Toxic Byproducts Generated in Disinfected Drinking Water Contaminated with Pharmaceuticals

Final Report for Illinois/Indiana Sea Grant College Program Seed Grant

Michael J. Plewa, Ph.D., Professor of Genetics
Principal Investigator
University of Illinois at Urbana-Champaign
September 27, 2010

INTRODUCTION

The lack of access to safe drinking water for over 1.2 billion people is already one of the most pervasive problems in the world today (Shannon et al. 2008). Unfortunately, a new, growing contamination problem may emerge as one of the most serious public health concerns yet, affecting both developing and developed nations. Widespread chemical contamination from pharmaceutical, industrial, personal care, and agricultural agents are finding their way into the drinking water supply, posing serious threats to public health. Already pharma-compounds including hormones and endocrine disrupters are thought to be the potential cause of the feminization of children and loss of fertility of males. This is a harbinger for future health problems, mirroring drastic changes observed in nature due to these agents. Of even more worry is that recent studies show contrast agents used in medical imaging not only make their way into sanitary and then drinking water systems, but the very act of disinfecting the water creates some of the most potent geno- and cytotoxic disinfection byproduct compounds ever measured. What is even more frightening is that we know very little about the toxicity of thousands of pharma- and their decomposition products, nor how to remove them. Current treatment methods do not degrade many of the pharma-contaminants, and may generate more toxic byproducts. In order for the U.S. EPA to regulate these compounds in our drinking water, practitioners first must be able to sense them, know how toxic they are to humans, and then be able to mitigate them. Unfortunately, the basic science of pharma-product interactions in water and treatment systems is not known.

OBJECTIVES

We recently observed that a widely distributed, pharmaceutical contaminant can be modified into byproducts when chlorine disinfection of drinking water is conducted. The conversion of a non-toxic pharmaceutical contaminant into toxic byproducts associated with water disinfection is a new and worrisome discovery. The primary objective of this project was to analyze source water contaminated with the X-ray imaging contrast pharmaceutical iopamidol before and after disinfection with chlorine. The specific objectives were to, (1) determine if this contaminant pharmaceutical was directly cytotoxic and genotoxic in mammalian and human cells, (2) determine if iopamidol was converted into byproducts after chlorine disinfection that are cytotoxic and genotoxic in mammalian and human cells, and (3) determine if there was a correlation between the formation of iopamidol-mediated iodinatated drinking water disinfection

byproducts and toxicity. These results were and continue to be used as a foundation for a proposal to a federal agency to fund this important research in pharmaceutical contamination of drinking water sources.

PROJECT SUMMARY

Abstract

Iodinated X-ray contrast media (ICM) were investigated as a source of iodine in the formation of iodo-trihalomethane (iodo-THM) and iodo-acid disinfection by-products (DBPs), both of which are highly genotoxic and/or cytotoxic in mammalian cells. ICM are widely used at medical centers to enable medical imaging of soft tissues (e.g., organs, veins, blood vessels), they are almost completely excreted in urine or feces within 24 h, and they are not well removed in wastewater treatment plants, such that they have been found at elevated concentrations in rivers and streams (up to 100 µg/L). Naturally occurring iodide in source waters is believed to be a primary source of iodine in the formation of iodo-DBPs, but a previous 23-city iodo-DBP occurrence study also revealed appreciable levels of iodo-DBPs in some drinking water treatment plants that had no detectable iodide or very low iodide in their source waters. When 10 of the original 23 cities' source waters were re-sampled, four ICM were found—iopamidol, iopromide, iohexol, and diatrizoate—with iopamidol most frequently detected, in 6 of the 10 plants sampled, up to 2700 ng/L. Subsequent controlled laboratory reactions of iopamidol with chlorine and monochloramine in buffered deionized water (in the absence of natural organic matter (NOM)), produced only very low levels of iodo-DBPs; however, when reacted in real source waters (containing NOM), chlorine and monochloramine produced significant levels of iodo-THMs and iodo-acids, up to 212 nM for dichloroiodomethane and 3.0 nM for iodoacetic acid, respectively, for chlorination. The pH behavior was different for chlorine and monochloramine, such that iodo-DBPs were generally higher at higher pH (8.5) for chlorine, but were higher at lower pH (6.5) for monochloramine. Iodate was also formed and was maximized at pH 7.5 and 8.0. Extracts from chloraminated natural source waters with and without iopamidol as well as from chlorinated natural source water with iopamidol were the most cytotoxic samples in mammalian cells. Source water with iopamidol but no disinfection were the least cytotoxic. While extracts from chlorinated and chloraminated natural source waters were genotoxic, the addition of iopamidol enhanced their genotoxicity. Therefore, while ICM are not toxic in themselves, their presence in source waters may be a source of concern because of the formation of highly toxic iodo-DBPs in chlorinated or chloraminated drinking water.

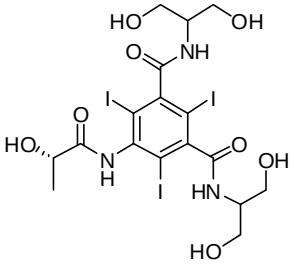
Background

In a previous 23-city occurrence study, we measured the widespread presence of iodinated disinfection by-products (iodo-DBPs)—iodo-acids and iodo-trihalomethanes (iodo-THMs)—in chloraminated and chlorinated drinking water in the United States and Canada at µg/L levels (up to 10.2 µg/L or 1.7 µg/L for individual iodo-THMs or iodo-acids, respectively (Richardson et al. 2008). Iodo-DBPs are highly genotoxic and cytotoxic, with iodoacetic acid being the most genotoxic DBP identified to date in mammalian cell systems (Plewa et al. 2004). The primary source of iodine in iodo-DBPs is believed to be from natural iodide in source waters. However, natural iodide levels were very low or not detected in some cases such that iodo-DBP formation could not be accounted for by natural iodide concentrations in the source waters (Richardson et

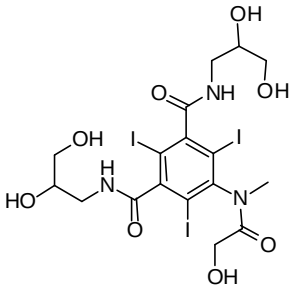
al. 2008). Therefore, we investigated other potential sources of iodine that could contribute to iodo-DBP formation.

Iodinated X-ray contrast media (ICM) are widely used to enable medical imaging of soft tissues (e.g., organs, veins, blood vessels). ICM are large molecules (~600-700 Da) with triiodobenzoic acid analogues in their basic structures (Figure 1). Global consumption of ICM is approximately 3.5×10^6 kg/year; a single application can be up to 200 g. ICM are designed to be inert, with 95% unmetabolized and eliminated in urine and feces within 24 h (Perez et al. 2006). Iodine atoms in ICM cause increased absorption of X-ray radiation. Individual ICM differ mainly in their side chains, which contain hydroxyl, carboxyl, and amide moieties to impart elevated polarity and aqueous solubility (Krause and Schneider 2002).

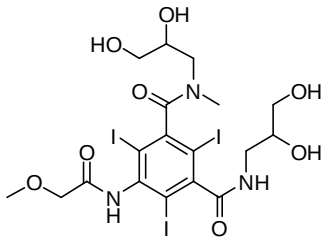
Iopamidol
62883-00-5
776.85 Da



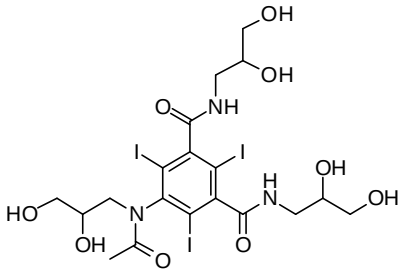
Iomeprol
78649-41-9
776.85 Da



Iopromide
73334-07-3
790.87 Da



Iohexol
66108-95-0
820.88 Da



Diatrizoate
737-31-5
613.77 Da

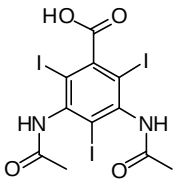


Figure 1.Chemical structures, CAS number and molecular weights of ICM commonly used for medical imaging**.**

Due to incomplete removal in wastewater treatment plants, ICM have been found at elevated concentrations in rivers and streams (Carballa et al. 2004; Hirsch et al. 2000; Oleksy-Frenzel et al. 2000; Putschew and Jekel 2001; Putschew and Jekel 2006). Concentrations as high as 100 µg/L have been detected in a creek containing more than 50% wastewater (Ternes and Hirsch 2000). ICM have also been found in groundwater and drinking water because they are partially recalcitrant during soil-aquifer passage, and are not completely removed by activated carbon

filtration or ozonation (Drewes et al. 2001; Drewes et al. 2003; Hirsch et al. 2000; Putschew et al. 2001; Sacher et al. 2001; Schittko et al. 2004; Ternes et al. 2003). ICM are primary contributors to the total organic halogen burden in clinical wastewater (Gartiser et al. 1996). More than 90 % of the adsorbable organic iodine in wastewater and surface water can be attributed to ICM (Gartiser et al. 1996; Kummerer et al. 1998; Putschew and Jekel 2001; Putschew and Jekel 2006; Putschew et al. 2001; Sprehe et al. 2001).

Many DBPs are formed by the reaction of disinfectants with natural organic matter (NOM), but anthropogenic contaminants can also react with disinfectants to form DBPs. Contaminant DBPs were reported for pharmaceuticals, personal care products, estrogens, pesticides, textile dyes, alkylphenol surfactants, UV filters, and diesel fuel (Richardson 2009). These contaminants have activated aromatic rings that readily react with oxidants. Recently DBPs were identified from the chlorination of the antacid cimetidine (Buth et al. 2007) and from the reaction of chlorine dioxide with beta-lactam antibiotics (Navalon et al. 2008). Contaminants with activated benzene rings or other functional groups can react with chlorine and other oxidants and are potential DBP precursors.

Because of the widespread presence of ICM and the relatively high levels observed in surface waters, we investigated them as a potential source of the iodine in iodo-THM and iodo-acid DBPs in chlorinated and chloraminated drinking water.

Materials and Methods for the Toxicology of ICMs

Mammalian Cell Cytotoxicity and Genotoxicity

Mammalian cell cytotoxicity and genotoxicity measurements were conducted on organic concentrates of source waters (20 L each) spiked with iopamidol (10 µM) that were treated with chlorine or monochloramine. Controls included raw source waters spiked with iopamidol (no oxidant) and raw source waters treated with chlorine or monochloramine (no iopamidol). Treated waters were concentrated using XAD resins (40 mL XAD-8 over 40 mL XAD-2), as described in a previously published procedure (Pressman et al. 2010). Chinese hamster ovary (CHO) cells, line AS52, clone 11-4-8 were used for the cytotoxicity and genotoxicity analyses of the water concentrates (Wagner et al. 1998). These assays have been described in the literature (Plewa and Wagner 2009; Wagner and Plewa 2009). For chronic cytotoxicity (72-h exposure), a series of concentrations were analyzed with 4-8 replicates per concentration. Each experiment was repeated. A concentration-response curve was generated and regression analysis was used to calculate the %C½ value. This value is analogous to the LC_{50} and is the concentration that induced a cell density that was 50% of the negative control. A one-way analysis of variance (ANOVA) test was conducted to determine whether the water concentrate induced a significant level of cell killing. If a significant F value ($P \leq 0.05$) was obtained, a Holm-Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic was maintained as 0.8 at $\alpha = 0.05$. To determine the acute genotoxicity of the water concentrates, single-cell gel electrophoresis (SCGE) was employed; it quantitatively measures genomic DNA damage induced in individual nuclei of treated cells (Tice et al. 2000; Wagner and Plewa 2009). CHO cells were exposed for 4 h at 37°C, 5% CO_2. Each experiment included a negative control, a positive control (3.8 mM ethylmethanesulfonate), and 9 water extract concentrations. The concentration range was determined by measuring acute cytotoxicity with a vital dye. After

treatment, cells were harvested, embedded in an agarose microgel, and lysed; the DNA was denatured and electrophoresed under alkaline conditions. Using Komet 3.1 software, the primary measure of DNA damage was the % tail DNA which is the amount of DNA that migrated from the nucleus into the agarose gel. Within the concentration range that allowed for 70% or greater viable cells, a concentration-response curve was generated, and a regression analysis was used to fit the curve. The SCGE genotoxic potency value was determined as the midpoint of this curve. The % tail DNA value for each microgel was determined, and the data were averaged among all of the microgels for each water extract concentration. The % tail DNA values were analyzed with an ANOVA test. If a significant F value ($P \leq 0.05$) was obtained, a Holm-Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic was maintained as 0.8 at $\alpha = 0.05$.

Toxicological Results and Discussion

Mammalian cell cytotoxicity and genotoxicity results supported the formation of toxic iodo-DBPs from iopamidol. Concentration-response curves for experiments that measured chronic CHO cell cytotoxicity with chlorine or chloramine as the disinfectant are presented in Figure 2A and 2B, respectively. Comparative chronic CHO cell cytotoxicity demonstrated that extracted ACC water was one of the least toxic samples (%C½ = 158.1×, Table 1) as well as an extraction of a pure water blank (data not shown). ACC water with iopamidol from two different sampling experiments expressed an average %C½ value of 127.4× which suggests that these organic extracts are slightly more cytotoxic than the ACC source water extracts (Table 2). A much greater effect was observed from the disinfection of ACC water in the absence of iopamidol. Disinfection with chlorine or chloramine increased the cytotoxicity of the extracts by 4.5-fold or 7.1-fold, respectively based on their %C½ values (Figure 2A, 2B, Table 1). Finally extracts from reaction mixtures containing iopamidol and chlorine in ACC water was slightly more cytotoxic than the corresponding source waters treated with chlorine (Figure 2A). For chloramine disinfection, there appears to be little effect on chronic cytotoxicity with or without iopamidol (Figure 2B, Table 1). Experiments that measured genomic DNA damage with chlorine or chloramine as the disinfectant are presented in Figures 2C and 2D, respectively. Organic extracts from ACC water alone, or ACC water with iopamidol were negative or very weakly genotoxic. After disinfection by chlorine or chloramine, the ACC source water extracts expressed significant genotoxicity because of DBP formation. Notably, the addition of iopamidol in ACC water disinfected with chlorine or chloramine resulted in a 1.7-fold or 1.3-fold increase in genotoxicity, respectively (Figure 2C, 2D, Table 1). During the past decade we demonstrated that iodinated DBPs are generally more cytotoxic and genotoxic than their brominated or chlorinated analogs. This trend holds true for DBP classes including the THMs (Plewa and Wagner 2009) halo acids (Plewa et al. 2010; Plewa et al. 2004), haloacetonitriles (Muellner et al. 2007), and haloacetamides (Plewa et al. 2008). Recent comparative human cell toxicogenomic analyses of the monohaloacetic acids demonstrated that iodoacetic acid modified the expression of more human genes associated with adverse health outcomes than bromo- or chloroacetic acid (Attene-Ramos et al. 2010). The fact that iopamidol can generate iodo-DBPs after disinfection and that iodo-DBPs demonstrate higher levels of toxicity support concerns that ICMs may have adverse impacts upon the public health and the environment when they are released into wastewaters.

Table 1. CHO Cell Chronic Cytotoxicity and Acute Genotoxicity of Water Concentrates					
CHO Cell Chronic Cytotoxicity Results					
Water Samples [a]	Conc. Factor Range (×-Fold)	%C½ Value (LC_{50}) [b]	R^2 [c]	Lowest Toxic Conc. Factor [d]	ANOVA Statistic [e]
ACC Water + Iopamidol	10 - 200	85.9×	0.98	50.0×	$F_{10, 117} = 58.1$; $P \leq 0.001$
ACC Water + HOCl	5 - 50	35.0×	0.96	30.0×	$F_{12, 168} = 34.3$; $P \leq 0.001$
ACC Water + Iopamidol + HOCl	5 - 50	23.5×	0.99	10.0×	$F_{10, 105} = 159$; $P \leq 0.001$
ACC Water + Iopamidol	10 - 350	168.9×	0.99	100×	$F_{10, 77} = 314$; $P \leq 0.001$
ACC Water + NH_2Cl	5 - 50	22.4×	0.99	20×	$F_{10, 77} = 378$; $P \leq 0.001$
ACC Water + Iopamidol + NH_2Cl	5 - 50	23.5×	0.98	20×	$F_{10, 77} = 563$; $P \leq 0.001$
ACC Water	10 - 350	158.1×	0.99	75×	$F_{10, 77} = 185$; $P \leq 0.001$
CHO Cell Acute Genomic DNA Damage (SCGE) Results					
Water Samples	Conc. Factor Range (×-Fold)	SCGE Genotox Potency Value [f]	R^2 [g]	Lowest Genotox Conc. Factor [h]	ANOVA Statistic
ACC Water + Iopamidol	40-1000	NA [i]	NA	NS [j]	$F_{13, 20} = 1.39$; $P = 0.25$
ACC Water + HOCl	40 - 480	285×	0.75	240×	$F_{12, 41} = 17.2$; $P \leq 0.001$
ACC Water + Iopamidol + HOCl	40 -240	166×	0.95	160×	$F_{10, 39} = 21.2$; $P \leq 0.001$
ACC Water + Iopamidol	120-1000	NA	NA	800×	$F_{9, 49} = 3.52$; $P \leq 0.002$
ACC Water + NH_2Cl	120-1000	760×	0.88	800×	$F_{21, 98} = 6.92$; $P \leq 0.001$
ACC Water + Iopamidol + NH_2Cl	120-1000	588×	0.93	520×	$F_{21, 38} = 25.3$; $P \leq 0.001$
ACC Water	120 - 1000	NA	NA	NS	$F_{9, 50} = 1.69$; $P = 0.12$

[a] Athens-Clark County (ACC) water with and without disinfection and with and without iopamidol (IPM). [b] The %C½value is the concentration factor of the extract determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative control. [c] The coefficient of determination for the regression analysis upon which the %C½ value was calculated. [d] Lowest toxic concentration factor of the water concentrate in the concentration-response curve that induced a significant reduction in cell density 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. [f] The SCGE genotoxic potency value is the concentration factor that was calculated, using regression analysis, at the midpoint of the curve within the concentration range that expressed above 70% cell viability. [g]The coefficient of determination for the regression analysis upon which the genotoxic potency value was calculated. [h] Lowest genotoxic concentration factor of the water concentrate in the concentration-response curve that induced significant genomic DNA damage as compared to the negative control. [i] Not applicable. [j] Not significant.

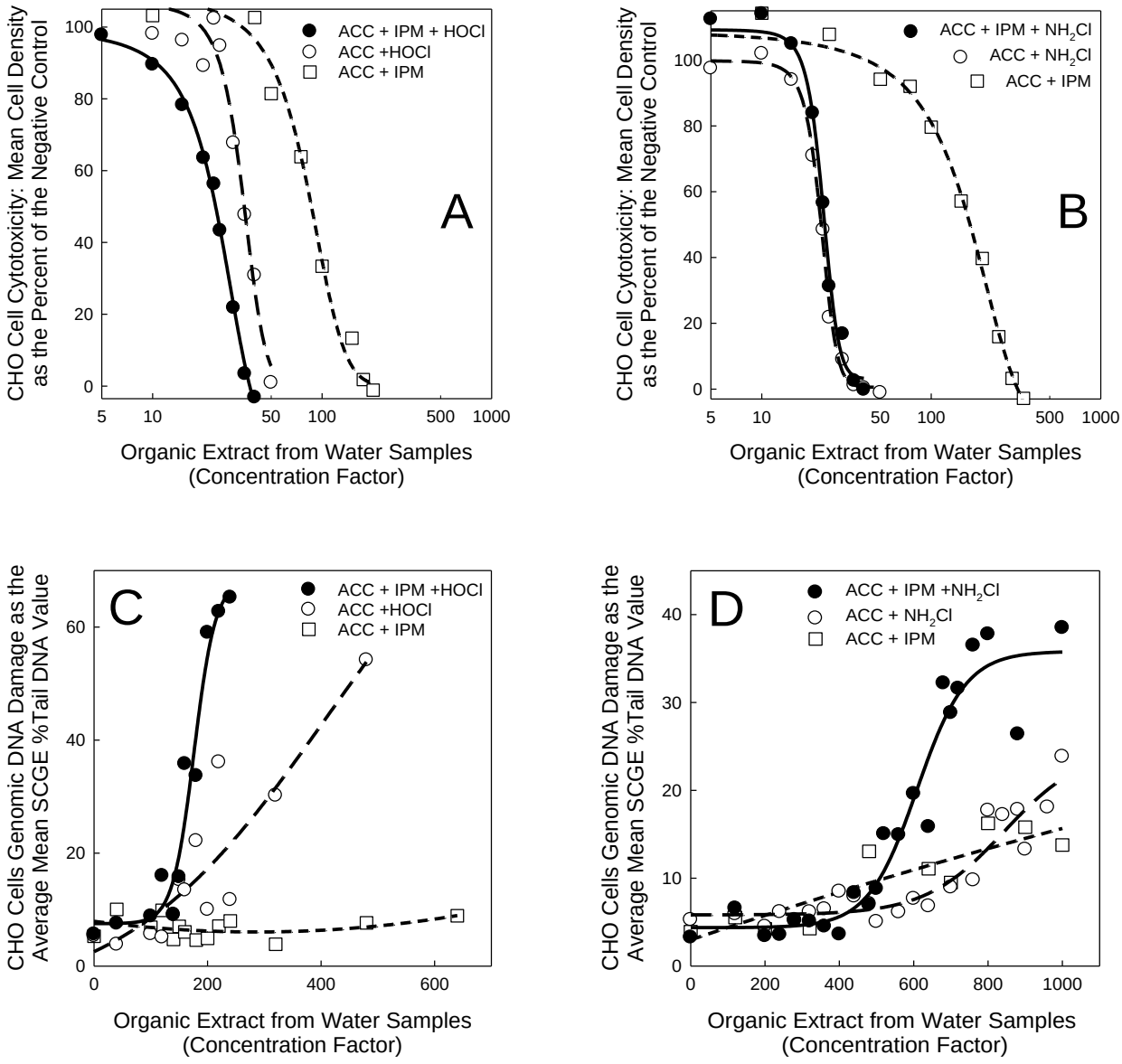


Figure 2. (2A) Concentration-response curves of CHO cell chronic cytotoxicity of organic extracts of Athens-Clark County (ACC) source water with iopamidol (IPM), ACC water after chlorination and ACC water plus iopamidol plus chlorination. (2B) Concentration-response curves of CHO cell chronic cytotoxicity of organic extracts of ACC source water with IPM, ACC water after chloramination and ACC water plus IPM plus chloramination. (2C) Concentration-response curves of CHO cell acute genotoxicity of organic extracts of ACC water with IPM, ACC water after chlorination and ACC water plus IPM plus chlorination. (2D) Concentration-response curves of CHO cell acute genotoxicity of organic extracts of ACC water with IPM, ACC water after chloramination and ACC water plus IPM plus chloramination.

Future Research and Implications

As indicated in the previous 23-city iodo-DBP occurrence study, natural iodide is probably still the most important source of iodine in the formation of iodo-DBPs, especially for chloraminated drinking waters (*21*). However, it is evident from the current study that the ICM, iopamidol, can also be a source of iodine in these DBPs for both chlorinated and chloraminated drinking water.

PROPOSALS EMANATED FROM THIS SEED FUNDING

1. Water Research Foundation Proposal (declined)
Title: Toxic Byproducts Generated in Disinfected Drinking Water Contaminated with Pharmaceuticals

Elizabeth D. Wagner, Ph.D.
University of Illinois at Urbana-Champaign

Michael J. Plewa, Ph.D.
University of Illinois at Urbana-Champaign

2. NSF Proposal (declined) This proposal is currently being revised for resubmission.
Title: Formation Mechanisms of Iodinated Disinfection By-Products from X-Ray Contrast Media

Thomas Ternes, Ph.D.,
Diploma Chemist, 11.04.1963,
Head of Water Chemistry department,
Bundesanstalt für Gewässerkunde
Germany

Stephen Duirk, Ph.D.
Department of Civil Engineering,
210 Auburn Science and Engineering Center
University of Akron, Akron, OH 44325, USA

Susan D. Richardson, Ph.D.
U.S. Environmental Protection Agency, National Exposure Research Laboratory, 960 College Station Road, Athens, GA 30605

Michael J. Plewa, Ph.D.
College of Agricultural, Consumer and Environmental Sciences, Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, Phone: (217)-333-3614

UTILITY OF SEED FUNDING TO DEVELOP A FUTURE PROGRAM

This seed funding from the Illinois Indiana Sea Grant College Program allowed us to establish a team and begin the analytical chemistry and analytical chemistry research on this important topic. We have prepared a draft manuscript that we shall refine and submit for publication in a

leading peer-reviewed journal. In addition we have a forthcoming presentation to be given at the 41st Annual Meeting of the Environmental Mutagen Society.

Although we have not been successful in sequestering external funding for this project, we are in the process of resubmitting a proposal to the NSF.

STUDENTS SUPPORTED

Jennifer Osiol
Graduate student in the Laboratory of Dr. Michael Plewa

PUBLICATION/PRESENTATIONS

1. Osiol, J.L., Duirk, J.S., Ternes, T.A., Richardson, S.D., Wagner E.D., Plewa, M.J. 2010Genotoxicity of X-Ray Contrast Agent-Contaminated Water after Disinfection. Environ. Molecular Mutagenesis 51. (Abstract) In Press.
2. Duirk, S.E., Lindell, C., Cornelison, C.C.,Ternes, T.A., Attene-Ramos. M., Osiol, Wagner, E.D., Plewa, M.J., Richardson, S.A. 2010, Formation of Toxic Iodinated Disinfection By-Products from Compounds Used in Medical Imaging, manuscript in preparation.

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