

SEA GRANT PROJECT SUMMARY FORM (90-2)

INSTITUTION: University of Illinois at Urbana-Champaign

ICODE:

TITLE: Toxic Byproducts Generated in Disinfected Drinking Water Contaminated with Pharmaceuticals

PROJECT NUMBER:

REVISION DATE:

PROJECT STATUS:

INITIATION DATE: 09/30/2009

COMPLETION DATE: 09/30/2010

SUB PROGRAM:

PRINCIPAL INVESTIGATOR: Dr. Michael Plewa

EFFORT: 0.5

AFFILIATION: University of Illinois

AFFILIATION CODE:

ASSOCIATE INVESTIGATOR:

EFFORT:

AFFILIATION:

AFFILIATION CODE:

SEA GRANT FUNDS: \$

STATE MATCHING FUNDS: \$

LAST YEAR'S SEA GRANT FUNDS: \$

LAST YEAR'S MATCHING FUNDS: \$

PASS-THROUGH FUNDS: \$

LAST YEAR'S PASS-THROUGH FUNDS: \$

RELATED PROJECTS:

PARENT PROJECTS: R/WF-09-06

SEA GRANT STRATEGIC PLAN CLASSIFICATION: Water Quality/Pharmaceuticals

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. 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 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 is to analyze source water contaminated with the X-ray imaging contrast pharmaceutical iopamidol before and after disinfection with chlorine. The specific objectives are to, (1) determine if this contaminant pharmaceutical is directly cytotoxic and genotoxic in mammalian and human cells, (2) determine if iopamidol is converted into byproducts after chlorine disinfection that are cytotoxic and genotoxic in mammalian and human cells, and (3) determine if there is a correlation between the formation of iopamidol-mediated iodinated drinking water disinfection byproducts and toxicity. These results will be used as a foundation for a proposal to a federal agency (NIH, NSF or EPA) to fund this important research in pharmaceutical contamination of drinking water sources.

METHODOLOGY: We shall employ mammalian and human cell biological assays to quantitatively measure chronic cytotoxicity and the induction of genomic DNA damage induced by the X-ray contrast pharmaceutical agent iopamidol. Iopamidol belongs to a class of drugs that are employed to enhance the resolution of soft tissues by X-ray or MRI scanning and are widely used by the medical industry (Figure 1) [1].

Media and Reagents:

All laboratory chemicals will be reagent grade or higher. All laboratory glassware and plastic ware will be purchased from reputable vendors. RNase-free and DNase-free reagents, pipet tips, plasticware and glassware will be kept separated from the general laboratory supply.

Chinese hamster ovary cells: CHO cells are widely used in toxicology. The transgenic CHO cell line AS52 was derived from the parental K1-BH4 line. Clone 11-4-8 was isolated from AS52 by Dr. E. Wagner [2]. The cells exhibit normal morphology, express cell contact inhibition, grow as a monolayer without expression of neoplastic foci, express a stable chromosome complement and a consistent cell doubling time as well as functional p53 protein. Stock cultures of the CHO cells will be frozen in a solution of 90% fetal bovine serum (FBS):10% dimethylsulfoxide (DMSO) (v/v) and stored at -80°C . Cells will be grown on glass culture plates in Hams F12 medium plus 5% FBS at 37°C in a humidified atmosphere of 5% CO_2 . CHO cells will be transferred when the culture becomes confluent.

Human FHs Cells: Human embryonic small intestine cell line FHs 74int. FHs cells will be obtained from the American Type Culture Collection. These cells are non-neoplastic, diploid, reverse transcriptase negative, adherent, and exhibit cell contact inhibition. These non-transformed cells are not immortal and new cultures will be required periodically. FHs cells will be maintained in modified Dulbecco's Hybricare medium with 2 mM L-glutamine plus 10% fetal bovine serum (FBS), 1% antibiotic (10 units/mL penicillin G sodium, 10 $\mu\text{g}/\text{mL}$ streptomycin sulfate, 25 $\mu\text{g}/\text{mL}$ amphotericin B, 0.85% saline) and 30 ng/mL human epidermal growth factor at 37°C in a humidified atmosphere of 5% CO_2 [3].

Iopamidol Samples: Iopamidol will be obtained from Dr. Susan Richardson, U.S. Environmental Protection Agency, National Exposure Research Laboratory, Athens, Georgia. Dr. Richardson will chlorine-disinfect Athens/Clark County source water with and without 10 μM iopamidol. The organic disinfection byproducts (DBPs) from 20 L will be isolated and concentrated on XAD columns [4] and sent to our laboratory at the University of Illinois. We will also analyze iopamidol directly. *In this proposal we refer to iopamidol and the organic extracts of iopamidol in disinfected water as iopamidol samples.*

Chronic Cytotoxicity Assay: *Hypothesis* – Iopamidol in water will express differential chronic cytotoxic responses in mammalian and human cells before and after chlorine disinfection. The chronic cytotoxicity assay measures the level of cytotoxicity as a function of the concentration of the test agent over a 72 h period [5]. A 96-well flat-bottomed microplate will be used to evaluate a series of iopamidol concentrations. One column of 8 wells will serve as the blank control (medium only); the concurrent negative control column will consist of 3×10^3 cells plus medium. The wells of the remaining columns will contain cells, medium and a known concentration of iopamidol sample in a total of 200 μL . The wells will be covered with a sheet of sterile AlumnaSeal™ and the cells will be incubated for 72 h at 37°C at 5% CO_2 . After the treatment time, the medium from each well will be aspirated and the cells will be fixed and stained with a 1% crystal violet solution. The microplate will be washed, 50 μL of DMSO/methanol (3:1 v/v) will be added to each well, and the plate will be analyzed at 595 nm with a BioRad microplate reader; the absorbancy of each well will be recorded and stored on a spreadsheet file. This assay was calibrated and there is a direct relationship between the absorbancy of the crystal violet dye associated with the cells and the number of viable cells [5]. The mean blank-corrected absorbancy value of the negative control will be adjusted to 100%; the absorbancy for each treatment group well will be converted into a percentage of the negative control. For each concentration of the iopamidol sample, 8 replicate wells will be analyzed per experiment, and the experiments will be repeated 2-4x. These data will be used to generate a concentration-response curve for each iopamidol sample. Regression analysis of the concentration-response curve will calculate the LC_{50} value (the iopamidol

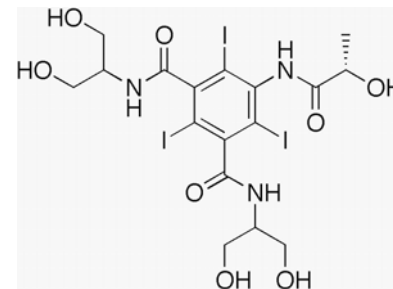


Figure 1. Chemical structure of iopamidol.

sample concentration that induces a cell density that is 50% of the negative control). The data from the cytotoxicity experiments will be transferred to Excel spreadsheets and for each iopamidol sample a one-way analysis of variance (ANOVA) test will be conducted to determine if the iopamidol sample induced a significant level of cell killing at a specific concentration. If a significant F value of $P \leq 0.05$ is obtained, a Holm-Sidak multiple comparison versus the control group analysis will be conducted. The power of the test statistic will be maintained as ≥ 0.8 at $\alpha = 0.05$.

Genotoxicity Assay: *Hypothesis* – Iopamidol in water will express differential genotoxic responses in mammalian and human cells before and after chlorine disinfection. Single Cell Gel Electrophoresis (SCGE) is a molecular genetic assay that quantitatively measures the level of genomic DNA damage, including single and double strand breaks, incomplete excision repair sites and alkali-labile sites, induced in individual nuclei of cells [6]. The day before treatment, 4×10^4 cells will be added to each microplate well in 200 μL medium and incubated. The next day the cells will be washed with Hank's balanced salt solution (HBSS) and treated with a series of concentrations of an iopamidol sample in medium without FBS in a total volume of 25 - 50 μL for 4 h at 37°C, 5% CO_2 . The wells will be covered with sterile AlumnaSeal™. With each experiment a negative control, a positive control (3.8 mM ethylmethanesulfonate, EMS) and 9 concentrations of an iopamidol sample will be conducted concurrently. After incubation the cells will be washed and harvested. To measure acute cytotoxicity a 10 μL aliquot of cell suspension will be mixed with 10 μL of 0.05% trypan blue vital dye in phosphate-buffered saline (PBS). To reduce artifacts from high levels of cytotoxicity, SCGE data will not be used if the acute cytotoxicity exceeds 30%. The remainder of the cell suspension from each well will be embedded in a layer of low melting point agarose prepared with PBS and placed upon previously coated SCGE slides. After the microgels solidify on ice, a final layer of 0.5% low melting point agarose will be placed upon the previous layers. The cellular membranes will be removed by an overnight immersion in lysing solution at 4°C. The microgels will be placed in an alkaline buffer (pH 13.5) in an electrophoresis tank at 4°C and the DNA will be denatured for 20 min and then electrophoresed at 25 V, 300 mA (0.72 V/cm) for 40 min. The microgels will be neutralized with Tris buffer, pH 7.5, rinsed, dehydrated in cold methanol, dried at 50°C and stored at room temperature. For microscopic analysis the microgels will be hydrated in cold water for 20 min and stained with 65 μL of ethidium bromide (20 $\mu\text{g}/\text{mL}$). The microgels will be rinsed and analyzed with a Zeiss fluorescence microscope with an excitation filter of 546/10 nm and a barrier filter of 590 nm. For each experiment 2 microgels will be prepared per treatment group and nuclei will be analyzed in each microgel using a charged coupled device camera. A computerized image analysis system (Komet version 3.1, Kinetic Imaging Ltd., Liverpool, UK) will be employed to determine the tail moment (integrated value of migrated DNA density multiplied by the migration distance) of the nuclei as a measure of DNA damage. The digitalized data will be automatically transferred to a computer based spreadsheet for subsequent statistical analysis. The experiments will be repeated 3 \times for each iopamidol sample. The median tail moment value for each microgel will be calculated and transferred to a data spreadsheet with the acute cytotoxicity of the treated cells. For each iopamidol sample a concentration-response curve will be generated. The data will be regressed and the SCGE genotoxic potency value will be calculated. The SCGE genotoxic potency value represents the midpoint of the curve within the concentration range that expressed above 70% cell viability. For statistical analysis the median tail moment value for each microgel will be determined as described above and the data will be averaged amongst all of the microgels for each iopamidol concentration. The averaged median tail moment values obtained from repeated experiments will be analyzed with a one-way ANOVA test [7]. If a significant F value of $P \leq 0.05$ is obtained, a Holm-Sidak multiple comparison versus the control group analysis will be conducted. The power of the test statistic will be maintained as ≥ 0.8 at $\alpha = 0.05$.

RATIONALE: The contamination of wastewaters, surface and ground waters by X-ray contrast pharmaceuticals have been widely studied in Germany where annually 500 metric tons of these pharmaceuticals are consumed annually [1]. Iodinated X-ray contrast media exhibit high biochemical stability and are excreted by patients usually within 24 h of treatment. In the environment iodinated X-ray contrast media are the main contributors to the burden of total absorbable organic halogens in clinical wastewater. In a recent study on the occurrence and toxicity of iodinated DBPs, (I-DBPs) we discovered that several drinking water plants generated highly toxic I-DBPs yet they did not contain free iodine in their source waters [8]. However, the source waters of these utilities were contaminated with iopamidol. Dr. Richardson discovered that if iopamidol was added to a drinking water source water and chlorinated, I-DBPs were generated (Richardson, unpublished data). This suggests that highly toxic I-DBPs [9] may be generated in the process of drinking water disinfection if the intake water is contaminated with these X-ray contrast pharmaceuticals. We recently conducted a preliminary experiment in which Athens/Clark Co source water was chlorinated, with and without iopamidol. The results demon-

strated that the sample that contained the iopamidol induced increased genotoxicity in mammalian cells (Plewa, unpublished data) (Figure 2). These remarkable data indicate that a non-toxic, ubiquitous, pharmaceutical water contaminant can be transformed into byproducts that are highly genotoxic. We propose to generate sufficient preliminary data with this IL-IN Sea Grant Development Project for use in the development of a research proposal to a federal agency.

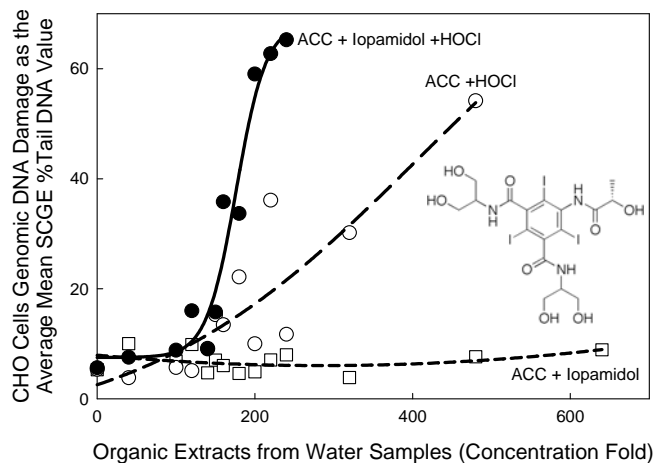


Figure 2. CHO cell SCGE analysis for genomic DNA damage induced by the samples received from Dr. Richardson's laboratory. The samples are: (1) Athens/Clark Co source water plus 10 μ M iopamidol (open squares), (2) Athens/Clark Co source water plus HOCl (open circles), and (3) Athens/Clark Co source water plus 10 μ M iopamidol plus HOCl (closed circles). The values listed on the X-axis are concentration fold numbers of the original 10 L water samples. Thus a concentration of 400 \times represents a 400-fold concentration of the organics isolated from the water sample. All the genotoxicity data presented here were derived from concentrations that did not induce >10% acute cytotoxicity.

References

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2. Wagner, E. D.; Rayburn, A. L.; Anderson, D.; Plewa, M. J., Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells. *Environ. Mol. Mutagen.* **1998**, *32*, (4), 360-368.
3. Mueller, M. G.; Attene-Ramos, M. S.; Hudson, M. E.; Wagner, E. D.; Plewa, M. J., Human cell toxicogenomic analysis of bromoacetic acid: a regulated drinking water disinfection by-product. *Environ. Mol. Mutagen.* **2009**, *In Press*.
4. Crumley, G., XAD resin extraction disinfectant by-products in drinking water: EPA SOP RSB-003.0 In U. S. Environmental Protection Agency, Ed. Athens, GA, 2003; pp 1-3.
5. Plewa, M. J.; Kargalioglu, Y.; Vanker, D.; Minear, R. A.; Wagner, E. D., Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. *Environ. Mol. Mutagen.* **2002**, *40*, (2), 134-142.
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7. Lovell, D. P.; Thomas, G.; Dubow, R., Issues related to the experimental design and subsequent statistical analysis of in vivo and in vitro comet studies. *Teratogen. Carcinogen. Mutagen.* **1999**, *19*, (2), 109-119.
8. Richardson, S. D.; Fasano, F.; Ellington, J. J.; Crumley, F. G.; Buettner, K. M.; Evans, J. J.; Blount, B. C.; Silva, L. K.; Waite, T. J.; Luther, G. W.; McKague, A. B.; Miltner, R. J.; Wagner, E. D.; Plewa, M. J., Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. *Environ. Sci. Technol.* **2008**, *42*, (22), 8330-8338.
9. Plewa, M. J.; Wagner, E. D.; Richardson, S. D.; Thruston, A. D., Jr.; Woo, Y. T.; McKague, A. B., Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts. *Environ. Sci. Technol.* **2004**, *38*, (18), 4713-4722.

SEA GRANT BUDGET FORM 90-4

GRANTEE: University of Illinois			GRANT/PROJECT NO.:		
PRINCIPAL INVESTIGATOR: Dr. Michael Plewa Co-PI:			DURATION: 12 months Cumm 09/30/09-09/30/10		
A. SALARIES AND WAGES:		man-months			
	No. of People	Amount of Effort	Sea Grant Funds	Matching Funds	
1. Senior Personnel					
a. (Co) Principal Investigator	1	0.5	0		6783
b. Associate (Faculty or Staff):	0	0	0		0
	Subtotal:	1	0.5	0	6783
2. Other Personnel					
a. Professionals:	0	0	0		0
b. Research Associates:	0	0	0		0
c. Res. Asst./Grad Students:	0	0	0		0
d. Prof. School Students:	0	0	0		0
e. Pre-Bachelor Student(s):	0	0	0		0
f. Secretarial-Clerical:	0	0	0		0
g. Technicians:	0	0	0		0
h. Other:	0	0	0		0
	Total Salaries and Wages	0	0	0	0
B. FRINGE BENEFITS:					
Total Personnel (A and B)			0		0
C. PERMANENT EQUIPMENT:					
			0		0
D. EXPENDABLE SUPPLIES AND EQUIPMENT:					
			6300		0
E. TRAVEL:					
1. Domestic			0		0
2. International			0		0
Total Travel			0		0
F. PUBLICATION AND DOCUMENTATION COSTS:					
			0		0
G. OTHER COSTS:					
Communications			0		0
Copying			0		0
Postage/Mailing			0		0
Contractual Services			0		0
Membership/Sponsorship Fees			0		0
Training/Continuing Education			0		0
Project/Person Recognition			0		0
Housing/Board/Research			0		0
Tuition Remission			0		0
Other: 10. Other			0		0
Other: 11. Other			0		0
Total Other Costs:			0		0
TOTAL DIRECT COST (A through G):			6300		0
INDIRECT COST					
On Campus	58.50%		3688		0
Off Campus	0.00%		0		0
Total Indirect Cost:			3688		
TOTAL COSTS			9986		6783

Budget Justification (Form 90-4)

2009 Development Pre-Proposal

Dr. Michael J. Plewa, PI, University of Illinois at Urbana-Champaign

Title: **Toxic Byproducts Generated in Disinfected Drinking Water Contaminated with Pharmaceuticals**

The justification for the budget of this 2009 Illinois-Indiana Sea Grant Development Pre-Proposal is listed below. The total direct costs are \$6300.

1. Human FHs 74int cells. These cells are non-transformed fetal intestinal cells that are not immortalized. Thus a culture must be purchased for each experiment. Cost plus shipping and handling @450. Four cultures will be required at a cost of \$1800.
2. Fetal bovine serum (highest quality) is necessary to provide growth factors for the CHO cells and the human FHs cells @500/500 ml. We will require 1500 ml at a cost of \$1500.
3. Cloned human growth factor. This reagent is specifically required for the human FHs cells. The cost is \$600.
4. Plastic/glassware and disposables, computer supplies and materials for safety, \$900.
5. Chemical reagents, cell culture media, N₂ and CO₂ gas cylinders, \$700.
6. Zeiss fluorescent microscope replacement halogen burners (for SCGE experiments), \$800.

Please note that Dr. Plewa will conduct the experiments listed in the pre-proposal and he will prepare the resulting research grant proposals to the federal agencies at no cost to the IL-IN Sea Grant Development project. Dr. Plewa's time is listed as matching funds.

The total indirect costs (F&A) are based on University of Illinois negotiated federal cost rate. Indirect costs is \$3686

Total direct and indirect funds = \$9986.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME		POSITION TITLE	
Michael J. Plewa		Professor of Genetics	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Loyola University, Chicago	B.S.	1969	Biology (Chemistry, Philosophy minors)
Illinois State University, Normal, IL	M.S.	1971	Biological Sci. (Genetics)
Illinois State University, Normal, IL	Ph.D.	1974	Biological Sci. (Genetics)
University of Illinois at Urbana-Champaign	Postdoctoral	1974-1976	Genetics

A. Positions and Honors**Positions and Employment**

1969-1974	Graduate Research Asst.	D.F. Jones Scholar, Research Corp., Illinois State University
1974-1976	Post doctorate in Genetics	D.F. Jones Fellow, Research Corp., University of Illinois
1976-1978	Assist. Res. Geneticist	Institute for Environmental Studies, University of Illinois
1978-1982	Assist., Assoc., Full Prof.	Institute for Environmental Studies, University of Illinois
1995-present	Professor, Assoc. Head	Dept. of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois
2007-present	Professor/Investigator	NSF STC of Advanced Materials for the Purification of Water with Systems (WaterCAMPWS), College of Engineering, UIUC.

Other Experience and Professional Membership

1992-1993	Research Geneticist	National Institute for Environmental Health Sciences, Research Triangle Park, NC
1997	Visiting Research Scholar	Research Center for Environmental Quality Management, Faculty of Engineering, Kyoto University, Kyoto, Japan
2002-2003	Honorary Professor	University of Bradford, UK

Honors

1986	University Scholar for distinction as a member of the faculty of the University of Illinois.
1991	University of Manitoba Distinguished Professor Lectureship
1992-1993	J. William Fulbright Senior Scholar, U.S. Information Agency, 1992-1993
1978-present	UIUC Center for Teaching Excellence Incomplete List of Teachers Ranked as Excellent (19x)
1999	Broadrick-Allen Award for Excellence in Honors Teaching, University of Illinois
2000	Campus Wide Award for Excellence in Guiding Undergraduate Research, University of Illinois,
2003	Illinois State University Alumnus Achievement Award, 2003
2008	Phi Kappa Phi Honor Society, Chapter 46, President
2009	NACTA Teacher Fellow Award (national award)
2009	Environmental Mutagen Society, President-Elect, 40 th Annual meeting Program Chair

B. Selected peer-reviewed publications (of 185)

Rundell, M. S.; Wagner, E. D.; Plewa, M. J., The comet assay: genotoxic damage or nuclear fragmentation?
Environ. Mol. Mutagen. **2003**, *42*, (2), 61-67.

Principal Investigator/Program Director (Last, first, middle):

- Plewa, M. J.; Wagner, E. D.; Jazwierska, P.; Richardson, S. D.; Chen, P. H.; McKague, A. B., Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity. *Environ. Sci. Technol.* **2004**, *38*, (1), 62-68.
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- Plewa, M.J. and E.D. Wagner. Drinking Water Disinfection By-Products: Comparative Mammalian Cell Cytotoxicity and Genotoxicity. *Encyclopedia of Environmental Health*, **2009** (In Press).
- Wagner, E.D. and M.J. Plewa. Utility of a Microplate-Based Comet Assay, in A. Dhawan and D. Anderson, Eds, *The Comet Assay in Toxicology*, Royal Soc. Chem, London. **2009** (In Press).
- Gichner, T., E.D. Wagner and M.J. Plewa. The Comet Assay in Plant Systems, in A. Dhawan and D. Anderson, Eds, *The Comet Assay in Toxicology*, Royal Soc. Chem, London. **2009** (In Press).
- Plewa, M. J.; Wagner, E. D., *Quantitative Comparative Mammalian Cell Cytotoxicity and Genotoxicity of Selected Classes of Drinking Water Disinfection By-Products*. Water Research Foundation: Denver, CO, **2009** (In Press). *Book manuscript to be published with an ISBN number*.
- Yeatts, S.D., C. Gennings, E.D. Wagner, J.E. Simmons, and M.J. Plewa. Detecting departure from additivity along a fixed-ratio ray with a piecewise model for dose and interaction thresholds. *J. Agricultural, Biological, and Environmental Statistics*. **2009**. IN PRESS.
- Muellner, M.G., M.Hudson, M. Attene-Ramos, E.D. Wagner, M.J. Plewa. Human Cell Toxicogenomic Analysis of Bromoacetic Acid: A Regulated Drinking Water Disinfection By-product. **2009**. *Environmental and Molecular Mutagenesis* (IN PRESS).
- Walse, S.S., M.J. Plewa, W.A. Mitch. Exploring amino acid side chain decomposition using enzymatic digestion and HPLC-MS: combined lysine transformations in chlorinated waters. **2009** *Analytical Chemistry* (ACCEPTED).
- Yeatts, S.D., C. Gennings, E.D. Wagner, J.E. Simmons, and M.J. Plewa. **2009**. Detecting departure from additivity along a fixed-ratio ray with a piecewise model for dose and interaction thresholds. *J. Agricultural, Biological, and Environmental Statistics*. (IN PRESS)
- Komaki, Y., Pals, J., Wagner, E.D., Marinas, B.J., Plewa, M.J. Mammalian Cell DNA Damage and Repair Kinetics of Monohaloacetic Acid Drinking Water Disinfection By-Products. **2009**. (Submitted).
- Levac, D., W. Mitch, E.D. Wagner, M. J. Plewa. Genotoxicity of water concentrates from swimming pools and hot tubs after continuous disinfection. Manuscript in preparation.