WATER DISINFECTION BY-PRODUCTS AND THEIR SAFETY

Brian S. Cummings, Ph.D.
Associate Professor
Georgia Cancer Coalition Distinguished Scholar
Director: Interdisciplinary Toxicology Program
Lilly Teaching Fellow
Pharmaceutical and Biomedical Sciences
College of Pharmacy
University of Georgia

Cummings Research-Disclosures

- **Role of phospholipase A_2 (PLA_2) in prostate cancer**
  - **Calcium independent PLA_2 ($iPLA_2$)**
  - **Secretory PLA_2 ($sPLA_2$)**
  - **Phospholipase A_2 receptor (PLA2R)**

- **Lipidomics**
  - **PLA_2 mediated changes in the lipidome in prostate tumors during transformation and treatment**
  - **Changes in various tissues after oxidative stress**
  - **Changes in the lipidome in different pathologies (drugs of abuse)**
  - **Lots of ESI-MS**

- **Renal Toxicology**
  - **Role of epigenetics in the nephrotoxicity of environmental oxidants and chemotherapeutics**
  - **Epigenetic changes induced by nephrotoxic water disinfection by-products (DBPs)**

National Institutes of Health

NIBIB

Georgia Cancer Coalition

CDMRP

Georgia Cancer Coalition

Georgia Research Alliance

American Water Works Association

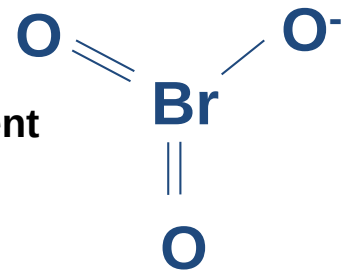
Water Disinfection Byproducts (DBPs)

- **Water disinfection**
  - **Protects 260 million Americans from pathogens**
  - **Oxidizes organic elements and produces byproducts**
- **DBPs**
  - **Formed when disinfects used in treatment plants react with naturally occurring material**
    - **Chlorination = Trihalomethanes, haloacetic acid, chlorate**
    - **Chlorine Dioxide = Chlorite, chlorate**
    - **Chloramine = Chlorate**
    - **Ozonation = Bromate**

von Gunten and Hoigne, *Env Sci & Tech*, 1994
Kurokawa et al., *Environ Health Perspect*, 1990
Wolf et al., *Tox Path*, 1998
DeAngelo et al., *Tox Path*, 1998
Kasai et al., *Carcinogenisis*, 1987
Zhang et al., *Toxicol*, 2010

Bromate

- A source water disinfection byproduct of the ozonation process.



- Ozonation is a widely used method in Europe and Asia, and to a limited extent in the U.S.A.

- BrO_3^- is designated as a probable human carcinogen by the International Agency for Research on Cancer (IARC 1987, US EPA, 1986).

- Regulated levels established by the USEPA – 0.01 ppm (Maximum Containment Level) – which is usually less than what is found after ozonation.

  -EPA RrD = Urinary = Renal effects (urothelial hyperplasia)
  -PoD = 1.1 mg/kg-day
  -UF = 300

US EPA 1999
Richardson SD *et al* 2007, *Mutation Research* 636: 178-242

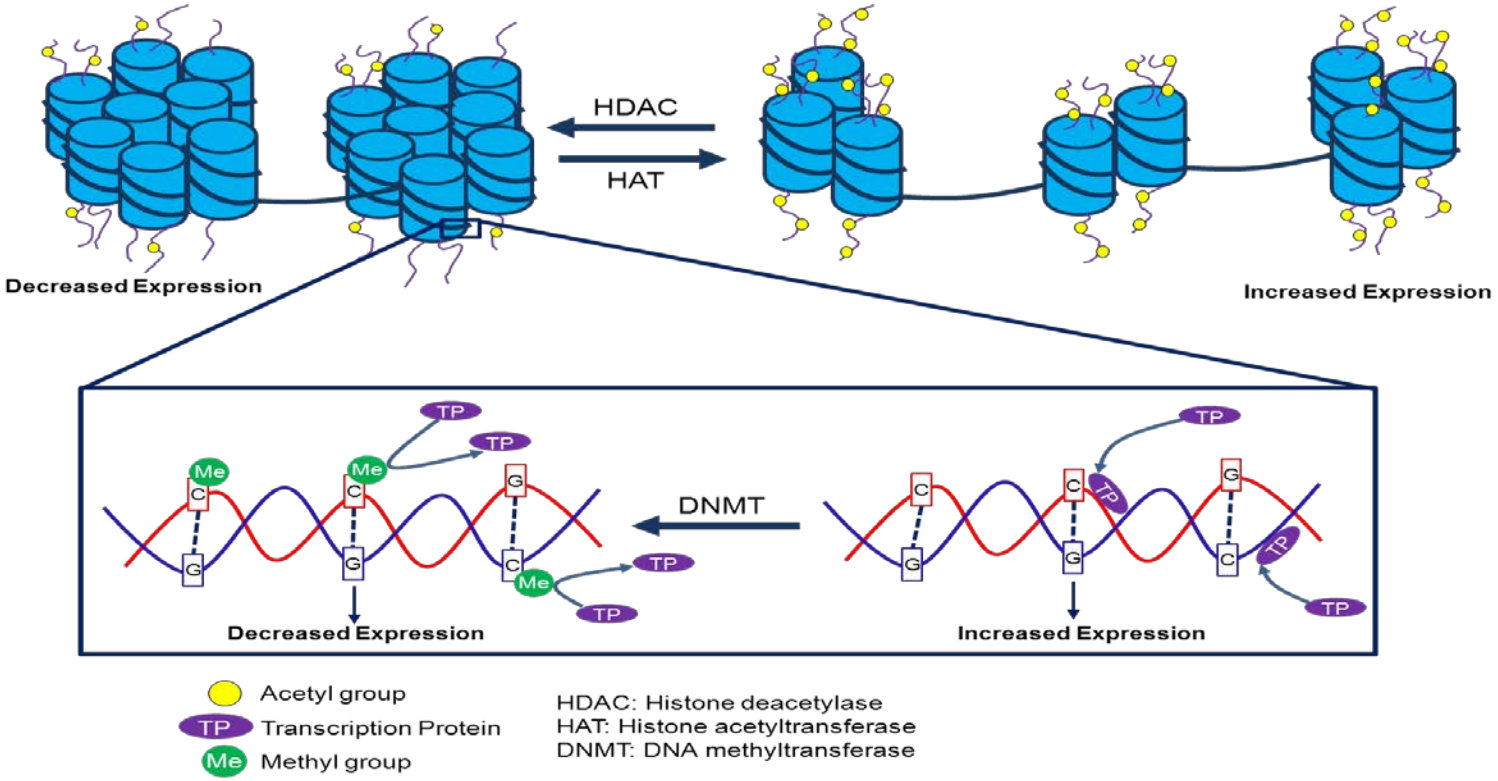
Epigenetic Alterations

- **Heritable changes in gene expression that are NOT associated with sequence changes**

- **Epigenetic events include**
  - **DNA methylation**
  - **Histone modification**
  - **Nonfunctional RNA (microRNA)**

Goodman et al., *Toxicol Sci*, 2010

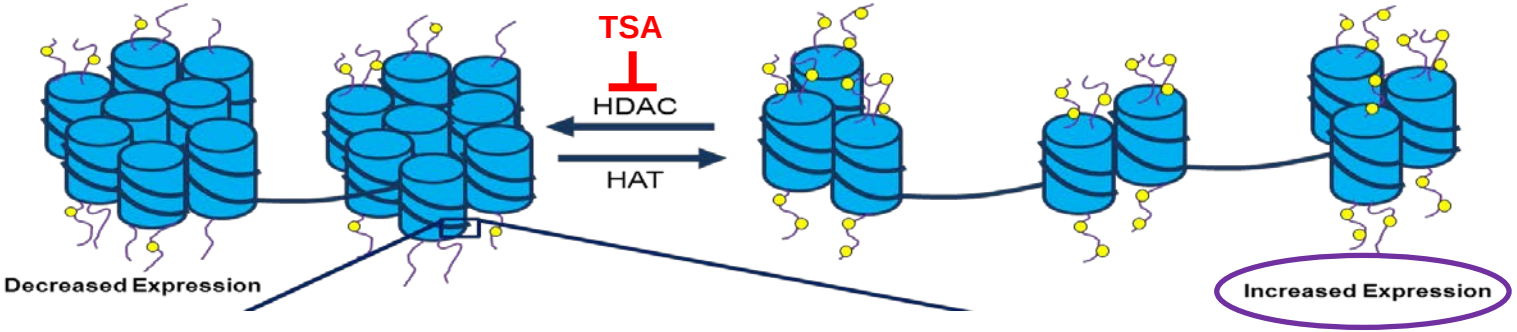
Epigenetics and Gene Expression



acetyl group: acetyl-coenzyme A
methyl group: S-adenosyl methionine (SAM)

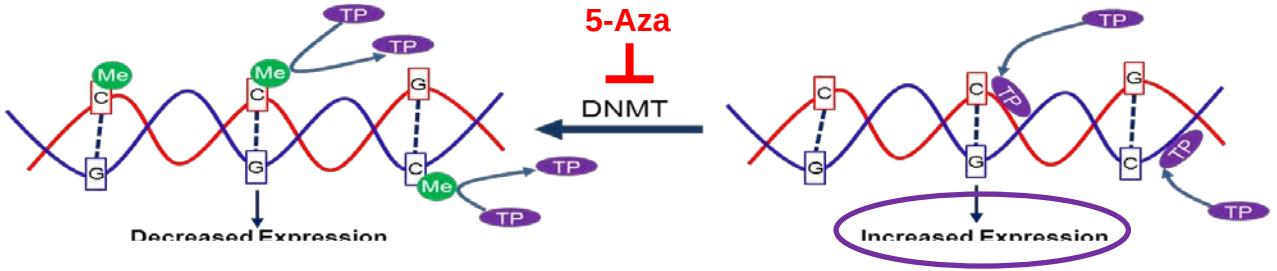
Epigenetic Inhibitors

- **Histone deacetylase (HDAC) inhibitors**
  - **Trichostatin A (TSA)**
  - **Prevent deacetylation of histone**
  - **Prevents gene silencing**
  - **Hyperacetylation**



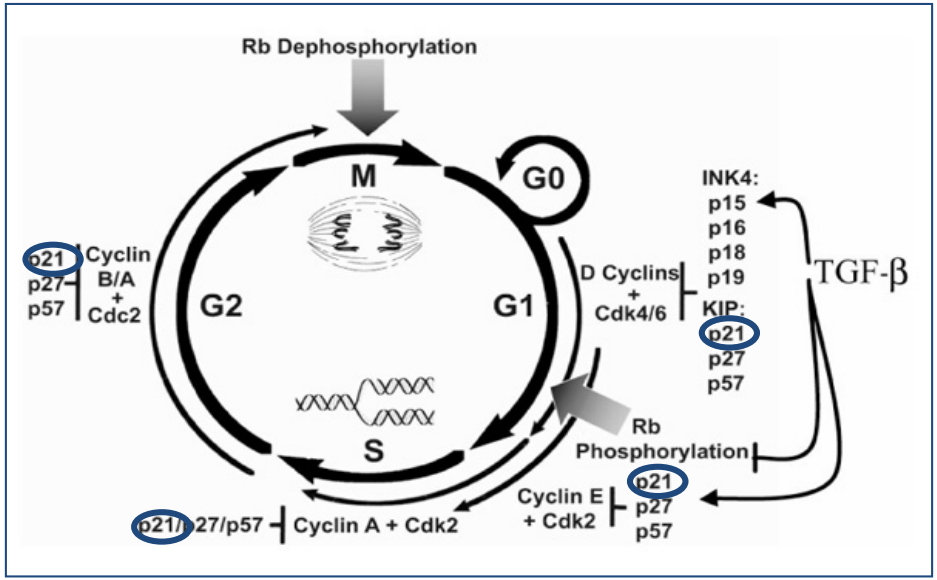
Epigenetic Inhibitors

- **DNA methyltransferase (DMNT) inhibitors**
  - **5-Azacytidine (5-Aza)**
  - **Prevents formation of 5-methylcytosine**
  - **Prevents gene silencing**



p21

- **p21 is a cyclin-dependent kinase inhibitor that regulates cell cycle progression at the G1 and S phases.**
- **The expression of this gene is controlled by both tumor suppressor protein p53-dependent and -independent pathways.**



Donovan J *et al* 2000, *Cancer Res.* 2:116-124

Bromate, p21 and Epigenetics

High doses of BrO_3^- induces DNA damage and 8-OHdG production (a measure of oxidative stress) *in vitro*.

BrO_3^- induces toxicity in human and rat kidney cells.

Inhibition of DNA methylation or histone deacetylation increased p21 expression and altered cell death.

Changes in p21 expression after exposure to low-dose BrO_3^- correlated to changes in DNA methylation within the coding region.

TOXICOLOGICAL SCIENCES 2014
doi: 10.1093/toxsci/kfu138
Advance Access publication July 11, 2014

Toxicological Sciences

Epigenetic Changes in p21 Expression in Renal Cells after Exposure to Bromate

N.E. Scholpa,[3] X. Zhang,[3,2] R.T. Kolli, and B.S. Cummings[1]

Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, 30602, Georgia

[1]To whom correspondence should be addressed. Fax: +706-542-5358. E-mail: bsc@rx.uga.edu.

Received June 2, 2014; accepted June 25, 2014

This study tested the hypothesis that bromate ($KBrO_3$)-induced renal cell death is mediated by epigenetic mechanisms. Global DNA methylation, as assessed by 5-methylcytosine staining, was not changed in normal rat kidney cells treated with acute cyto-

BrO_3^-	Bromate
Br^-	Bromide
Cdkn1a	Cyclin-dependent kinase inhibitor 1a
DBP	Disinfection byproduct
DNMT	DNA methyltransferase

BrO_3^--induced increase in p21 expression is regulated by epigenetic mechanisms.

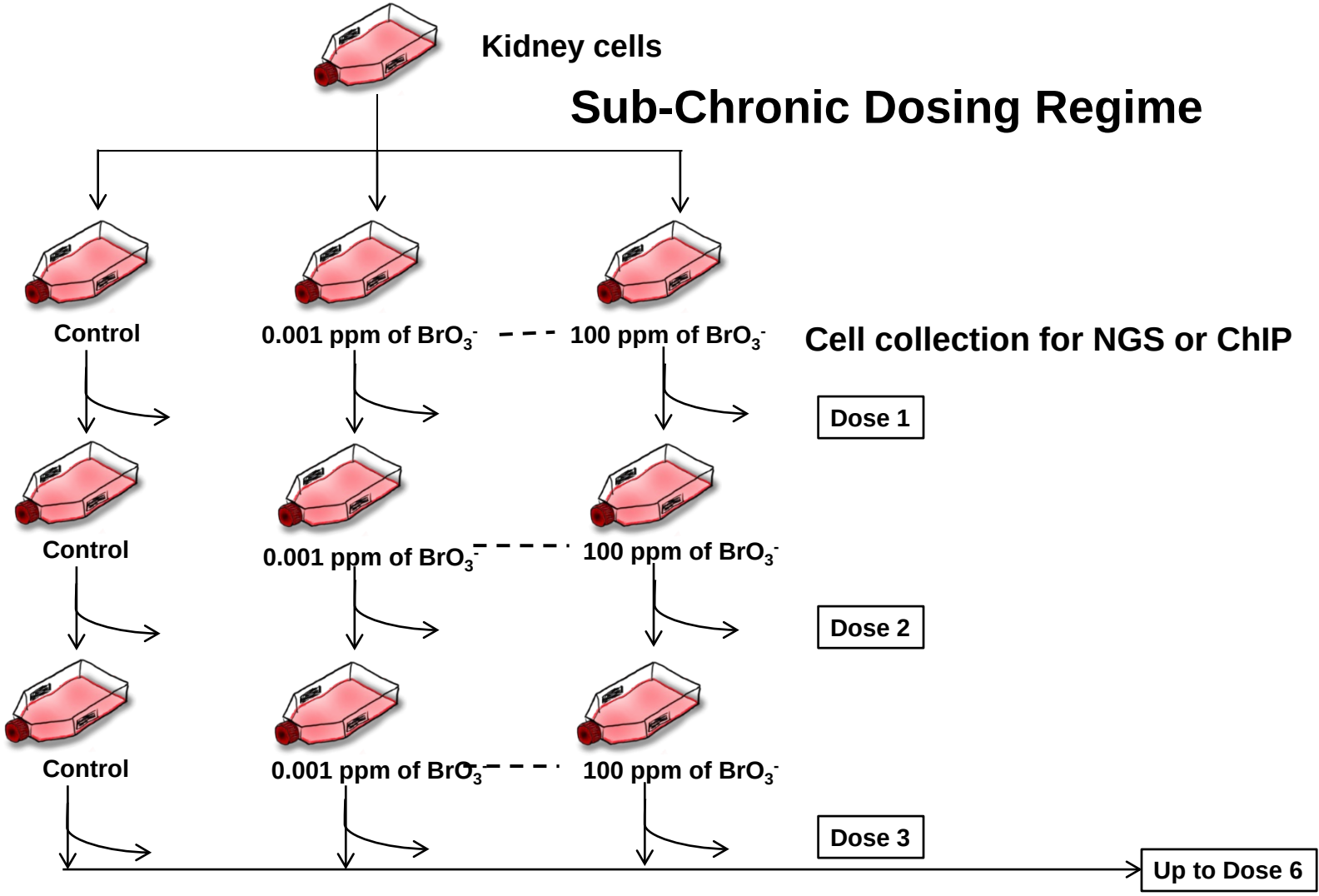
Questions

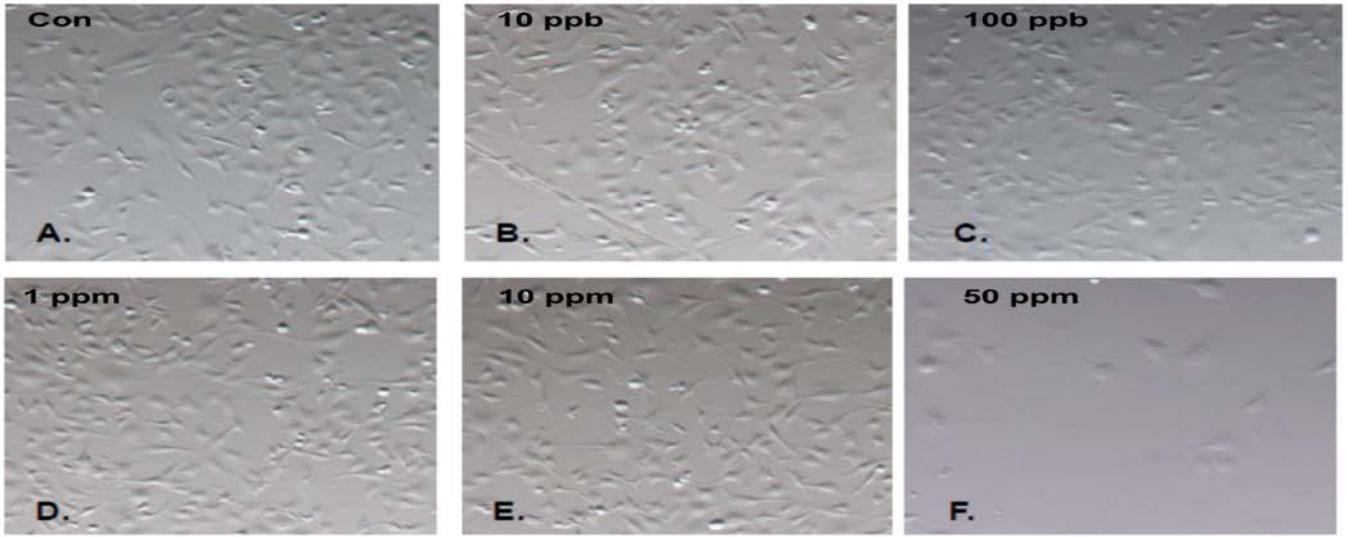
1. **Should epigenetic be used to assess the safety of DBPs?**

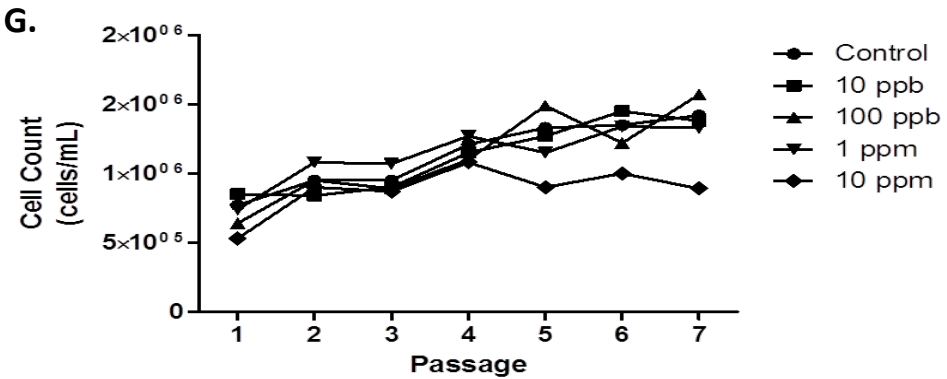
2. **What are the difference between rats and humans with regards to epigenetics?**

3. **What does this mean for risk assessment?**

Will address the above questions using p21 and bromate as an model

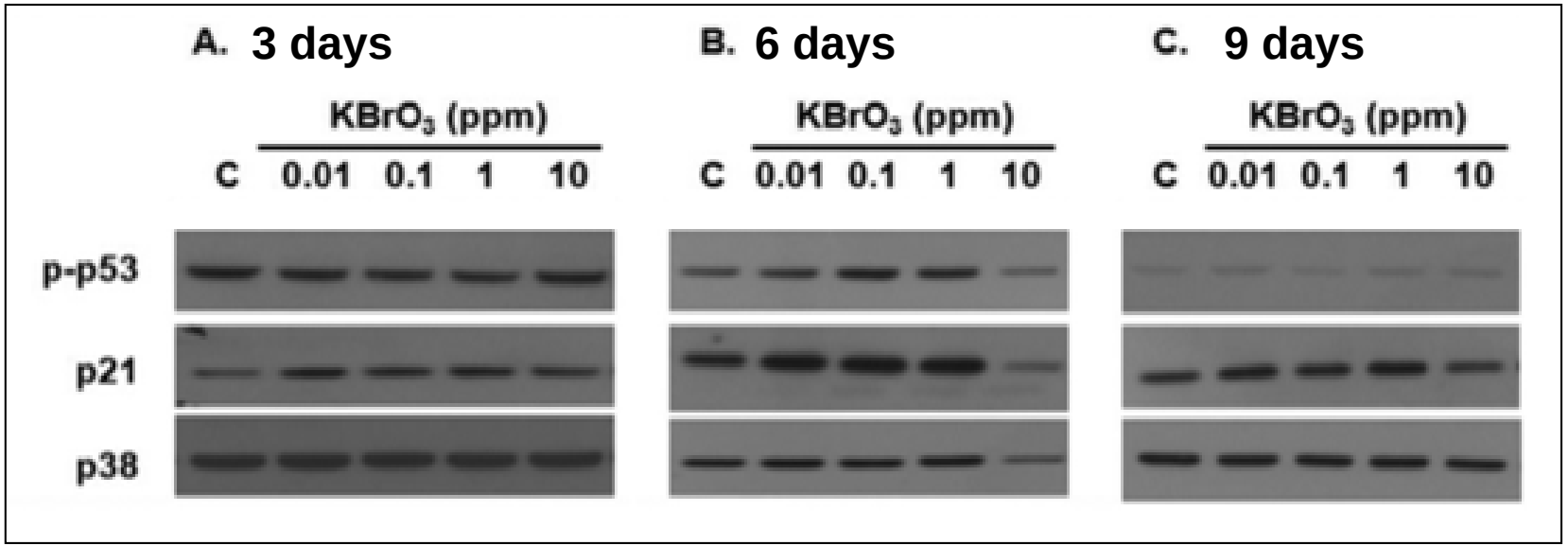

Kidney cells
Sub-Chronic Dosing Regime
Control
0.001 ppm of BrO_3^-
100 ppm of BrO_3^-
Cell collection for NGS or ChIP
Dose 1
Control
0.001 ppm of BrO_3^-
100 ppm of BrO_3^-
Dose 2
Control
0.001 ppm of BrO_3^-
100 ppm of BrO_3^-
Dose 3
Up to Dose 6





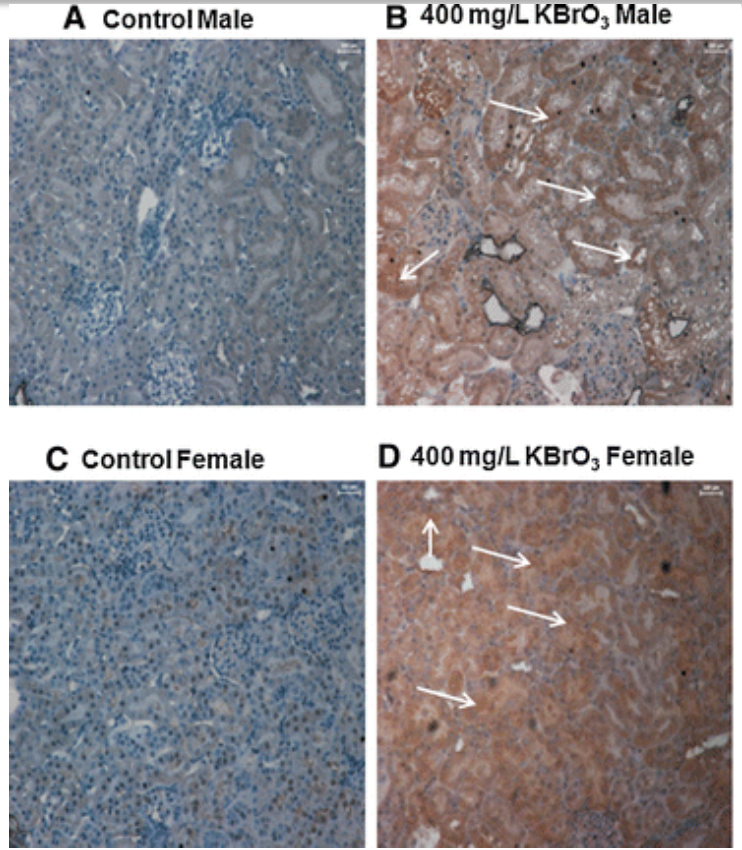
Scholpa et. al, *Toxicol Sci*, 2014

Effect of Bromate on p21 Expression in Renal Cells

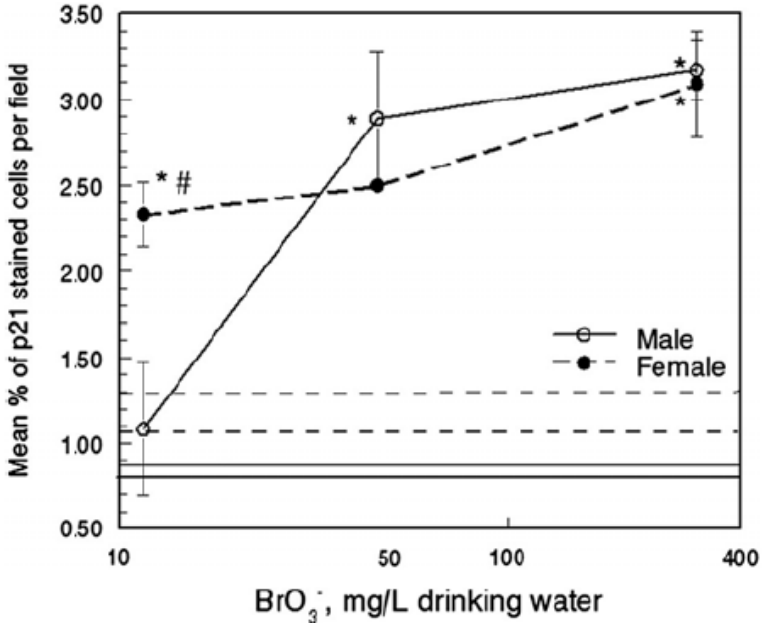


Normal Rat Kidney Cells
Bromate MCL = 0.01 ppm
Scholpa et. al, *Toxicol Sci*, 2014

Effect of Bromate on p21 Expression in Renal Cells



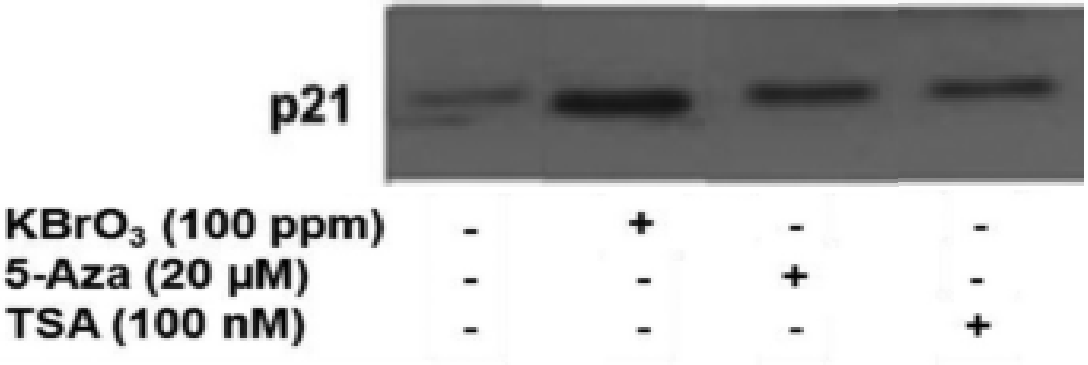
28 day exposure



Kolisetty et al., *Arch Toxicol*, 2013, Kolisetty et al, *Toxicol Appl Pharm,* 2013

Effect of Epigenetic Inhibitors on p21 Expression in Renal Cells

NRK Cells
72 hour Exposure



5-Aza = DNA Methyltransferase
TSA = Histone deacetylase inhibitor

Scholpa et. al, *Toxicol Sci,* 2014

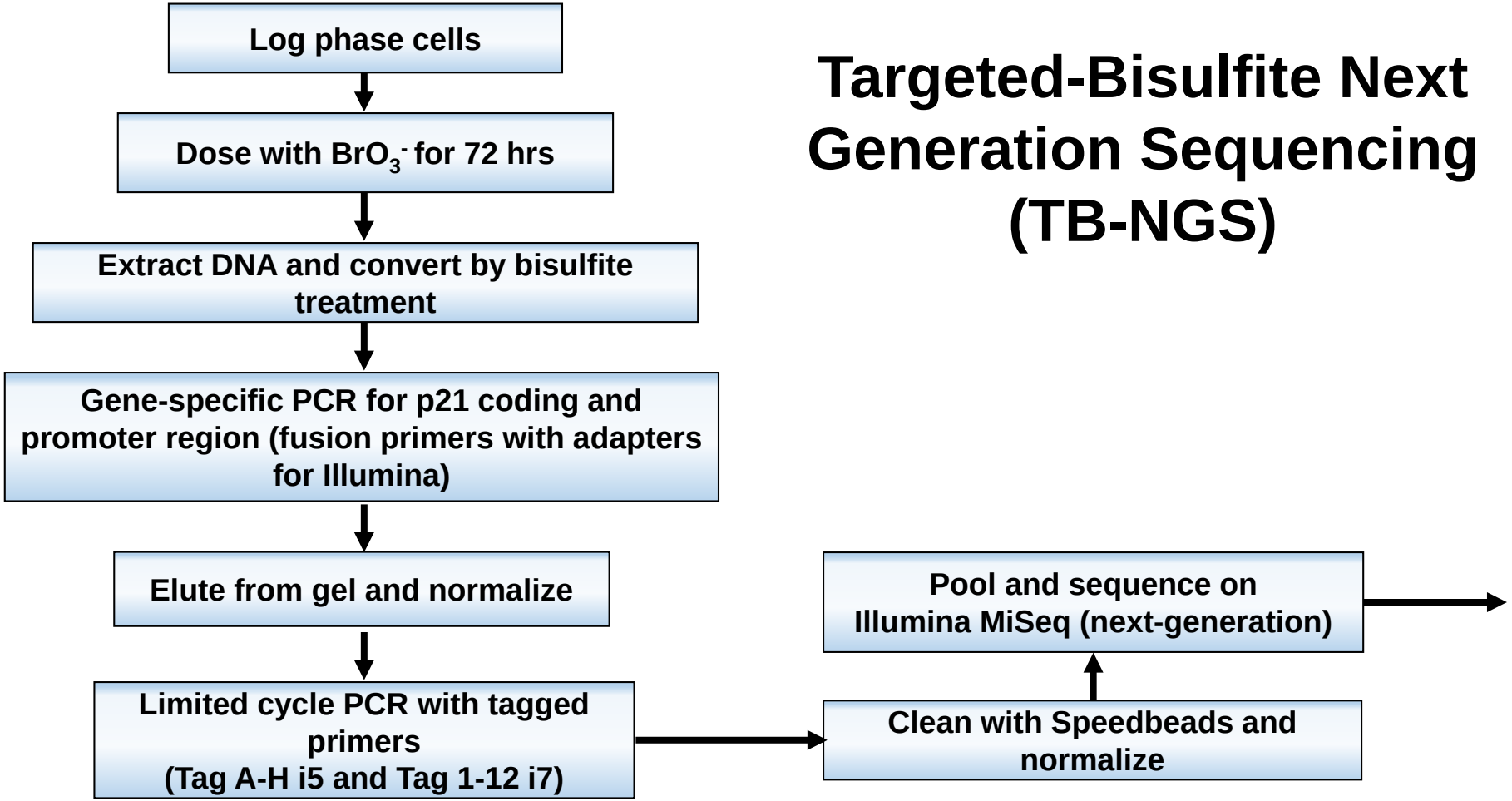
Summary Part 1

- **Bromate increases the expression of p21 in renal cells after both acute and sub-chronic exposures**
- **Increases in p21 expression *in vitro* occurred at doses that that are as low as the MCL (0.01 ppm)**
- **Bromate-induced p21 expression was altered by epigenetic inhibitors**
- **What is mechanism mediating bromate-induced epigenetic changes in p21?**
- **What are the differences between rats and humans?**
- **What does this mean for risk assessment?**

How Does Bromate Alter p21 Expression?

- **Identify the specific changes in DNA induced by bromate**
- **Epigenetic changes**
  - **DNA Methylation?**
  - **Histone acetylation?**
- **Problem**
  - **Assessment of DNA methylation is laborious and time consuming**
  - **Need a high through put more accurate way to assess DNA methylation of targeted genes**

Targeted-Bisulfite Next Generation Sequencing (TB-NGS)



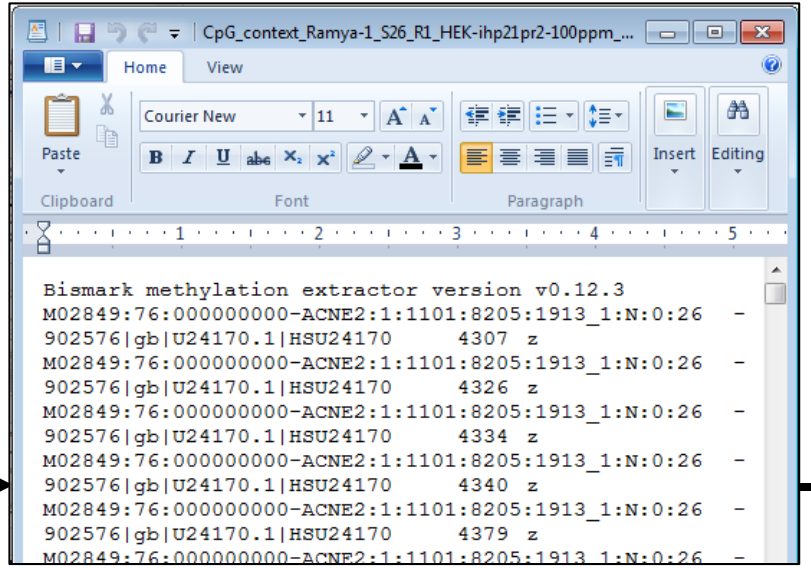
DNA Methylation Analysis Using Bismark Bisulfite Mapper

Prepare reference genome folder

Convert reference genome to bisulfite converted version.

Align the sequence reads from MiSeq to reference.

Extract percent methylation output.



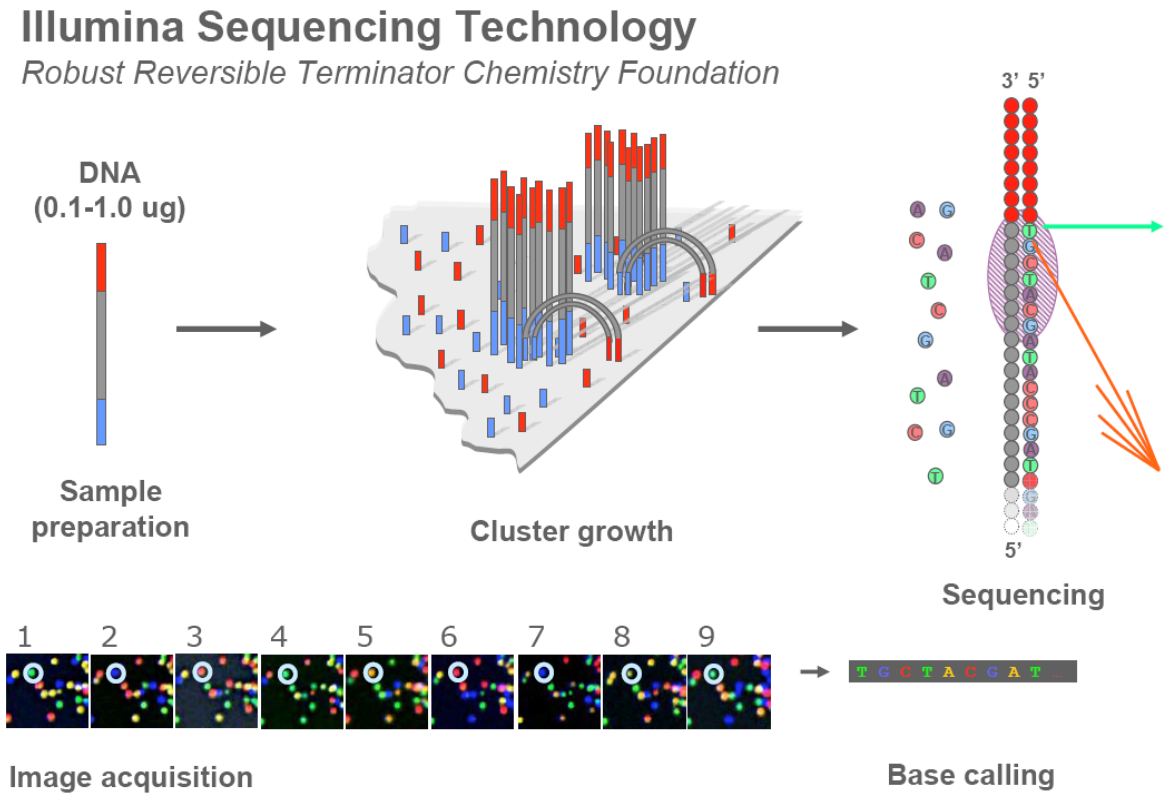
A)

B)

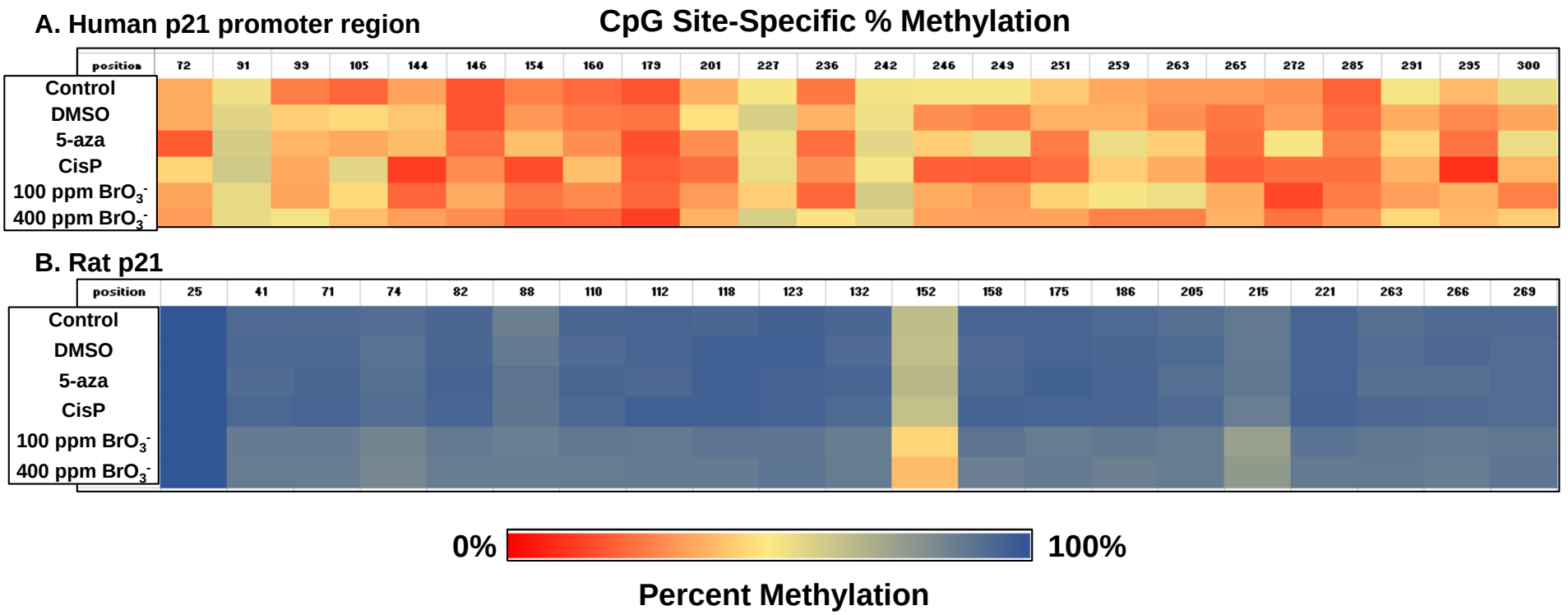
Tagged i5

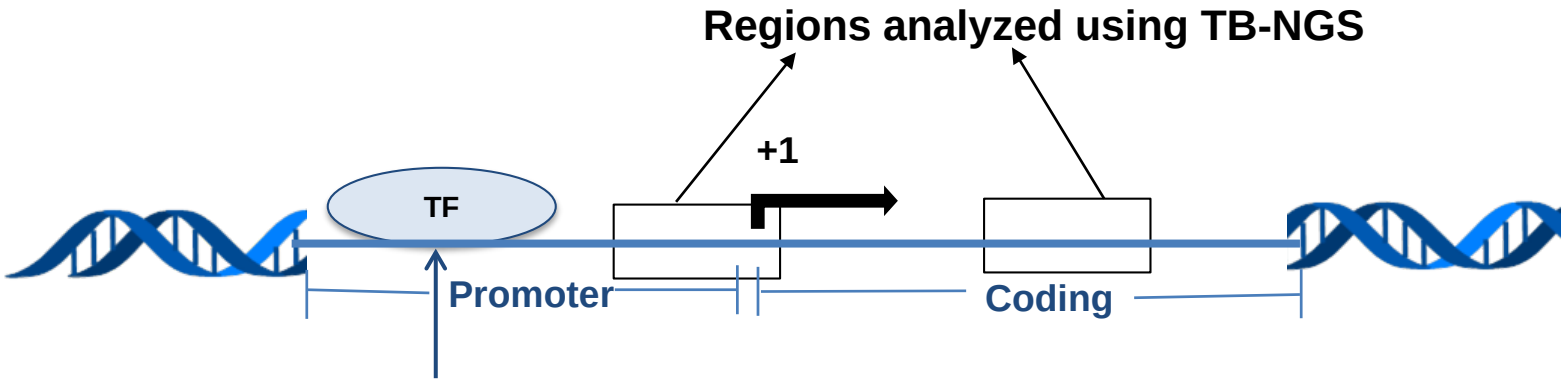
Tagged i7	A	B	C	D
1	A-1	B-1	C-1	D-1
2	A-2	B-2	C-2	D-2
3	A-3	B-3	C-3	D-3
4	A-4	B-4	C-4	D-4

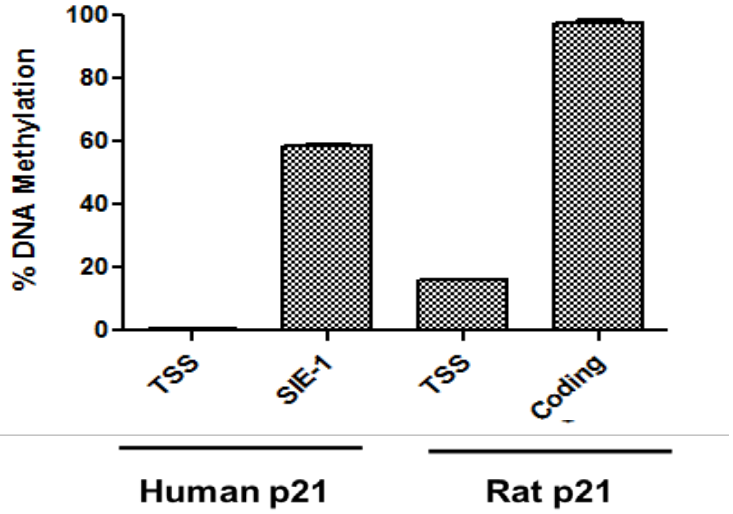
Sequencing using Illumina MiSeq

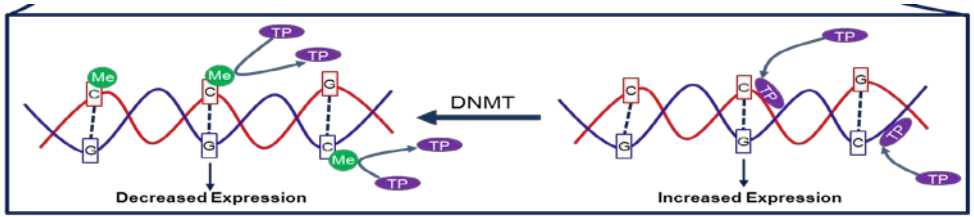


Heat-Map of Site-Specific Percent DNA Methylation Changes




Regions analyzed using TB-NGS
+1
TF
Promoter
Coding
Transcription Factor
Binding Site


% DNA Methylation
100
80
60
40
20
0
TSS
SIE-1
TSS
Coding
Human p21
Rat p21


TP
Me
DNMT
Decreased Expression
Increased Expression

Bromate-Induced Change in DNA Methylation in Human p21

20%

% DNA Methylation

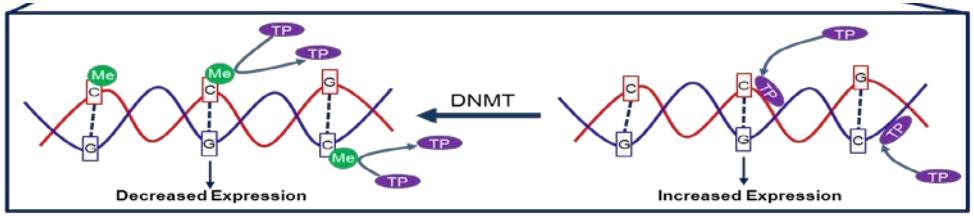
80%

A. Dose 1 = 3 days

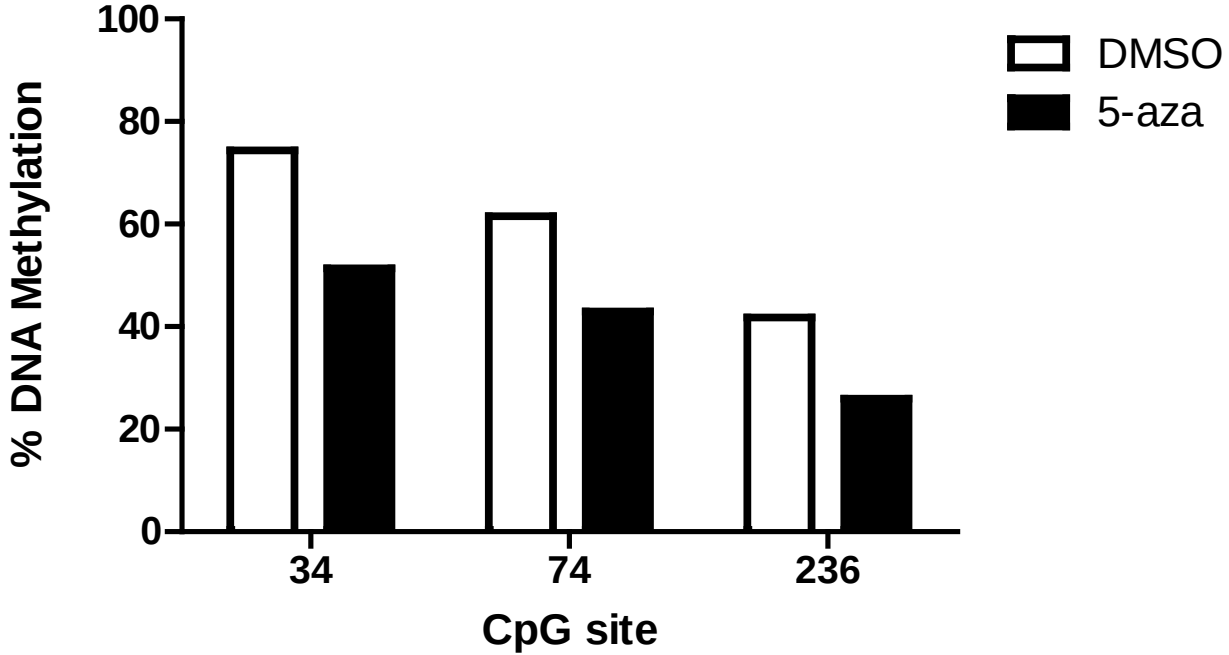
position	34	74	236
Water	78.03	66.59	35.78
0.001	74.39	71.95	39.18
0.01	74.81	71.94	46.29
0.1	74.69	71.01	42.12
1	79.10	67.16	42.49
10	69.97	70.55	41.10
100	74.66	60.96	42.66
DMSO	70.56	72.58	43.85
5-aza	67.26	58.41	37.00

B. Dose 6 = 18 days

position	34	74	236
Water	76.86	68.99	48.29
0.001	76.57	67.42	45.68
0.01	74.93	64.92	44.78
1	78.48	69.10	43.72
10	76.18	68.69	41.72
DMSO	74.37	61.53	41.76
5-aza	51.40	42.99	25.94

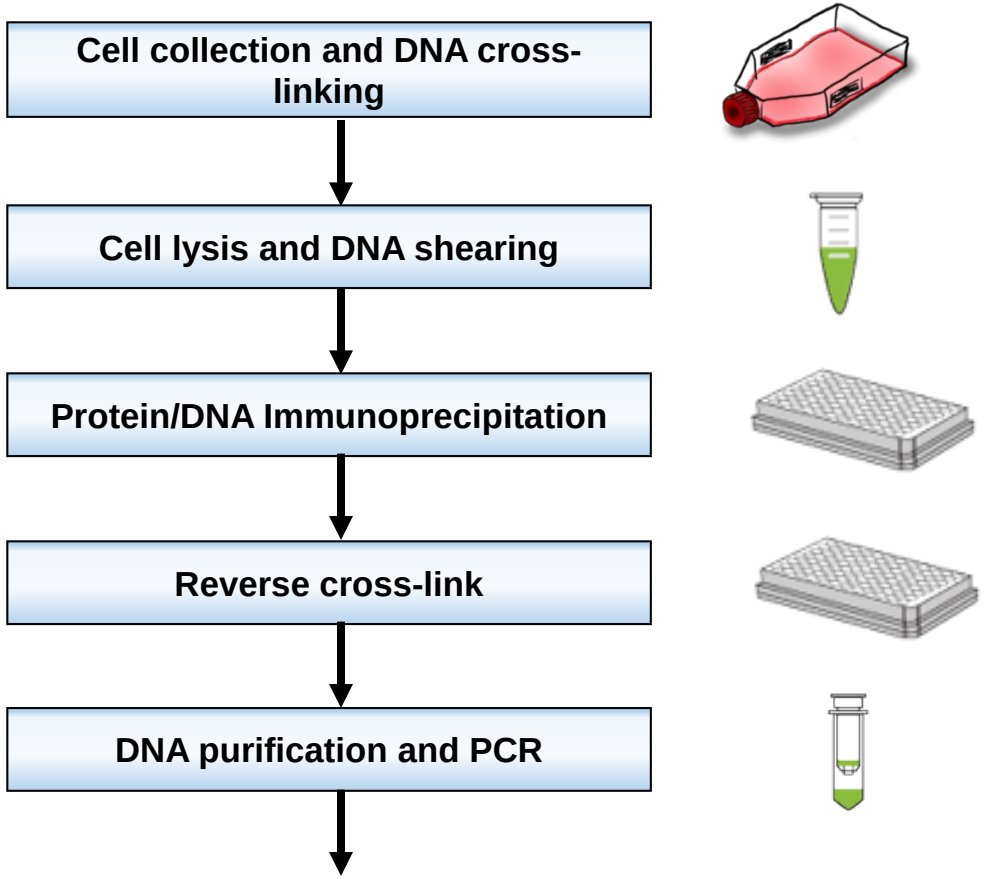


5-Aza-Induced Change in DNA Methylation in Human p21

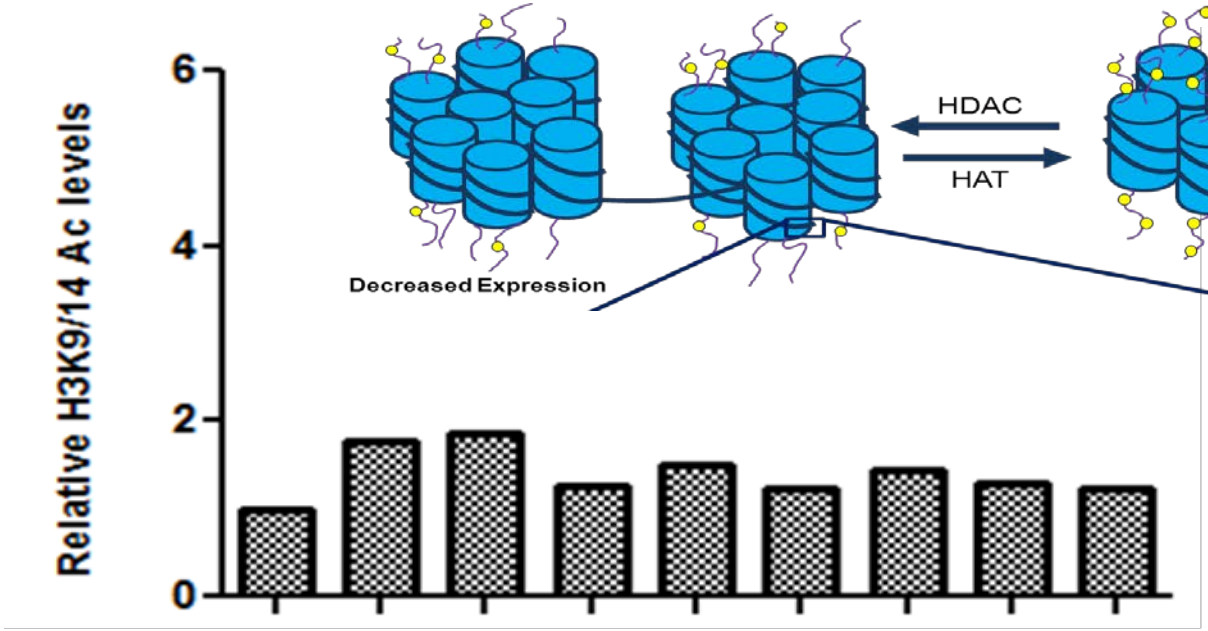


Bromate does not appear to induce change in DNA methylation in p21

Bromate-Induced Change in p21 Histone Acetylation (ChIP Assay)



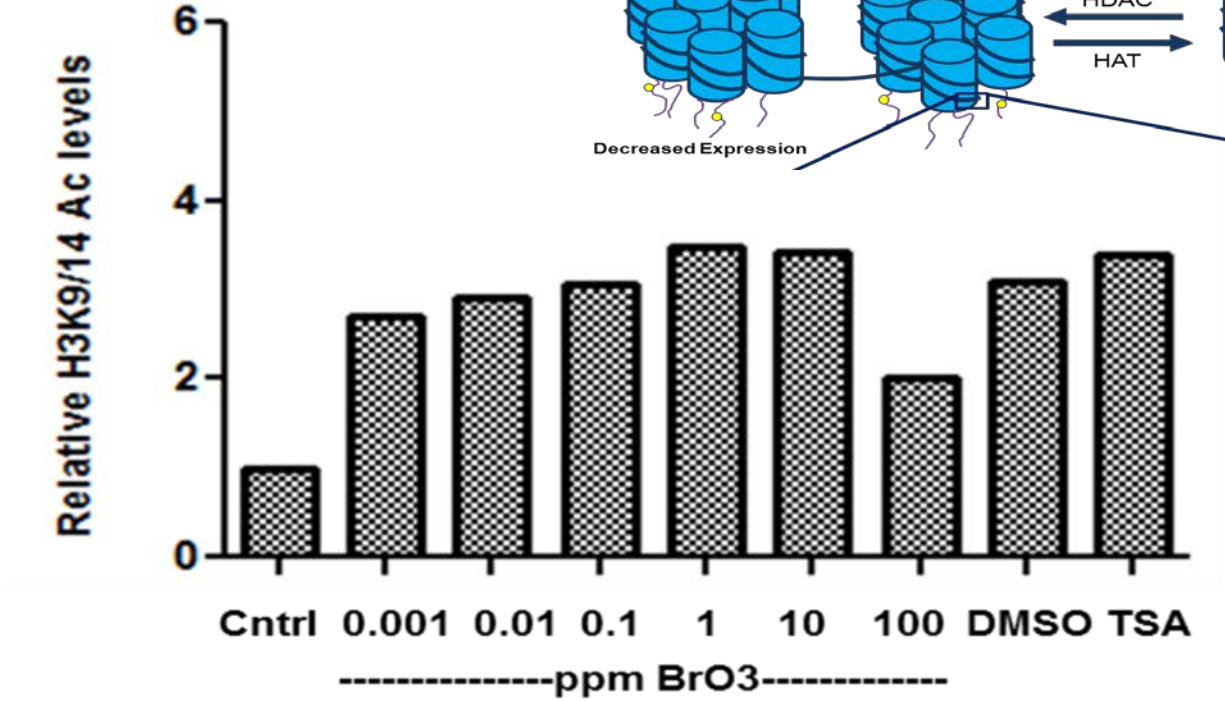
Acetylation of H3K9/14 in the p21 Promoter Region in Rat Kidney Cells

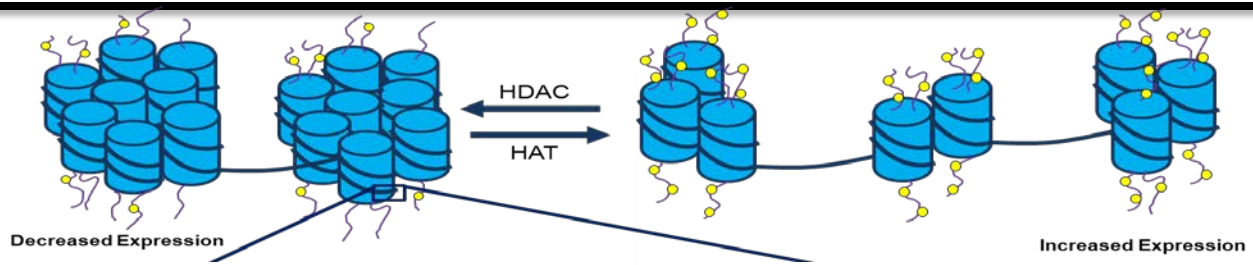


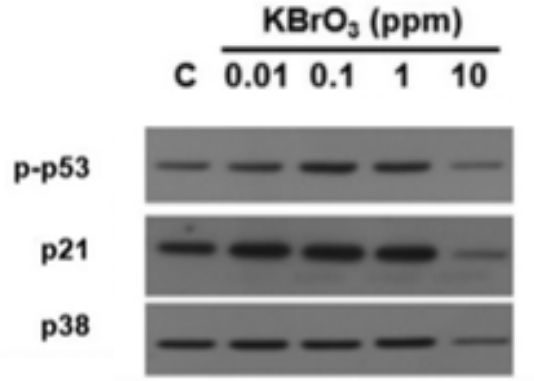
Dose 1 = 3 days

Acetyl Histone of H3K9/14 in the p21 Promoter Region in Rat Kidney Cells

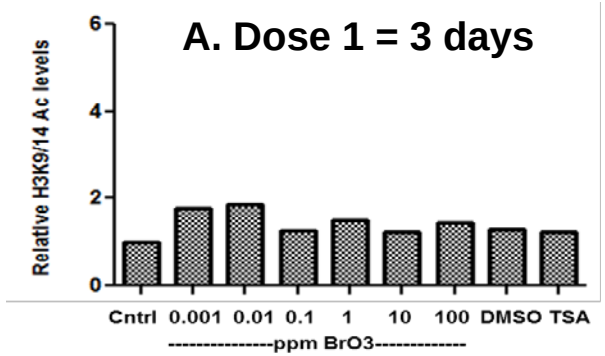
Dose 2 = 6 days

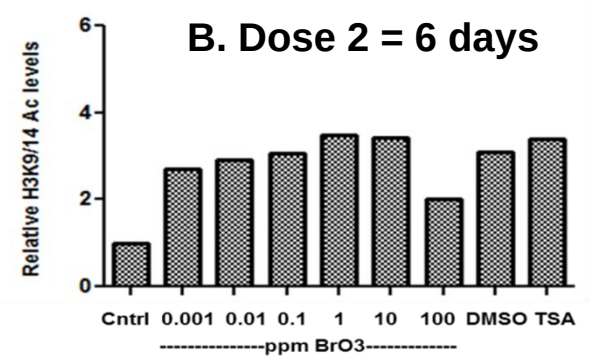


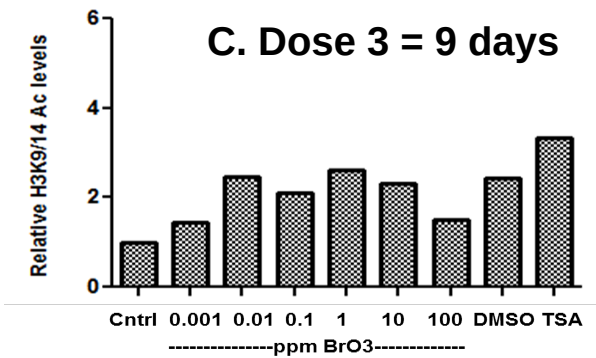


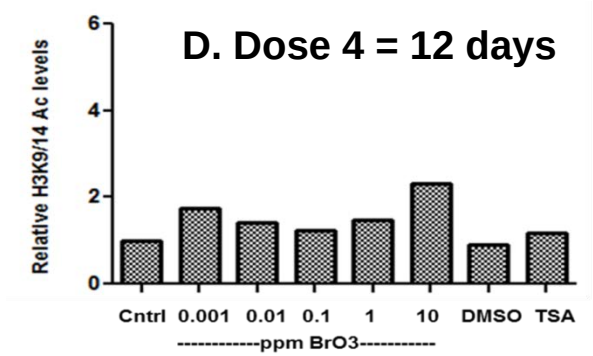


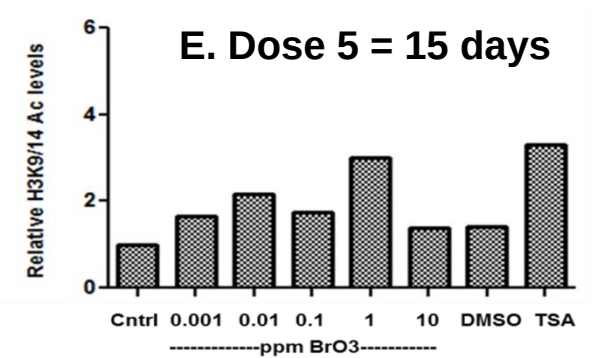
Acetyl Histone of H3K9/14 in the p21 Promoter Region in Rat Kidney Cells

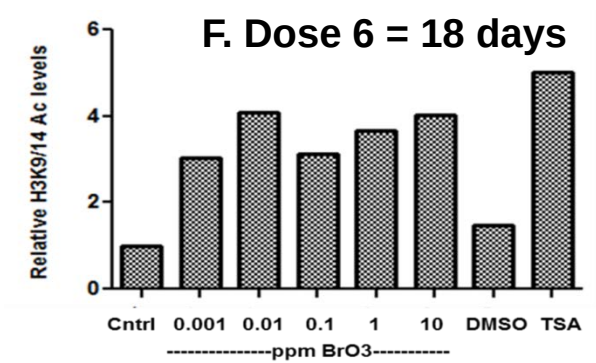












Summary Part 2

- **Bromate increases the acetylation of histone in p21 in renal cells after both acute and chronic exposures**
- **Increases in p21 histone acetylation in vitro occurred at doses that that are as low as the MCL (0.01 ppm)**
- **Bromate increase in p21 expression appear to be regulated by histone acetylation not DNA methylation**

Goodman et al., *Toxicol Sci*, 2010

Questions

- **What is mechanism mediating bromate-induced epigenetic changes in p21?**
  - **Histone acetylation**
- **What are the difference between rats and humans?**
  - **Significance difference in basal DNA methylation in p21 between rats and humans**
  - **Acetylation data is still being tabulated**

Goodman et al., *Toxicol Sci*, 2010

Questions

- **What does this mean for risk assessment?**
  - **Differences exist in the epigenetic regulation of p21 between rats and humans**
  - **Increases in p21 expression in the kidney is protective against ischemia reperfusion and chemotherapeutic toxicity**
    - **Epigenetic changes in p21 may be protective and not part of the mechanism of toxicity**

Key Takeaways-Epigenetics and DBPs

- **Epigenetic changes in rats do not always translate to humans**
  - **At this moment, you can't use epigenetic changes in rats to assess the risk of bromate in humans**
- **An epigenetic change isn't always an adverse change**
  - **EPA Bromate PoD = 1.1 mg/kg-day Urothelial hyperplasia**
  - **Epigenetic change = 0.01 mg/L-3 day = p21 expression**
  - **My big worry is if someone uses this epigenetic marker *in vivo* as a PoD**

Cummings Research-Acknowledgements-People

Cummings Laboratory

- Nagendra Kolisetty, Ph.D.
- Natalie Scholpa, Ph.D.
- Xiaoling Zhang, Ph.D.
- S. Muralidharaha
- Leah J. Costyn
- Ramya Kolli

Others

- Joseph A. Cutruvo
- Richard J Bull
- Don Delker (Utah)

FDA-NCTR

- Jeff Fisher, Ph.D.

Nevada Las Vegas

- Oscar Quinones
- Shayne Snyder

American Water Works Association