicity and Genotoxicity of Disinfection by-Products. Water Research Foundation.

Plewa, M.J., Kargalioglu, Y., Vankerk, D., Minear, R.A., Wagner, E.D., 2002. Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. Environ. Mol. Mutagen. 40, 134–142.

Qi, Y., Shang, C., Lo, I.M., 2004. Formation of haloacetic acids during monochloramination. Water Res. 38, 2375–2383.

Reckhow, D.A., Singer, P.C., Malcolm, R.L., 1990. Chlorination of humic materials: byproduct formation and chemical interpretations. Environ. Sci. Technol. 24, 1655–1664.

Richardson, S.D., Fasano, F., Ellington, J.J., Crumley, F.G., Buettner, K.M., Evans, J.J., Blount, B.C., Silva, L.K., Waite, T.J., Luther, G.W., 2008. Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. Environ. Sci. Technol. 42, 8330–8338.

Ringhand, H.P., Meier, J.R., Kopfler, F.C., Schenck, K.M., Kaylor, W.H., Mitchell, D.E., 1987. Importance of sample pH on recovery of mutagenicity from drinking water by XAD resins. Environ. Sci. Technol. 21, 382–387.

Schenck, K.M., Meier, J.R., Ringhand, H.P., Kopfler, F.C., 1990. Recovery of 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone from water samples on XAD resins and the effect of chlorine on its mutagenicity. Environ. Sci. Technol. 24, 863–867.

Seidel, C.J., McGuire, M.J., Summers, R.S., Via, S., 2005. Have utilities switched to chloramines? J. Am. Water Works Assoc. 97, 87–97.

Shin, G.-A., Sobsey, M.D., 2003. Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. Appl. Environ. Microbiol. 69, 3975–3978.

Stover, E., Haas, C., Rakness, K., Scheible, O., 1986. Design Manual: Municipal Wastewater Disinfection. US Environmental Protection Agency.

Sun, Y.-X., Wu, Q.-Y., Hu, H.-Y., Tian, J., 2009. Effect of bromide on the formation of disinfection by-products during wastewater chlorination. Water Res. 43, 2391–2398.

Tang, G., Adu-Sarkodie, K., Kim, D., Kim, J.-H., Teefy, S., Shukairy, H.M., Mariñas, B.J., 2005. Modeling *Cryptosporidium parvum* oocyst inactivation and bromate formation in a full-scale ozone contactor. Environ. Sci. Technol. 39, 9343–9350.

US Environmental Protection Agency, 2006. National primary drinking water regulations: stage 2 disinfectants and disinfection byproducts rule. Fed. Regist. 71, 387–493.

Von Gunten, U., 2003. Ozonation of drinking water: part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res. 37, 1469–1487.

Wagner, E.D., Rayburn, A.L., Anderson, D., Plewa, M.J., 1998. Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells. Environ. Mol. Mutagen. 32, 360–368.

Wagner, E.D., Plewa, M.J., 2017. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review. J. Environ. Sci. 58, 64–76.

Wert, E.C., Rosario-Ortiz, F.L., Drury, D.D., Snyder, S.A., 2007. Formation of oxidation byproducts from ozonation of wastewater. Water Res. 41, 1481–1490.

Wu, Q.-Y., Li, Y., Hu, H.-Y., Sun, Y.-X., Zhao, F.-Y., 2010. Reduced effect of bromide on the genotoxicity in secondary effluent of a municipal wastewater treatment plant during chlorination. Environ. Sci. Technol. 44, 4924–4929.

Yang, Y., Komaki, Y., Kimura, S.Y., Hu, H.-Y., Wagner, E.D., Mariñas, B.J., Plewa, M.J., 2014. Toxic impact of bromide and iodide on drinking water disinfected with chlorine or chloramines. Environ. Sci. Technol. 48, 12362–12369.

Zeng, T., Plewa, M.J., Mitch, W.A., 2016. N-nitrosamines and halogenated disinfection byproducts in US full advanced treatment trains for potable reuse. Water Res. 101, 176–186.