Mammalian and Human Cell Toxicology of Emerging Drinking Water Disinfection Byproducts

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August 27, 2010

waterCAMPWS

Safe Drinking Water: Benefits and Risks

- Drinking water disinfection was a major public health triumph of the 20th century. The disinfectants greatly reduced the incidence of typhoid, cholera and other waterborne diseases.
- Each day water utilities in the U.S.A. produce over 1.3×10^{10} liters of high quality, safe drinking water to over 270 million people.

Richardson SD, Simmons JE, Rice G. 2002. Disinfection by-products: the next generation. Environ Sci Technol 36:1198A-1220A.

Safe Drinking Water: Benefits and Risks

- However, there is an unintended consequence of disinfection, the generation of **disinfection by-products (DBPs).**
- DBPs are toxic compounds formed during drinking water disinfection as a result of the reaction between organic materials and disinfectants.

After 36 Years How Can DBPs Be Emerging Contaminants?

- Decreasing supplies of pristine waters → use of impaired waters
  - Algal impacts
  - Wastewater impacts and wastewater use (California and Florida)
  - Use of impaired waters → more organic-N → Nitrogenous DBP precursors

Schreiber IM and Mitch WA. 2006. Occurrence and fate of nitrosamines and nitrosamine precursors in wastewater impacted surface waters using boron as a conservative tracer. Environ Sci Technol 40:3203-3210.

After 36 Years How Can DBPs Be Emerging Contaminants?

- U.S. EPA Stage 2 DBP Rule to reduce THMs/HAAs
  - Utilities switching to ozonation and chloramination.
  - Unexpected consequences → Promote N-DBP and Iodo-DBP formation.
- U.S. EPA and WRF recommend more research in N-DBPs, I-DBPs, and I-N-DBPs
  - EPA Workshop 2005; AWWA meeting 2005, 2006; Environ. Mutagen Society Symposium 2008; Gordon Research Conference 2006, 2009.
  - The first comprehensive review of DBP occurrence, genotoxicity and carcinogenicity: *Mutation Research, 2007.*

Richardson SD, Plewa MJ, Wagner ED, Schoeny R, DeMarini DM. 2007. Occurrence, genotoxicity, and carcinogenicity of emerging disinfection by-products in drinking water: a review and roadmap for research. Mutation Res 636:178-242 .

Solutions to the Impediments for a Comparative Toxicology of DBPs

- Structure-Function Activity studies to define high priority DBPs
- U.S. EPA Nationwide Occurrence Study
- New analytical biological approaches that integrate with the analytical chemistry of DBPs

Woo Y-T, Lai D, McLain JL, Manibusan MK, Dellarco V. 2002. Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ Health Perspect 110 (Supp 1):75-87.

Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D., Jr., The occurrence of a new generation of disinfection by-products. *Environ. Sci. Technol.* **2006,** *40*, (23), 7175-7185.

Krasner, S. W., The formation and control of emerging disinfection by-products of health concern. *Philos. Transact.R. Soc. A* **2009,** *367*, (1904), 4077-4095.

Plewa, M. J.; Wagner, E. D.; Muellner, M. G.; Hsu, K. M.; Richardson, S. D., Comparative mammalian cell toxicity of N-DBPs and C-DBPs. In *Occurrence, formation, health effects and control of disinfection by-products in drinking water*, Karanfil, T.; Krasner, S. W.; Westerhoff, P.; Xie, Y., Eds. American Chemical Society: Washington, D.C., 2008; Vol. 995, pp 36-50.

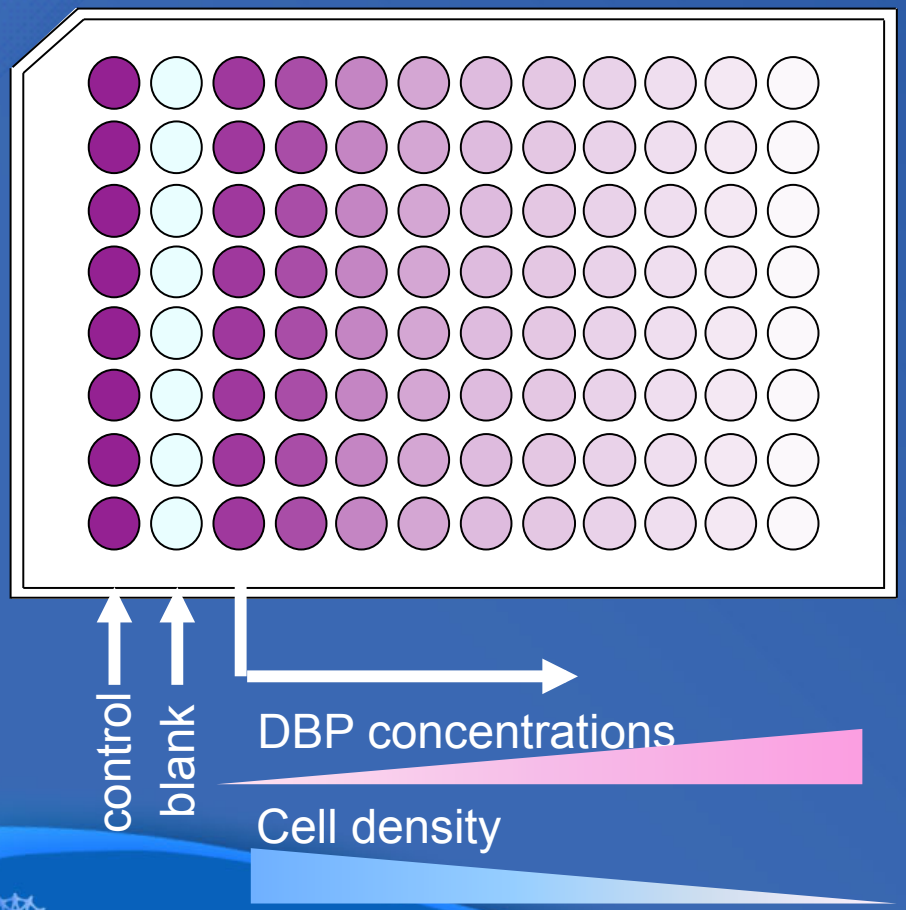
In Vitro Mammalian Cell DBP Toxicity Database

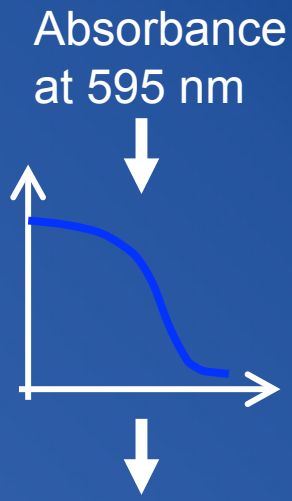
- Select DBPs within chemical classes from U.S. EPA.
- Analyze the direct-acting cytotoxicity and genomic genotoxicity of individual DBPs with Chinese hamster ovary (CHO) cells.
- Determine the cytotoxic and genotoxic rank order of the DBPs.
- Develop a quantitative and comparative DBP toxicity database.
- Conduct comparative toxicology, structure activity relationship analysis, chemical class analysis.

Plewa, M. J.; Wagner, E. D., *Mammalian cell cytotoxicity and genotoxicity of disinfection by-products. Water Research Foundation:* Denver, CO, 2009; p 134.

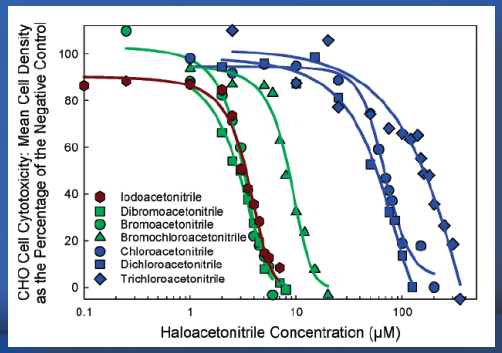
CHO Cell
Chronic Cytotoxicity

Measurement of CHO Cell Chronic Cytotoxicity

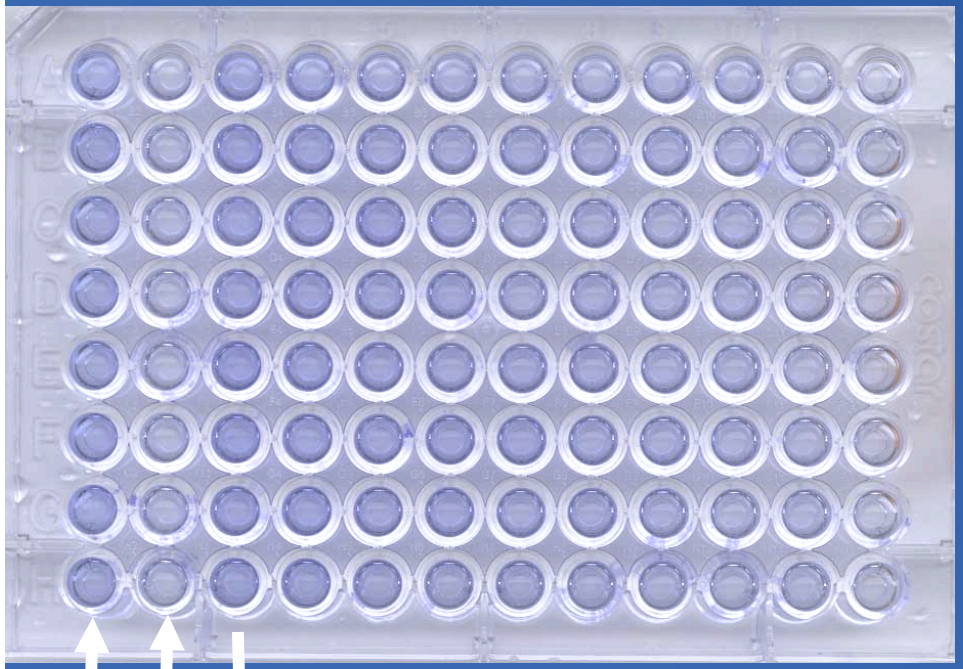




The absorbancy data are blank-corrected and expressed as the percentage of the mean absorbency of the concurrent negative control



In vitro CHO Cell Chronic Cytotoxicity Assay (72-h exposure)



Wash cells, fix with MeOH, stain, wash and scan on microplate reader (595 nm)

↓

Blank correct data

↓

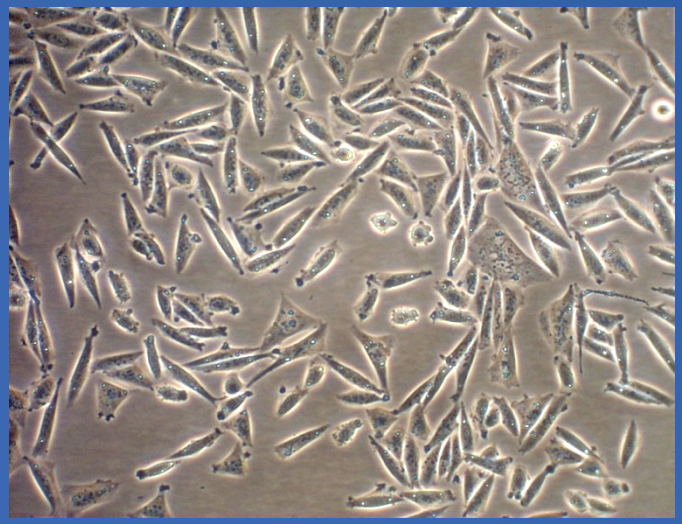
Normalize data to a percentage of the concurrent negative control

↓

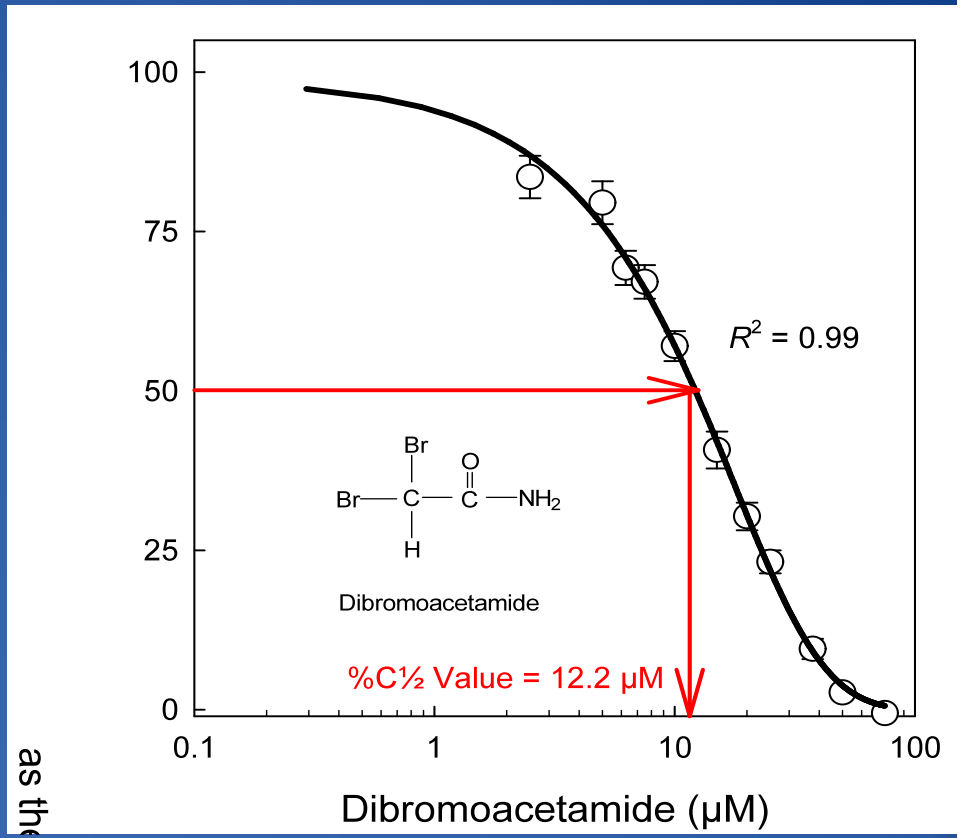
Save data as a spreadsheet file

In vitro Chronic Cytotoxicity Using CHO Cells

CHO Cells, AS52, Clone 11-4-8

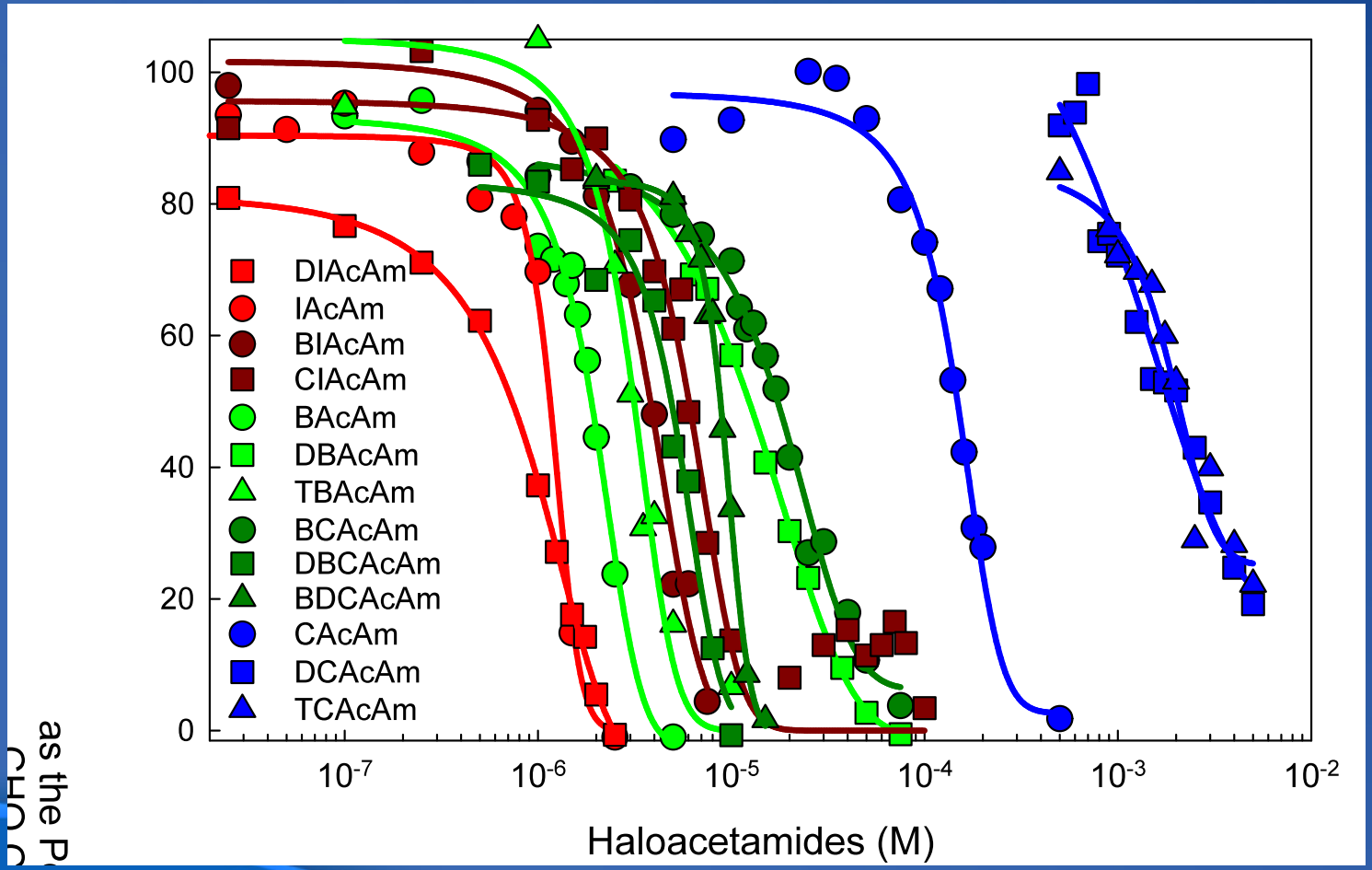


The %C½ value (LC_{50}) is the concentration of each test agent that reduced the CHO cell density by 50% as compared to the negative control.



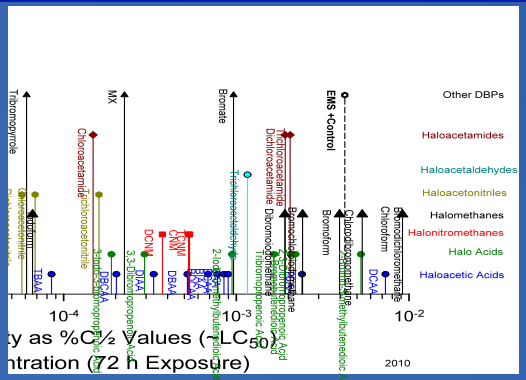
Wagner, E. D.; Rayburn, A. L.; Anderson, D.; Plewa, M. J., Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells. *Environ. Mol. Mutagen.* **1998,** *32*, (4), 360-368.

Comparative Chronic Cytotoxicity of Haloacetamides



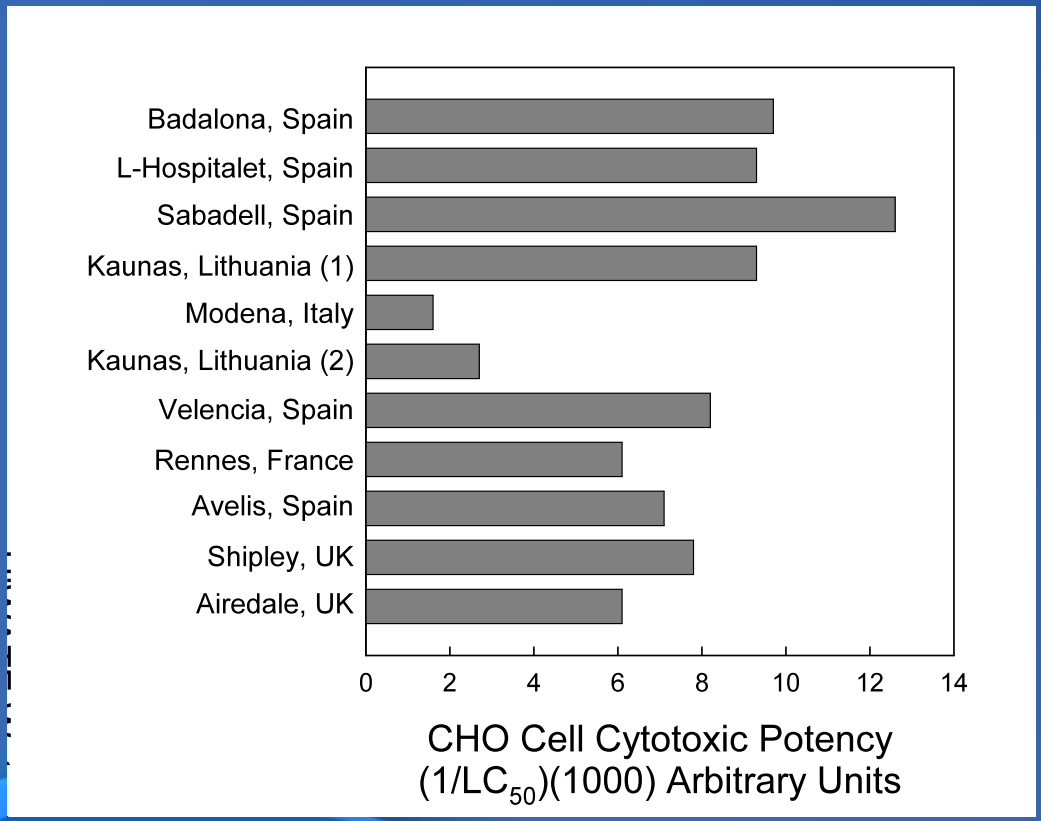
Plewa, M. J.; Muellner, M. G.; Richardson, S. D.; Fasano, F.; Buettner, K. M.; Woo, Y. T.; McKague, A. B.; Wagner, E. D., Occurrence, synthesis and mammalian cell cytotoxicity and genotoxicity of haloacetamides: An emerging class of nitrogenous drinking water disinfection by-products. *Environ. Sci. Technol.* 2008, *42*, (3), 955-961.

Comparative DBP CHO Cell Chronic Cytotoxicity Database



Plewa, M. J.; Wagner, E. D., *Mammalian cell cytotoxicity and genotoxicity of disinfection by-products.* Water Research Foundation: Denver, CO, 2009; p 134.

What About Organic Extracts From Real Drinking Water?



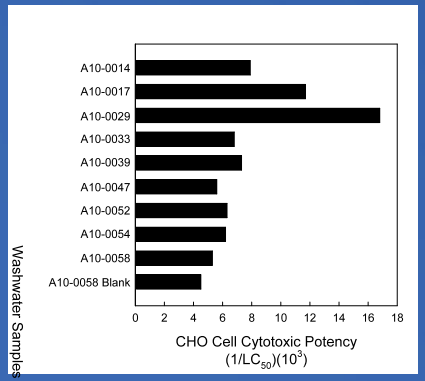
CHO cell cytotoxicity of organics extracted from drinking waters in the European Union HiWATE Program.

We are working to determine if there is a correlation between in vitro toxicity and adverse pregnancy outcomes in a large drinking water epidemiological program.

(Clara Jeong, Susan Richardson, Minolas Kogevinas, Mark Nieuwenhuijsen)

Nieuwenhuijsen, M. J. et al.,Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE. *J. Water Health* ***2009,*** 7, (2), 185-207.

Cytotoxicity of Wastewaters From Amine Based Carbon Capture Technologies



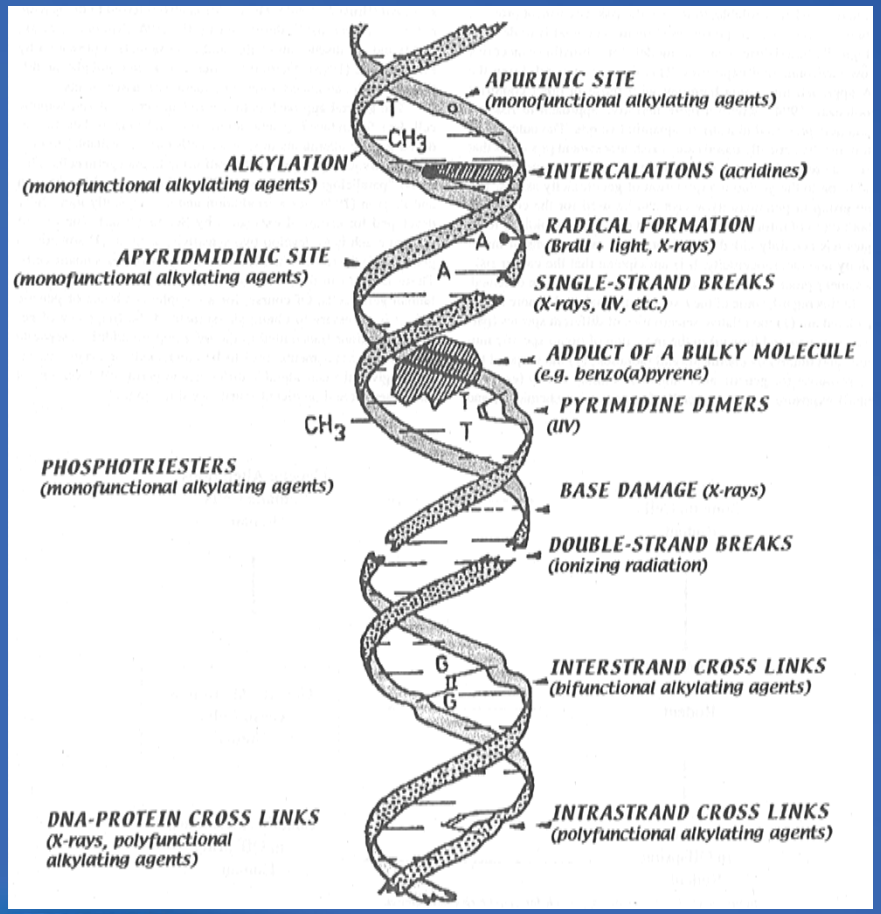
Optimization of an engineering process using toxicological information.

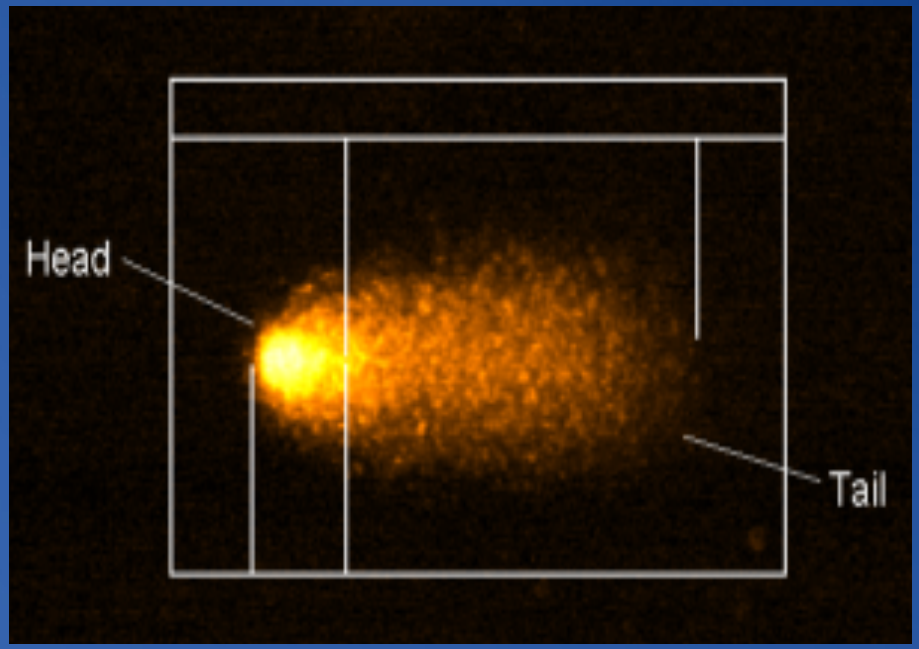
The figure represents the cytotoxic characteristics of washwaters from a carbon capture pilot plant as a function of altering the inputs and operating conditions of the plant.

(Jen Osiol, Bill Mitch)

Genomic DNA Damage

DNA Damage Analysis Using Single Cell Gel Electrophoresis: the Target is the Genome

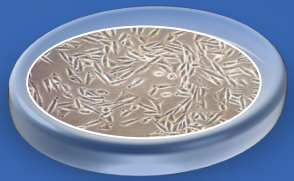


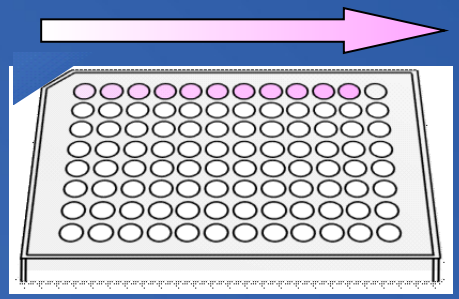


The **tail moment** is the integrated value of DNA density multiplied by the migration distance. The **% tail DNA** is the amount of DNA that has migrated into the gel from the nucleus.

Wagner, E. D.; Plewa, M. J., Microplate-based comet assay. In *The Comet Assay in Toxicology*, Dhawan, A.; Anderson, D., Eds. Royal Society of Chemistry: London, 2009; pp 79-97.

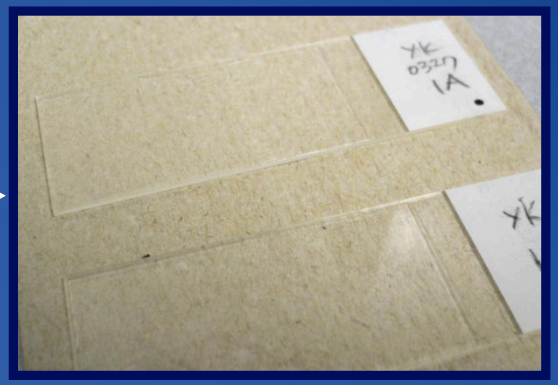
Single Cell Gel Electrophoresis (SCGE) / Comet assay





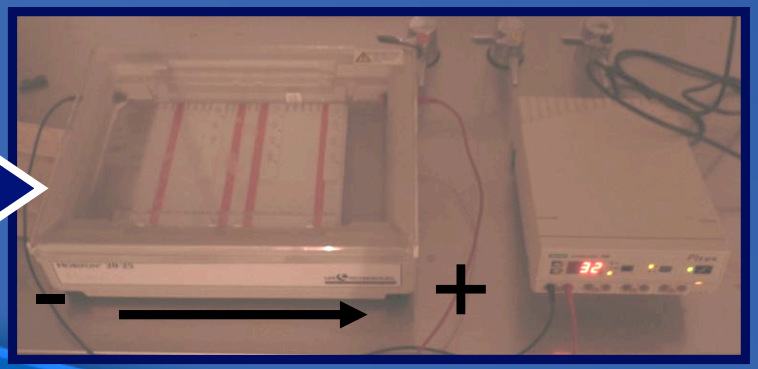
Cell treatment 4 h at 37°C & 5% CO_2

Cell treatment with DBPs



Microgel preparation

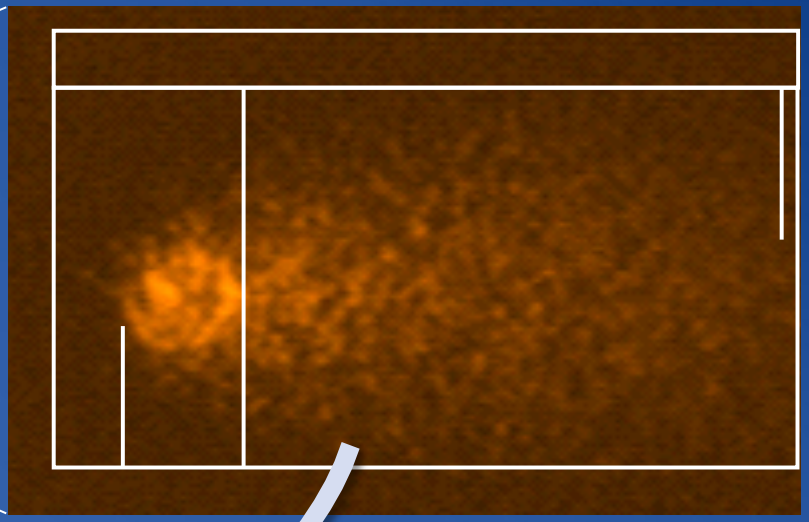
Cell lysis

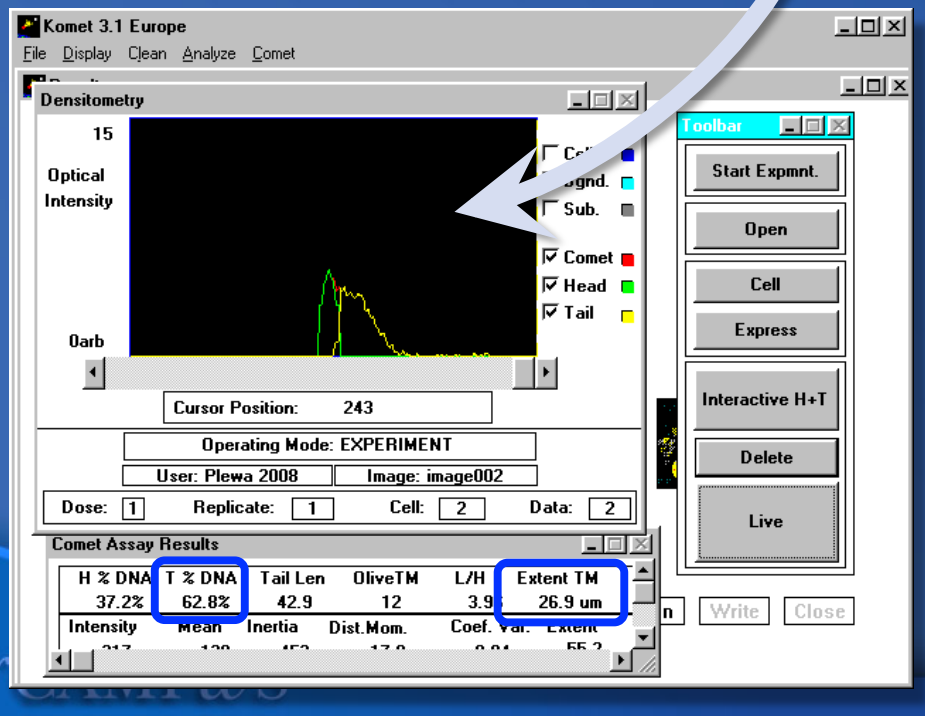


Electrophoresis (pH > 13)

Image Analysis

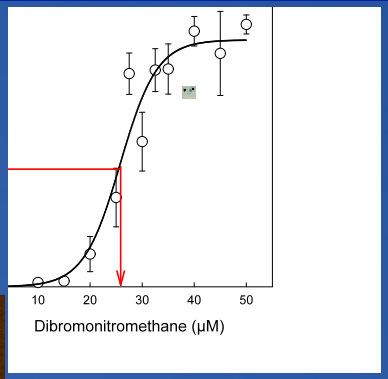
Image analysis

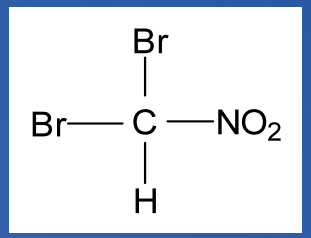




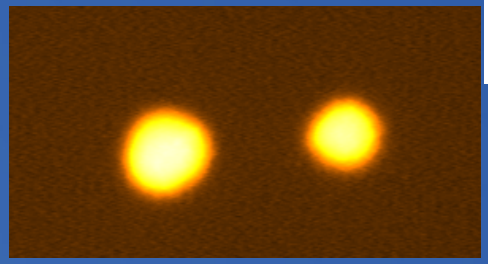
Genomic DNA Damage Induced by DBNM

Plewa MJ, Wagner ED, Jazwierska P, Richardson SD, Chen PH, McKague AB. 2004. Halonitromethane drinking water disinfection by-products: chemical characterization and mammalian cell cytotoxicity and genotoxicity. Environ Sci Technol 38:62-68.

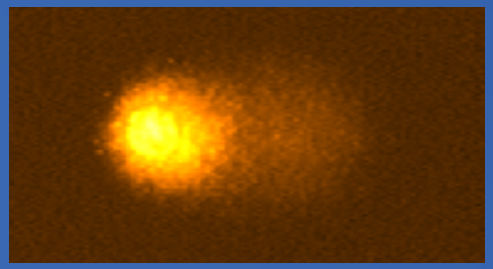




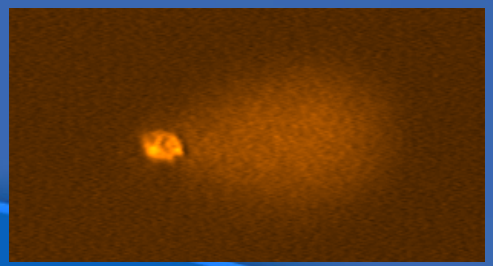
Control



30 μM DBNM

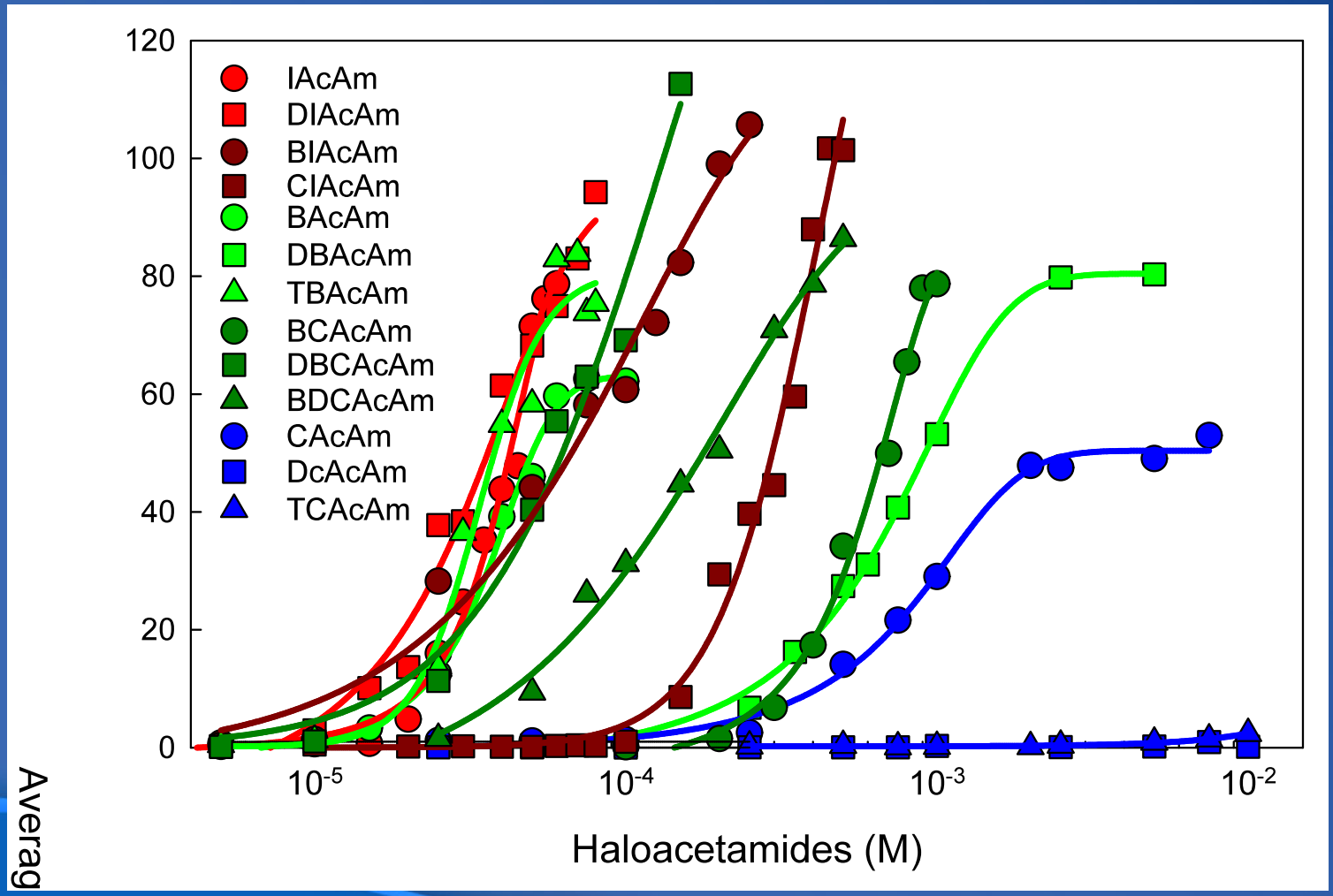


40 μM DBNM



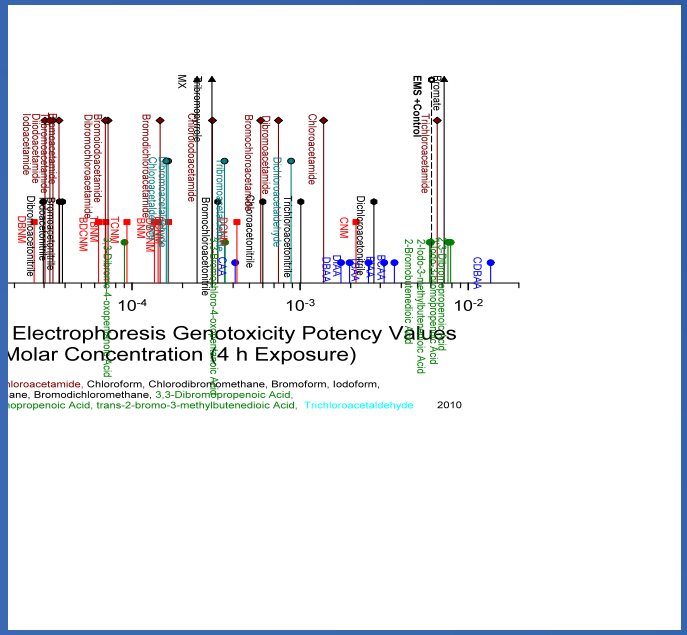
SCGE Genotoxic Potency = 26.2 μM

Comparative Genotoxicity of Haloacetamides



Plewa, M.J.; Muellner, M.G.; Richardson, S.D.; Fasano, F.; Buettner, K.M.; Woo, Y.T.; McKague, A.B.; Wagner, E.D. *Environ. Sci. Technol.* 2008, *42*, (3), 955-961.

Comparative DBP CHO Cell Acute Genotoxicity Database



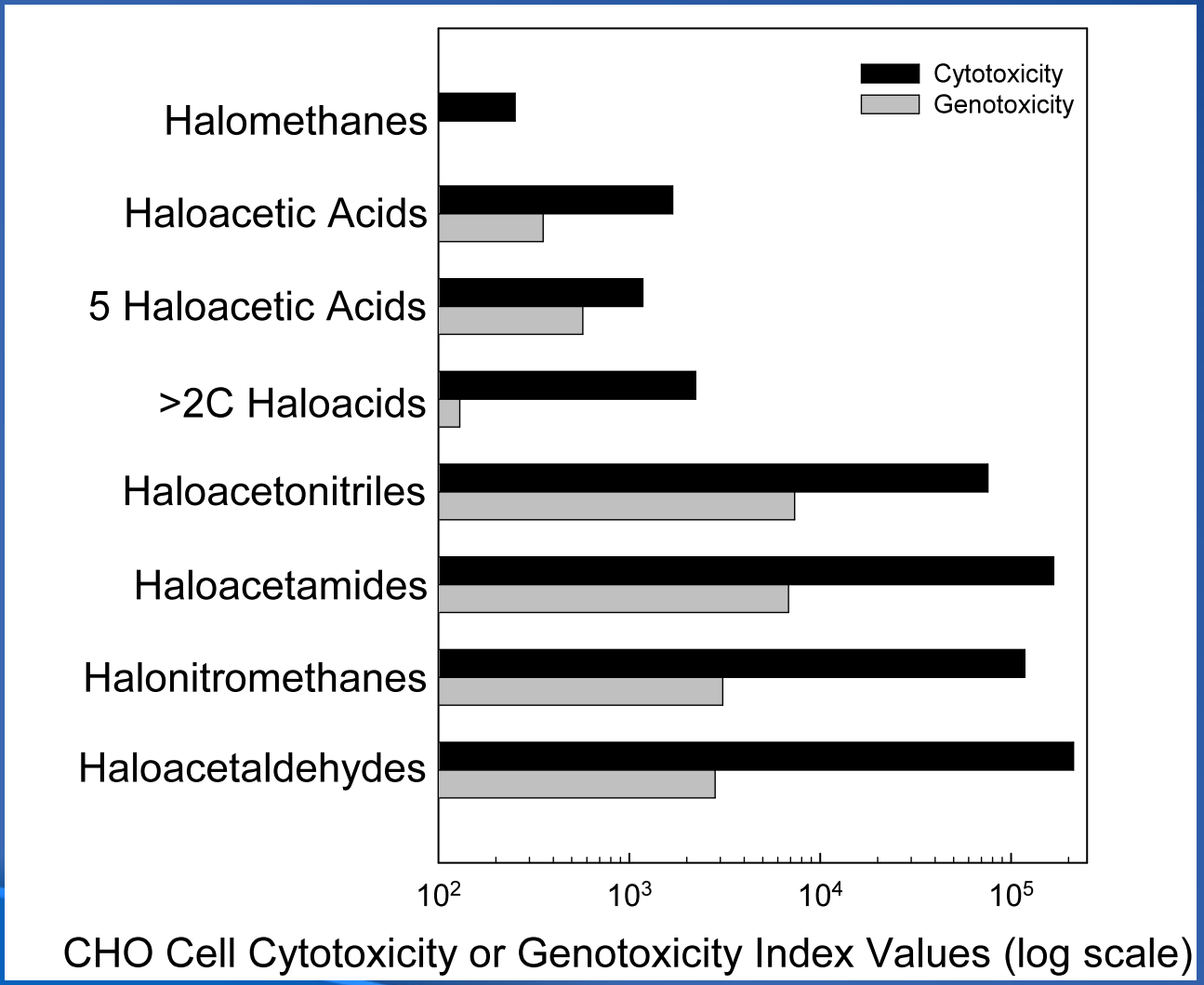
Plewa, M. J.; Wagner, E. D., *Mammalian cell cytotoxicity and genotoxicity of disinfection by-products.* Water Research Foundation: Denver, CO, 2009; p 134.

Conclusions I

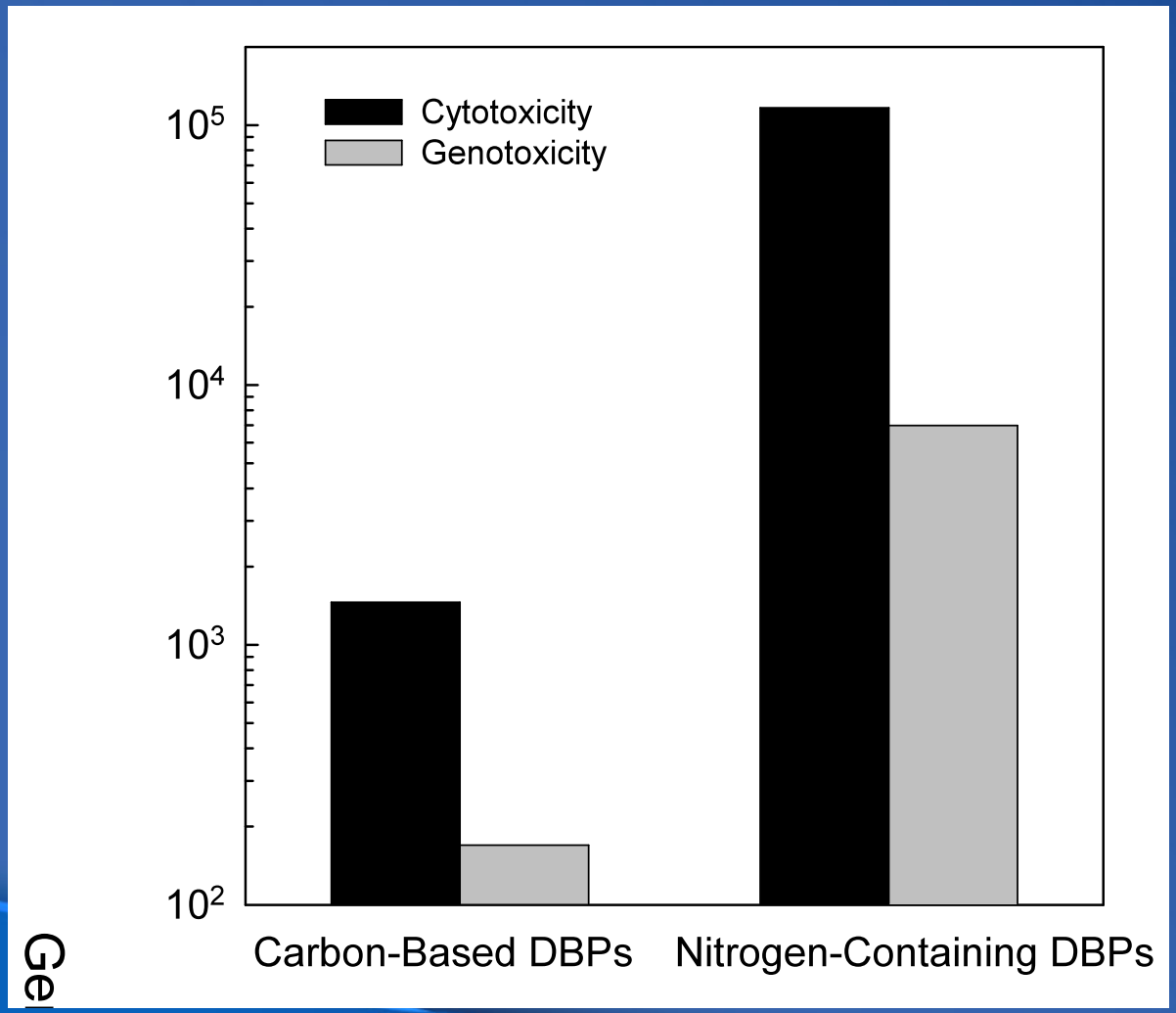
- With our current database, >70 DBPs were compared on a level toxicological playing field.
- We can quantitatively compare the chronic cytotoxicity of DBPs using their %C½ values.
- We can quantitatively compare the genotoxicity of DBPs using the SCGE Genotoxic Potency values.
- We can compare classes or specific groups of DBPs based on their Toxicity Index. Within a class, the reciprocal of the averaged median %C½ values is the cytotoxicity index value and the reciprocal of the averaged median genotoxic potency values is the genotoxicity index value.

Genomic DNA Damage

Comparison of the Cytotoxicity and Genotoxicity of DBP Classes



Toxicity Index of DBP Classes: (C-DBPs versus N-DBPs)

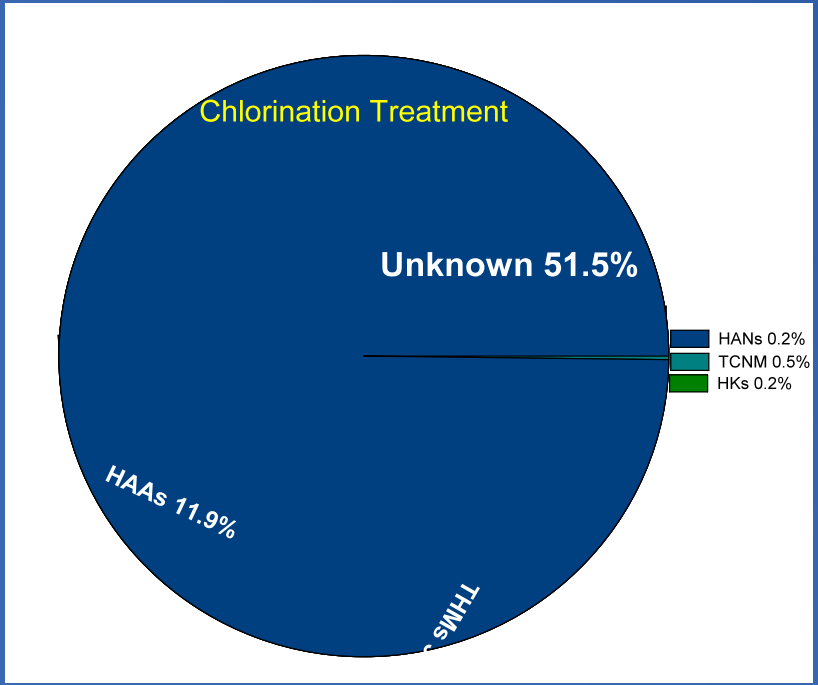


The Problem of Unexpected Consequences

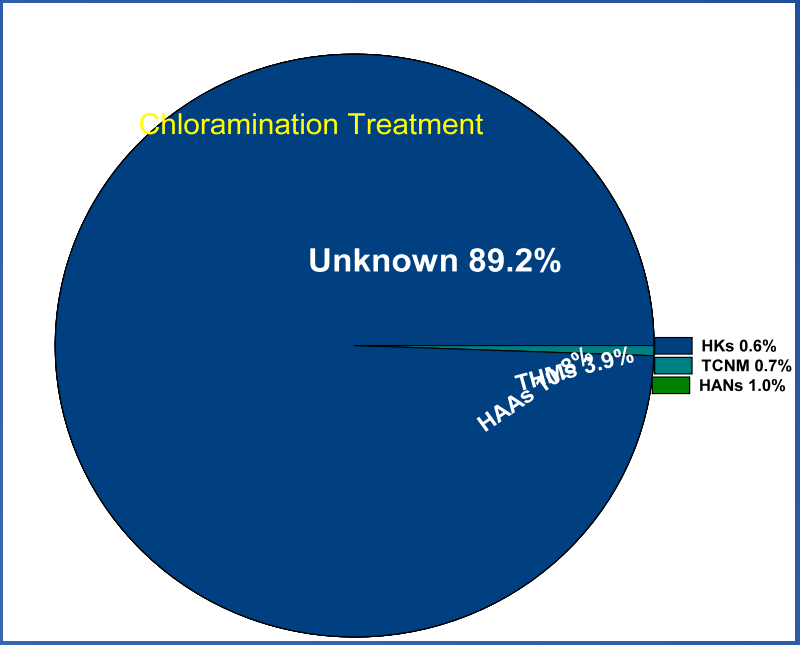
- U.S. EPA Stage 2 DBP Rule to reduce THMs/HAAs
  - Utilities switching to ozonation and chloramination.
  - Unexpected consequences → Promote N-DBP and Iodo-DBP formation.

Change the Disinfectant: Change the Spectra of DBPs

TOX 572 µg/L



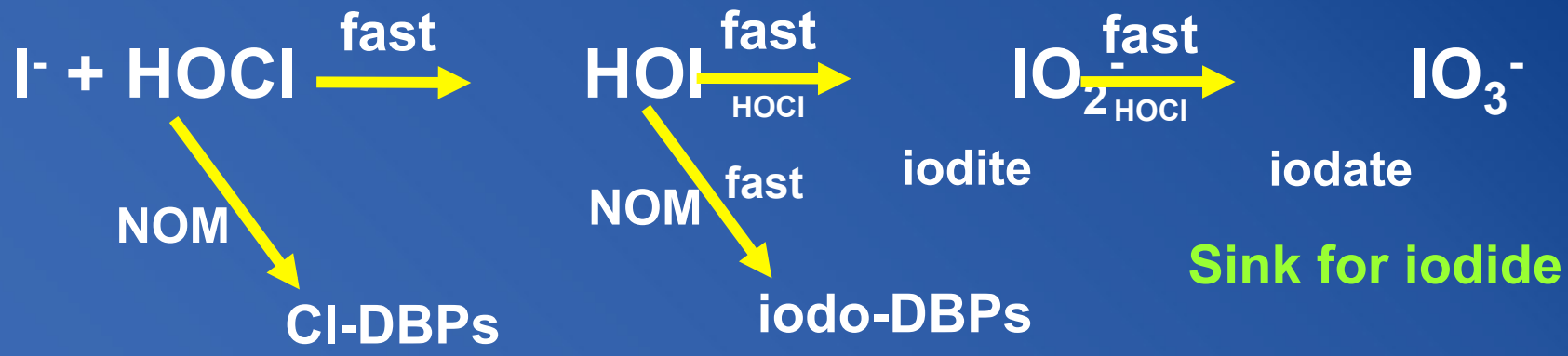
TOX 155 µg/L



Zhang, X., S. Echigo, R.A. Minear and M.J. Plewa. 2000. ACS Symposium Series 761. American Chemical Society, Washington, D.C.

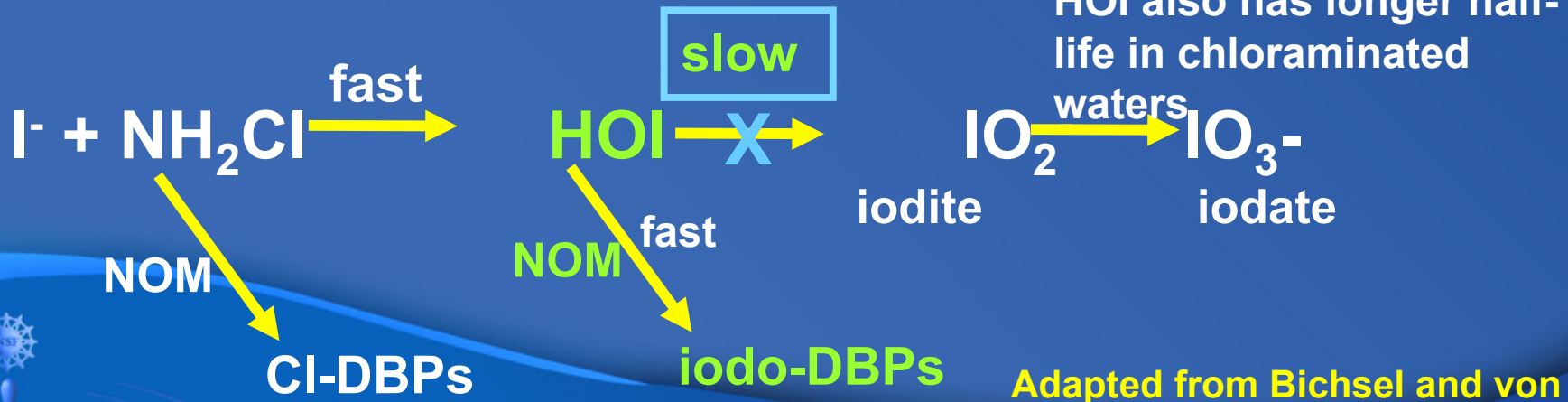
Iodo-DBP Formation Is Maximized with Chloramines

Chlorine:



HOCl also competes for the reaction with NOM, so much lower iodo-DBPs with chlorine

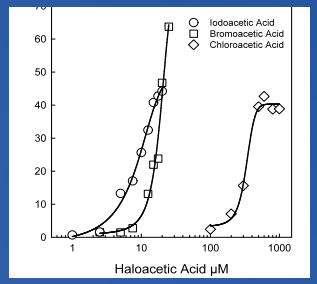
Chloramines:



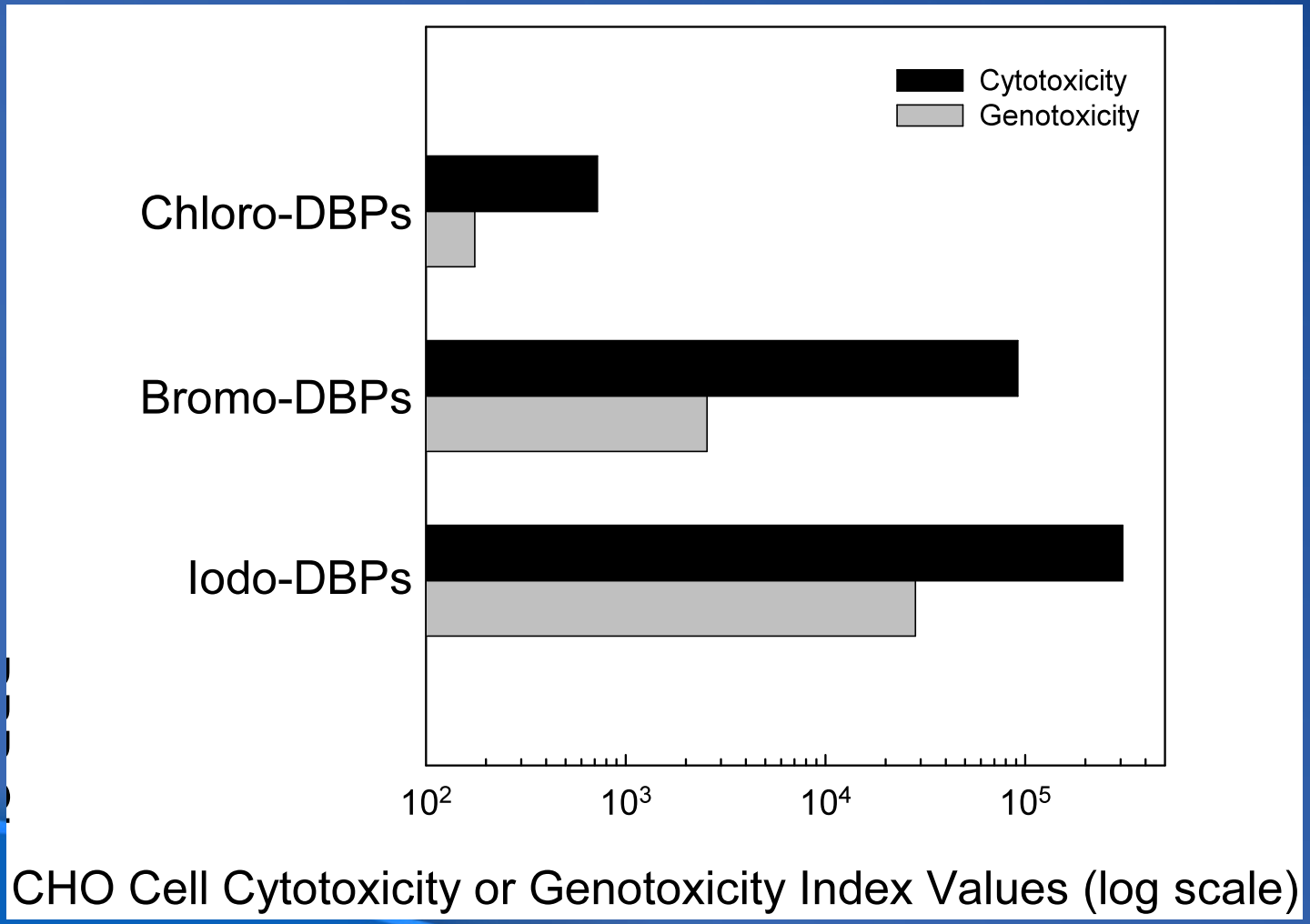
Adapted from Bichsel and von Gunten, 1999 and 2000

Comparative *in vitro* Toxicology of Iodo-DBPs

- Cytotoxicity and genotoxicity indices were calculated for 18 iodinated, brominated, or chlorinated haloacetic acids, haloacetamides, acetonitriles or THMs.
- When this balanced design of DBPs was analyzed, the iodinated DBPs were substantially more toxic than their brominated or chlorinated analogues.



Toxicity Index of DBP Classes: (impact of the halogen leaving group)



Conclusions II

- The current U.S. EPA-regulated DBP classes (THMs and HAAs) are substantially less toxic than emerging DBPs.
- Iodinated-DBPs are far more toxic than their brominated or chlorinated analogs.
- N-DBPs are much more toxic than C-DBPs.
- The occurrence of these emerging DBPs are on the rise because of changes in source water quality and the increased use of alternative water disinfectants.
- These emerging DBPs may pose adverse health risks.

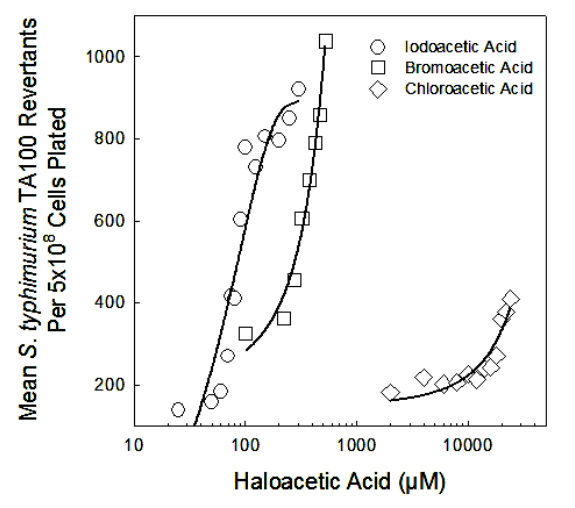
Richardson, S. D.; Fasano, F.; Ellington, J. J.; Crumley, F. G.; Buettner, K. M.; Evans, J. J.; Blount, B. C.; Silva, L. K.; Waite, T. J.; Luther, G. W.; McKague, A. B.; Miltner, R. J.; Wagner, E. D.; Plewa, M. J., Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. *Environ. Sci. Technol.* **2008,** *42*, (22), 8330-8338.

Mechanisms of DBP Toxicity

Mechanisms of DBP Toxicity

- Continue with SAR analysis of DBP chemical classes such as the aldehydes (Clara Jeong).
- Develop and implement molecular technologies to obtain mechanisms of toxicity by DBPs. Oxidative stress, enzyme inhibition and other cellular mechanisms (Justin Pals, Justin Ang).
- Measure the kinetics of DNA repair of genomic damage induced by DBPs (Yukako Komaki, Justin Pals).
- Identify DBP-induced modulation of human gene expression using toxicogenomics (Matias Attene-Ramos, Justin Pals).

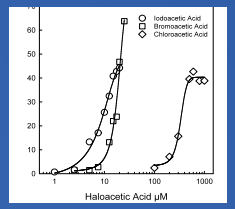
Genotoxicity: IAA>BAA>>CAA



Salmonella

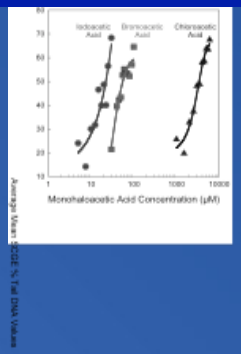
Kargalioglu, Y.; McMillan, B. J.; Minear, R. A.; Plewa, M. J., Analysis of the cytotoxicity and mutagenicity of drinking water disinfection by-products in *Salmonella typhimurium*. *Teratogen. Carcinogen. Mutagen.* **2002,** *22*, (2), 113-128.

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.



CHO Cells

Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J., Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. *Envion. Sci. Technol.* **2010**, In Press.



Mouse teratogenicity: IAA>BAA>>CAA

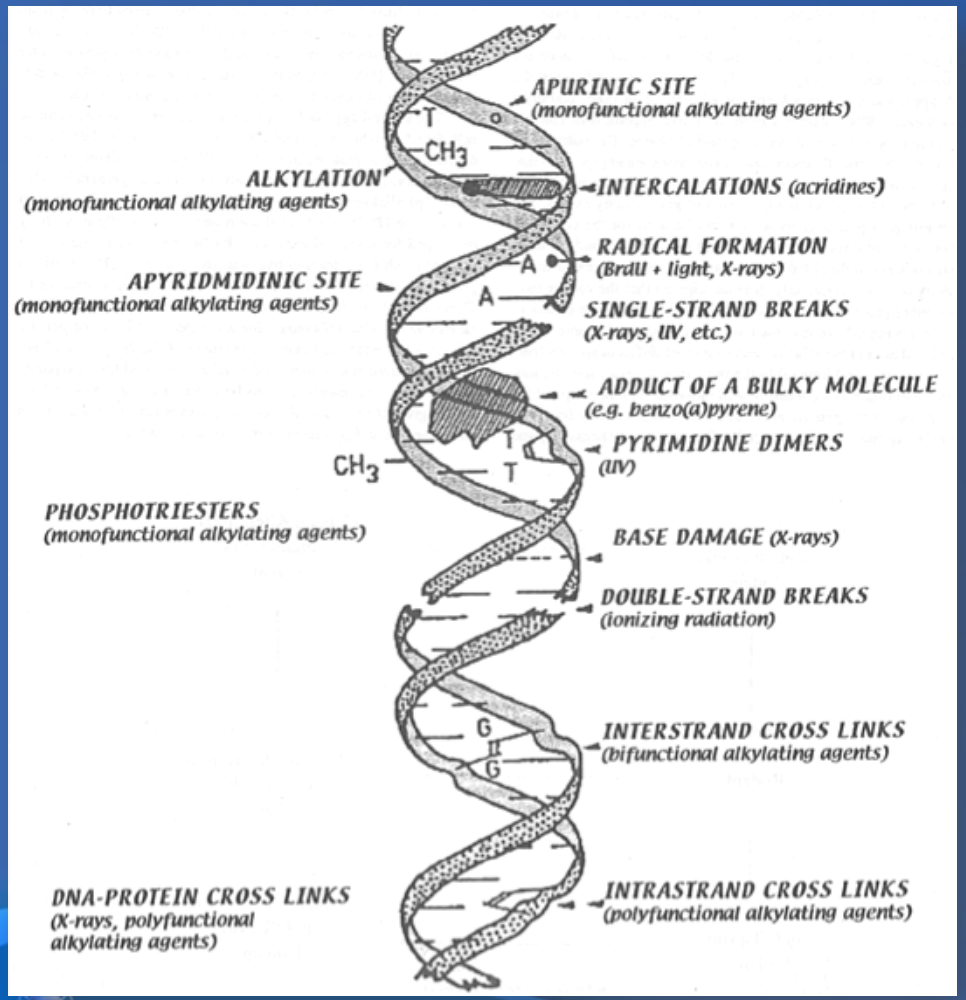
Hunter, E. S., 3rd; Rogers, E. H.; Schmid, J. E.; Richard, A., Comparative effects of haloacetic acids in whole embryo culture. *Teratology* **1996,** *54*, (2), 57-64.

Human Cells

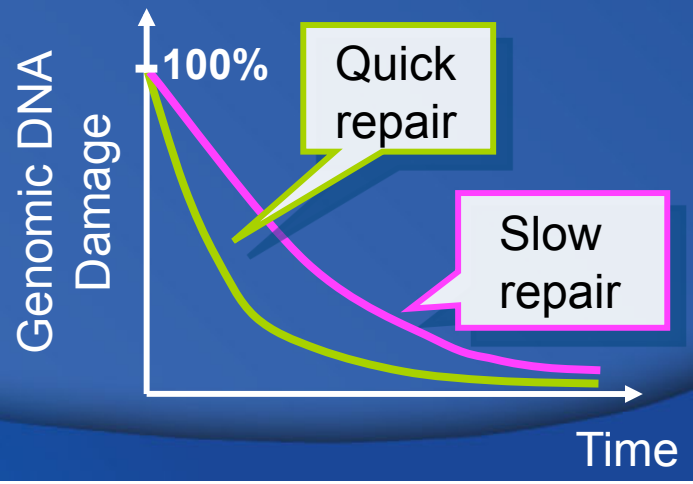
Mechanisms of DBP Toxicity

- Structure Activity Relationship (SAR) analysis of DBPs and toxic response.
- Correlation analysis of physiochemical characteristics and toxic responses.
- Direct interaction of DBPs with DNA versus the inhibition of metabolic enzymes leading to the generation of toxic reactive oxygen species.

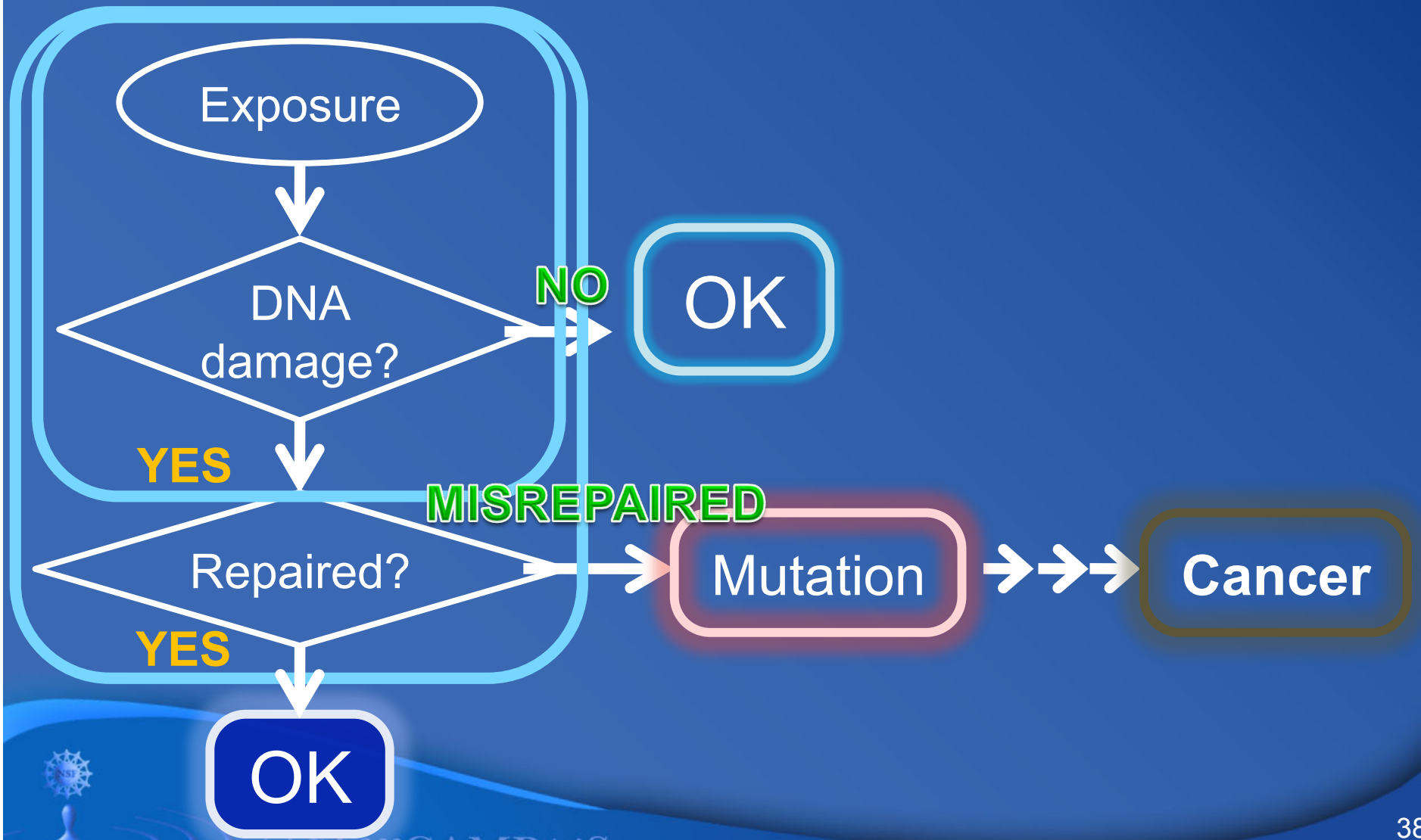
DNA Repair Kinetics



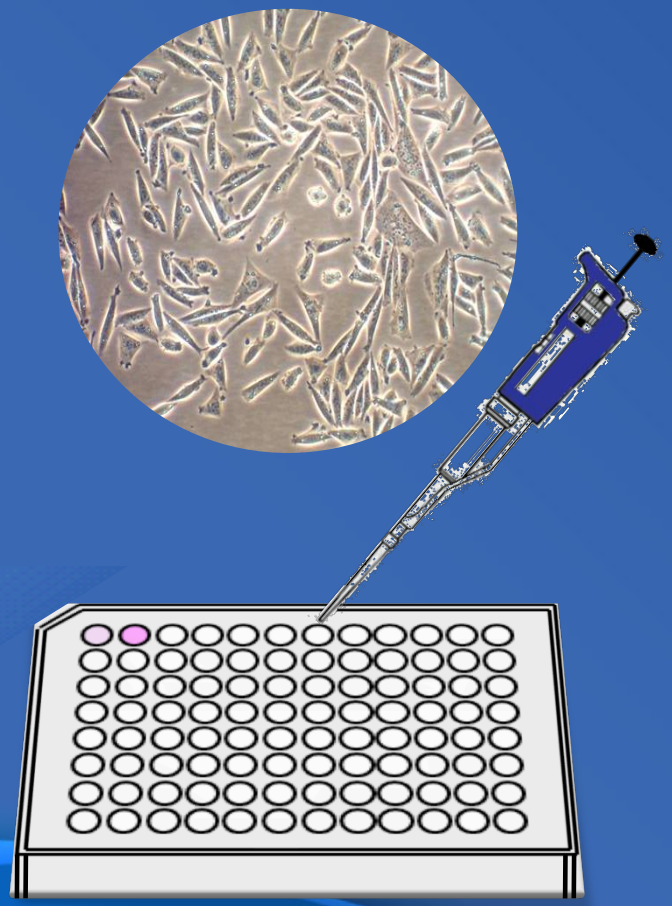
- Each lesion has different rate of repair
- Comparison between DNA repair kinetics provides implication of specific genomic damage



DNA damage induction vs. repair



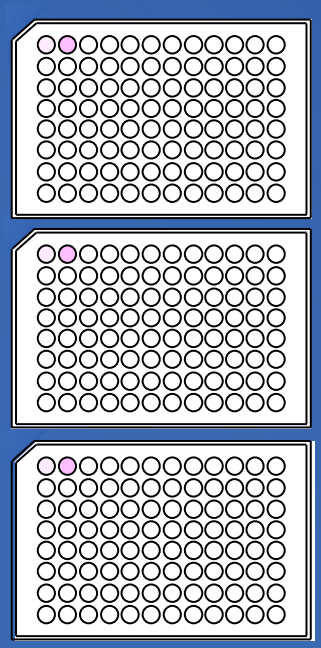
DNA Repair Kinetics - Measurement of DNA damage



Chinese hamster ovary (CHO) cells line AS 52, clone 11-4-8

- Low spontaneous mutation frequency and low chromosomal aberration frequency.
- High responsiveness to DBPs.
- Cells are DNA repair competent.
- The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without expression of neoplastic foci.

DNA Repair Kinetics

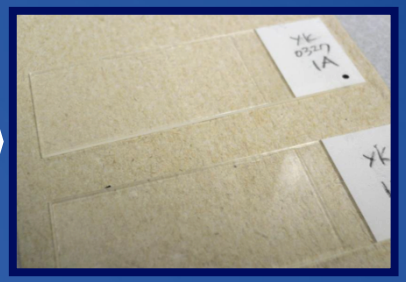


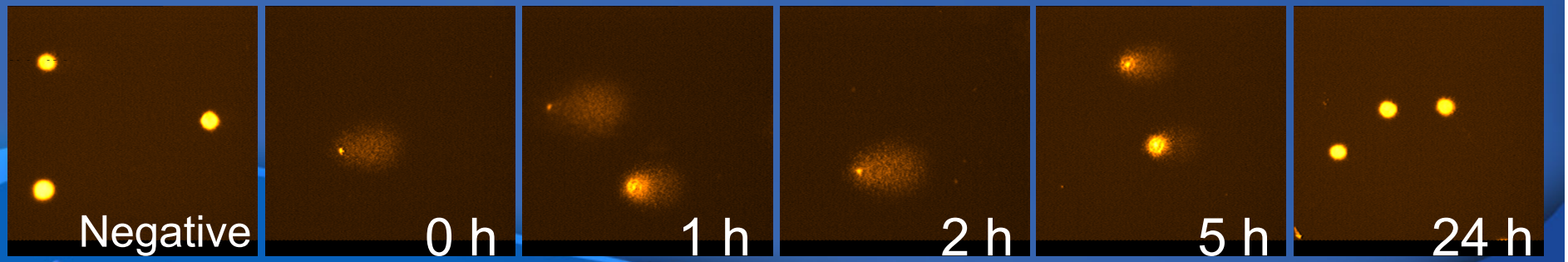
Cell treatment with DBPs

Cell treatment 4 h
at 37°C & 5% CO_2

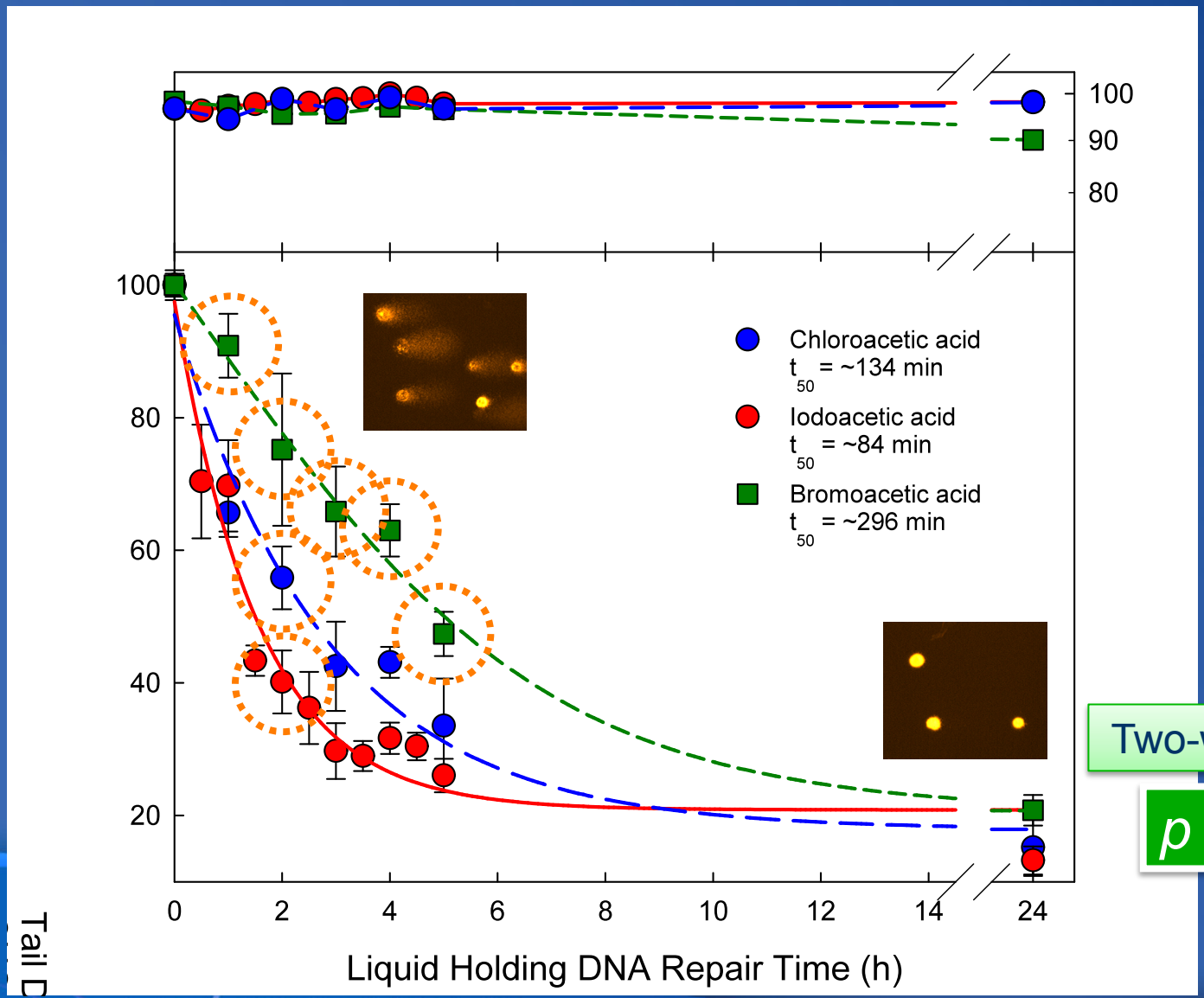
Microgel preparation

Liquid holding





DNA repair kinetics curve



Yukako Komaki

Two-way ANOVA

$p < 0.05$

DNA damage induction vs. DNA repair

- The pattern of DNA damage Iodo > Bromo>> Chloro is related to the halogen leaving group.
- The rate of DNA repair of induced genomic damage may be dependent on control of the cell cycle to allow cells to proceed with DNA repair metabolic processes. The pattern is:

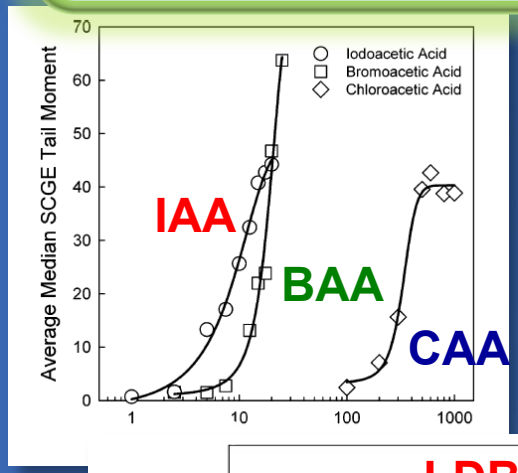
Iodo ≈ Chloro > Bromo

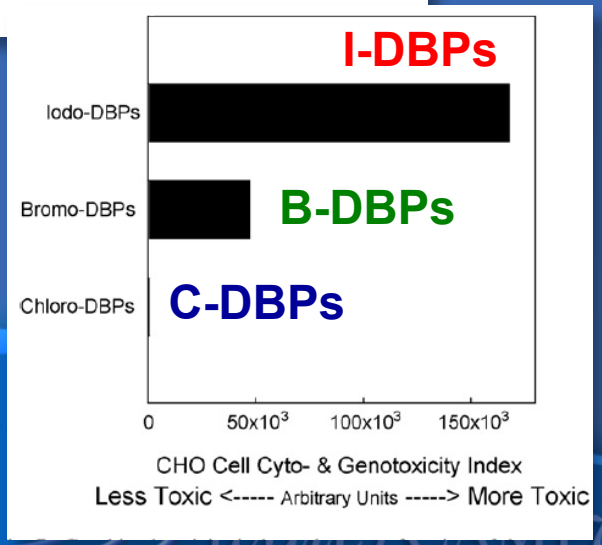
Hypothesis:

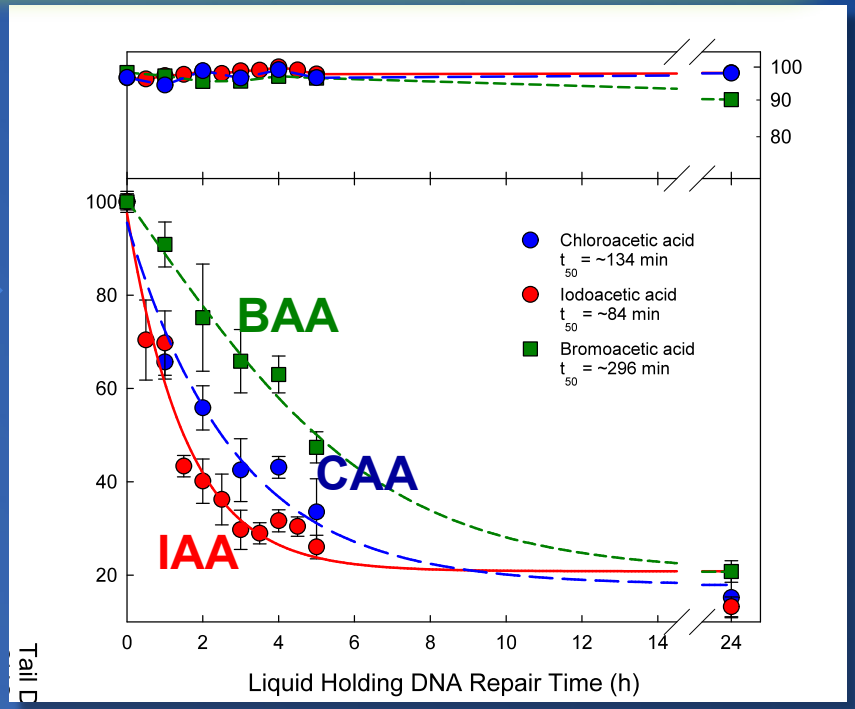
Brominated DNA lesions may not induce cell cycle arrest, resulting in reduced DNA repair efficiency.

Genotoxicity Risk Management

Induction of DNA damage + Rate of DNA repair







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. *Environ. Sci. Technol.* ***2009,*** 43, (21), 8437–8442.

Conclusions III

- We developed and calibrated a SCGE-mediated DNA repair procedure.
- Bromoacetic acid-induced DNA lesions required the longest time for DNA repair.
- A significant difference in the DNA repair kinetics was expressed for BAA as compared to IAA or CAA.
- Different DNA repair kinetic curves by the three haloacetic acids indicate that the halogen atoms may play an important role in not only the induction of DNA damage but also its repair.

Human Cell Toxicogenomics

Human Cell Toxicogenomics

- Toxicogenomics is the combination of genetic microarray technology and toxicological methods and is the study of the relationship between the structure and activity of the genome and the adverse biological effects of toxic agents.
- The goal of toxicogenomics is to understand mechanisms of toxicity and to identify gene expression patterns that lead to adverse health effects.
- With comparative toxicogenomics human biomarkers may emerge.

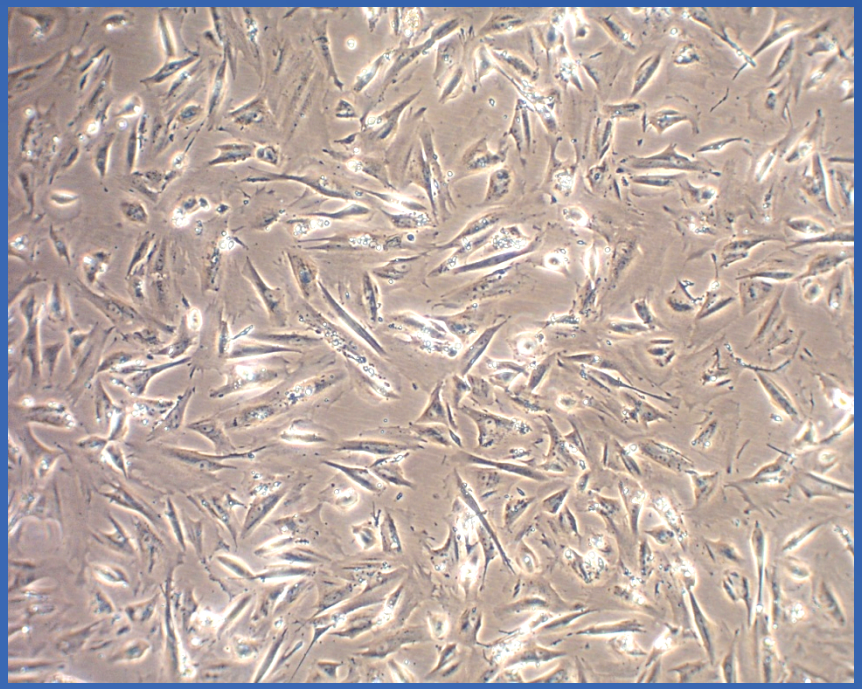
Suggestions for *in vitro* Toxicogenomic Experimental Designs

- Use nontransformed cells; this is more expensive and labor intensive than using tumor cell lines, but the data may be more representative of real world experience.
- Decide on the experimental design; include the type of gene array, the number of replicates, the exposure times, and the statistical analysis to be applied to the data.
- Conduct paired concurrent controls for each treatment group and time period.
- Conduct experiments at non-cytotoxic concentrations. If not, you will be isolating RNA from dead or dying cells.
- Use concentrations that generate equivalent biological responses.
- Carefully evaluate the amount and quality of RNA extracts and equalize RNA concentrations across control and treatment groups for each exposure time group.
- Interpret your data with a focus on functional gene groups and metabolic pathways.

Human Cell Toxicogenomic Analysis of DBPs

- Our goal is to identify genes with modified expression associated with human DNA damage/repair and toxic response.
- Bromoacetic acid is the most toxic, regulated DBP in our database.
- We treated non-transformed human FHs cells with a non-cytotoxic concentration of BAA that induced genomic DNA damage.

Human Cell Toxicogenomic Analysis of DBPs

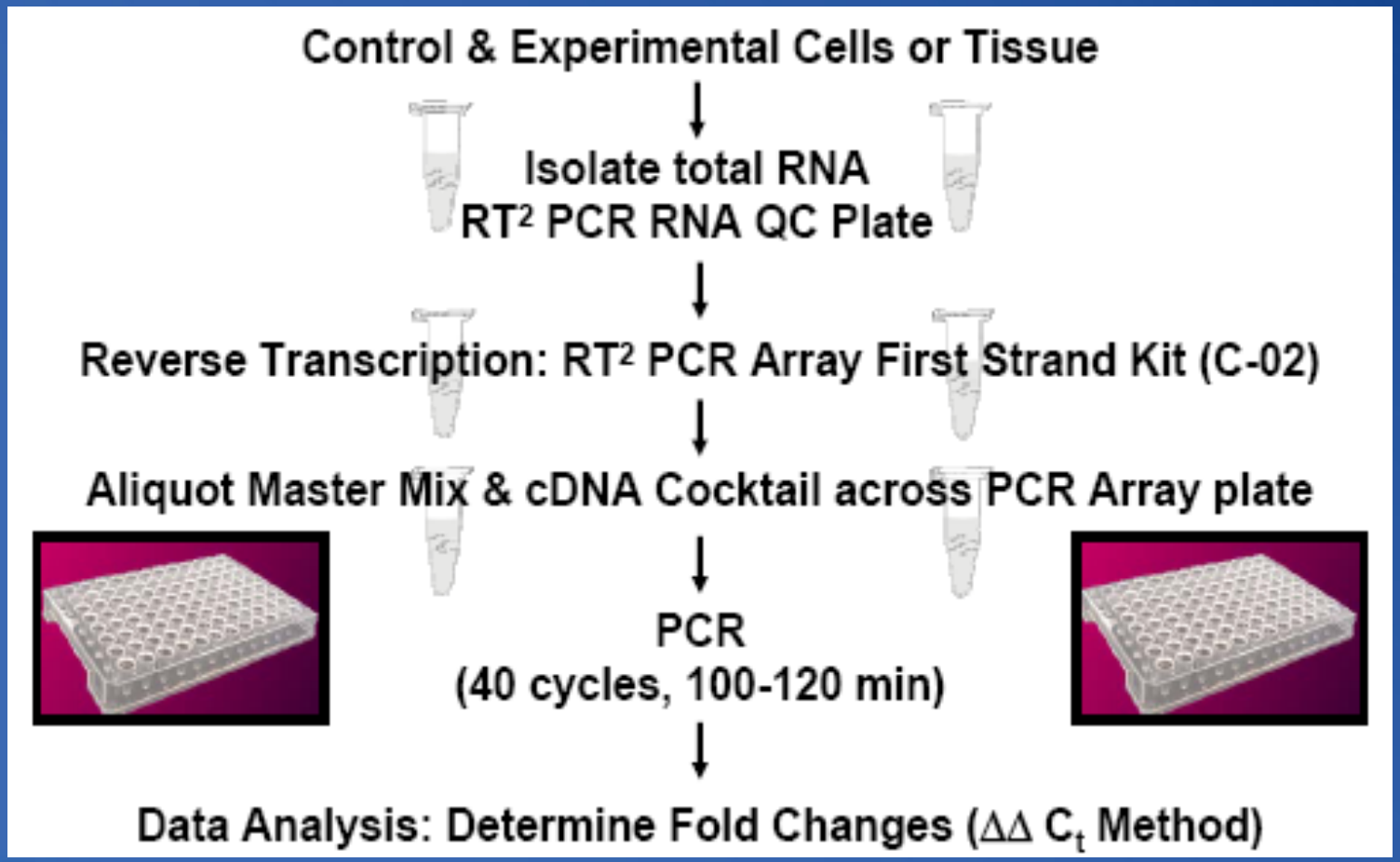


Human embryonic FHs cells

- Non-transformed human embryonic intestinal cells (FHs int) were exposed to a nontoxic concentration of bromoacetic acid (60 µM) for 30 min or 4 h.
- Treatments were in 6-well plates with concurrent paired negative controls.
- Experiments were replicated 3x with the identical clone of cells.

Attene-Ramos, M. S.; Nava, G. M.; Muellner, M. G.; Wagner, E. D.; Plewa, M. J.; Gaskins, H. R., DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells. *Environ. Mol. Mutagen.* **2010,** *In press*.

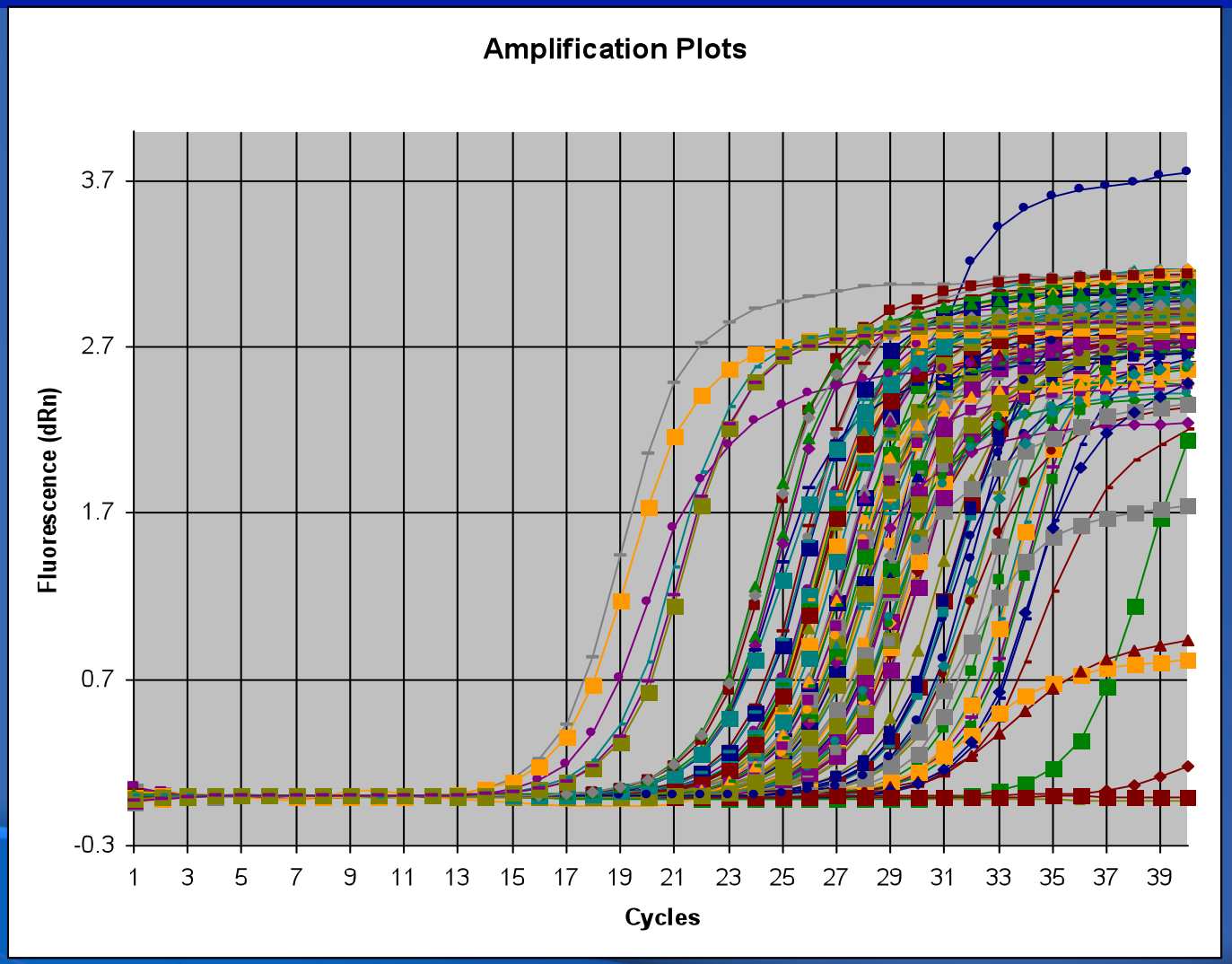
Human Cell Toxicogenomic Experimental Design: PCR Gene Arrays



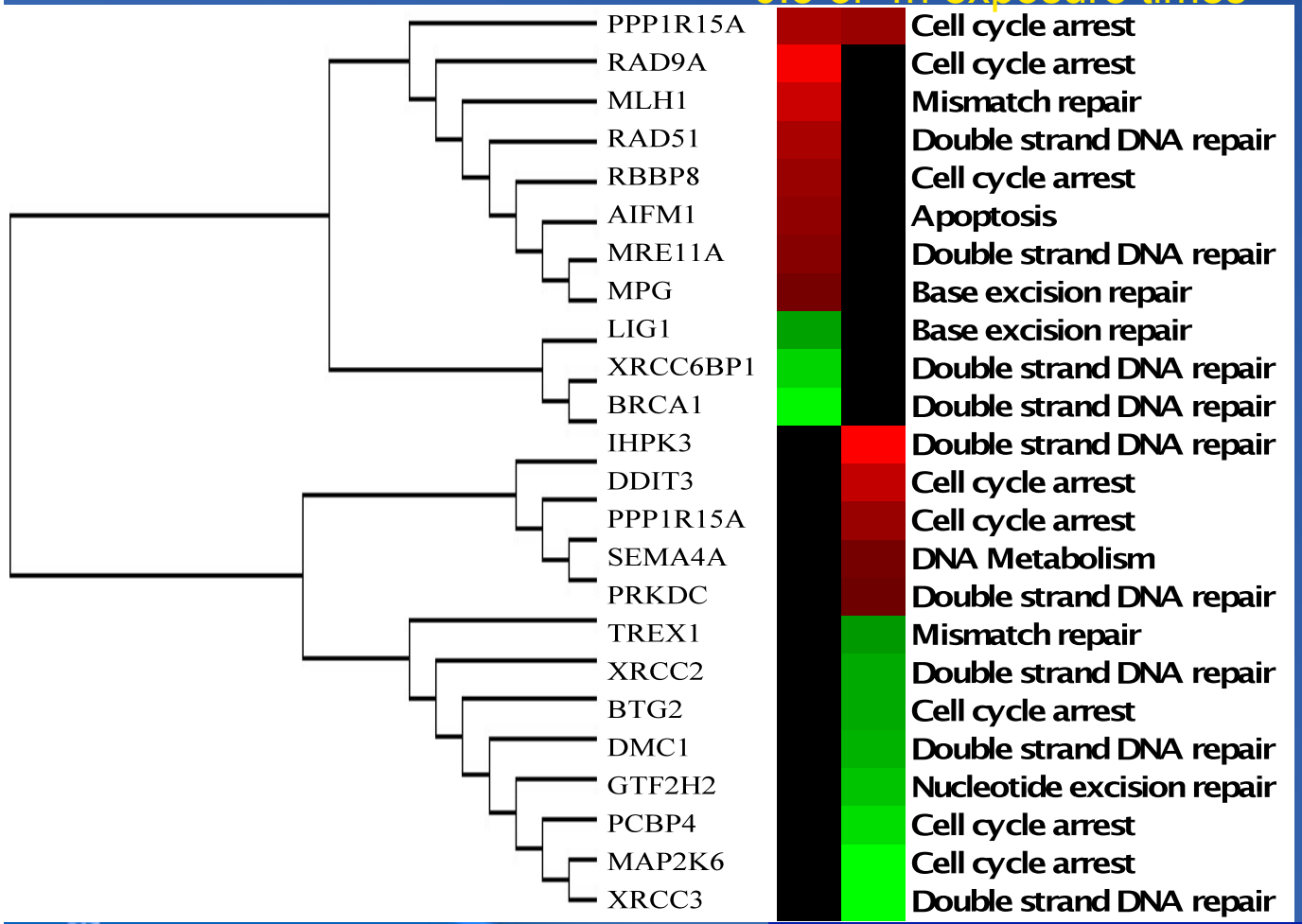
Statistical Analysis using the Rank Product method.

Bretling R. et al., 2005. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Letters 573:83-92.

Human DNA Damage/Repair PCR Array Amplification Plots



Biological Mechanisms of Bromoacetic Acid Induced Genomic Damage



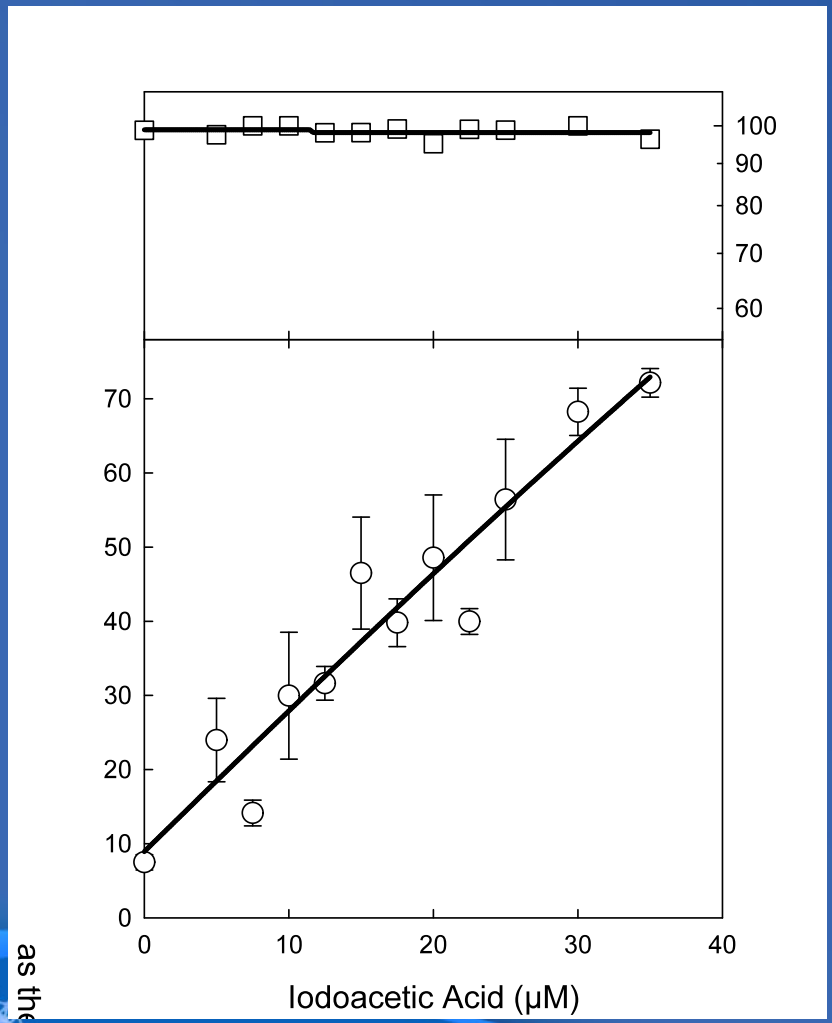
Of the DNA repair genes with altered expression, many are involved in double strand DNA breaks.

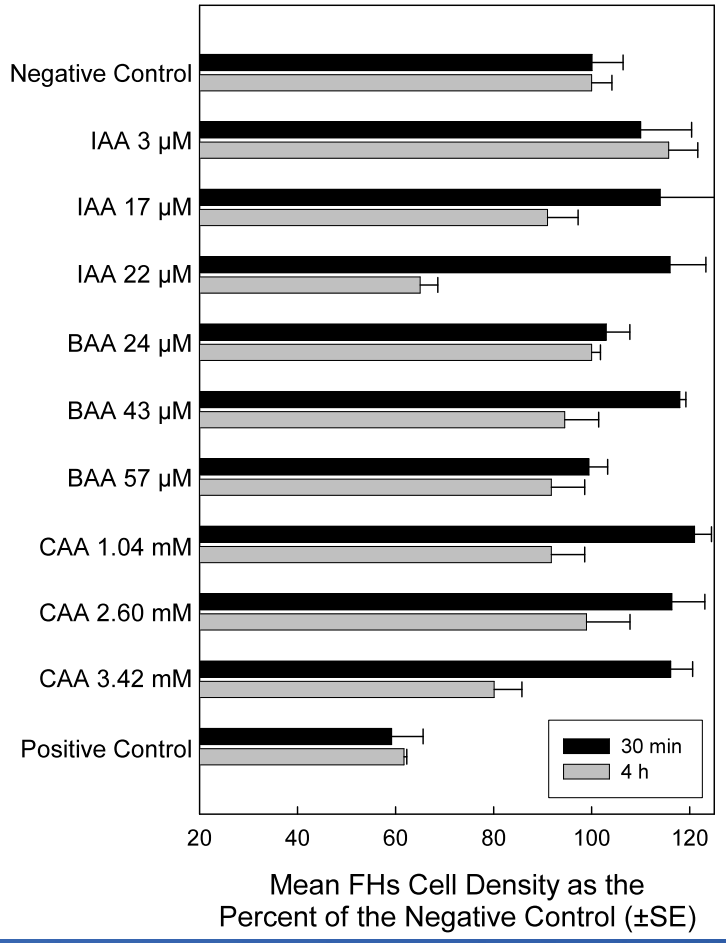
Muellner, M. G.; Attene-Ramos, M. S.; Hudson, M. E.; Wagner, E. D.; Plewa, M. J., Human cell toxicogenomic analysis of bromoacetic acid: a regulated drinking water disinfection by-product. *Environ. Mol. Mutagen.* ***2010,*** 51, 205-214.

Comparative Toxicogenomics of the Monohaloacetic Acids

- Conduct cytotoxicity, genotoxicity and plating efficiency experiments.
- Conduct the entire study using a single cloned culture of nontransformed human FHs cells.
- Run repeated independent arrays with repeated concurrent negative controls.
- Post gene array data in Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo)

Acute Cytotoxicity Versus Growth Rate

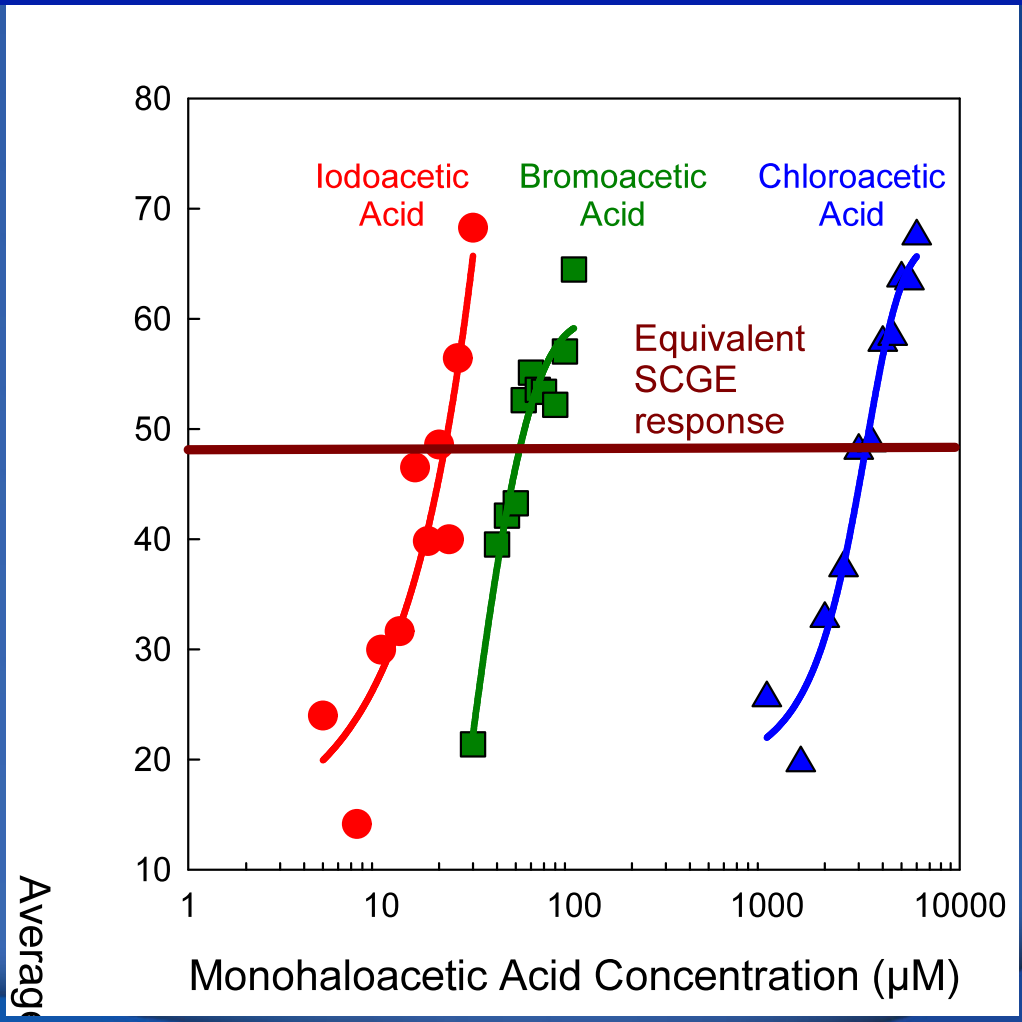




Note: BAA does not express a reduction in cell cycle.

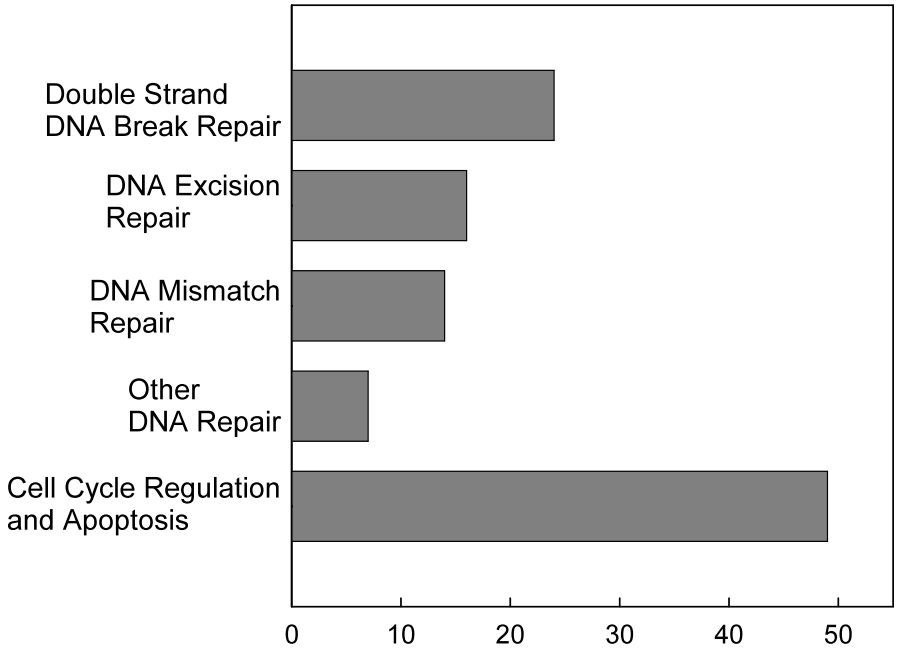
Comparative Toxicogenomics of the Monohaloacetic Acids

We calculated an equivalent concentration for each HAA that induced an equivalent SCGE biological response: 17 µM IAA, 57 µM BAA, 3.42 mM CAA.



Toxicogenomics of CAA, BAA, IAA

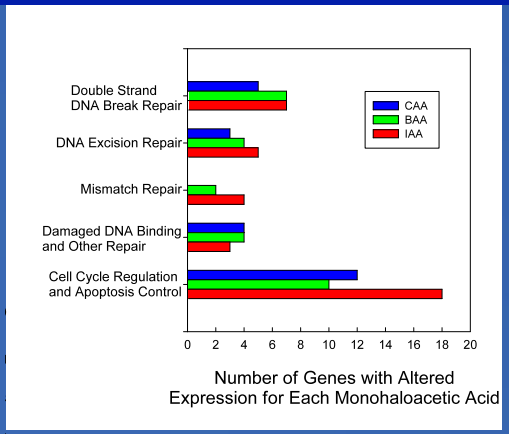
- We employed the DNA Damage/Repair gene array for the monoHAAs.
- The global response for the major functional groups show that double strand DNA break repair is involved for all 3 HAAs.



Number of Genes with Altered Expression for the Monohaloacetic Acids

Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J., Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. *Envion. Sci. Technol.* **2010,** In Press.

Altered Gene Expression for Each MonoHAA



Note: BAA alters the expression of the fewest number of genes for cell cycle arrest (as compared to CAA and IAA).

DAVID Pathway Analysis

MonoHAA-induced transcriptome profiles analyzed using the Database for Annotation, Visualization and Integrated Discovery						
Pathway	BAA 30 min	CAA 30 min	IAA 30 min	BAA 4 h	CAA 4 h	IAA 4 h
ATM signaling pathway	X		X	X	X	X
Cell cycle control				X	X	
Cyclins and cell cycle regulation					X	
FC Epsilon RI signaling pathway						X
MAPK signaling pathway			X	X		X
p53 signaling pathway			X	X		X
Role of BRCA1, BRCA2 and ATR in cancer susceptibility and dsDNA repair pathways	X		X	X	X	

Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J., Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. *Envion. Sci. Technol.* **2010,** In Press.

Conclusions IV

- Human toxicogenomic analysis generates mechanistic data at low DBP concentrations.
- CAA, BAA, and IAA at non-cytotoxic concentrations induce a modulation in genes involved in ds-DNA break repair; this suggests that these HAAs cause ds-DNA breaks.
- CAA, BAA and IAA alter the expression of human *XRCC3* (ds-DNA break repair gene).
- People carrying the *XRCC3-241* polymorphism have higher rates of bladder cancer. Bladder cancer is associated with exposure to disinfected water.
- Perhaps there are subpopulations that are more susceptible to monoHAA-induced genomic damage.
- BAA has the lowest rate of DNA repair (compared to IAA and CAA) and it expresses a lower level of cell cycle arrest (both in plating efficiency and in gene array analyses). The halogen may play a role in repair efficiency.
- Genotoxic risk management may need to consider both the induction of DNA damage and the rate of DNA repair.

Andrew, A. S.; Karagas, M. R.; Nelson, H. H.; Guarrera, S.; Polidoro, S.; Gamberini, S.; Sacerdote, C.; Moore, J. H.; Kelsey, K. T.; Demidenko, E.; Vineis, P.; Matullo, G., DNA repair polymorphisms modify bladder cancer risk: a multi-factor analytic strategy. Hum. Hered. 2008, 65, (2), 105-118.

Take Home Message

- Biologists, chemists and engineers must form functional interdisciplinary teams to address problems posed by hazardous DBPs and other micropollutants in water.
- Systematic, comparative *in vitro* toxicology must be integrated as a feed-back information loop into new engineering methods to remove and degrade micropollutants and disinfect water.
- The biological mechanisms of toxicity of emerging DBPs should be included with molecular epidemiology studies.
- We must develop systems to prevent unintended toxic consequences as we move forward in the implementation of new methods to desalinate, decontaminate and disinfect water.

Students and Colleagues

- Dr. Yahya Kargalioglu
- Dr. Mark Muellner
- Ms. Nancy Hsu
- Dr. Eduardo Cemeli
- Mr. Justin Pals
- Ms. Yukako Komaki
- Dr. Matias Attene-Ramos
- Ms. Clara Jeong
- Ms. Angelica Lagunas
- Mr. Justin Ang
- Ms. Jen Osiol

- Dr. Susan Richardson
- Dr. Yin-Tak Woo
- Dr. Bruce McKague
- Dr. Benito Mariñas
- Dr. Jane Ellen Simmons
- Dr. William Mitch

Funded in part by AwwaRF Grants 554, 3089, & 4132, U.S. EPA Grant CR83069501, Illinois-Indiana Sea Grant R/WF-09-06, WaterCAMP*WS* NSF Center Grant CTS-0120978, NSF Grant 06-51333, and Statoil Grant.

M. Muellner was supported by T32 ES07326 NIH Predoctoral Fellowship.