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The impact of iodinated X-ray contrast agents on formation and toxicity of disinfection by-products in drinking water

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ABSTRACT

The presence of iodinated X-ray contrast media (ICM) in source waters is of high concern to public health because of their potential to generate highly toxic disinfection by-products (DBPs). The objective of this study was to determine the impact of ICM in source waters and the type of disinfectant on the overall toxicity of DBP mixtures and to determine which ICM and reaction conditions give rise to toxic by-products. Source waters collected from Akron, OH were treated with five different ICMs, including iopamidol, iopromide, iohexol, diatrizoate and iomeprol, with or without chlorine or chloramine disinfection. The reaction product mixtures were concentrated with XAD resins and the mammalian cell cytotoxicity and genotoxicity of the reaction mixture concentrates was measured. Water containing iopamidol generated an enhanced level of mammalian cell cytotoxicity and genotoxicity after disinfection. While chlorine disinfection with iopamidol resulted in the highest cytotoxicity overall, the relative iopamidol-mediated increase in toxicity was greater when chloramine was used as the disinfectant compared with chlorine. Four other ICMs (iopromide, iohexol, diatrizoate, and iomeprol) expressed some cytotoxicity over the control without any disinfection, and induced higher cytotoxicity when chlorinated. Only iohexol enhanced genotoxicity compared to the chlorinated source water.

Introduction

Disinfection by-products (DBPs) in drinking water are formed through the reaction between disinfectants, natural organic matter (NOM), bromide, and iodide. Among various factors influencing the spectrum of DBPs in finished water, the composition of the source water can play an important role, especially at point sources where large amounts of wastewater contaminants are being discharged. In general, iodinated DBPs (iodo-DBPs) are known to be more cytotoxic and genotoxic than their chlorinated or brominated analogues (Plewa et al., 2004; Richardson et al., 2007). Naturally occurring iodide in source waters was thought to be the only precursor in iodo-DBPs formation, and it was shown that increase in natural iodide leads to higher level of iodo-DBPs formed (Bichsel and von Gunten, 2000; Richardson et al., 2008). However, there is a new concern that iodine-containing pharmaceuticals could also serve as precursors to highly toxic iodo-DBPs in drinking water

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(Duirk et al., 2011; Kormos et al., 2011; Wendel et al., 2014; Yang et al., 2016).

Iodinated X-ray contrast media (ICM) are widely used at hospitals and medical centers for tissue imaging, such as organs and blood vessels. The molecular structure of ICM consists of 2,4,6-triiodinated benzoic derivatives, with molecular weights varying between 600 and 900 Da, depending on the type of amide side chains (Fig. 1). The iodine atoms are responsible for the absorption of X-rays, and the compounds are designed to be persistent and polar so that they can be excreted within few hours after application. Typically, 95% of non-metabolized ICM are eliminated through urine and feces within 24 hr after application (Perez et al., 2006). ICM are not completely removed during the wastewater treatment, allowing them to enter source waters and serve as sources of iodine to form iodo-DBPs (Duirk et al., 2011; Richardson et al., 2008; Ternes and Hirsch, 2000; Wendel et al., 2014; Yang et al., 2016; Ye et al., 2014).

Few ICM occurrence studies in source waters have been performed in the United States. Duirk et al., examined the source waters for commonly used ICM from ten of the 23 cities in which iodide was not detected from a previous iodo-DBP occurrence study, and detected four ICM, including iopamidol, iopromide, iohexol, and diatrizoate (Fig. 1) (Duirk et al., 2011). Iopamidol was detected most frequently with a maximum concentration of 2.7 μg/L. Controlled laboratory reactions of these ICM were then conducted using chlorine and chloramine disinfection, and iodo-DBPs were found, including iodo-trihalomethanes and iodo-acids (Duirk et al., 2011). The chemical mechanism involved in the formation of iodo-DBPs by ICM is different from the mechanism involving naturally occurring iodide. Reactions with iopamidol appear to involve an initial attack by chlorinated oxidants at one of amide iopamidol side chains resulting in the release of iodine, which reacts with NOM to form iodo-DBPs (Wendel et al., 2014, 2016).

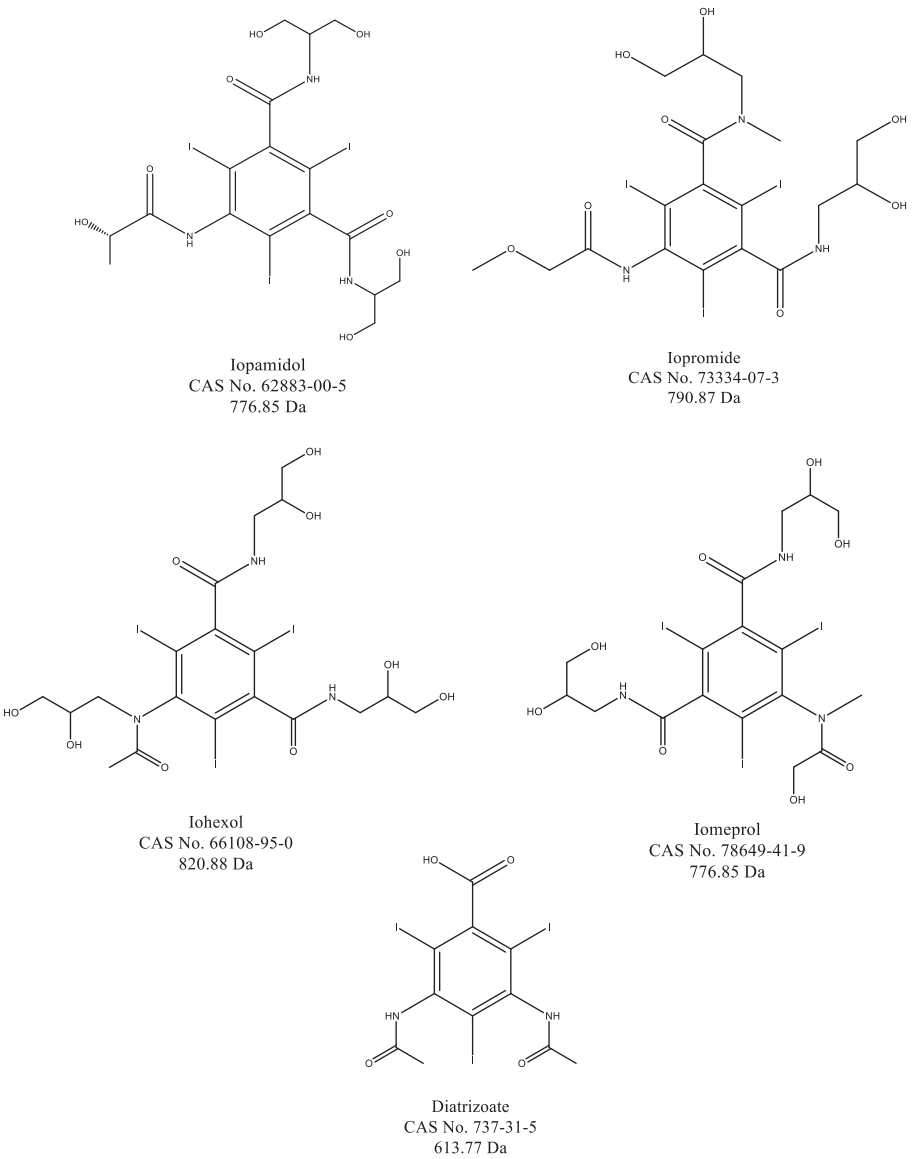

Fig. 1 – Chemical structures of five common iodinated X-ray contrast media.

Many studies focused on the involvement of iopamidol after chlorination or chloramination on the chemistry of the transformation products and the generation of mammalian cell cytotoxic and genotoxic DBPs (Duirk et al., 2011; Wendel et al., 2014, 2016). However, it is unclear whether different ICM undergo unique transformation processes when they react with disinfectants to generate different DBP mixtures. The overall hypothesis of this study is that oxidizing disinfectants react with non-toxic ICM to form toxic low molecular weight iodo-DBPs and higher molecular weight DBPs of unknown toxicity in NOM-containing source waters. To test this hypothesis, controlled laboratory reactions of five ICMs (iopamidol, diatrizoate, iopromide, iomeprol, and iohexol) with disinfectants were conducted to simulate drinking water treatment. The specific objectives of this study were to (i) disinfect source waters with chlorine or chloramine and with or without iopamidol, and determine the relative *in vitro* chronic cytotoxicity and acute genotoxicity in mammalian cells for each reaction concentrate, (ii) disinfect the source waters with chlorine and with or without the presence of four ICM (iopromide, iohexol, diatrizoate, iomeprol), and determine the relative *in vitro* chronic cytotoxicity and acute genotoxicity in mammalian cells for each reaction concentrate, and, (iii) analyze for the impact of disinfectant types and individual ICM on overall toxicity.

1. Materials and methods

1.1. Chemicals and reagents

General reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO) and Fisher Scientific Co. (Itasca, IL). Ham's F12 medium and fetal bovine serum (FBS) were purchased from Fisher Scientific Co. (Itasca, IL).

1.2. Characterization of source water and sample preparation

Source waters for this study were obtained from Akron, OH. The characteristics of source water are summarized in Table 1. Total organic carbon (TOC) concentrations were measured using a Shimadzu TOC analyzer (Shimadzu Scientific, Columbia, MD, USA) and calibrated according to Standard Method 505A (APHA, 2005). The ultraviolet absorbance at 254 nm (UV_{254}) and spectral characteristics of the NOM were measured with Shimadzu UV 1601 ultraviolet (UV)–visible spectrophotometer in accordance with Standard Method 5910 (APHA, 2005). The specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was calculated from the relation: $SUVA_{254} = UV_{254}/DOC$ (dissolved organic carbon). Bromide and iodide were analyzed using a Dionex ICS-3000 ion chromatograph system (Dionex Corporation, Sunnyvale, CA, USA). Source waters were filtered through 0.45 μm Whatman nylon membrane filters (West Chester, PA, USA) and stored at 4°C prior to use. No iodo-acids were detected when directly measured from those samples. Total iodo-trihalomethanes for the chlorine experiment was 273.5 ± 13.9 nmol/L and monochloramine experiment was 9.0 ± 1.2 nmol/L. To determine the overall cytotoxicity and genotoxicity of reaction concentrates with chlorine or chloramine and with or without iopamidol addition, 20 L of source water was collected for each reaction condition (Table 2). To determine the overall cytotoxicity and genotoxicity of reaction concentrates with chlorine and with or without four different ICM (iopromide, iohexol, iomeprol and diatrizoate), 20 L of source water was used for each reaction condition (Table 3). Briefly, 20 L reactors of Akron source water with 5 μmol/L iopamidol, 100 μmol/L chlorine or monochloramine, at pH 7.5, and 10 mmol/L phosphate buffer were allowed to react for 72 hr in the dark prior to extraction. Controls included: deionized waste, Akron source water, Akron source water spiked with iopamidol, and Akron source water treated with chlorine or monochloramine without iopamidol. After reaction, water samples were extracted using XAD2 and XAD8 resins, eluted with ethyl acetate, and concentrated to 2 mL for the biological experiments as described previously (Richardson, 2011). The ethyl acetate extracts were solvent-exchanged into dimethyl sulfoxide (DMSO). These samples were stored in Supelco glass vials with Teflon cap liners at –20°C.

Table 1 – Characteristics of source waters from Akron Water Treatment Plant.

Parameter	Akron source water
DOC (mg/L C)	5.57
Bromide (μmol/L)	<0.50
Iodide (μmol/L)	<0.50
UV_{254} (cm^{-1})	0.121
$SUVA_{254}$ (L/(mg·m))	2.17

SUVA: specific ultraviolet absorbance; DOC: Dissolved organic carbon; UV: Ultraviolet.

1.3. Chinese hamster ovary (CHO) cells

The CHO cell line AS52 was used for the biological assays (Hsie et al., 1975a, 1975b; Tindall and Stankowski, 1989; Tindall et al., 1984; Wagner et al., 1998a, 1998b). CHO cells were maintained on glass culture plates in Ham's F12 medium containing 5% FBS, 1% antibiotics (100 U/mL sodium penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B in 0.85% saline), and 1% glutamine at 37°C in a humidified atmosphere of 5% CO_2.

Table 2 – Akron source water (SW) with and without iopamidol (IDOL) and disinfected with either chlorine (HOCl) or monochloramine (NH_2Cl).

Sample [a]	Source water	Iopamidol	Disinfection
SW	Akron	NA [b]	NA
SW + IDOL	Akron	5 μmol/L	NA
SW + HOCl	Akron	NA	$[Cl_2]_T$ = 100 μmol/L
SW + HOCl + IDOL	Akron	5 μmol/L	$[Cl_2]_T$ = 100 μmol/L
SW + NH_2Cl	Akron	NA	$[NH_2Cl]_T$ = 100 μmol/L
SW + NH_2Cl + IDOL	Akron	5 μmol/L	$[NH_2Cl]_T$ = 100 μmol/L

[a] Experiments were performed on 20 L of water sources. Each sample was extracted using ethyl acetate and concentrated to a final volume of 2 mL.
[b] NA: not applied.

Table 3 – Akron source water (SW) with four iodinated X-ray contrast media (ICM), with, and without chlorine (HOCl) disinfection.

Sample [a]	ICM	ICM concentration	Disinfection
SW + Iopromide	Iopromide	5 μmol/L	NA [b]
SW + HOCl + Iopromide	Iopromide	5 μmol/L	$[Cl_2]_T$ = 100 μmol/L
SW + Iohexol	Iohexol	5 μmol/L	NA
SW + HOCl + Iohexol	Iohexol	5 μmol/L	$[Cl_2]_T$ = 100 μmol/L
SW + Diatrizoate	Diatrizoate	5 μmol/L	NA
SW + HOCl + Diatrizoate	Diatrizoate	5 μmol/L	$[Cl_2]_T$ = 100 μmol/L
SW + Iomeprol	Iomeprol	5 μmol/L	NA
SW + HOCl + Iomeprol	Iomeprol	5 μmol/L	$[Cl_2]_T$ = 100 μmol/L

[a] Experiments were performed on 20 L of water sources. Each sample was extracted using ethyl acetate and concentrated to a final volume of 2 mL.
[b] NA: not applied.

1.4. CHO cell chronic cytotoxicity assay

This assay measures the reduction in cell density as a function of the water sample concentrates over a period of approximately three-four cell divisions (72 hr). Chronic cytotoxicity to CHO cells was measured using an assay we previously developed for the analysis of DBPs (Plewa et al., 2002; Plewa and Wagner, 2009). For each experiment, a series of dilutions were prepared by diluting the concentrates with F12 culture medium on the day of the experiment and rapidly transferring into the wells with CHO cells for treatment. These dilution series represent a range of concentration factors for the organics in the original water. Flat-bottom, tissue culture 96-well microplates were employed; 4 replicate wells were prepared for each concentration of each water sample extract. Eight wells were reserved for the blank control consisting of 200 μL of F12 + 5% FBS. The negative control consisted of 8 wells containing 100 μL of a titered CHO cell suspension (3×10^4 cells/mL) plus 100 μL F12 + 5% FBS. The wells for the remaining columns contained 3000 CHO cells, F12 + 5% FBS, and a known concentration of a water sample organic extract (200 μL). To prevent sample evaporation or cross contamination between wells due to volatilization of the organic extract, a sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) was pressed over the wells before the microplate was covered. To distribute the cells uniformly, the microplate was placed on a rocking platform for 10 min, and then placed in a tissue culture incubator for 72 hr. After incubation, each well was gently aspirated, fixed in 100% methanol for 10 min, and stained for 10 min with a 1% crystal violet solution in 50% methanol. The plate was gently washed in tap water, inverted and tapped dry upon paper towels, and 50 μL of DMSO/methanol (3:1, V/V) was added to each well for 10 min. The plate was analyzed in a microplate reader at 595 nm. The data were automatically recorded and transferred to an Excel spreadsheet on a microcomputer connected to the microplate reader. The blank-corrected absorbance value of the negative control (cells with medium only) was set at 100%. The absorbance for each treatment group was converted into a percentage of the negative control. For each organic extract concentration, 4–8 replicate wells were analyzed per experiment, and the experiments were repeated 2–3 times. A concentration-response curve was generated for each water sample extract, and a regression analysis was conducted for each curve. The lethal dose (LC_{50}) values were calculated from each regression analysis, where the LC_{50} represents the concentration factor that induced a 50% reduction in cell density as compared to the concurrent negative control. A DMSO control demonstrated that no significant cytotoxicity was induced throughout the solvent concentration used in these experiments.

1.5. Single cell gel electrophoresis (SCGE) assay

SCGE is a molecular genetic assay that quantitatively measures the level of genomic deoxyribonucleic acid (DNA) damage induced in individual nuclei of treated cells (Fairbairn et al., 1995; Rundell et al., 2003; Tice et al., 2000). We employed the microplate SCGE method (Wagner and Plewa, 2009). The day before treatment, 4×10^4 CHO cells were added to each microplate well in 200 μL of F12 + 5% FBS and incubated. The next day, the cells were washed with Hank's balanced salt solution (HBSS) and treated with a series of concentrations of an organic extract from the concentrates in F12 medium without FBS in a total volume of 25 μL for 4 hr at 37°C, 5% CO_2. The wells were covered with sterile AlumnaSeal ™. After incubation, the cells were washed 2× with HBSS and harvested with 50 μL of 0.01% trypsin +53 μmol/L EDTA. The trypsin was inactivated with 70 μL of F12 + FBS. Acute cytotoxicity was measured from a 10 μL aliquot of cell suspension mixed with 10 μL of 0.05% trypan blue vital dye in phosphate-buffered saline (PBS) (Phillips, 1973). SCGE data were not used if the acute cytotoxicity exceeded 30%. The remaining cell suspension from each well was embedded in a layer of low melting point agarose prepared with PBS upon clear microscope slides that were previously coated with a layer of 1% normal melting point agarose prepared with deionized water and dried overnight. The cellular membranes were removed by an overnight immersion in lysing solution (2.5 mol/L NaCl, 100 mmol/L Na_2EDTA, 10 mmol/L Tris, 1% sodium sarcosinate, 1% Triton X-100, and 10% DMSO) at 4°C. The microgels were placed in an alkaline buffer (1 mmol/L Na_2EDTA, 300 mmol/L NaOH, pH 13.5) in an electrophoresis tank, and the DNA was denatured for 20 min. The microgels were electrophoresed at 25 V, 300 mA (0.72 V/cm) for 40 min at 4°C. The microgels were neutralized with Tris buffer (pH 7.5), rinsed in cold water, dehydrated in cold methanol, dried at 50°C, and stored at room temperature in a covered slide box. The next day, the microgels were hydrated in cold water for 30 min and stained with 65 μL of

ethidium bromide (20 μg/mL) for 3 min. The microgels were rinsed in cold water and analyzed with a Zeiss fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. For each experiment, 2 microgels were prepared per treatment group. Randomly chosen nuclei (25 per microgel) were analyzed using a charged coupled device camera. A computerized image analysis system (Comet IV, Perspective Instruments, Ltd., Suffolk, UK) was employed to determine the SCGE %Tail DNA value of the nuclei as indices of DNA damage (Kumaravel and Jha, 2006). The digitalized data were automatically transferred to a computer-based spreadsheet for subsequent statistical analysis. Within each experiment, a negative control, a positive control (3.8 mmol/L ethylmethanesulfonate), and concentration series of an organic extract were analyzed concurrently. The experiments were repeated 2–3 times for each sample. Within each concentration factor range with >70% cell viability, a concentration-response curve was generated for each sample from repeated experiments, and non-linear regression analysis was conducted. The concentration factor that induces 50% of the genomic DNA to migrate from the nucleus (50%Tail DNA value) was calculated from each regression analysis.

1.6. Statistical analysis

For the cytotoxicity assay, a one-way analysis of variance (ANOVA) test was conducted to determine if the sample extract induced a statistically significant level of cell death at a specific concentration. If a significant F value ($P \leq 0.05$) was obtained, a Holm–Sidak multiple comparison *versus* the control group analysis was performed to identify the lowest cytotoxic concentration. The power of the test statistic ($1 - \beta$) was maintained as ≥0.8 at $\alpha = 0.05$. For the SCGE assay, the %Tail DNA values are not normally distributed which limits the use of parametric statistics (Box et al., 1978). The mean %Tail DNA value for each microgel was calculated and these values were averaged among all of the microgels for each sample concentration. A one-way ANOVA test was conducted on these averaged %Tail DNA values (Lovell and Omori, 2008). If a significant *F* value of $P \leq 0.05$ was obtained, a Holm–Sidak multiple comparison *versus* the control group analysis was conducted with the power ≥0.8 at $\alpha = 0.05$. A bootstrap statistical approach was used to generate a series of multiple LC_{50} values and %Tail DNA per sample; for each LC_{50} value, a cytotoxicity index (CTI) value was calculated as $(LC_{50}^{-1})(10^3)$ and for each 50%Tail DNA value, a genotoxicity index (GTI) value was calculated as $(50\%\text{Tail DNA}^{-1})(10^4)$. These values were then analyzed using an ANOVA test to determine significant differences among the sample (Efron, 1987; Singh and Xie, 2008).

2. Results and discussion

2.1. Iopamidol generated an enhanced level of CHO cell cytotoxicity and genotoxicity in conjunction with chlorine or chloramine disinfection

2.1.1. CHO cell chronic cytotoxicity analyses

CHO cell cytotoxicity concentration–response curves for each condition were generated. Table 4 presents the LC_{50} concentration factor for each sample and the lowest concentration factor that induced a significant increase from the negative control. A statistical analysis for each cytotoxicity concentration-response curve and an ANOVA test against the negative control is also presented in Table 4. The mean CTIs are presented in Table 4. From an ANOVA test of the bootstrap mean CTI values, significant differences were resolved among the samples and are presented in Table 5.

Iopamidol induced a significant increase in cytotoxicity as compared to the source water, while no significant difference was found between the source water plus iopamidol and the source water disinfected with chloramine (Table 5). The chlorinated source water was significantly more cytotoxic than the source water alone or the source water disinfected with chloramine (NH_2Cl). Chlorinated source water plus iopamidol showed the highest CTI value (21) followed by the chlorinated source water without iopamidol (CTI = 19). A clear significant increase in cytotoxicity was induced by iopamidol in chloraminated water (CTI with iopamidol = 17, CTI without iopamidol = 7.7). Of importance is that the relative iopamidol-mediated increase in CHO cell cytotoxicity was much greater

Table 4 – Experimental group 1: comparative CHO cell chronic cytotoxicity of X-ray contrast agent iopamidol in Akron source water (SW) with and without iopamidol (IDOL) and disinfected with either chlorine (HOCl) or monochloramine (NH_2Cl).

Sample	LC_{50} (CF) [a]	r^2 [b]	Mean CTI [c]	Lowest cytotoxic conc. factor [d]	ANOVA test statistic [e]
SW	161	0.94	6.2	75	$F_{10, 50} = 53.5$; $P \leq 0.001$
SW + IDOL	124	0.96	8.1	75	$F_{9, 48} = 168$; $P \leq 0.001$
SW + HOCl	53.0	0.94	19	25	$F_{10, 45} = 141$; $P \leq 0.001$
SW + HOCl + IDOL	47.6	0.97	21	25	$F_{11, 122} = 142$; $P \leq 0.001$
SW + NH_2Cl	129	0.94	7.8	100	$F_{10, 50} = 72.3$; $P \leq 0.001$
SW + NH_2Cl + IDOL	60.3	0.99	17	25	$F_{10, 125} = 154$; $P \leq 0.001$

ANOVA: analysis of variance; CF: concentration factor.

[a] The LC_{50} value is the fold concentration factor of the water source control (WSC) sample, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls.

[b] r^2 is the coefficient of determination for the regression analysis upon which the LC_{50} value was calculated.

[c] CTI = cytotoxicity index, calculated as $(LC_{50})^{-1}(10^3)$.

[d] Lowest cytotoxic concentration was the lowest concentration factor of the sample in the concentration-response curve that induced a statistically significant reduction in cell density as compared to the concurrent negative controls.

[e] The degrees of freedom for the between-groups and residual associated with the calculated F-test result and the resulting probability value.

Table 5 – Test for significance among GTI values.

	SW	IDOL	HOCl	HOCl-IDOL	NH_2Cl	NH_2Cl-IDOL
SW						
IDOL						
HOCl						
HOCl-IDOL						
NH_2Cl						
NH_2Cl-IDOL						

Abbreviations; SW = Akron source water, IDOL = iopamidol, HOCl = source water plus chlorine disinfection, HOCl-IDOL = source water plus iopamidol plus chlorine disinfection, NH_2Cl = source water plus chloramine disinfection, NH_2Cl-IDOL = source water plus iopamidol plus chloramine disinfection; CTI: cytotoxicity index. In the pairwise comparisons, red indicates a significant difference between the paired groups, green indicates no significant difference between the paired groups.

when NH_2Cl was used as the disinfectant *versus* chlorine. The relative iopamidol-mediated percent increase in cytotoxicity over the chlorinated Akron source water was 11.3%, while the relative iopamidol-mediated cytotoxicity associated with chloramine disinfection was 114%. An explanation of these results may be that, compared to chlorine, chloramine disinfection enhances the formation of highly toxic iodo-DBPs in the presence of NOM (Bichsel and von Gunten, 1999, 2000; Krasner et al., 2006; Richardson et al., 2008). Previously, we proposed an extensive pathway for formation of iodo-DBPs from iopamidol (Wendel et al., 2014). These data demonstrate that iopamidol enhances the cytotoxicity of disinfected source water. The iopamidol-mediated increase in CHO cell toxicity was also observed in a previous study using source waters from Athens, GA (Duirk et al., 2011). Interestingly, the relative iopamidol-mediated percent increase in cytotoxicity over the chlorinated Athens source water was 49%, while there was no change in iopamidol-mediated cytotoxicity associated with chloramine disinfection (changed less than 5%), which suggests that the increased cytotoxicity depends on the NOM composition in source water (Athens, GA *versus* Akron, OH).

2.1.2. CHO cell acute genotoxicity analyses

The CHO cell SCGE genotoxicity concentration–response curves for each condition were generated. Table 6 presents a statistical analysis for each genotoxicity concentration-response curve and a test against the negative control. Table 6 presents the 50%Tail DNA concentration factors and the lowest concentration factor that induced a significant increase over the negative control. The mean GTIs are presented in Table 6. From an ANOVA test of the bootstrap mean GTI values, significant differences were resolved among the sample; these differences are presented in Table 7.

The presence of iopamidol enhanced the genotoxicity of the source waters. No significant difference was observed between the source water and the source water after chloramination (Table 7). The chlorinated source water was significantly more genotoxic than the source water alone or the chloraminated source water (Fig. 3). An increase in genotoxicity was induced by iopamidol alone as compared to the source water. Chlorinated water with iopamidol was significantly more genotoxic than the chlorinated source water alone. A significant increase in genotoxicity was induced by iopamidol in chloraminated water, but the GTI for chloraminated water with iopamidol (35.8) was smaller than the GTI for chlorinated water only (44.8). The relative iopamidol-mediated percent increase in genotoxicity over the chlorinated Akron source water was 64%, while the relative iopamidol-mediated percent increase in genotoxicity over the chloraminated water was 97%. As with the CHO cytotoxicity data, an explanation of these results may be that chloramine disinfection enhances the generation of highly toxic iodo-DBPs in the presence of NOM (Bichsel and von Gunten, 1999, 2000; Krasner et al., 2006; Richardson et al., 2008). These data demonstrate that besides affecting cytotoxicity, iopamidol enhances the genotoxicity of disinfected source water. These results were in agreement with previously published work (Duirk et al., 2011) and indicated that the iopamidol-mediated increased genotoxicity was independent of source water. Overall, the data suggest that iodo-DBPs, which are more toxic than chlorinated or brominated DBPs, (Plewa and Wagner, 2009; Richardson et al., 2007) were generated from

Table 6 – Comparative CHO cell acute genotoxicity of X-ray contrast agent iopamidol in Akron source water (SW) with and without iopamidol (IDOL) and disinfected with either chlorine (HOCl) or monochloramine (NH_2Cl).

Sample	50%Tail DNA (CF) [a]	r^2 [b]	Mean GTI [c]	Lowest genotoxic conc. factor [d]	ANOVA test statistic [e]
SW	680	0.99	14.7	650	$F_{14, 39} = 12.0$; $P \leq 0.001$
SW + HOCl	223	0.96	44.8	175	$F_{9, 40} = 20.4$; $P \leq 0.001$
SW + IDOL	527	0.58	19.0	250	$F_{10, 43} = 3.35$; $P \leq 0.003$
SW + HOCl + IDOL	136	0.99	73.5	100	$F_{6, 21} = 32.8$; $P \leq 0.001$
SW + NH_2Cl	545	0.99	18.3	350	$F_{14, 27} = 65.6$; $P \leq 0.001$
SW + NH_2Cl + IDOL	279	0.98	35.8	250	$F_{12, 29} = 60.6$; $P \leq 0.001$

ANOVA: analysis of variance; SCGE: single cell gel electrophoresis; DNA: deoxyribonucleic acid; CF: concentration factor.

[a] The SCGE 50% Tail DNA value is the WSC sample concentration factor determined from a regression analysis of the data that was calculated to induce a 50% SCGE Tail DNA value.

[b] r^2 is the coefficient of determination for the regression analysis upon which the SCGE %Tail DNA value was calculated.

[c] GTI = genotoxicity index, calculated as $(50\%\text{Tail DNA})^{-1}(10^4)$.

[d] The lowest genotoxic concentration was the lowest concentration factor of the WSC sample in the concentration-response curve that induced a statistically significant amount of genomic DNA damage as compared to the negative control.

[e] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test result and the resulting probability value.

Table 7 – Test for significance among GTI values.

	SW	IDOL	HOCl	HOCl-IDOL	NH_2Cl	NH_2Cl-IDOL
SW						
IDOL						
HOCl						
HOCl-IDOL						
NH_2Cl						
NH_2Cl-IDOL						

Abbreviations: SW = Akron source water, IDOL = iopamidol, HOCl = source water plus chlorine disinfection, HOCl-IDOL = source water plus iopamidol plus chlorine disinfection, NH_2Cl = source water plus chloramine disinfection, NH_2Cl-IDOL = source water plus iopamidol plus chloramine disinfection; GTI: genotoxicity index. In the pairwise comparisons, red indicates a significant difference between the paired groups, green indicates no significant difference between the paired groups.

iodide released from iopamidol upon disinfection, leading to enhanced cytotoxicity and genotoxicity.

2.2. Other ICM generated enhanced level of CHO cell cytotoxicity and genotoxicity in conjunction with chlorine disinfection at different extents

2.2.1. CHO cell chronic cytotoxicity analyses

For the experiments with the remaining ICMs, Table 8 presents a statistical analysis within each cytotoxicity concentration-response curve and an ANOVA test against the negative control. Table 8 also presents the LC_{50} concentration factors and the lowest concentration factor that induced a significant increase from the negative control. The mean bootstrap CTIs (±SE) are presented in Fig. 2. The significant differences in CTI values among the concentrates are presented in Table 9. Cytotoxicity significantly increased for all chlorinated groups compared to their non-chlorinated groups. Based on the CTI values, iopromide showed the lowest increase (41% higher compared to the non-chlorinated water with iopromide) while iohexol showed the highest

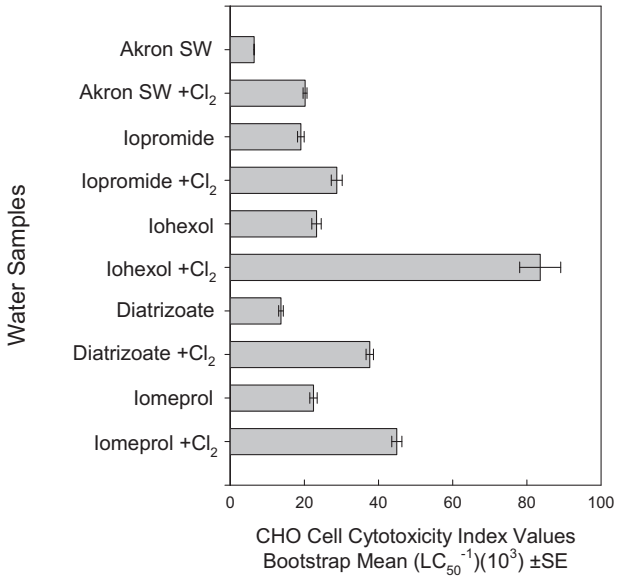

Fig. 2 – Chinese hamster ovary (CHO) cell chronic cytotoxicity index (CTI) values.

increase (224% higher compared to the non-chlorinated water with iohexol) among the four ICM pairs. The difference in cytotoxicity among the four ICMs suggests that different spectrum of ICM transformation products are generated during the reaction depending on the type of ICM. For further understanding, ICM transformation products in the reaction mixtures should be identified and their toxicities need to be determined.

2.2.2. CHO cell Acute genotoxicity analyses

For the experiments with the remaining ICM, Table 10 presents a statistical analysis for each genotoxicity concentration-response curve and a test against the negative control. The 50%Tail DNA concentration factors and the lowest concentration factor that induced a significant increase over the negative

Table 8 – Comparative CHO cell chronic cytotoxicity of X-ray contrast media iopromide, iohexol, diatrizoate and iomeprol in Akron source water (SW) samples with and without chlorine disinfection.

Sample	LC_{50} (CF) [a]	r^2 [b]	Lowest cytotoxic conc. factor [c]	ANOVA test statistic [d]
SW + IPRO	53.4	0.92	25	$F_{10, 117} = 39.7$; $P \leq 0.001$
SW + IPRO + HOCl	38.0	0.93	15	$F_{11, 172} = 22.9$; $P \leq 0.001$
SW + IHX	46.6	0.96	20	$F_{16, 167} = 16.4$; $P \leq 0.001$
SW + IHX + HOCl	14.4	0.93	7.5	$F_{11, 124} = 26.1$; $P \leq 0.001$
SW + DTZ	74.0	0.87	20	$F_{15, 168} = 24.9$; $P \leq 0.001$
SW + DTZ + HOCl	26.9	0.96	15	$F_{11, 124} = 37.7$; $P \leq 0.001$
SW + IOME	44.0	0.96	10	$F_{10, 85} = 26.7$; $P \leq 0.001$
SW + IOME + HOCl	22.9	0.99	7.5	$F_{15, 164} = 31.9$; $P \leq 0.001$

CHO: Chinese hamster ovary.

[a] The LC_{50} value is the fold concentration factor of the WCS sample, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls.

[b] r^2 is the coefficient of determination for the regression analysis upon which the LC_{50} value was calculated.

[c] Lowest cytotoxic concentration was the lowest concentration factor of the WSC in the concentration–response curve that induced a statistically significant reduction in cell density as compared to the concurrent negative controls.

[d] The degrees of freedom for the between-groups and residual associated with the calculated F-test result and the resulting probability value.

Table 9 – Test for significance among CTI values.

	SW	HOCl	IPRO	IPRO-HOCl	IHX	IHX-HOCl	DTZ	DTZ-HOCl	IOME	IOME-HOCl
SW										
Cl										
IPRO										
IPRO-HOCl										
IHX										
IHX-HOCl										
DTZ										
DTZ-HOCl										
IOME										
IOME-HOCl										

Abbreviations; SW = Akron source water, HOCl = source water plus chlorine disinfection, IPRO = iopromide, IPRO–HOCl = iopromide plus chlorine disinfection, IHX = iohexol, IHX–HOCl = iohexol plus chlorine disinfection, DTZ = diatrizoate, DTZ–HOCl = diatrizoate plus chlorine disinfection, IOME = iomeprol, IOME–HOCl = iomeprol plus chlorine disinfection; CTI: cytotoxicity index. All treated waters contain Akron source waters. In the pairwise comparisons, red indicates a significant difference between the paired groups, green indicates no significant difference between the paired groups.

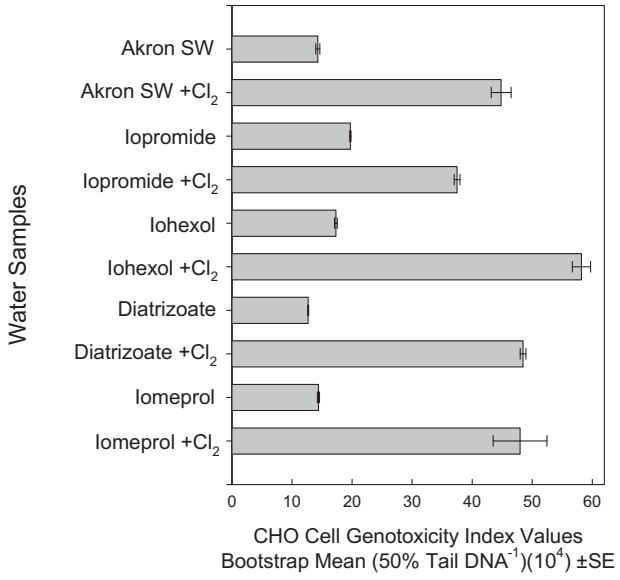

Fig. 3 – CHO cell acute genotoxicity index (GTI) values.

control are presented in Table 10. The mean bootstrap GTIs (± SE) are presented in Fig. 3. Significant differences of GTI values among concentrates are presented in Table 11.

The genotoxicity of iopromide, iohexol, diatrizoate or iomeprol in source water were not significantly different from the source water. Chlorination of ICM containing waters significantly increased the genotoxicity of waters compared to their ICM alone pairs. Only iohexol enhanced the genotoxicity compared to the chlorinated source water. Interestingly, iopromide expressed reduced genotoxicity as compared to the chlorinated source water (Fig. 3).

3. Conclusions

In this study, the impact of ICM on complex DBP mixture toxicity was investigated in source waters collected from Akron, OH with or without disinfection. When evaluated for mammalian cell cytotoxicity and genotoxicity, iopamidol in water disinfected with chlorine or chloramines was clearly the most responsive in generating adverse biological responses. The relative iopamidol-mediated increase in CHO cell cytotoxicity and genotoxicity was much greater when chloramine was used as the disinfectant, as compared to chlorine. Four other ICMs (iopromide, iohexol, diatrizoate, and iomeprol) expressed some cytotoxicity over the control, and expressed higher cytotoxicity when chlorinated. Of these four ICM, only iohexol expressed an enhanced genotoxicity compared to the chlorinated source water control, while iopromide reduced the genotoxicity. For further interpretation, individual DBPs, as well as ICM transformation products in the reaction mixtures, should be identified and their toxicities needs to be determined.

Table 10 – Comparative CHO cell acute genotoxicity of X-ray contrast media iopromide, iohexol, diatrizoate and iomeprol in Akron source water (SW) samples with and without chlorine disinfection.

Sample	50%Tail DNA (CF) [a]	r^2 [b]	Lowest genotoxic conc. factor [c]	ANOVA test statistic [d]
SW + IPRO	509	0.99	460	$F_{9, 34} = 19.7$; $P \leq 0.001$
SW + IPRO + HOCl	267	0.96	250	$F_{11, 38} = 44.6$; $P \leq 0.001$
SW + IHX	562	0.95	480	$F_{14, 39} = 7.79$; $P \leq 0.001$
SW + IHX + HOCl	169	0.98	140	$F_{8, 40} = 28.9$; $P \leq 0.001$
SW + DTZ	790	0.99	700	$F_{13, 58} = 30.9$; $P \leq 0.001$
SW + DTZ + HOCl	207	0.99	140	$F_{12, 49} = 193.8$; $P \leq 0.001$
SW + IOME	706	0.95	700	$F_{12, 31} = 7.39$; $P \leq 0.001$
SW + IOME + HOCl	211	0.89	150	$F_{9, 32} = 18.8$; $P \leq 0.001$

Abbreviations: SW = Akron source water, HOCl = chlorine disinfection, IPRO = iopromide, IHX = iohexol, DTZ = diatrizoate, IME = iomeprol, and DNA = deoxyribonucleic acid.

[a] The SCGE 50% Tail DNA value is the WSC sample concentration factor determined from a regression analysis of the data that was calculated to induce a 50% SCGE Tail DNA value.

[b] r^2 is the coefficient of determination for the regression analysis upon which the SCGE %Tail DNA value was calculated.

[c] The lowest genotoxic concentration was the lowest concentration factor of the WSC sample in the concentration–response curve that induced a statistically significant amount of genomic DNA damage as compared to the negative control.

[d] The degrees of freedom for the between-groups and residual associated with the calculated F-test result and the resulting probability value.

Table 11 – Test for significance among GTI values.

	SW	HOCl	IPRO	IPRO-HOCl	IHX	IHX-HOCl	DTZ	DTZ-HOCl	IOME	IOME-HOCl
SW										
Cl										
IPRO										
IPRO-HOCl										
IHX										
IHX-HOCl										
DTZ										
DTZ-HOCl										
IOME										
IOME-HOCl										

Abbreviations; SW = Akron source water, HOCl = source water plus chlorine disinfection, IPRO = iopromide, IPRO–HOCl = iopromide plus chlorine disinfection, IHX = iohexol, IHX–HOCl = iohexol plus chlorine disinfection, DTZ = diatrizoate, DTZ–HOCl = diatrizoate plus chlorine disinfection, IOME = iomeprol, IOME–HOCl = iomeprol plus chlorine disinfection; GTI: genotoxicity index. All treated waters contain Akron source waters. In the pairwise comparisons, red indicates a significant difference between the paired groups, green indicates no significant difference between the paired groups.

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