Linking Mass Spectrometry with Toxicology for Emerging Water Contaminants

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3 Ways to Link MS with Toxicology

- Toxicity assays used to test new environmental contaminants previously identified using MS

- Toxicity assays used in conjunction with MS
  - fractions (size or polarity, etc.) are collected and tested for toxicity
  - toxicity of mixture compared to individual known contaminants

- Toxicity assays used to test environmental degradation products or treatment products of contaminants, so their risk can be determined

Will show some examples from my own work,
but also the work of others, to illustrate this

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Drinking Water DBPs

Concern over possible human health risk:

- Epidemiologic studies: risk of bladder cancer; some cause cancer in laboratory animals
- Recent concerns about possible reproductive & developmental effects (from epi studies)

Goal: Comprehensively identify DBPs formed from different disinfectants, test for toxicity, understand their formation, minimize or eliminate in drinking water

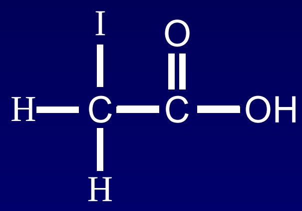
Only 11 DBPs Regulated in U.S.

DBP	MCL (μg/L)
Total THMs	80
5 Haloacetic acids	60
Bromate	10
Chlorite	1000

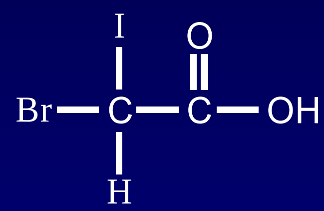
Little known about occurrence, toxicity of unregulated DBPs

Regulated DBPs do not cause bladder cancer in animals!

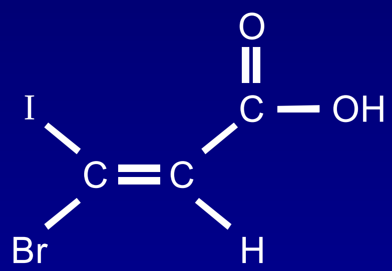
New Iodo-Acids Identified in Nationwide Occurrence Study



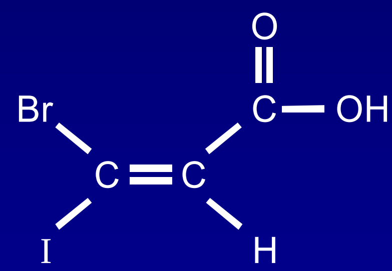
Iodoacetic acid



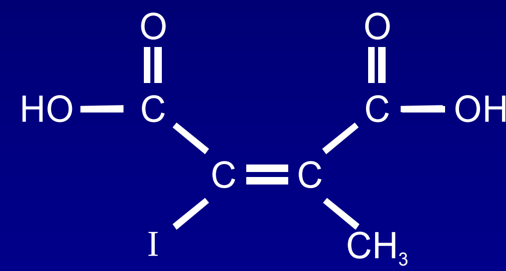
Bromoiodoacetic acid



(Z)-3-Bromo-3-iodopropenoic acid



(E)-3-Bromo-3-iodopropenoic acid

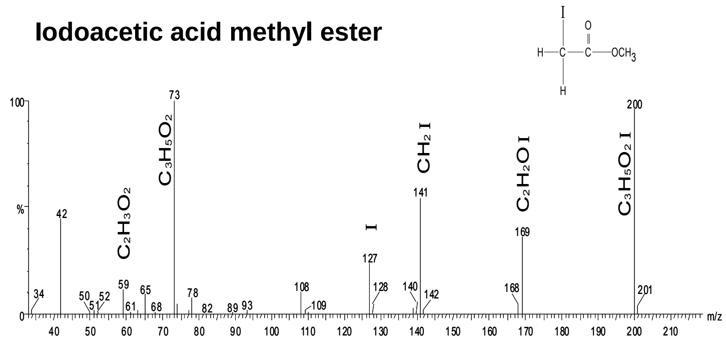


(E)-2-Iodo-3-methylbutenedioic acid

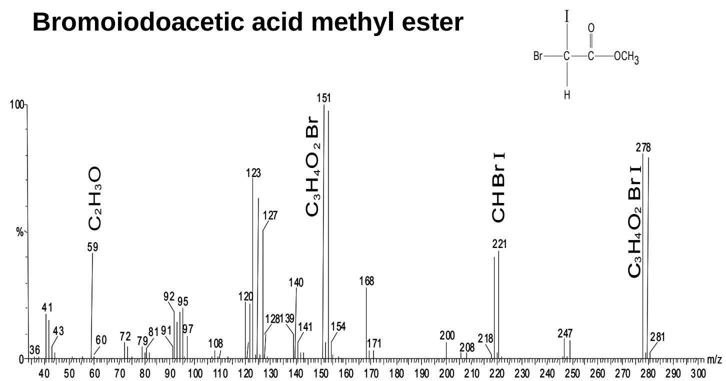
Krasner, Weinberg, Richardson, et al., *Environ. Sci. Technol*. 2006, 40, 7175-7185.

EI Mass Spectra of Iodo-Acids

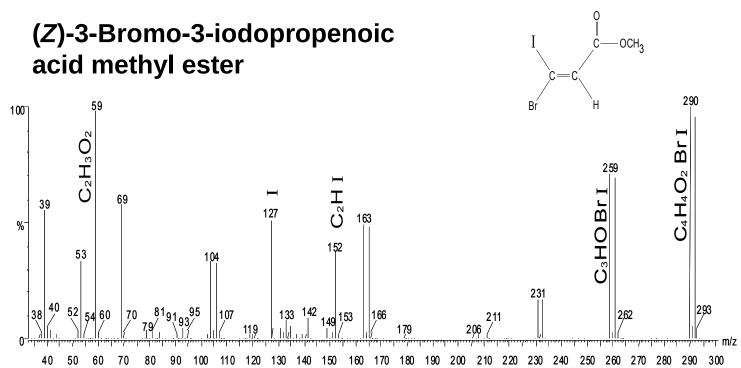
Iodoacetic acid methyl ester



Bromoiodoacetic acid methyl ester



(*Z*)-3-Bromo-3-iodopropenoic acid methyl ester



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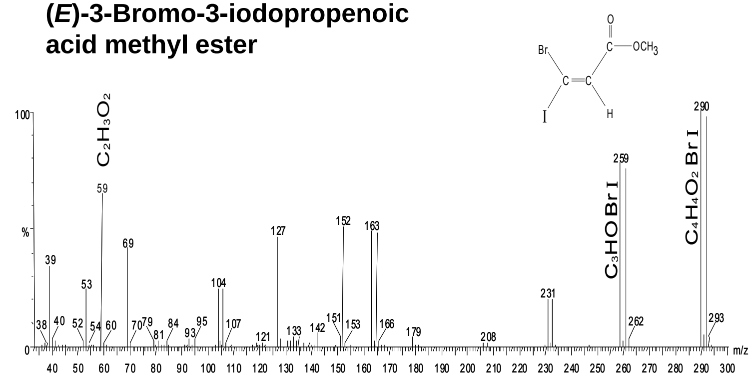

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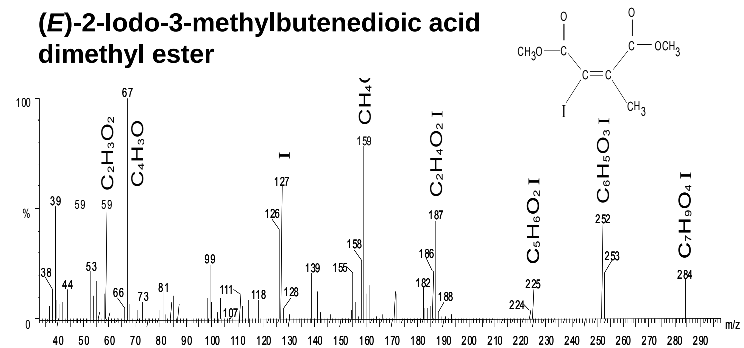
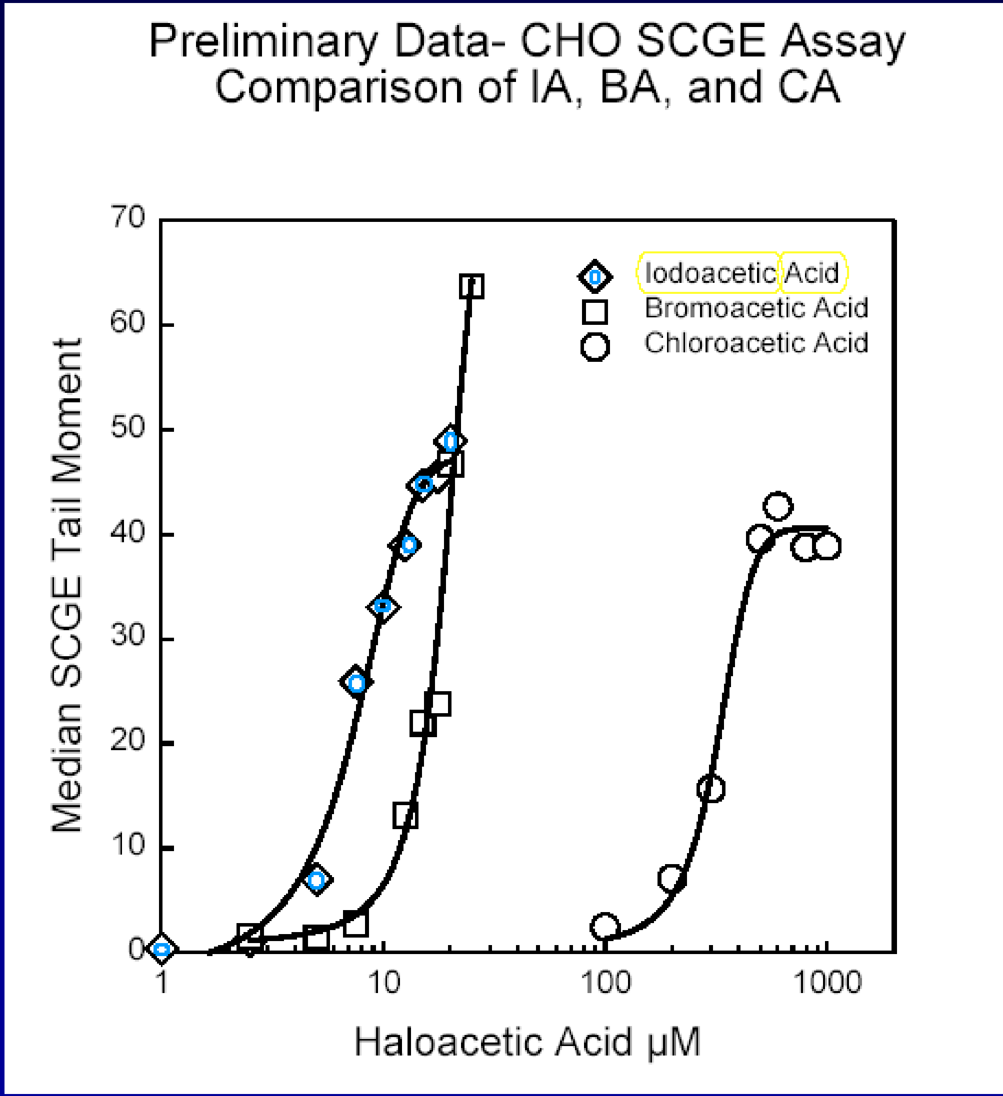


Genotoxicity of Iodoacetic acid



Michael Plewa

Plewa et al., *Environ. Sci. Technol.* 2004

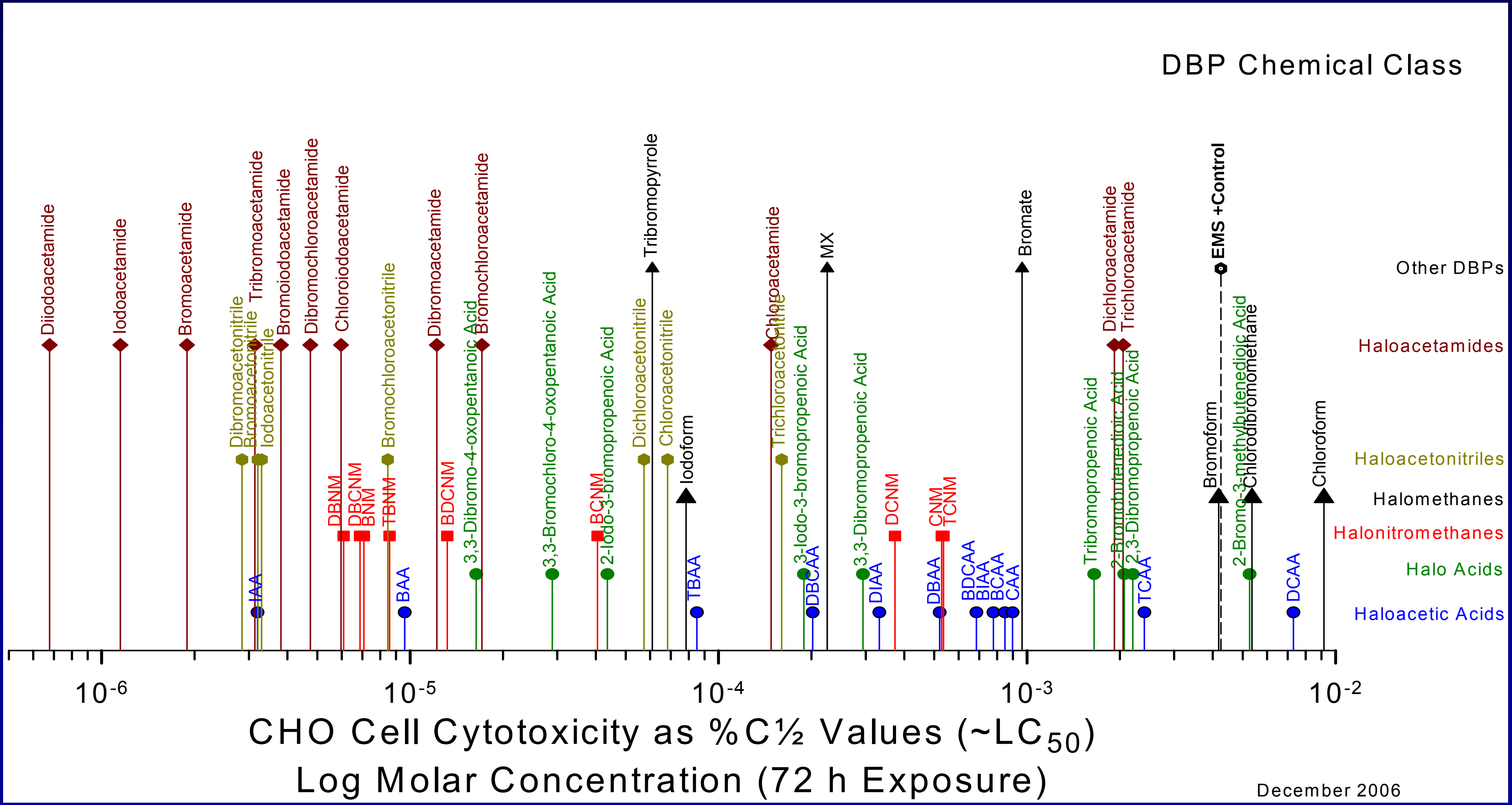
IA also caused developmental effects in mouse embryos (Hunter et al., 1995)

Iodo-DBP Occurrence Study

- 23 City occurrence study (U.S. and Canada)
- GC/NCI/MS and GC/high resolution EI-MS used for quantification
- Found up to 1.7 ppb iodo-acids; 10.2 ppb iodo-THMs (individually)
- Iodo-THMs measured for cytotoxicity/genotoxicity for the first time; also reported cytotoxicity/genotoxicity for other iodo-acids
- Study highlighted potential issues with chloraminated drinking water

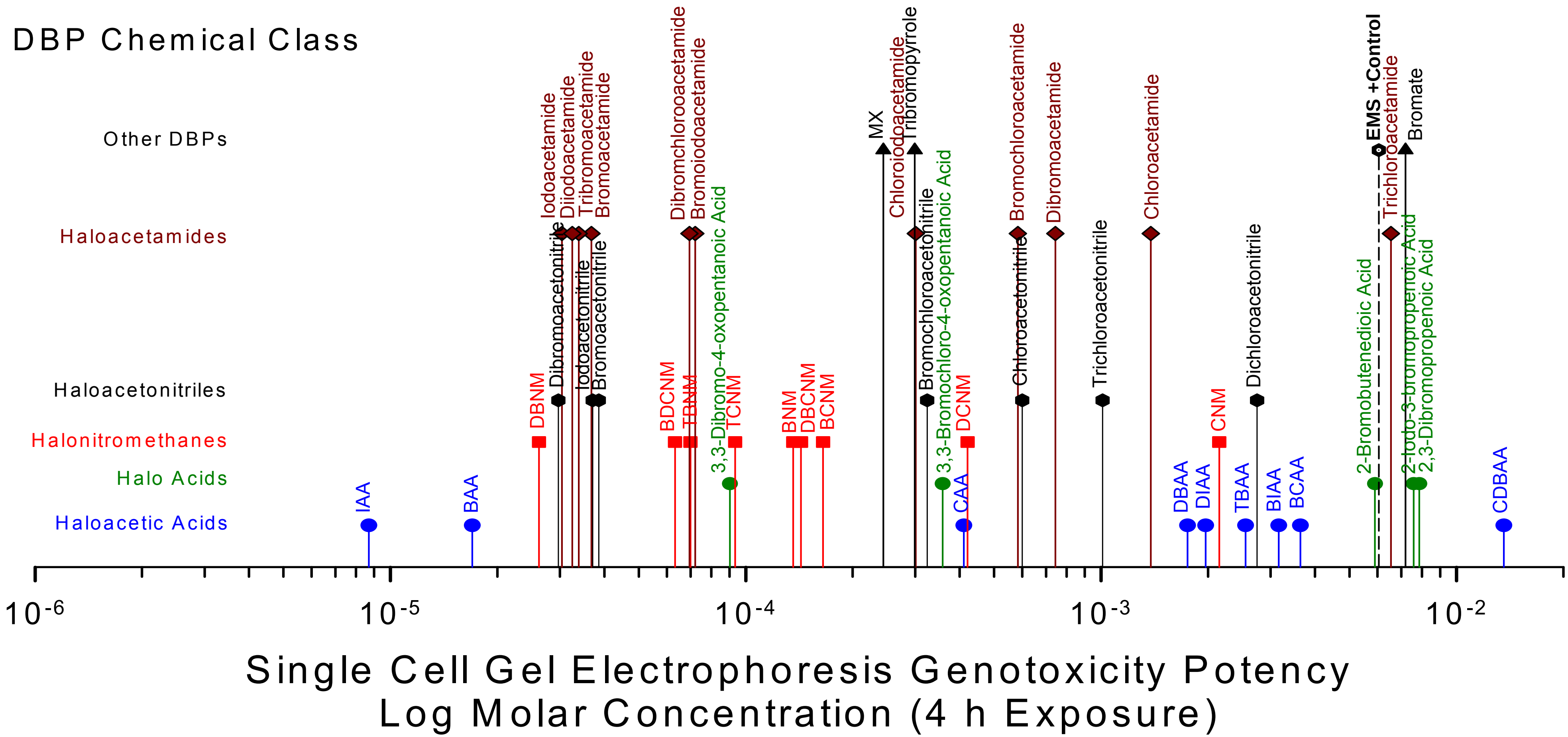
Richardson et al., *Environ. Sci. Technol.* 2008, 42, 8330-8338.

Cytotoxicity of DBPs



Data courtesy of Michael Plewa, University of Illinois

Genotoxicity of DBPs



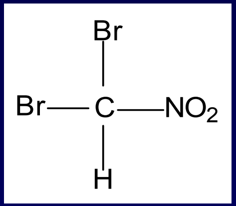
Not Genotoxic: DCAA, TCAA, BDCAA, Dichloroacetamide, Chloroform
Chlorodibromomethane, 3,3-Dibromopropenoic Acid,
3-Iodo-3-bromopropenoic Acid, 2,3,3,Tribromopropenoic Acid

December 2006

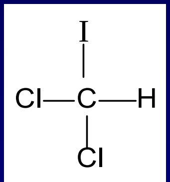
Data courtesy of Michael Plewa, University of Illinois

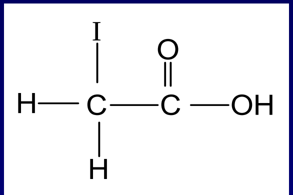
Emerging DBPs

- Halonitromethanes (up to 3 ppb; highly genotoxic); new *in vivo* effects; increased with preozonation
  Krasner, Weinberg, Richardson, et al., *ES&T* 2006, 40, 7175-7185.

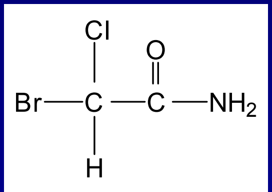


- Iodo-THMs and Iodo-Acids (iodo-THMs up to 15 ppb; iodo-acids up to 1.7 ppb; both classes highly cytotoxic or genotoxic); increased with chloramination
  Richardson et al., *ES&T* 2008, 42, 8330.

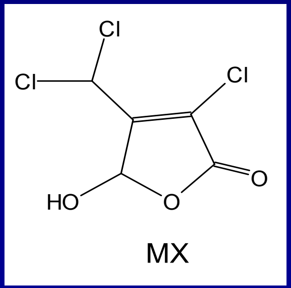




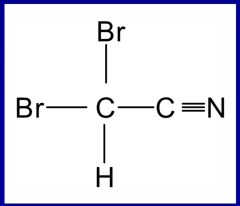
- Haloamides (up to 14 ppb; highly genotoxic) may be increased with chloramination



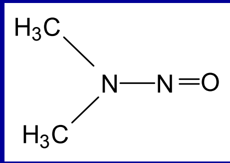
- Halofuranones (up to 2.4 ppb for total MX analogues; genotoxic, carcinogenic); chloramination can also form



- Haloacetonitriles (up to 41 ppb; ~10% of THM4; genotoxic, cytotoxic); may be increased with chloramination



- Nitrosamines (up to 180 ppt; probable human carcinogens) increased with chloramination



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Four Lab Study: Linking DBPs measured to overall toxicity effects

***In vitro*:**

- Reproductive/developmental
- Mutagenicity
- Carcinogenicity
- Neurotoxicity
- Metabolism

***In vivo*:**

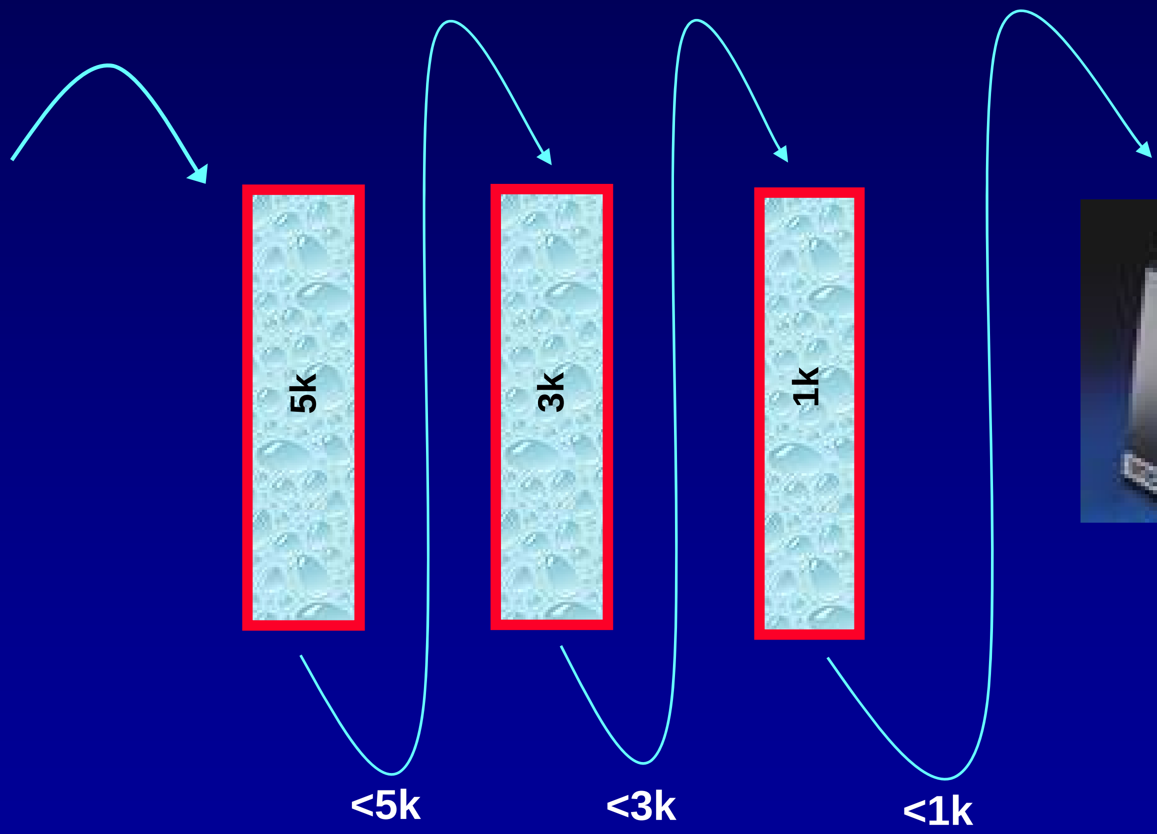
- Reproductive/developmental
- Mutagenicity/carcinogenicity
- Immunotoxicity
- Hepatic/renal toxicity
- Neurotoxicity/developmental neurotoxicity
- Kinetics/metabolism

Jane Ellen Simmons

Purpose: To address concerns related to potential health effects from exposure to complex mixtures of DBPs

High Molecular Weight DBP Research

Ultrafiltration membrane device

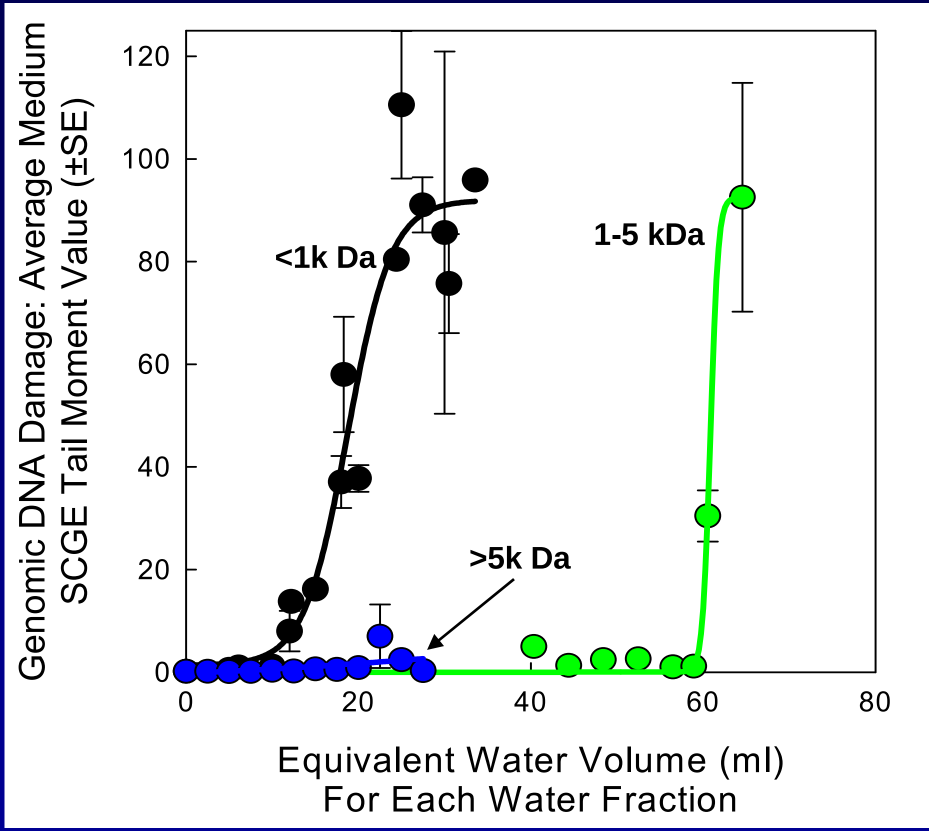


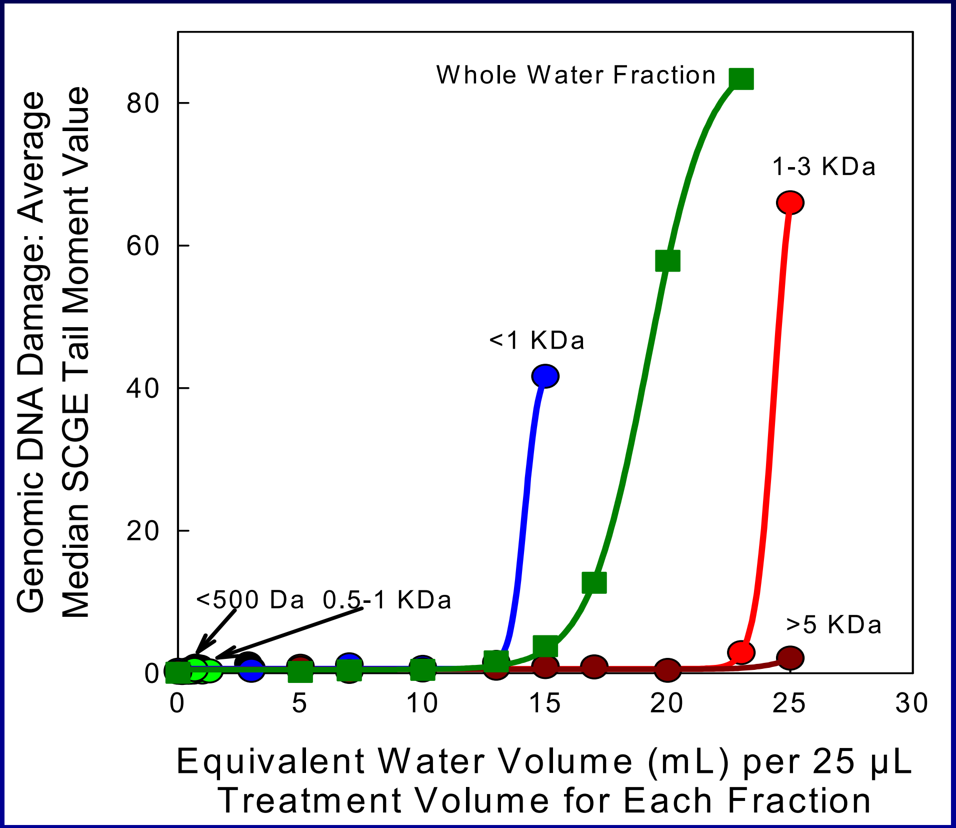
500 MW

Fractions collected: >5kDa 3-5kDa 1-3kDa <1kDa 500-1kDa <500Da

MS and Toxicity Characterization of drinking water fractions

Genotoxicity of Ultrafiltration Fractions



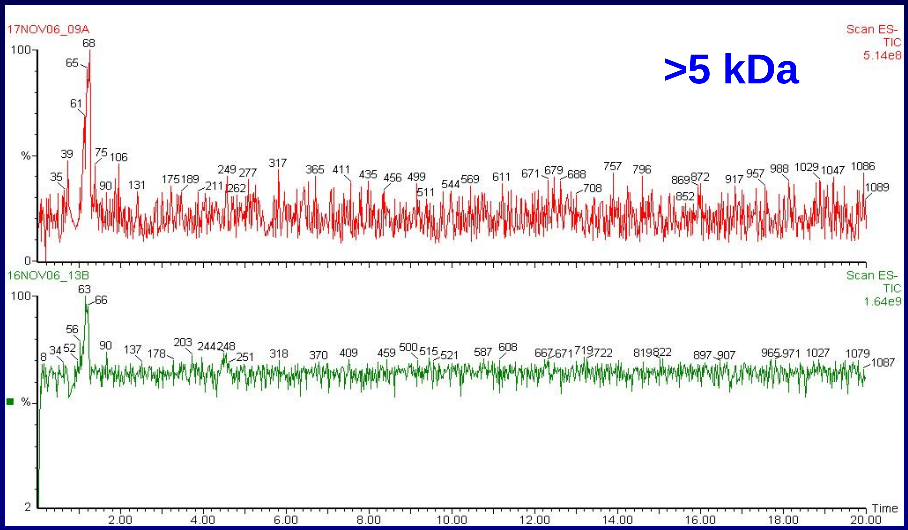


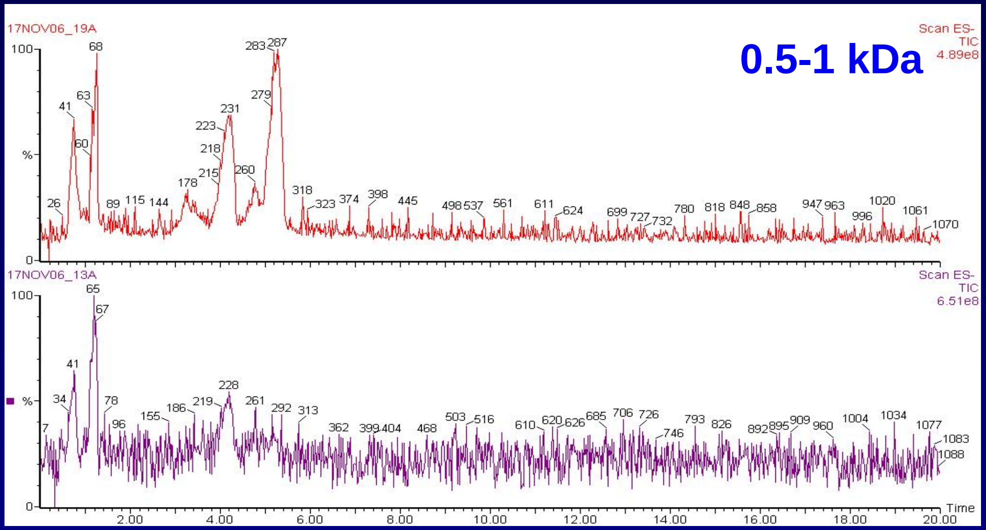
Plant 1 (Chloramination, high Br)

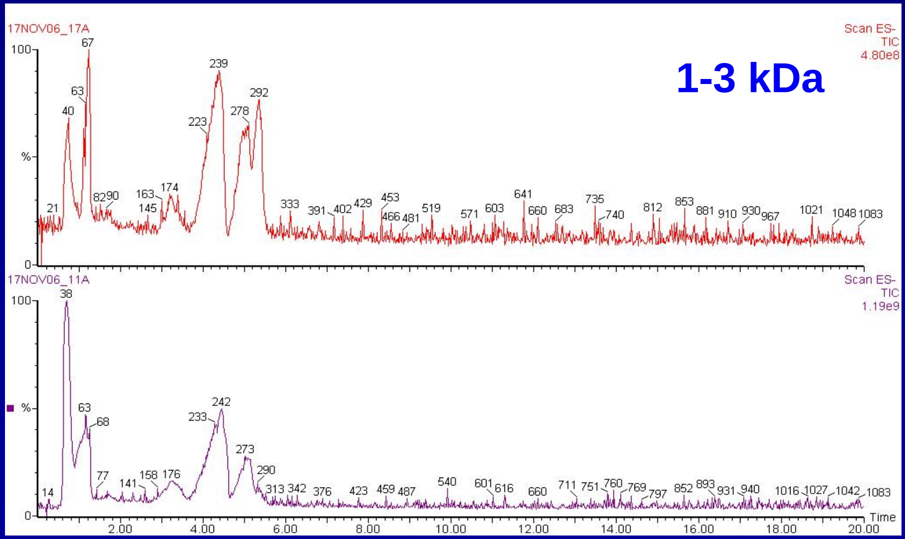
Plant 2 (Chlorination, low Br)

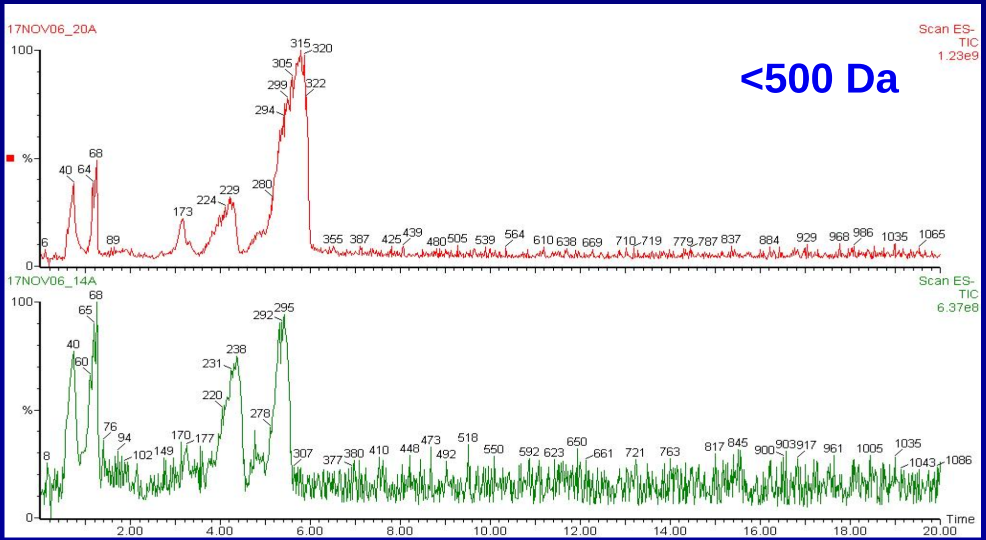
Corresponding raw waters not genotoxic

Chlorinated & Chloraminated Drinking Water
LC / full scan ESI-MS



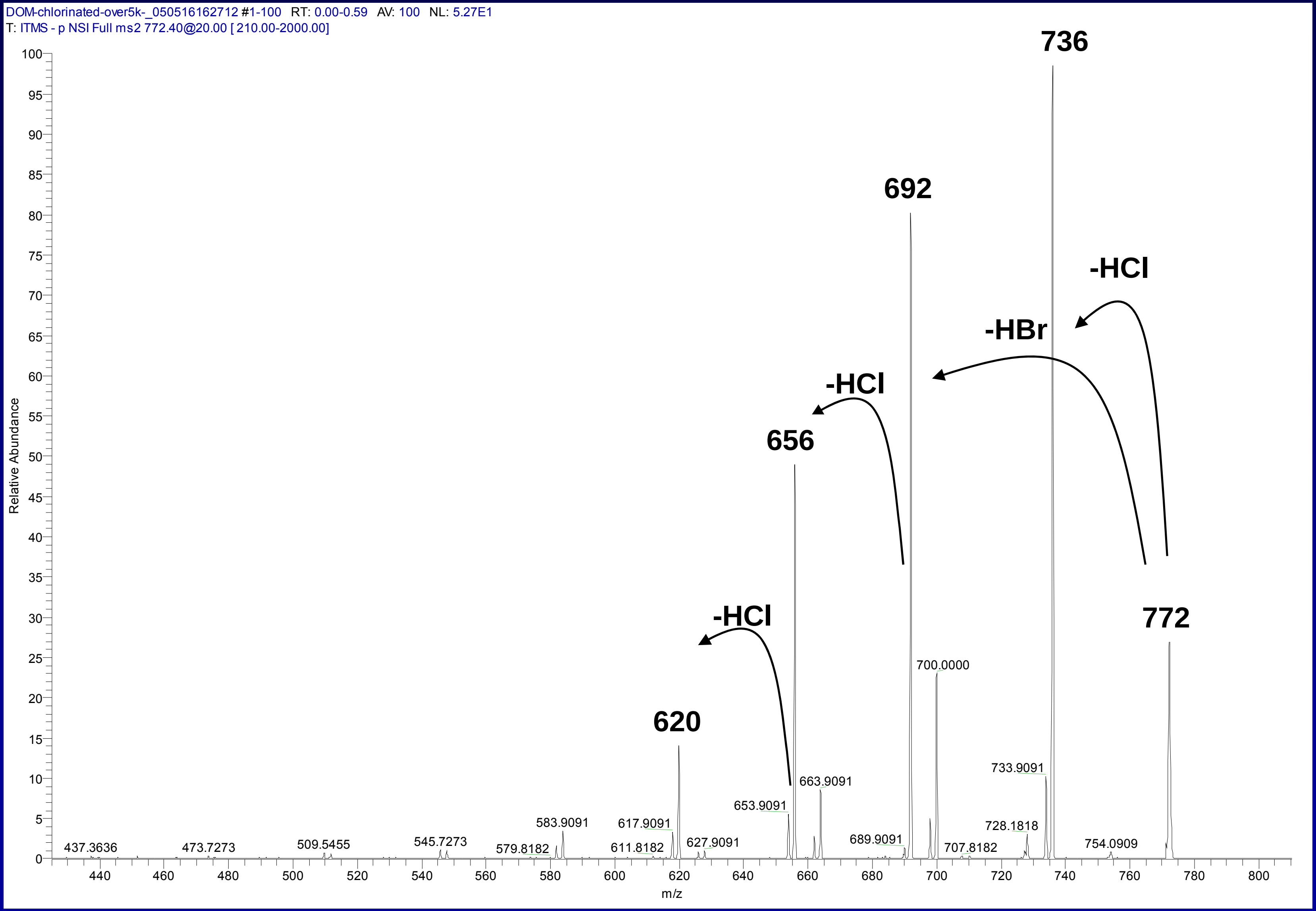




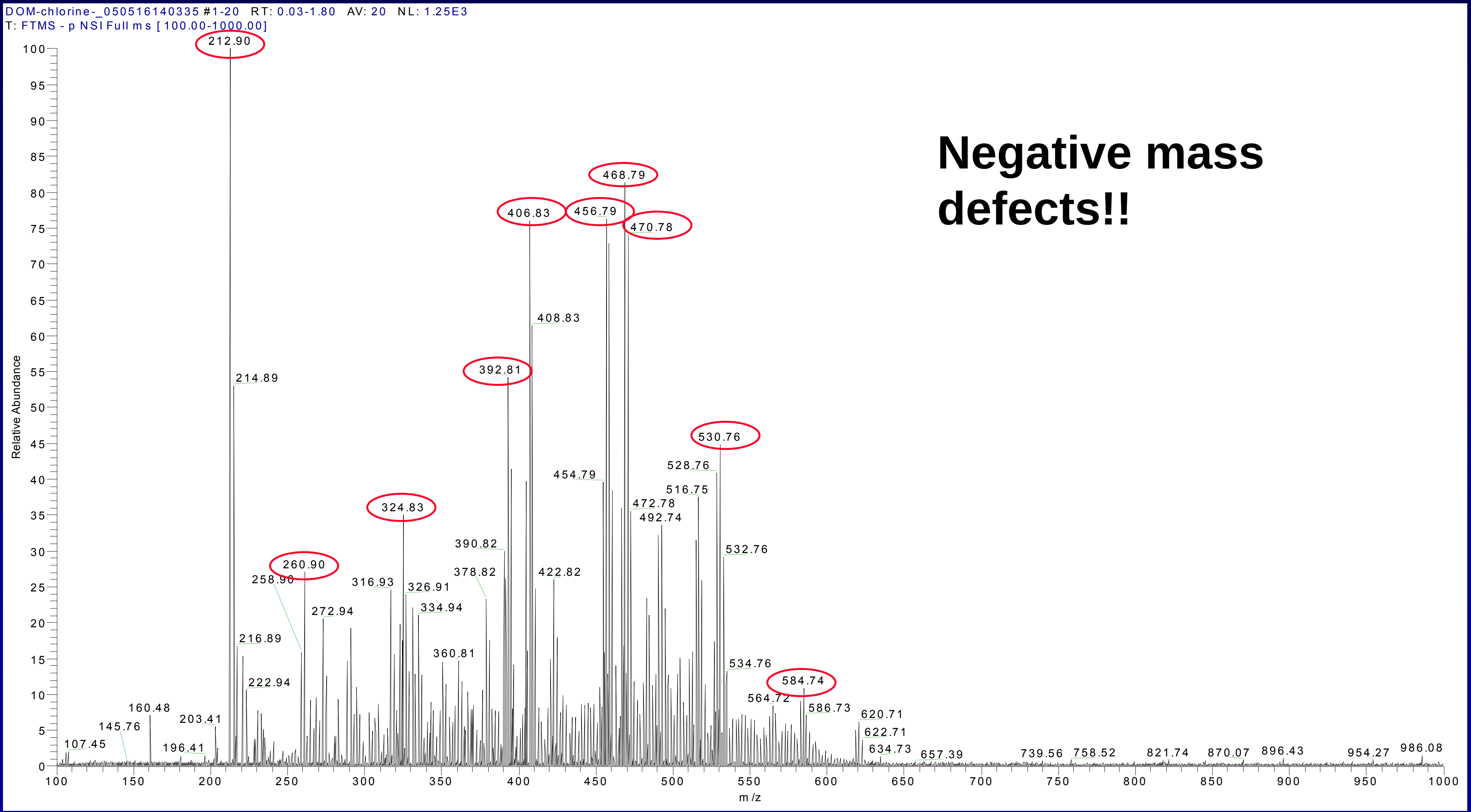


Background subtracted, amino LC column, m/z 20-2000

Daughters (LTQ) of m/z 772



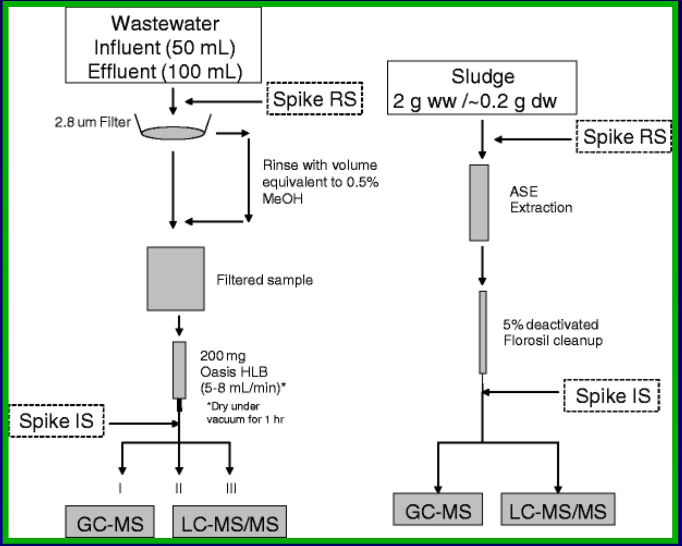
Neg. Ion ESI-MS (FT-MS) of Chlorinated Dissolved Organic Matter



Toxicity Identification Fractionation (TIF)

estrogenic compounds in wastewaters and sludge

- Fractionated with C18 SPE cartridge
- Fraction I: nonylphenol (NP), its mono (NPEO1) and diethoxylate (NPEO2)
- Fraction II: bisphenol A (BPA) and synthetic and natural hormones
- Fraction III: hormone conjugates
- Three fractions analyzed in parallel using GC/MS and LC/MS/MS and recombinant yeast assay (RYA)
- Investigated removal in wastewater treatment and accumulation in sludge



Silvia Lacorte

Fernandez, M. P.; Noguerol, T. N.; Lacorte, S.; Buchanan, I.; Pina, B. *Anal. Bioanal. Chem*. 2009, *393*, 957-968.

University of Alberta (Canada), IBMB-CSIC & IIQAB-CSIC (Barcelona, Spain)

Toxicity Identification Fractionation (TIF)

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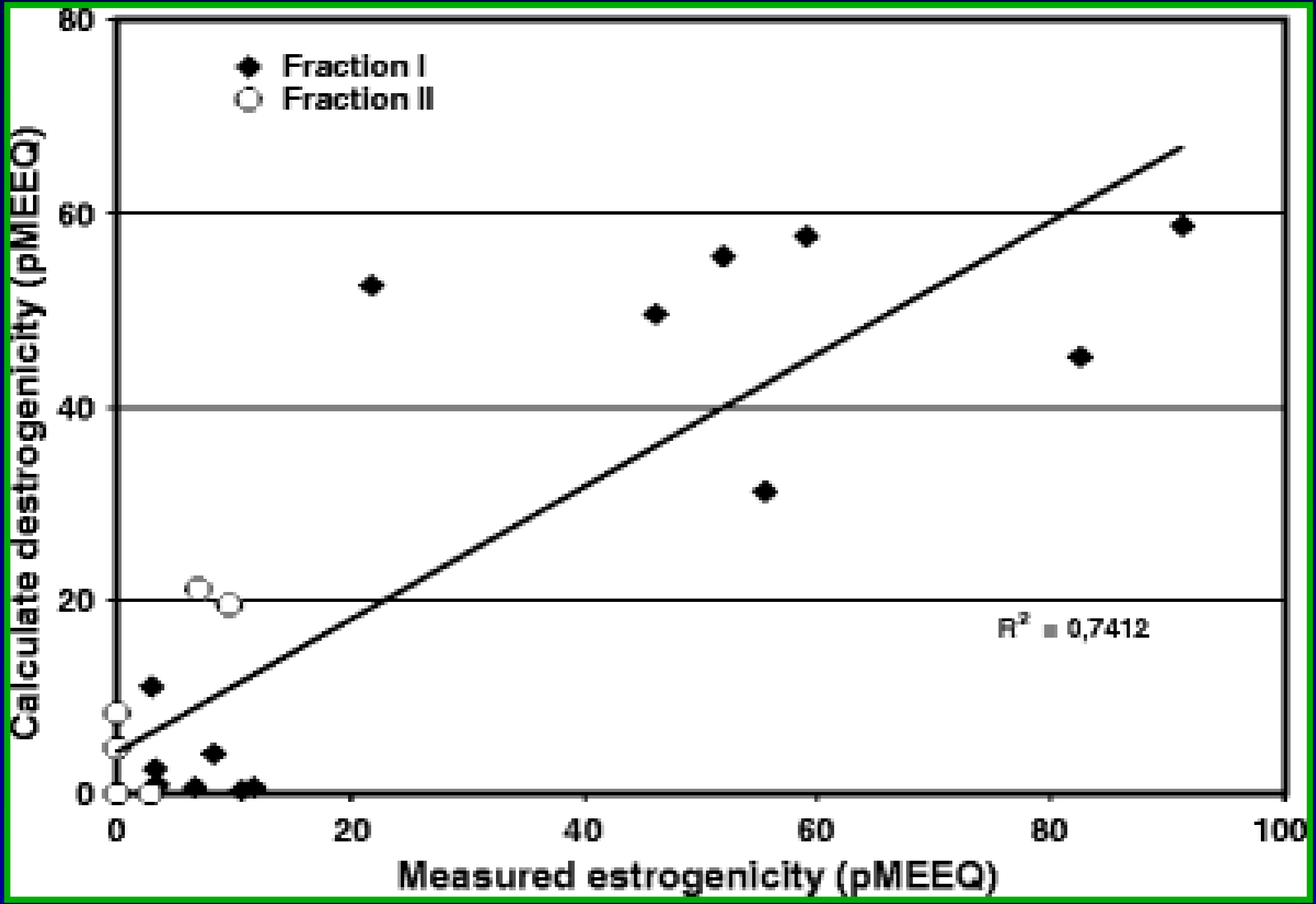

Correlation between measured and calculated estrogenic activity for Fraction I and Fraction II

Fernandez, M. P.; Noguerol, T. N.; Lacorte, S.; Buchanan, I.; Pina, B. *Anal. Bioanal. Chem*. 2009, *393*, 957-968.

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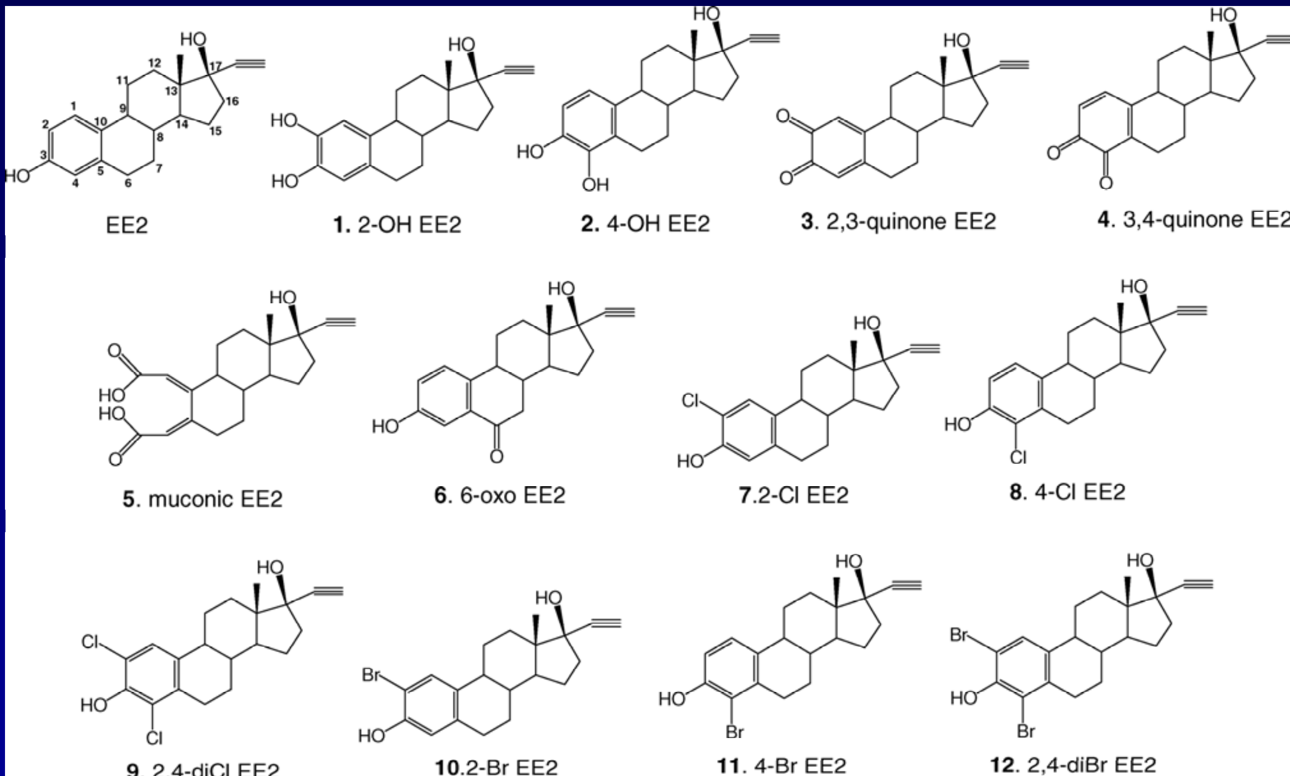
Treatment of Pharmaceuticals in Wastewater

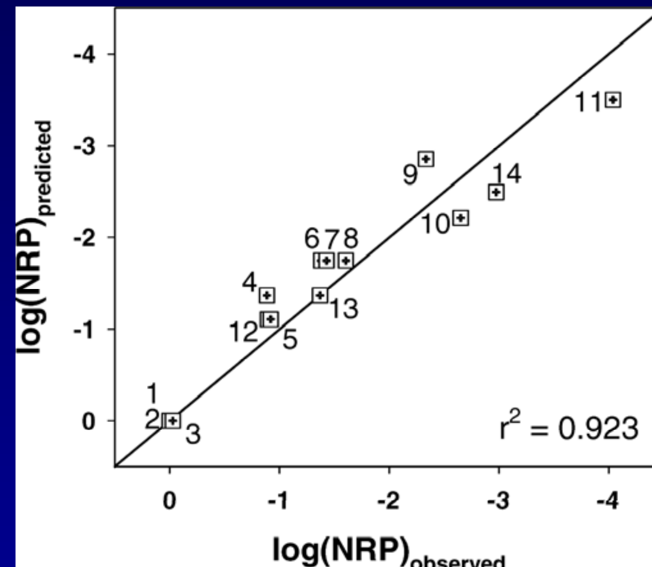
- 17α-Ethinylestradiol (EE2) treated with chlorine, bromine, ozone, hydroxyl radical, chlorine dioxide, and ferrate to test removal/transformation in wastewater treatment
- Initial transformation products show substantially lower in vitro estrogenic activity (<13% of EE2)
- QSAR shows substituents changed on phenolic moiety affect relative in vitro estrogenic activity
- Initial products that still exhibit residual estrogenic activity are quickly further transformed by most of the tested oxidants
- Therefore, oxidative wastewater treatment may be a powerful tool to remove estrogenic activity induced by steroid estrogens

Urs von Gunten

Lee, Escher, von Gunten. *Environ. Sci. Technol*. 2008**,** *42* (17), 6333-6339.

Initial Transformation Products

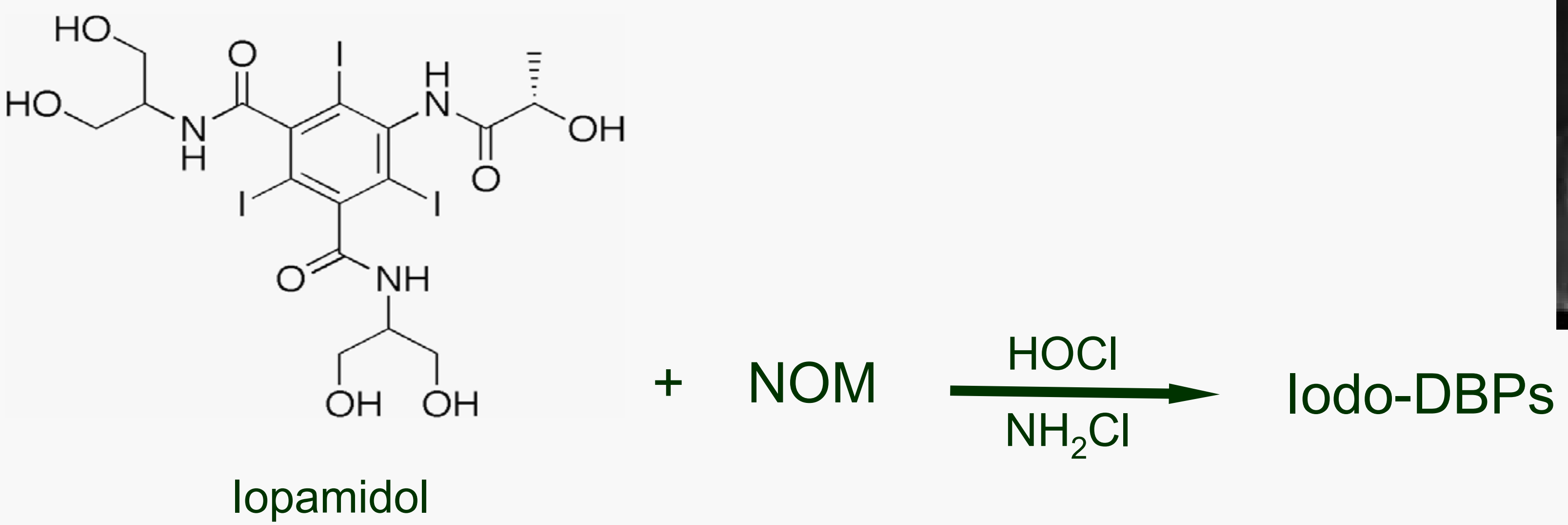




Predicted vs. observed normalized relative potency

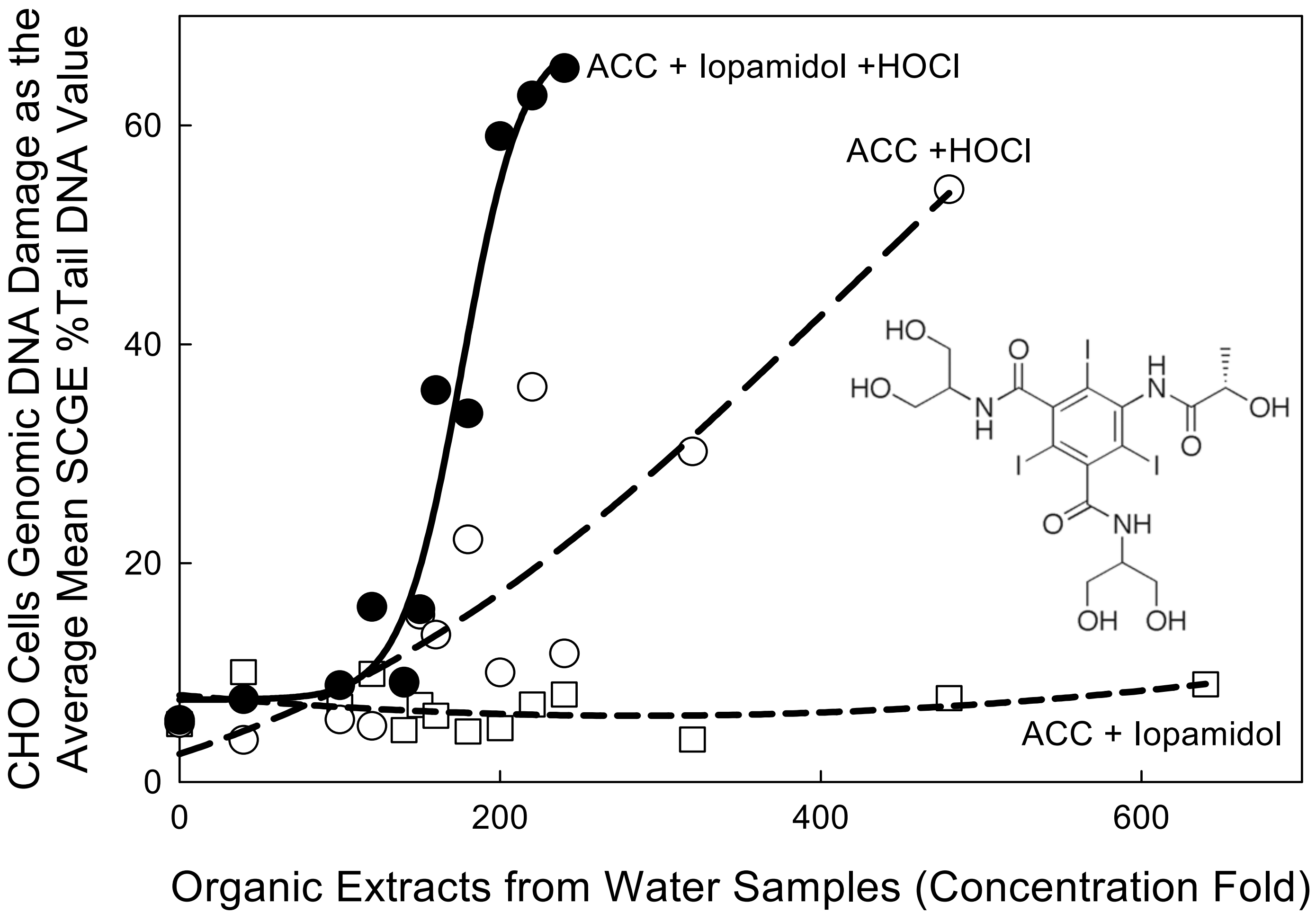
Lee, Escher, von Gunten. *Environ. Sci. Technol*. 2008, *42* (17), 6333-6339.

Formation of iodo-DBPs from X-ray contrast media



Thomas Ternes

Genotoxicity of Chlorinated Waters Containing Iopamidol



Michael Plewa

ICM in U.S. Drinking Water Sources (ng/L)

	Iopamidol	Iomeprol	Iopromide	Iohexol	Diatrizoate
Plant 1	11	ND	ND	ND	ND
Plant 2	510	ND	24	120	93
Plant 4	110	ND	6	49	ND
Plant 10	ND	ND	ND	ND	ND
Plant 11	100	ND	ND	85	ND
Plant 12	280	ND	ND	120	ND
Plant 13	ND	ND	ND	ND	ND
Plant 15	2700	ND	25	ND	ND
Plant 17	ND	ND	ND	ND	ND
Plant 19	ND	ND	ND	ND	ND

Courtesy of Thomas Ternes, Federal Institute of Hydrology, Germany

ICM measured using LC/ESI-MS/MS; DLs = 5-20 ng/L

Acknowledgments

Michael Plewa, Univ. Illinois

Jane Ellen Simmons, U.S. EPA, NHEERL, NC

Silvia Lacorte, IBMB-CSIC & IIQAB-CSIC, Barcelona, Spain

Urs von Gunten, EAWAG, Switzerland

Thomas Ternes, Federal Institute of Hydrology, Germany

Steve Duirk, Cristal Lindell, Chris Cornelison, U.S. EPA-Athens