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Changes in mutagenicity and acute toxicity of solutions of iodinated X-ray contrast media during chlorination

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HIGHLIGHTS

- Among the five ICM tested, only iopamidol was decomposed by chlorine.
- Mutagenicity and acute toxicity increased with chlorination of iopamidol.
- Three candidate iopamidol TPs were hypothesized to induce mutagenicity.
- QSAR analysis did not contradict the mutagenicities of the candidate TPs.

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ABSTRACT

In the present study, the effects of chlorination on the mutagenicity (assessed via the Ames assay) and acute toxicity (assessed via a bioluminescence inhibition assay) of solutions containing one of five commonly used iodinated X-ray contrast media (ICM) (iopamidol, iohexol, iopromide, iomeprol, and diatrizoate) were investigated. Of the five ICM tested, only iopamidol was degraded by chlorine. Chlorination of the iopamidol-containing solution induced both mutagenicity and acute toxicity, which increased with chlorination time (up to 96 h). The areas of five out of 54 peaks detected on the LC/MS total ion chromatogram had good correlation ($r^2 > 0.90$) between peak area and observed mutagenicity. To identify possible contributors to the observed mutagenicity, the Ames assay and LC/MS analysis were conducted on samples collected at 48-h chlorination time and extracted under different pH conditions. Of the five peaks, one peak was detected in the sample extracted at pH 7, but this sample was not mutagenic, indicating that the peak was not related to the observed mutagenicity. MS/MS analysis with an orbitrap mass spectrometer of the remaining four peaks revealed that two of the peaks represented the same TP (detected in negative and positive ion modes). Finally, three TPs were identified as suspected contributors to the mutagenicity induced by the iopamidol-containing solution after chlorination: 5-[(1,3-dihydroxypropan-2-yl)carbamoyl]-3-[(3-hydroxypropanoyl)oxy]-2,4-diiodobenzoic acid; *N*-(1,3-dihydroxypropan-2-yl)-3-(2,3-dioxopropyl)-2,4,6-triiodobenzamide; and 3-[(1,3-dihydroxypropan-2-yl)carbamoyl]-5-[(3-hydroxybutanoyl)oxy]-2,4,6-triiodobenzoic acid. Prediction of the mutagenicity potential of these three TPs with a battery of four quantitative structure–activity relationship models did not contradict our conclusion that these TPs contributed to the observed mutagenicity.

1. Introduction

Many of the pharmaceuticals used today in hospitals and private households are metabolized in the liver by drug-metabolizing enzymes. However, some are excreted into the feces and urine in the unchanged form. Sewage water contaminated with these pharmaceuticals is treated at wastewater treatment plants by means of biodegradation processes such as activated sludge treatment before being discarded into environmental waters, where it may be taken up by drinking-water treatment plants downstream. Although, the concentrations of pharmaceuticals in raw water sources are generally very low, this contamination is still considered to pose a risk to human health.

Iodinated X-ray contrast media (ICM; triiodinated benzene derivatives) are widely used in medical X-ray imaging. ICM have been detected in environmental waters at concentrations between several hundred nanograms per liter (Seitz et al., 2006; Boleda

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et al., 2011) and several micrograms per liter (Ternes and Hirsch, 2000; Duirk et al., 2011), which are concentrations a couple of orders of magnitude higher than those of other pharmaceuticals. These high concentrations are attributable to the high dosages of ICM used (up to 200 g-ICM/administration) (Pérez and Barceló, 2007) and the high biological stability of ICM in the human body (e.g., iopamidol, a widely used ICM, binds very little to plasma proteins and is therefore almost completely [>90%] excreted into the urine in its unmetabolized form within 72–96 h after administration (Mckinstry et al., 1984). The high biological stability of ICM also prevents them from being degraded by conventional biological wastewater treatment processes (Ternes and Hirsch, 2000). Therefore, the pollution of drinking water with ICM is a potential health risk. Because only limited amounts of ICM are removed by conventional drinking-water treatment processes such as coagulation, sedimentation, and sand filtration (Westerhoff et al., 2005; Suarez et al., 2009), or by advanced treatment processes such as ozonation or activated carbon adsorption (Kovalova et al., 2013), mainly due to the high hydrophilicity of ICM, it is likely that ICM contained within the water comes into direct contact with chlorine, which is added to finished water as a disinfectant.

ICM are widely accepted as safe, given the many toxicity tests required for their registration for clinical use and their long history of use, and given that the toxicities of their possible metabolites in the human body must be sufficiently investigated. However, the toxicities of transformation products (TPs) produced during the water chlorination process remain to be investigated. Recently, the change in cytotoxicity of an iopamidol-containing solution during chlorination was investigated in Chinese hamster ovary (CHO) cells, and the chlorination process was found to promote the cytotoxicity of iopamidol (Wendel et al., 2014); however, whether mutagenicity is increased by the chlorination process remains to be determined.

Here we investigated the change in mutagenicity of ICM-containing solutions during chlorination and attempted to identify possible contributors to the observed mutagenicity. First, Ames assays were conducted to evaluate the mutagenicity of each ICM-containing solution; this was followed by bioluminescence inhibition assays to evaluate acute toxicity. Second, TPs suspected to have contributed to the observed mutagenicity were identified by means of a correlation analysis between the areas of the TP peaks detected on the LC/MS charts and the observed mutagenicity. Third, the chemical structures of the TPs suspected to have contributed to the mutagenicity were determined by means of MS/MS analysis using an orbitrap mass spectrometer. Finally, the mutagenicity of each TP was predicted by using a battery of quantitative structure–activity relationship (QSAR) models.

2. Materials and methods

2.1. ICM

Four non-ionic ICM (iopamidol [Wako Pure Chemical Industries, Ltd., Osaka, Japan], iopromide [The United States Pharmacopeial Convention, Inc., Rockville, MD, USA], iohexol [LKT Laboratories, Inc., St. Paul, MN, USA], and iomeprol [Dr. Ehrenstorfer, GmbH, Augsburg, Germany]) and one ionic ICM (diatrizoate [MP Biomedicals, LLC, Solon, OH, USA]) were used as supplied without further purification. Each ICM stock solution was prepared by dissolving 200 mg of ICM in 100 mL of Milli-Q water (Milli-Q Advantage, Millipore Co., Bedford, MA, USA).

2.2. Batch chlorination experiments

To investigate the reactivity of each ICM with chlorine, batch chlorination experiments were conducted. Batches (500 mL) of phosphate buffer (100 mM, pH 7.0) were placed in beakers, and each beaker was spiked with ICM to a final concentration of 1 mg/L. Sodium hypochlorite solution was then added to each spiked solution at room temperature in the dark so that the residual chlorine concentration was 1 mg-Cl_2/L after 96 h of contact time. Samples (50 mL) were drawn from the beakers at 0, 12, 24, 48, 72, and 96 h, and the residual chlorine was immediately quenched with sodium ascorbate (for the quantification of ICM) or with sodium sulfite (for the quantification of dissolved organic carbon or released iodine). The concentrations of ICM and dissolved organic carbon in the samples were then immediately determined. As controls, the ICM-containing solutions were allowed to stand for 96 h without the addition of sodium hypochlorite, and the ICM concentration was determined as described above. Each batch experiment was conducted in triplicate.

Batch chlorination experiments were also conducted to investigate the change in toxicity of the ICM-containing solutions. Phosphate buffer (40 L, 100 mM, pH 7.0) was prepared in a glass vessel, and an ICM was added to the buffer to a final concentration of 100 mg L^{-1}. Sodium hypochlorite was then added to the solution at 800 mg-Cl_2 L^{-1}. The solution was divided into four in glass vessels and left at rest in the dark for chlorination. Samples (total 6 L at each sampling time) were withdrawn from the beakers at 0, 12, 24, 48, 72, and 96 h, and the residual chlorine was immediately quenched with sodium ascorbate. The samples were then subjected to a solid-phase extraction by using Isolute ENV+(Biotage AB, Uppsala, Sweden) as follows: 28.8 g of Isolute ENV + bulk solvent was packed in a solid-phase extraction reservoir (volume, 70 mL). The solid-phase extraction solvent was activated with the addition of 50 mL of methanol, 100 mL of Milli-Q water at pH 2, 50 mL of methanol, and 200 mL of Milli-Q water at pH 7 in this order. For the extraction of TPs, after adjusting the pH to 2 with HCl, 6 L of chlorinated ICM-containing solution was circulated through the solid-phase extraction reservoir by a pump (Sep-Pak Concentrator Plus, Waters Corporation, Milford, MA, USA) at a flow rate of 20 mL min^{-1} for 20 h. After sample extraction, the solid-phase extraction reservoir was washed with 100 mL of Milli-Q water at pH 7 and then dried by introducing nitrogen gas for 30 min. TPs trapped in the solid-phase extraction reservoir were eluted by introducing 200 mL of methanol at 1 mL min^{-1}. The eluate was divided into samples of 33 and 167 mL, which were dried under a nitrogen gas flow in a water bath at 45 °C. The two residue samples were redissolved in 2 mL of Milli-Q water and 10 mL of dimethyl sulfoxide, respectively, to obtain 500-fold concentrates. The concentrate in dimethyl sulfoxide was directly subjected to the mutagenicity assay (50, 100 and 200 μL $plate^{-1}$), whereas the concentrates in Milli-Q water was subjected to the bioluminescence inhibition assay after diluted with 20% NaCl solution at a ratio of 1:5000. The concentrations of the samples were chosen according to the sensitivities of the bioassays after preliminary experiments by using the samples at different concentrations.

ICM were directly quantified by means of liquid chromatography–electrospray–ionization–tandem mass spectrometry (LC–ESI/MS/MS, API3000, Applied Biosystems, Foster City, CA, USA) without extraction. To investigate the TPs generated during the chlorination of the ICM-containing solutions, the accurate masses of the TPs were determined by using a hybrid quadrupole-orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with a liquid chromatograph (UltiMate 3000 LC Systems, Thermo Fisher Scientific) after solid-phase extraction (500-fold). Iodoacetic acid and triiodoacetic acid were quantified by using an LC–MS/MS system (LC, UltiMate 3000 LC Systems; MS/MS, Q Exactive, Thermo Fisher Scientific) after solid-phase extraction (500-fold). Iodoform, dichloroiodomethane, and chloroiodomethane were quantified by using a GC–MS system

(QP2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with a capillary column (DB-1MS UI; length, 30 m; internal diameter, 0.25 mm; thickness, 0.25 μm) after solid-phase extraction (500-fold). Details of measurements are described in Supplementary Data.

2.3. Bioassays

Two types of bioassay were performed to investigate the change in mutagenicity of ICM-containing solutions during chlorination: bioluminescence inhibition assay for evaluation of acute toxicity, and Ames assay for evaluation of mutagenicity. Details of the bioassays were described in Supplementary Data.

2.4. QSAR analysis

To evaluate the *Salmonella* mutagenicity of the TPs identified by the MS/MS analysis, QSAR analyses were conducted by using four pieces of freely available QSAR software: Toxtree (version 2.6.6) was developed by Ideaconsult Ltd. (Sofia, Bulgaria) and was downloaded from http://toxtree.sourceforge.net/. CAESAR (version 2.1.12) was developed as part of the European Union-funded CAESAR project and was used on the VEGANIC platform (version 1.0.8, downloaded from http://www.vega-qsar.eu/). Lazar was developed by In Silico Toxicology GmbH (Basel, Switzerland) and is web-based software (http://www.in-silico.ch/). T.E.S.T. (version 4.1) was developed by the United States Environmental Protection Agency and was downloaded from http://www.epa.gov/nrmrl/std/qsar/qsar.html. T.E.S.T. included four types of QSAR methodology (i.e., hierarchical method, FDA method, nearest neighbor method, and consensus method) for the prediction of *Salmonella* mutagenicity. In the present study, we predicted the *Salmonella* mutagenicity of the TPs by using the consensus method because this methodology was shown in the user's guide to have achieved the best prediction results during external validation. The QSAR models used in the present study predicted *Salmonella* mutagenicity in the absence and/or the presence of metabolic activation, because the datasets for the QSAR models consist of the mutagenicity data both in the absence and the presence of the metabolic activation (Zeiger, 1987; Kazius et al., 2004; Hansen et al., 2009).

3. Results and discussions

3.1. Acute toxicity and mutagenicity of the ICM

None of the ICM tested induced acute toxicity up to a concentration of 100 mg L^{-1} (Fig. S1). Of the ICM tested, only iopromide has previously been evaluated for acute toxicity to *Vibrio fischeri*, and was also reported not to induce acute toxicity (Steger-Hartmann et al., 1999). Furthermore, no mutagenicity was observed in either of the Ames tester strains in either the absence or the presence of S9 mix (Fig. S2), indicating that none of the ICM tested induced either frameshift or base-pair-substitution mutagenicity. Morisetti et al. (1994) conducted an Ames assay on iomeprol in the presence or absence of metabolic activation and similarly reported that the compound did not induce mutagenicity, which agrees with our results.

3.2. Decomposition of ICM during chlorination

Fig. S3 shows the change in ICM concentration during chlorination. Without the addition of chlorine, no decrease in concentration was observed for any of the ICM tested, indicating that these ICM do not undergo hydrolysis at room temperature at pH 7. This supports the chemical stability of the ICM reported elsewhere. After the addition of chlorine, four of the five ICM (i.e., iohexol, iopromide, iomeprol, and diatrizoate) were not decomposed. However, iopamidol was decomposed by the addition of chlorine; the survival ratio of iopamidol gradually decreased with time, and at 96 h of chlorination approximately 20% of the initial iopamidol remained. Duirk et al. (2011) reported that during chlorination iopamidol donates iodine to natural organic matter (NOM) contained in river water, thereby producing iodo-disinfection TPs (iodo-disinfection byproducts [DBPs]). Although Duirk et al. (2011) did not directly confirm the decomposition of iopamidol during the chlorination process, their results imply that iopamidol is decomposed by chlorine. Duirk et al. (2011) also demonstrated that iohexol and iopromide did not form appreciable levels of iodo-DBPs during chlorination, which is most likely due to the non-reactivity of iohexol and iopromide with chlorine, as demonstrated by the present results. Wendel et al. (2014) recently reported that of the five ICM included in the present study, only iopamidol is degraded by chlorine, which again agrees with the present results. Because iopamidol was the only ICM that was decomposed by chlorine, we subsequently examined the changes in acute toxicity and mutagenicity for the iopamidol-containing solution only.

3.3. Change in acute toxicity of an iopamidol-containing solution during chlorination

To investigate the change in toxicity of an iopamidol-containing solution during chlorination, a batch chlorination test was conducted using a concentration of 100 mg L^{-1} of iopamidol. Fig. 1(a) shows the change in iopamidol concentration with chlorination time. Iopamidol concentration gradually decreased with time in a manner similar to that seen in the decomposition experiment with 1 mg L^{-1} of iopamidol (see Section 3.2). Furthermore, the total dissolved organic carbon concentration of the solution did not decrease with time (Fig. 1(b)), indicating that iopamidol was not mineralized by the reaction with chlorine but was instead transformed into organic TPs.

Fig. 2(a) shows the change in bioluminescence inhibition during chlorination of the iopamidol-containing solution. When the iopamidol concentration had decreased to 60% of its initial concentration (at 12 h of chlorination time), bioluminescence was inhibited by 20%, indicating that the sample induced acute toxicity and that the TPs produced by the reaction between iopamidol and chlorine likely contributed to this acute toxicity. At 48 h of contact time, bioluminescence inhibition had increased, despite the iopamidol concentration having decreased during the same period. At 96 h, although very little iopamidol remained, the bioluminescence inhibition was again increased.

Duirk et al. (2011) investigated the cytotoxicity of iopamidol during chlorination in the presence of NOM by using CHO cells and reported that cytotoxicity was only slightly induced by chlorination of river water containing iopamidol; these investigators attributed the increased cytotoxicity to iodo-DBPs such as iodoacetic acid produced from reactions between the NOM present and iodine (or possibly hypoiodous acid) released from the iopamidol. Although the endpoint of the bioluminescence inhibition assay used in the present study (30 min of contact time) was different from that of the CHO cell chronic cytotoxicity assay (72 h of contact time), the present results demonstrated, the present results demonstrated that TPs capable of inducing acute toxicity were produced from the reaction between iopamidol and chlorine even in the absence of NOM. We next measured the concentrations of two iodo-haloacetic acids (iodoacetic acid and triiodoacetic acid), two iodo-trihalomethanes (iodoform and dichloroiodomethane), and chloroiodomethane in the samples subjected to the

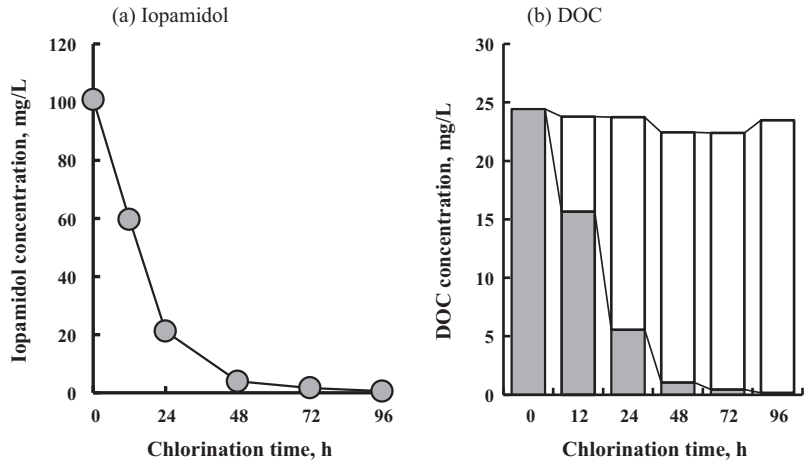


Fig. 1. Change in (a) iopamidol and (b) dissolved organic carbon (DOC) concentration during chlorination. Gray and white columns in panel (b) represent iopamidol-derived and transformation product-derived DOC, respectively.

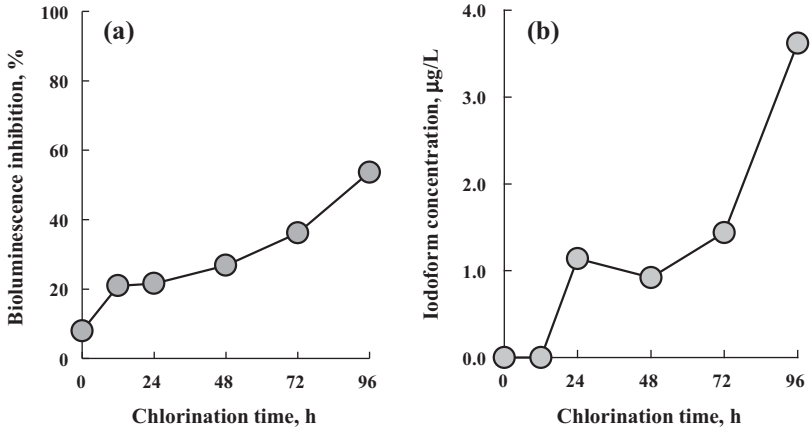


Fig. 2. (a) Change in cytotoxicity during chlorination of iopamidol-containing solution. The assay was conducted using a solution that initially contained 10 mg/L of iopamidol. (b) Change in iodoform concentration in the samples subjected to the bioluminescence inhibition assay.

bioluminescence inhibition assay. Dichloroiodomethane, chloroiodomethane, and triiodoacetic acid were not detected in any of the samples. However, iodoacetic acid was detected at 0.22 μg L^{-1} in the 48-h sample (Fig. S4), indicating that it was generated not only by the reaction between iodine and NOM (Duirk et al., 2011) but also possibly by the reaction between iodine and iopamidol or iopamidol-derived TPs. Iodoacetic acid has been reported to exhibit no acute toxicity to human small intestine epithelial cells at concentrations of 5–30 μM (Attene-Ramos et al., 2010), and the concentrations that reduced cell density by 50% as compared to negative control have been reported to be 303 and 2.95 μM for *S. typhimurium* and CHO cells, respectively (Plewa et al., 2004). Although the endpoints of their bioassays were different from ours (bioluminescence inhibition assay), we supposed that the cytotoxicity observed in the present study was unlikely caused by iodoacetic acid alone since the concentration detected in the present study (1.2 nM) was lower than these concentrations by three orders of magnitude.

In addition, Iodoform was detected after 24 h of chlorination (Fig. 2(b)). Iodoform (53 μM) has been reported to bring about 50% inhibition of bioluminescence (EC_{50}) in *V. fischeri* (Stalter et al., 2013). The maximum iodoform concentration detected in the present study was 3.6 μg L^{-1} (9.2 nM), which is much less than the EC_{50} reported by Stalter et al. (2013). Therefore, iodoform did not contribute to the cytotoxicity observed in the present study. Overall, compounds other than the iodo-trihalomethanes and iodo-haloacetic acids could have contributed to the cytotoxicity.

3.4. Change in mutagenicity of an iopamidol-containing solution during chlorination

Fig. 3 shows the change in mutagenicity of the iopamidol-containing solution during chlorination. In the frameshift-mutagenicity-detecting Ames tester strain (TA98, Fig. 3(a)), no mutagenicity was observed without metabolic activation. However, mutagenicity was increased dramatically at 24 h in the presence of metabolic activation, indicating that one or more indirect mutagenic TPs were produced during chlorination of the iopamidol-containing solution. Mutagenicity continued to increase at 96 h, even though very little iopamidol remained at 48 h. Duirk et al. (2011) reported that genotoxicity was observed in chlorinated iopamidol-containing river water because of DBP formation, but they did not investigate the direct reaction between iopamidol and chlorine. Although the endpoint of the bioassay conducted in the present study (mutagenicity) was different from that conducted by Duirk et al. (2011) (genotoxicity), our results clearly showed that mutagenic compounds were produced from the direct reaction between iopamidol and chlorine, even in the absence of NOM.

In the base-pair-substitution-mutagenicity-detecting Ames tester strain (TA100, Fig. 3(b)), no mutagenicity was observed in the

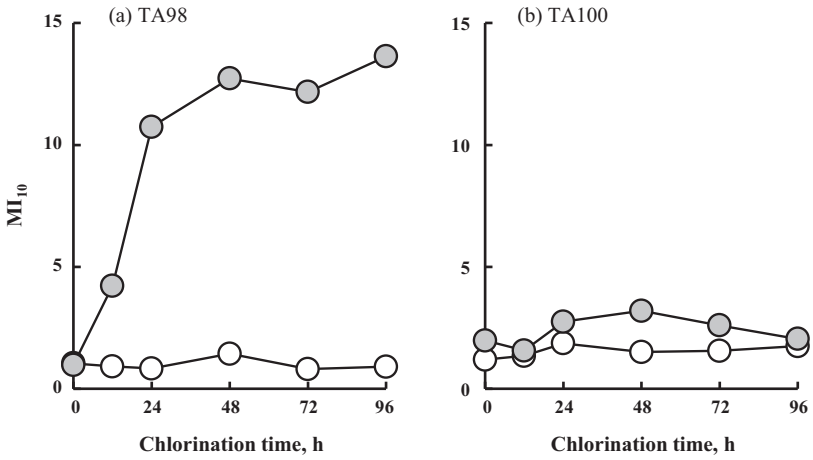


Fig. 3. Change in mutagenicity during chlorination of iopamidol-containing solution. White and gray circles represent data for experiments conducted without and with S9 mix, respectively. Mutagenicity is expressed as "MI_{10}", which is defined as mutagenicity intensity (i.e. the ratio of the number of His^+ revertants per plate to the number of spontaneous revertants per plate) at the dose of sample solution that had contained 10 mg of iopamidol before chlorination.

absence of metabolic activation, and only slight mutagenicity was observed in the presence of metabolic activation. The mutagenicity induced with the chlorinated iopamidol-containing solution was much larger in the TA98 strain than in the TA100 strain, indicating that the mutagenic TPs generated during chlorination induced frameshift mutations rather than base-pair-substitution mutations. Since mutagenicity was observed only in the presence of metabolic activation in both Ames tester strains, the TPs generated during the chlorination of the iopamidol-containing solution can be assumed to not directly react with DNA, but instead modify DNA after being metabolized by drug-metabolizing enzymes.

3.5. MS/MS–QSAR analysis of possible mutagenic TPs

Iodoacetic acid is known to be genotoxic (Richardson et al., 2008), and in the present study it was detected in the 48-h sample (Fig. S4), whose concentration was equivalent to 22 ng plate^{-1} in the Ames assay conducted in the present study. We employed the Ames assay on iodoacetic acid up to 5 μg plate^{-1}, but no mutagenicity was observed for TA98 tester strain with metabolic activation (Fig. S5). Therefore, it is unlikely that iodoacetic acid contributed to the observed mutagenicity.

Iodoform was also detected (Fig. 2(b)), whose maximum concentration was equivalent to 0.36 μg plate^{-1} in Ames assay conducted in the present study. We conducted the Ames assay on iodoform up to 5 μg plate^{-1}, but no mutagenicity was observed for TA98 tester strain with metabolic activation (Fig. S5). Haworth et al. (1983) has reported that $\geqslant$ 100 μg plate^{-1} of iodoform induced mutagenicity in the Ames TA98 strain with metabolic activation, but that the mutagenicity was not induced with $\leqslant$ 10 μg plate^{-1}. Therefore, it is unlikely that iodoform contributed to the observed mutagenicity.

To identify the TPs contributing to the observed mutagenicity, the TPs generated during chlorination were examined by means of LC/MS analysis. A total of 54 peaks with an individual area larger than 1/100th of the initial peak area of iopamidol at 0 h of contact time (i.e., before chlorination) were detected on the total ion chromatogram (combined positive and negative ion mode). A high correlation ($r^2 > 0.90$) between peak area and observed mutagenicity in Ames tester strain TA98 (with metabolic activation) was observed for five of the 54 peaks (Table S1, Fig. 4): 577.88 (Peak 16, $r^2 = 0.98$, negative ion mode), 579.90 (Peak 17, $r^2 = 0.96$, positive ion mode), 641.78 (Peak 21, $r^2 = 0.92$, negative ion mode), 719.81 (Peak 39, $r^2 = 0.93$, positive ion mode), and 763.80 (Peak 43, $r^2 = 0.91$, negative ion mode). Therefore, it is likely that these compounds contributed to the observed mutagenicity.

To further narrow down which TPs contributed to the observed mutagenicity, the 48-h sample was extracted at pH 7 and pH 2. The mutagenicity of the extracted samples was then evaluated with the Ames assay using tester strain TA98 and metabolic activation; LC/MS analysis was also performed on the two samples. Although the 48-h sample induced mutagenicity when the sample was extracted at pH 2, no mutagenicity was observed when the sample was extracted at pH 7 (Fig. S6). The LC/MS analysis revealed that Peaks 16, 17, and 21 were detected only in the sample extracted at pH 2 and that the area of Peak 39 was decreased by 97% by changing the extraction pH from 2 to 7. Furthermore, the areas of Peak 43 at pH 2 and at pH 7 were comparable. Therefore, it is likely that only Peaks 16, 17, 21, and 39 contributed to the observed mutagenicity. We next determined the chemical formulae of the peaks from the accurate mass and chemical structure data collected during the MS/MS analysis, and we determined that Peaks 16 and 17, detected in the negative and positive ion modes, respectively, were attributable to the same compound.

Finally, we concluded that the three TPs likely to have contributed to the observed mutagenicity were 5-[(1,3-dihydroxypropan-2-yl)carbamoyl]-3-[(3-hydroxypropanoyl)oxy]-2,4-diiodobenzoic acid (TP1; Peaks 16 and 17); *N*-(1,3-dihydroxypropan-2-yl)-3-(2,3-dioxopropyl)-2,4,6-triiodobenzamide (TP2; Peak 21); and 3-[(1,3-dihydroxypropan-2-yl)carbamoyl]-5-[(3-hydroxybutanoyl)oxy]-2,4,6-triiodobenzoic acid (TP3; Peak 39) (further details of the identification are shown in Figs. S7–S14). Proposed chemical structures of the TPs are shown in Table 1. Wendel et al. (2014) identified 19 TPs produced during the chlorination of iopamidol and proposed a reaction pathway for their formation; however, this reaction pathway does not include any of the TPs identified in the present study. This may be because of the difference in the chlorine/iopamidol molar ratio used in the present study (molar ratio, approx. 90) and that applied in the study described by Wendel et al. (2014) (molar ratio, 20).

To predict whether or not the three TPs were mutagenic, four QSAR models (Toxtree, CAESAR, Lazar, and T.E.S.T.) were applied to the detected TPs (Table 1). Although CAESAR and Lazar predicted that all three TPs would be non-mutagenic, T.E.S.T. predicted that TP1 and TP3 would be mutagenic, and Toxtree predicted that TP2 would be mutagenic. Although the presence of structural alerts by one QSAR model is highly suggestive, the lack of structural alerts in a molecule is not proof that the compound is devoid of

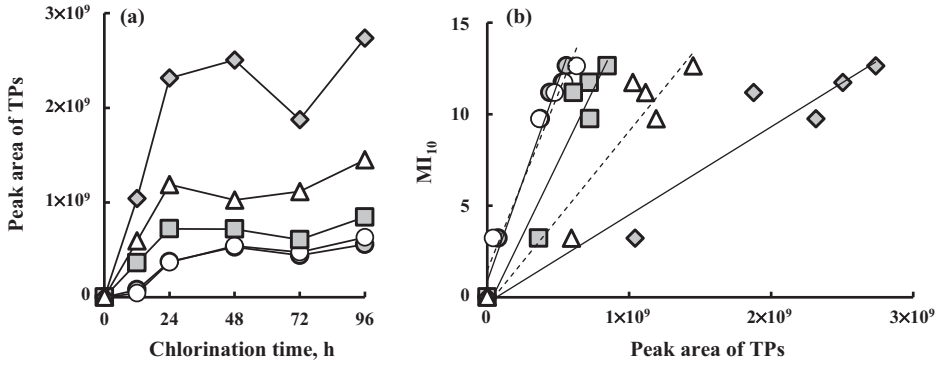
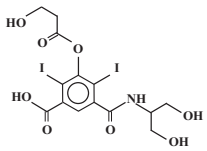
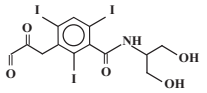
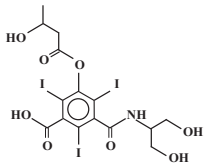


Fig. 4. Changes in the peak areas of transformation products (TPs) with chlorine contact time (a) and relationship between induced mutagenicity and TP peak area (b). Gray diamonds, Peak 39 (m/z = 719.81, positive ion mode); white triangles, Peak 43 (763.80, negative); gray squares, Peak 21 (641.78, negative); white circles, Peak 17 (579.90, positive); gray circles, Peak 16 (577.88, negative). Mutagenicity is expressed as "MI_{10}", which is defined as mutagenicity intensity (i.e. the ratio of the number of His^+ revertants per plate to the number of spontaneous revertants per plate) at the dose of sample solution that had contained 10 mg of iopamidol before chlorination.

Table 1
Chemical structures of TPs suspected to contribute to the observed mutagenicity and results of QSAR analysis of the TPs.

Chemical structure	IUPAC name	Chemical formula	Results of QSAR			
			Toxtree	CAESAR	Lazar	T.E.S.T.
	TP1 (Peak 16 and 17) 5-[(1,3-dihydroxypropan-2-yl) carbamoyl]-3-[(3-hydroxypropanoyl)oxy]-2,4-diiodobenzoic acid	$C_{14}H_{14}O_8NI_2$	Negative	Negative	Negative	Positive
	TP2 (Peak 21) *N*-(1,3-dihydroxypropan-2-yl)-3-(2,3-dioxopropyl)-2,4,6-triiodobenzamide	$C_{13}H_{12}O_5NI_3$	Positive	Negative	Negative	Negative
	TP3 (Peak 39) 3-[(1,3-dihydroxypropan-2-yl)carbamoyl]-5-[(3-hydroxybutanoyl)oxy]-2,4,6-triiodobenzoic acid	$C_{15}H_{16}O_8NI_3$	Negative	Negative	Negative	Positive

toxic potential (Nendza et al., 2013). To avoid false negative results, the European Chemicals Agency recommends that predictions be obtained from at least three different methods (ECHA, 2008). The false negative rate can also be reduced by combining two software tools, where the overall prediction is considered positive if either tool gives a positive prediction (Worth et al., 2010). In the present study, each TP was predicted to be mutagenic by one of the four QSAR models, which did not contradict our hypothesis that these TPs contributed to the mutagenicity induced by the iopamidol-containing solution during chlorination.

4. Conclusions

1. Iopamidol was decomposed by chlorination, whereas iohexol, iopromide, iomeprol, and diatrizoate were not.
2. The acute toxicity of an iopamidol-containing solution increased during chlorination, indicating that toxic TPs were produced by the reaction between iopamidol and chlorine.
3. The mutagenicity of an iopamidol-containing solution in Ames tester strain TA98 with metabolic activation increased during chlorination, indicating that TPs that indirectly induced frame-shift mutations were produced by the reaction between iopamidol and chlorine.
4. From a correlation analysis between the peak area of the TPs detected on the LC/MS total ion chromatogram and the induced mutagenicity, the following three TPs were considered to contribute to the induced mutagenicity: 5-[(1,3-dihydroxypropan-2-yl)carbamoyl]-3-[(3-hydroxypropanoyl)oxy]-2,4-diiodobenzoic acid; *N*-(1,3-dihydroxypropan-2-yl)-3-(2,3-dioxopropyl)-2,4,6-triiodobenzamide; and 3-[(1,3-dihydroxypropan-2-yl)carbamoyl]-5-[(3-hydroxybutanoyl)oxy]-2,4,6-triiodobenzoic acid.
5. QSAR analysis did not contradict our hypothesis that these three TPs contributed to the induced mutagenicity.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.03.082.

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