

Emerging Drinking Water Disinfection By-Products

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U.S. Environmental Protection Agency

Office of Research & Development

Drinking Water DBPs—What are the Issues?

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

Drinking Water DBPs: How are they formed?

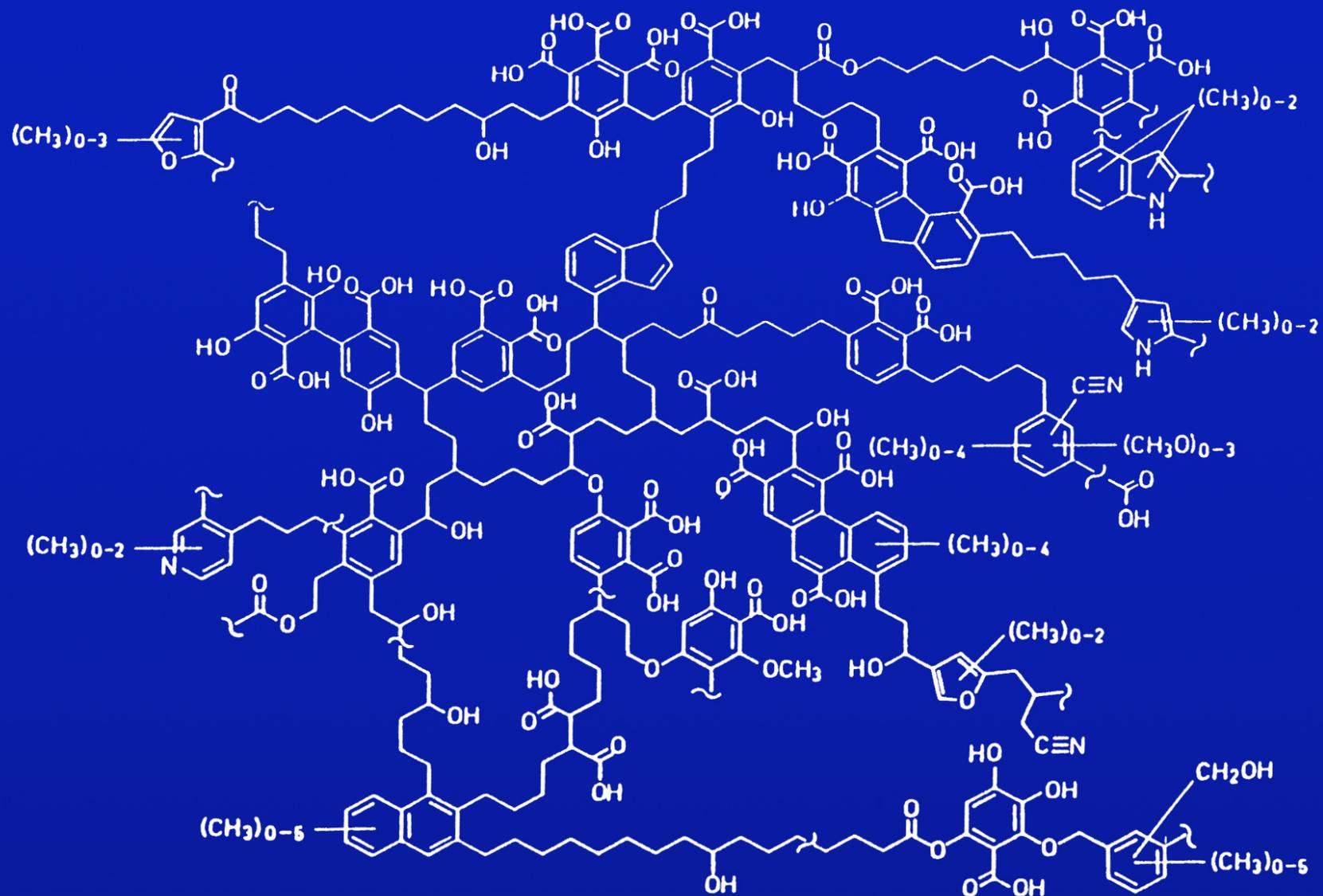


Fig. 12.7 Chemical network structure of humic acids according to Schulten and Schnitzer.⁷ Reproduced by permission of Springer-Verlag.

DBPs discovered in 1974

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1974 v. 23, Part 2 June

* Discovery of DBP

FORMATION OF HALOFORMS DURING CHLORINATION OF NATURAL WATERS

By
J. J. ROOK

Rotterdam Waterworks

Investigations have shown that haloforms are produced during chlorination of humic substances in natural waters. In view of possible physiological effects it is suggested that some caution might be needed in applying chlorination to such waters.

1. INTRODUCTION

Gas chromatographic headspace gas analysis, described earlier by the author¹, has been applied to different types of surface waters for routine quality control of water treatment at the Berenplaat plant. Treatment comprises storage, superchlorination, combined activated carbon addition and coagulation, filtration, cascade aeration and postchlorination.

This analytical method, which detects low polar volatiles, such as the lower alkanes, freons, chlorinated solvents and substituted benzenes and toluenes, has shown effective removal of such micropollutants during the 3 weeks' storage of the river water, the reduction varying from 40% in winter to over 90% in summer.

Interpretation of the results has been confused by the appearance of additional peaks in the chromatograms of chlorinated water. These have been identified as being due to the formation of various haloforms by chloro-bromination of naturally occurring humic substances.

Headspace gas chromatography of a given surface water produces a "fingerprint" of peaks on the chromatogram that does not usually change very much over long periods. Identification of the peaks requires the use of a mass spectrometer, chromatographic retention times alone not being sufficiently characteristic. Mass spectrometry has confirmed, at least for river Rhine water, that the variety of volatile micropollutants does not vary much from year to year, but there are seasonal changes.

Comparison of headspace fingerprints of water before and after breakpoint chlorination indicated that the volatile micropollutants passed this treatment step in diminished concentrations. A less reassuring, and initially puzzling, observation was the appearance of four new peaks, which were clearly produced by chlorination (Fig. 1). Fortunately the concentrations of the four new impurities in the water were significantly reduced in subsequent purification by adsorption on powdered activated carbon. Their concentrations were further reduced by volatilization while flowing through open channels, by filtration and finally by cascade aeration, the overall removal amounting to 60-70%.

This investigation sought to identify these by-products of chlorination and the cause of their formation. This meant tracing their origin in either impurities in chlorine or in the chlorination of precursor substances present in the water.

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Jon Rook

oxidation or removal of NH₃-N is only partial rather than total.
 For treatment it may be advantageous to follow breakpoint chlorination with dechlorination by carbon adsorption or by a sulfur compound. Carbon adsorbs any chloroorganics that may be produced during the breakpoint procedure. In the case of wastewater, this may solve any problems of toxicity to fish. In the case of water treatment, it may improve taste and characteristics of the treated water.

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DECEMBER 1974 ml-66 (12)

The Occurrence of Organohalides in Chlorinated Drinking Waters

T. A. Bellar, J. J. Lichtenberg, and R. C. Kroner

The national media have reported that the chlorination of water during treatment is responsible for the formation of potentially harmful chlorinated organic materials—notably chloroform—in the nation's water supplies. The following report by three research scientists from the Natl. Envir. Res. Ctr. of EPA describes that agency's research concerning these organohalides. The report concludes that the number of organohalides formed during the chlorination process does not constitute any immediate threat to the public health or welfare, but that more research into possible long-term effects is warranted.

A contribution submitted to the JOURNAL on Nov. 7, 1974, by T. A. Bellar, J. J. Lichtenberg, and R. C. Kroner (Active Member, AWWA), all of the Natl. Envir. Res. Ctr., EPA, Cincinnati, Ohio.

In recent years there has been great speculation and concern about the effect of chlorination upon organic materials contained in natural waters and wastewaters. Considering the widespread use of chlorine in water- and sewage-treatment processes, household and commercial laundering, paper-pulp bleaching, and related processes, it is easy to postulate the possible inadvertent, widespread production of chlorinated organic materials. There are an infinite number of organic materials commonly contained in natural waters and wastewaters that may react with free chlorine. For the most part mechanisms for these reactions have not been studied because rapid and precise analytical methods capable of monitoring the reaction products have not been available. Klopffer and Fairless,¹ Novak et al.,² Foloux,³ Gorb,⁴ and others⁵ have reported the presence of organohalides in finished water, but because of the nature of the studies made and the analytical methods used, no conclusions could be drawn as to the source of these compounds.

Recently the Methods Dev. and Qual. Assurance Lab. of the Natl. Envir. Res. Ctr. in Cincinnati has developed and reported on a procedure for isolating and measuring nanogram quantities of volatile and semi-volatile organic materials in wastewaters (see "Determining Volatile Organics at Microgram-Per-Liter Levels by Gas Chromatography" on

T. A. BELLAR ET AL. 709

Tom Bellar

>600 DBPs Identified

Halogenated DBPs

- Halomethanes
- Haloacids
- Haloaldehydes
- Haloketones
- Halonitriles
- Haloamides
- Halonitromethanes
- Halofuranones (e.g., MX)
- Oxyhalides (e.g., bromate)
- Many others

Non-halogenated DBPs

- Nitrosamines
- Aldehydes
- Ketones
- Carboxylic acids
- Others

>600 DBPs Identified

Halogenated DBPs

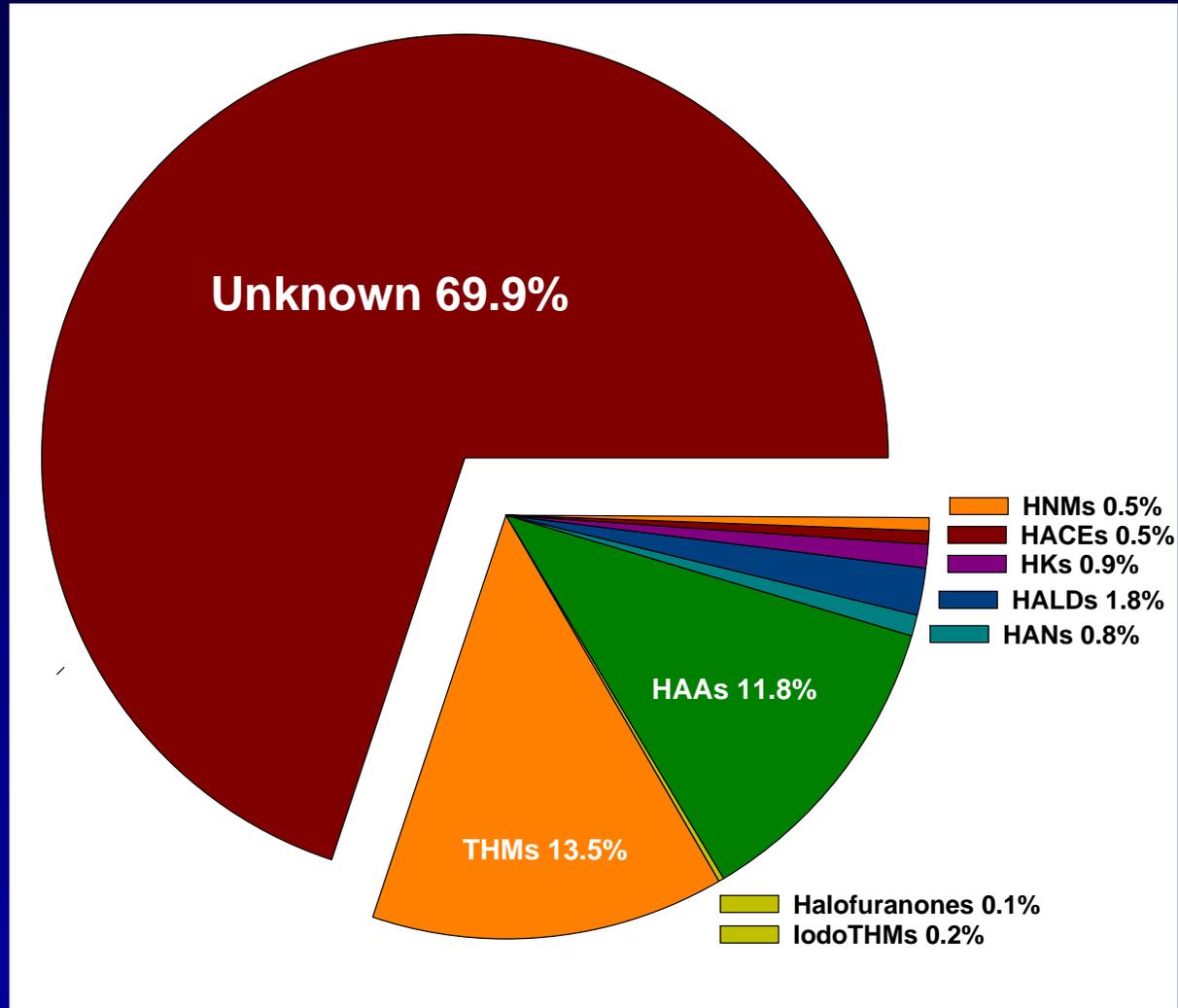
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- Many others

Non-halogenated DBPs

- Nitrosamines
- Aldehydes
- Ketones
- Carboxylic acids
- Others

N-DBPs

But, more than 50% still not known....



Nationwide Occurrence Study, Krasner et al., *Environ. Sci. Technol.* **2006**, *40*, 7175-7185.

~50% of TOX >1000 Da: Khiari, et al., Proc. 1996 AWWA Water Quality Technology Conference

Only 11 DBPs Regulated in U.S.

DBP	MCL ($\mu\text{g/L}$)
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!

Only 11 DBPs Regulated in U.S.

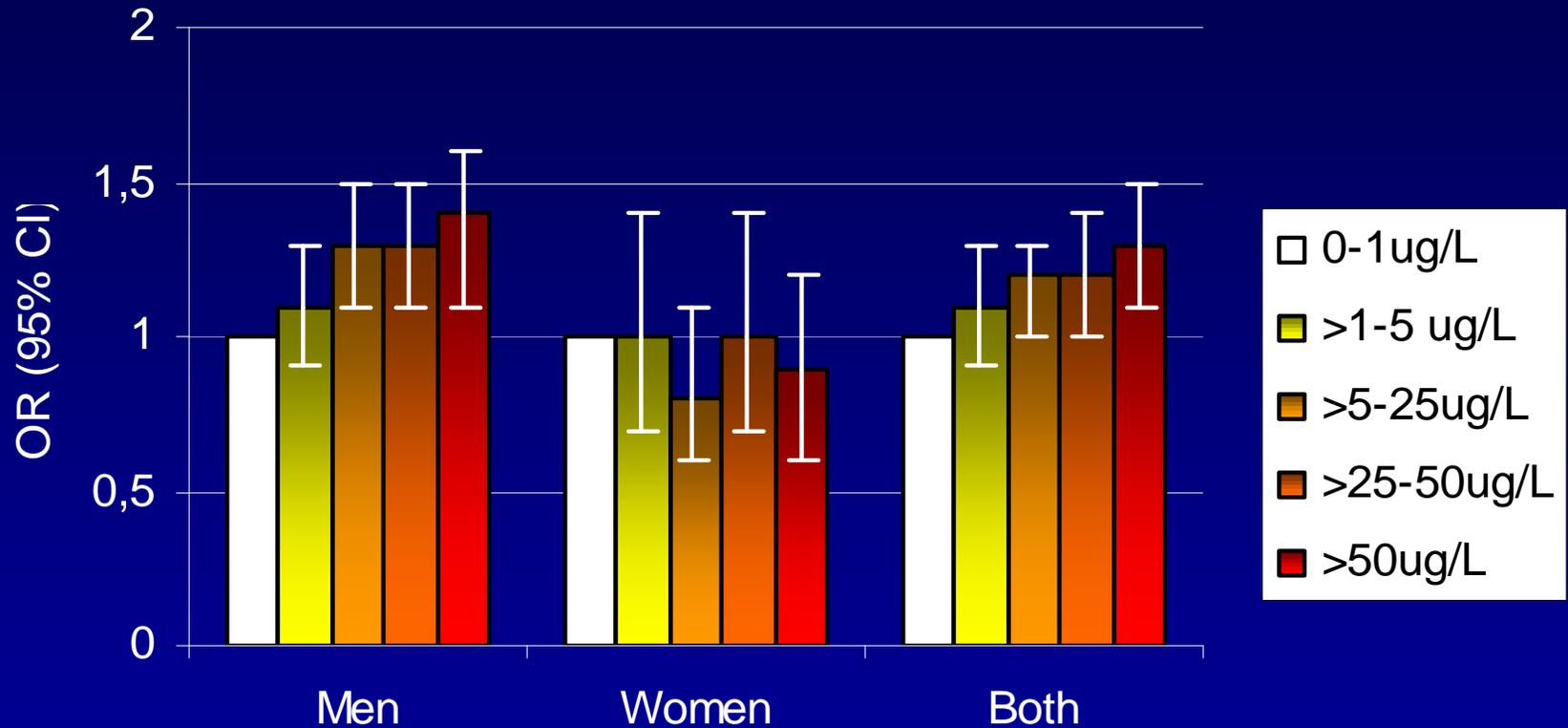
DBP	MCL ($\mu\text{g/L}$)
Total THMs	80
5 Haloacetic acids	60
Bromate	10
Chlorite	1000

One regulated DBP never tested for cancer

- Two unregulated DBPs are carcinogens

- Many unregulated DBPs more genotoxic than regulated ones

Bladder cancer and drinking water: Pooled analysis



OR adjusted by (sex), study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption and total fluid intake

Exposure routes

Inhalation

(shower, swimming pool, etc.)

Volatile DBP
e.g. THMs

Ingestion

(water, coffee, tea, water-based food and beverages)

All disinfection by-products

Dermal absorption

(swimming pool, bath, etc.)

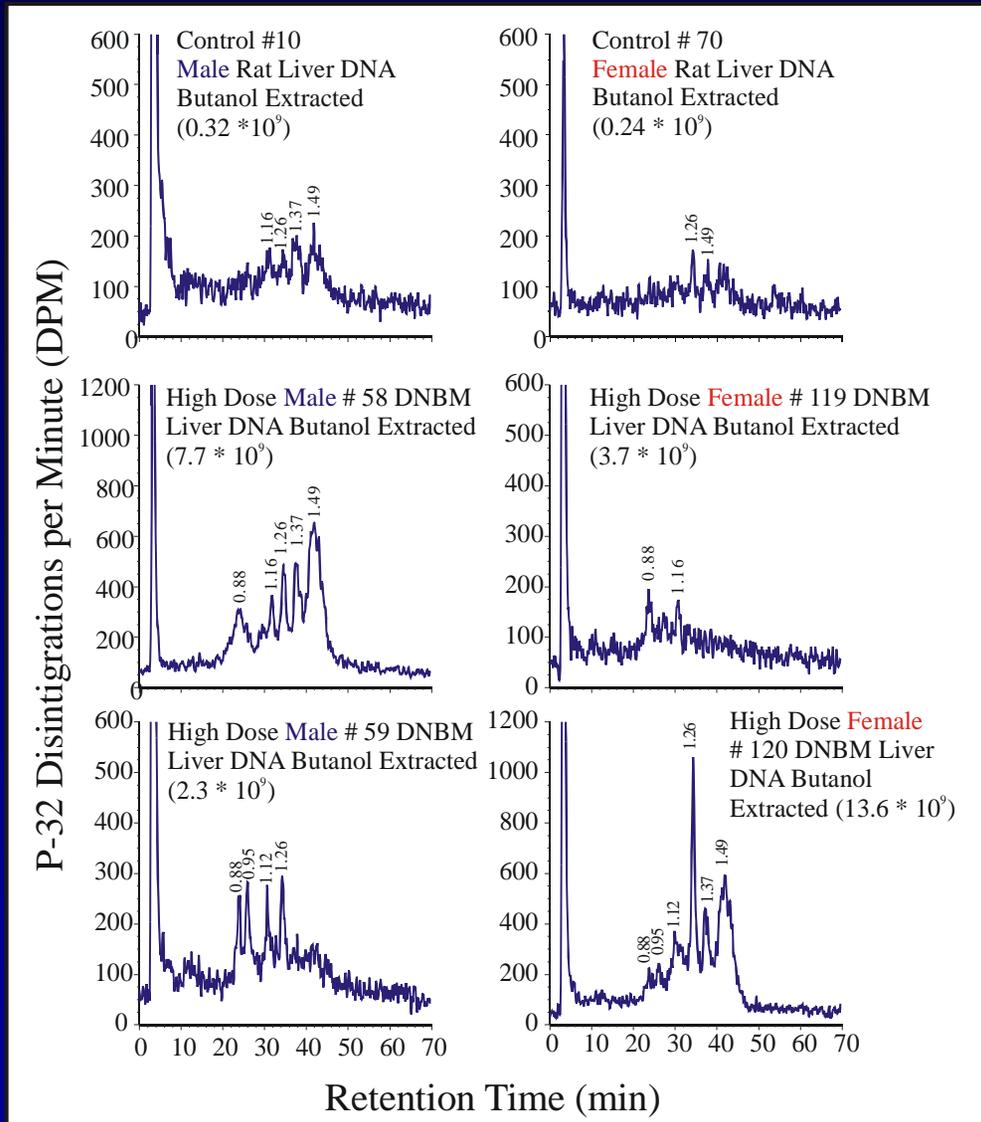
Permeable DBPs
e.g. THMs, haloketones, ...

**TOTAL
INTERNAL DOSE**

Unlike other contaminants that may or may not be present in drinking water...

**DBPs
are ubiquitous**

Dibromonitromethane—DNA Adducts



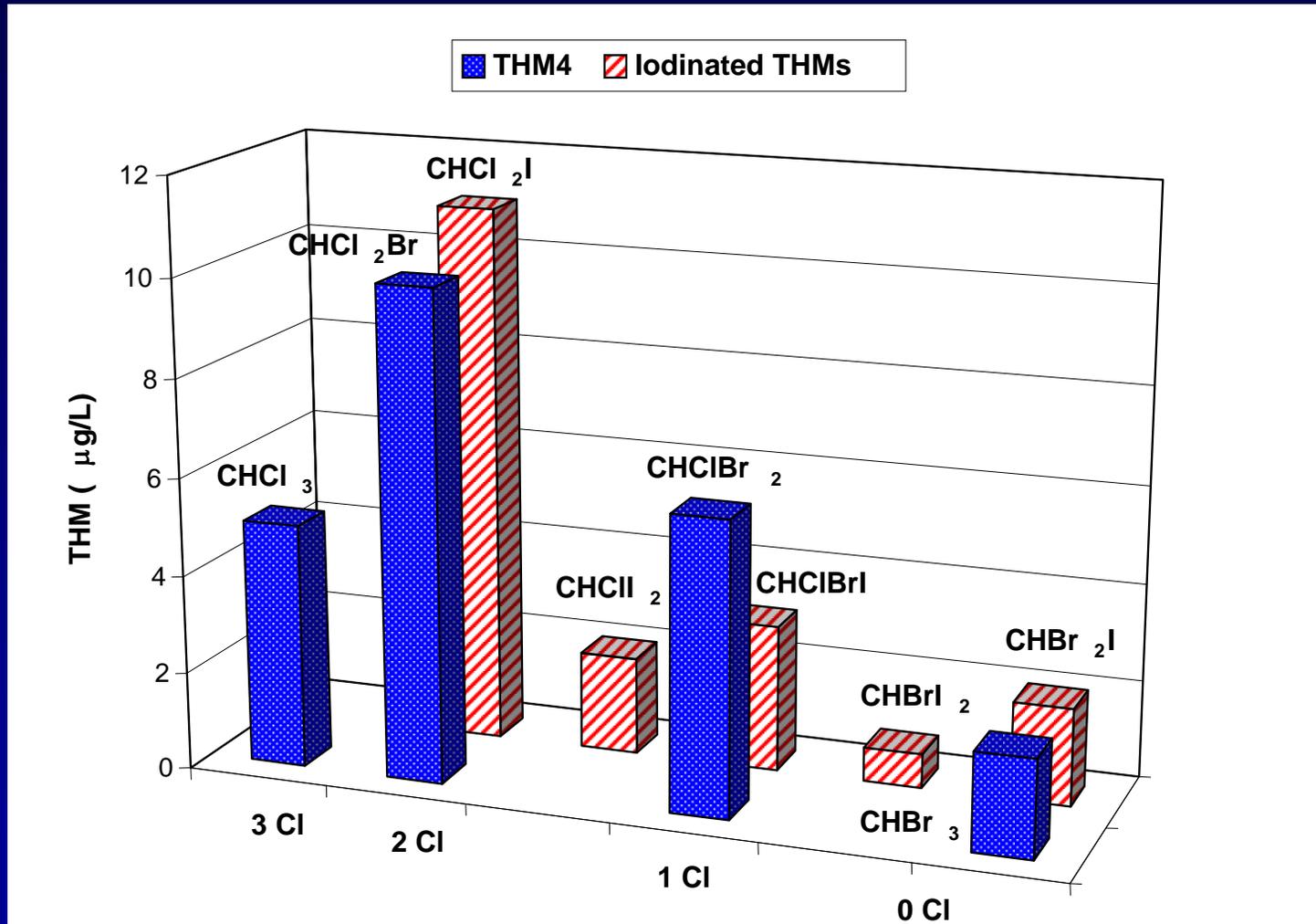
DBNM produces DNA adducts in the livers of rats after only 30 days of exposure

(in vivo, male and female rats)

Tony also now seeing effects in normal human colon cells

Data courtesy of Tony DeAngelo & Leon King, U.S. EPA, NHEERL, RTP, NC

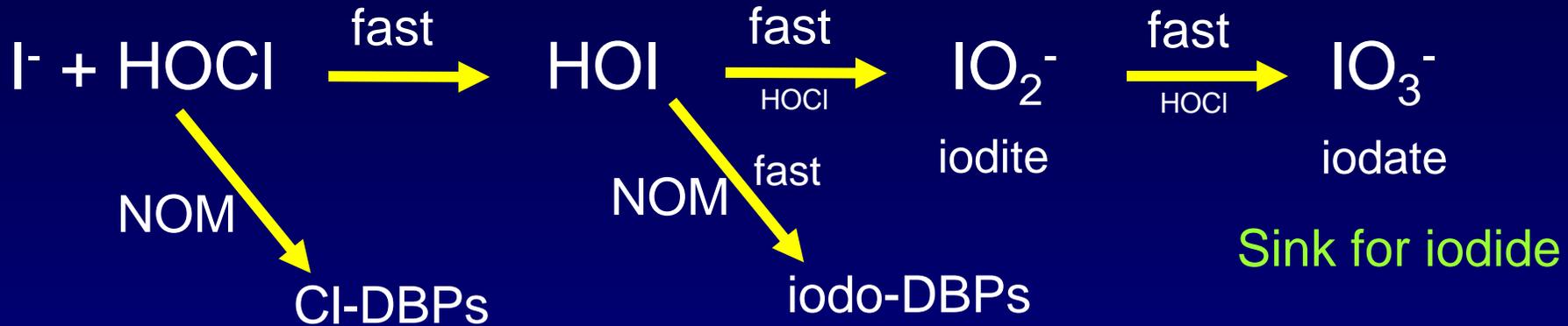
Iodo-THMs



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

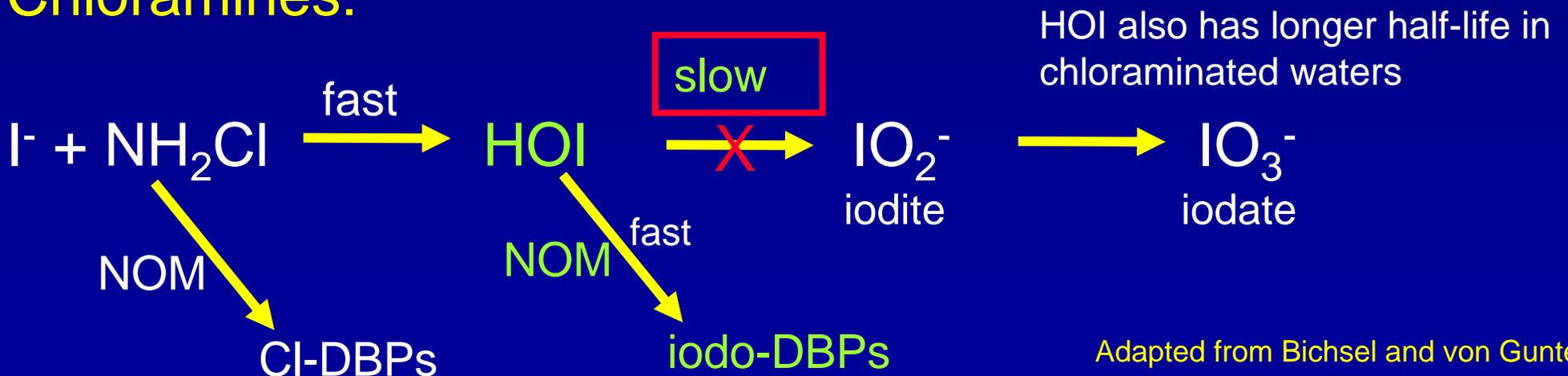
Iodo-DBPs Maximized with Chloramines

Chlorine:



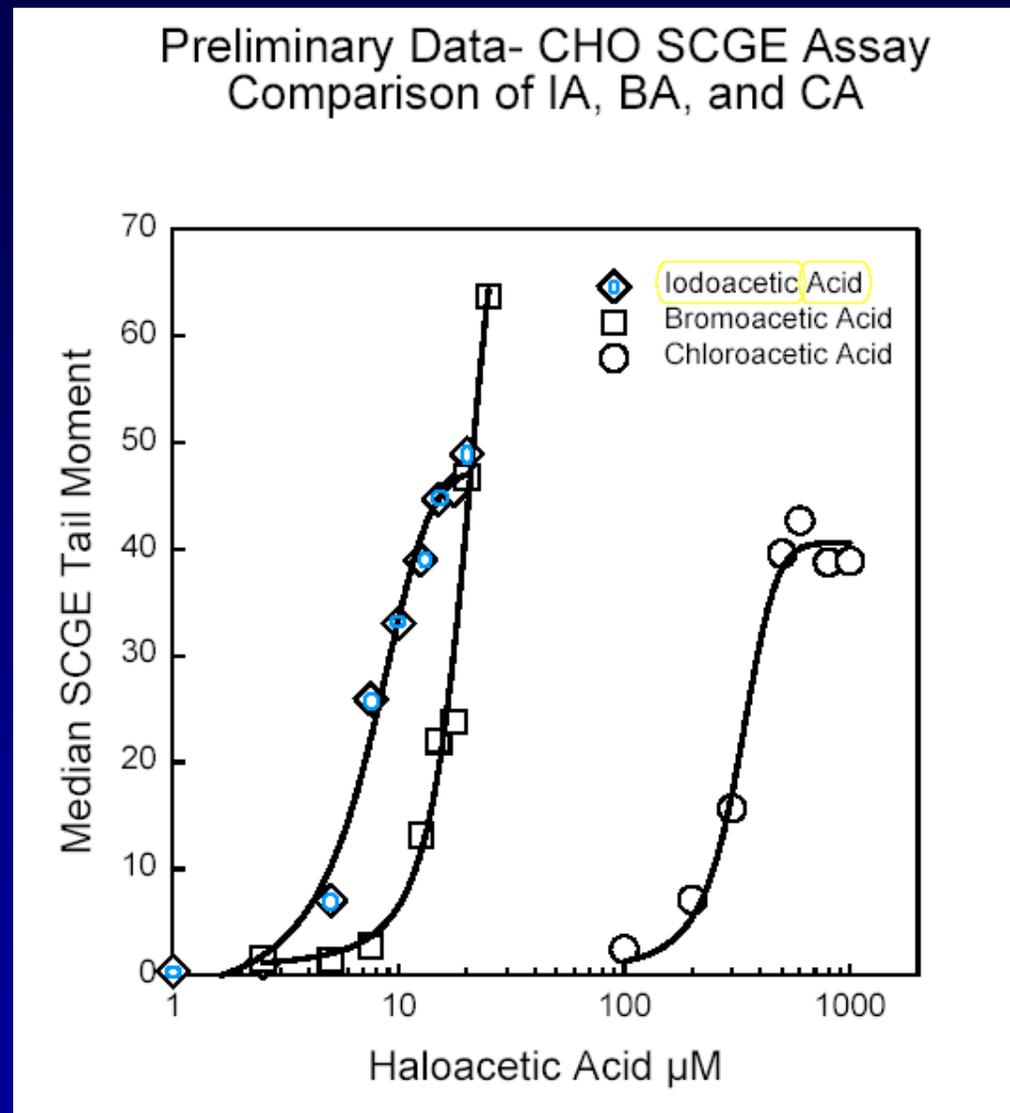
$HOCl$ also competes for rxn with NOM, so much lower iodo-DBPs with chlorine

Chloramines:



Adapted from Bichsel and von Gunten, 1999 and 2000

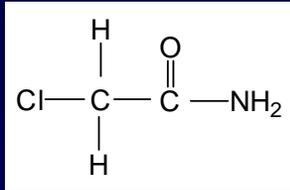
Genotoxicity of Iodoacetic acid



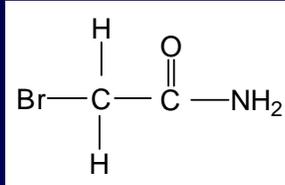
Plewa et al., *Environ. Sci. Technol.* 2004

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

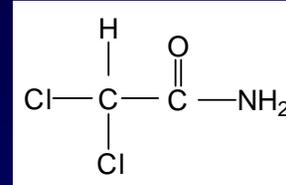
Haloamides



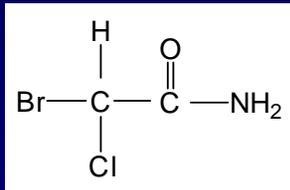
Chloroacetamide



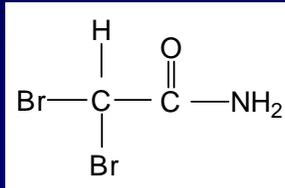
Bromoacetamide



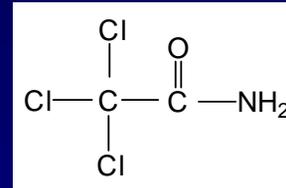
Dichloroacetamide



Bromochloroacetamide

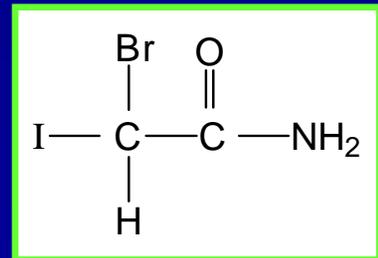


Dibromoacetamide



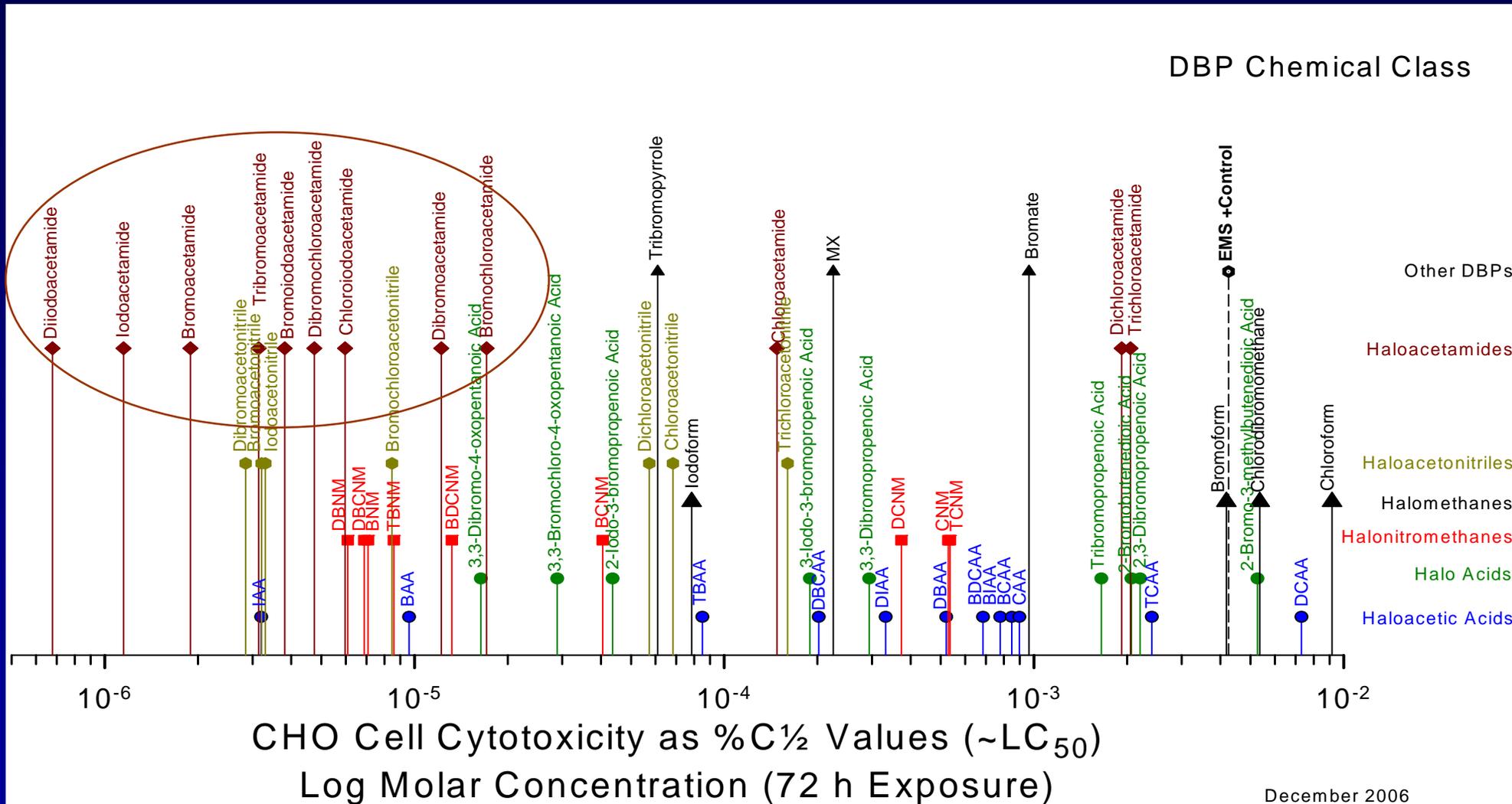
Trichloroacetamide

- New class of DBP recently identified
- Nationwide DBP Occurrence Study: up to 14 ug/L; NH_2Cl may increase their formation
- Highly genotoxic, cytotoxic
- New iodoamide DBP: Bromoiodoacetamide
 - Found in drinking water from 6 states



Plewa et al., *Environ. Sci. Technol.* 2008, 42, 955-961.

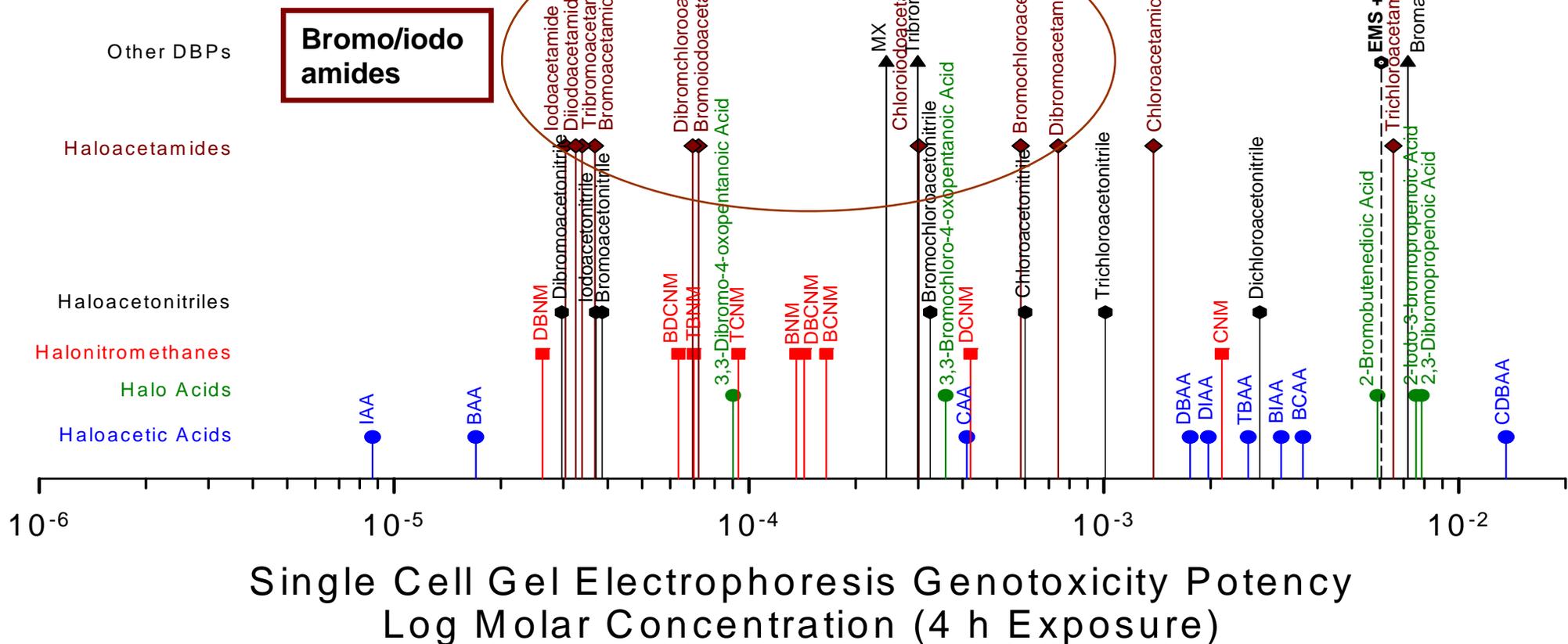
Haloamides--Cytotoxicity



Data courtesy of Michael Plewa, University of Illinois

Haloamides--Genotoxicity

DBP Chemical Class



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

But, all of this toxicity testing is for separate, individual DBPs...

DBPs
are really present as MIXTURES



>300 DBPs probably
present in glass of water



Four Lab Study

Integrated Disinfection By-products Mixtures Research: Toxicological and Chemical Evaluation of Alternative Disinfection Treatment Scenarios

A collaborative effort between:

NHEERL (National Health and Environmental Effects Research Laboratory), RTP

NERL (National Exposure Research Laboratory), Athens

NRML (National Risk Management Research Laboratory), Cincinnati

NCEA (National Center for Environmental Assessment), Cincinnati

Purpose:

To address concerns related to potential health effects from exposure to DBPs that cannot be addressed directly from toxicological studies of individual DBPs or simple DBP mixtures



In Vitro and *In Vivo* Toxicological Assays

In vitro:

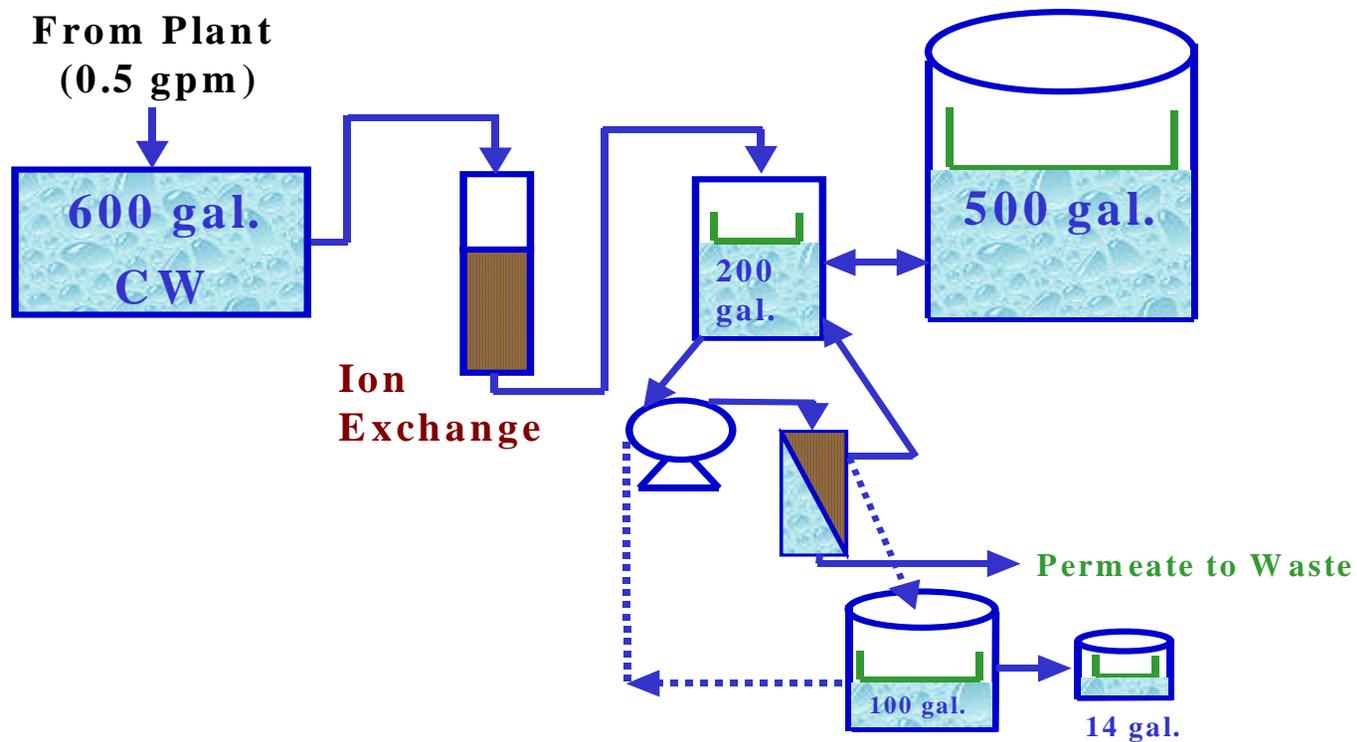
- Reproductive/developmental
- Mutagenicity
- Carcinogenicity
- Neurotoxicity
- Metabolism

In vivo:

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

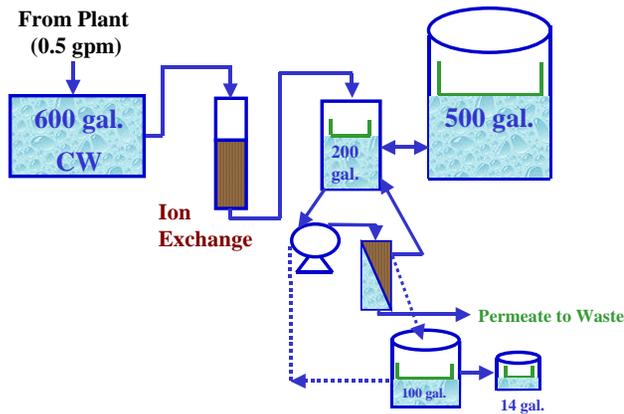


RO Concentration of DBPs



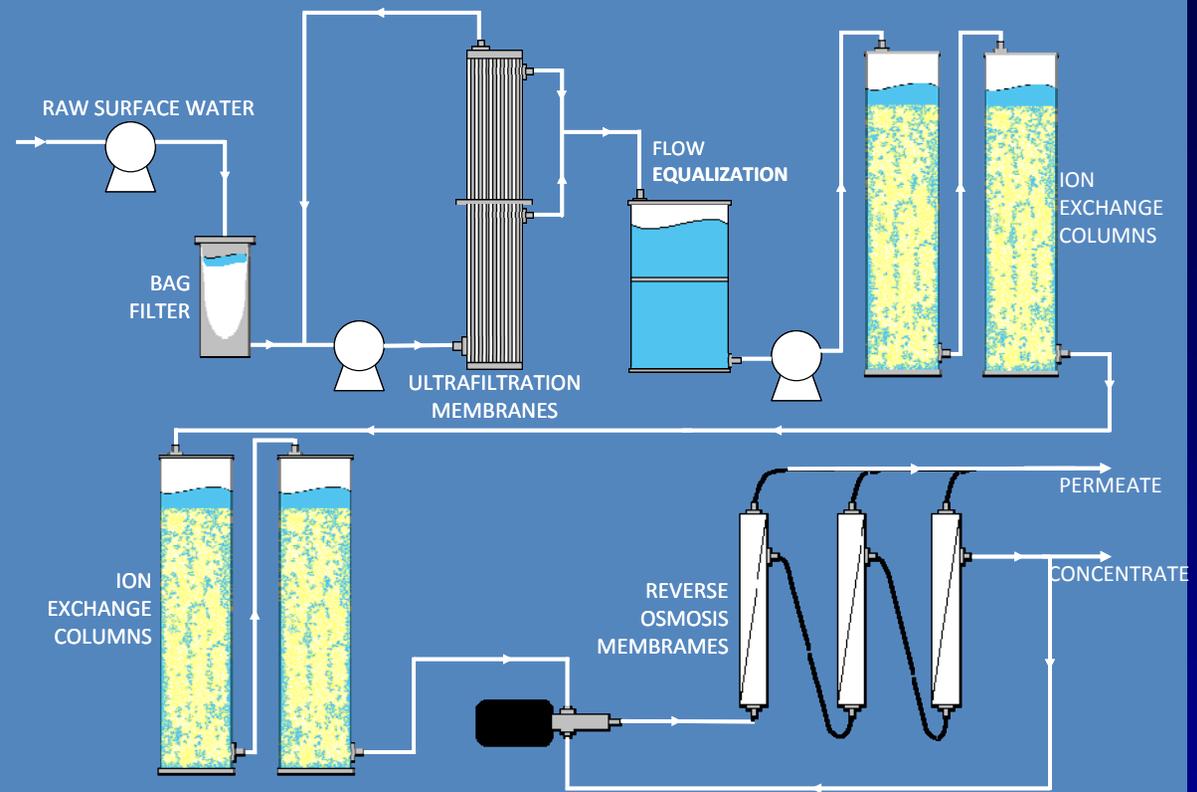
Water is concentrated by RO to maintain the water matrix for rats to be able to drink (can't give organic solvents to rats!)

RO Concentration of DBPs



1st Phase (1999-2001):
 Cl_2 & O_3 ; treated water first
 Concentrated after

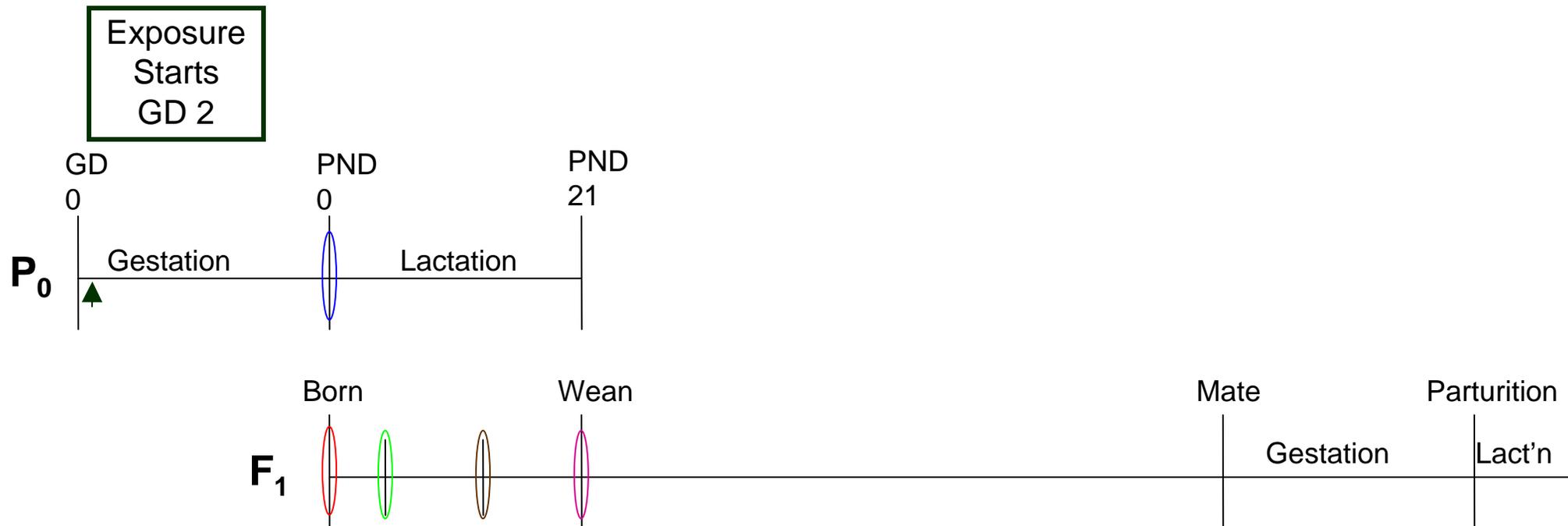
Full Study Concentration



Full Study (2006-2008):
 Concentrated NOM first
 Treated with Cl_2 after

1st Phase of study published: Richardson et al., *J. Toxicol. Environ. Health* 2008, 71, 1165-1186.
 Other 4-Lab papers also in this special issue

General Timeline for Animal Experiments



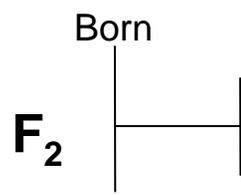
Gestation Length
Parturition

Litter Exams
Postnatal Day 0
Viability
Pup weight
Anogenital distance
• 12 litters/group

Litter Exams
Postnatal Day 6
Viability
Pup weight
Reduce litter size to 8
(4 males, 4 females)

Litter Exams
Day 13
Viability
Eye opening
Nipple retention

Litter Exams
Day 21
Viability
Pup weight
Wean
Assign fate



Results

- Good mix of Cl/Br DBPs produced
- Most DBPs fairly consistently produced among chlorination events
- Most DBPs are stable over time on the rats' cages

Toxicity Results

No effects for:

- Gestation length
- Prenatal viability
- Postnatal viability
- Pup Weight
- Eye opening
- Nipple retention
- Organ weights

Effects for:

- Delayed puberty in F1 females (<1 day)
- Delayed puberty also seen with regulated DBP mixtures
- Subtle puberty effect (at 136x) that appears consistent with dose-response curve of regulated DBPs at 500x, 1000x, 2000x
- Increased anogenital distance (AGD) in F1 males
- Males: Sperm counts down 50%
- Total litter loss (postnatal) in one F2 litter
- Vaginal prolapse in one P0 dam
- Dental malocclusion in one F1 litter
- Small neurotoxicity effects (motor activity, grip strength)
- Inhibited differentiation of human placental trophoblast cells and hCG secretion (both with chlorinated concentrate and regulated DBPs)
- Small increase in mutagenicity in chlorinated concentrate
- Mammary tumors (regulated DBP mixture)

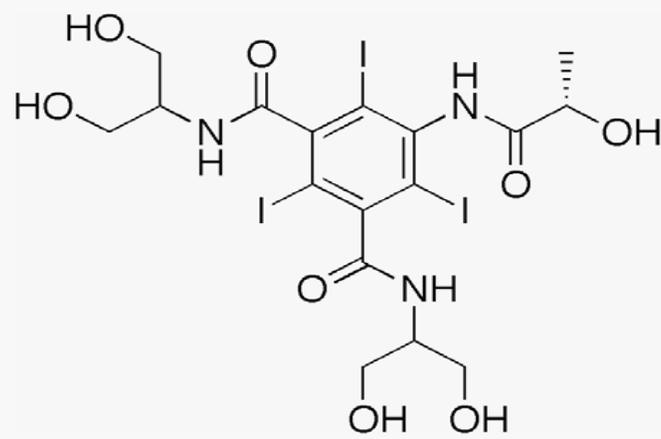


Conclusions

- A thorough examination of reproductive/developmental and other endpoints was predominantly negative
- Some small, subtle effects for chlorinated water concentrate (136x concentration factor may be “on the edge” of ability to see effects)
- Concentration offered by RO a bonus for detecting DBPs present at very low levels (e.g., MX, which is present in drinking water at ng/L levels)
- Combination of comprehensive, qualitative identification work and quantification of 75 DBPs allowed comprehensive assessment of DBPs present in water
- Most DBPs stable on rats’ cages and chlorination events were reproducible

Want to determine the statistical power in this study for detection of the subtle effects noted and do a follow-up study that includes Chlorine vs. Chloramines

Formation of iodo-DBPs from X-ray contrast media



Iopamidol

+ NOM



Iodo-DBPs



Richardson, Duirk, Lindell, Cornelison, Ternes, presented at Micropol Conference, June 2009

Iodo-DBP Occurrence Study

	Iodide ($\mu\text{g/L}$)	Sum iodo-acids ($\mu\text{g/L}$)	Sum iodo-THMs ($\mu\text{g/L}$)
Plant 2	1.0	0.37	4.9
Plant 4	ND	0.10	1.2
Plant 11	1.5	0.21	2.3
Plant 15	ND	0.17	2.4

Detection limit = $0.13 \mu\text{g/L}$

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

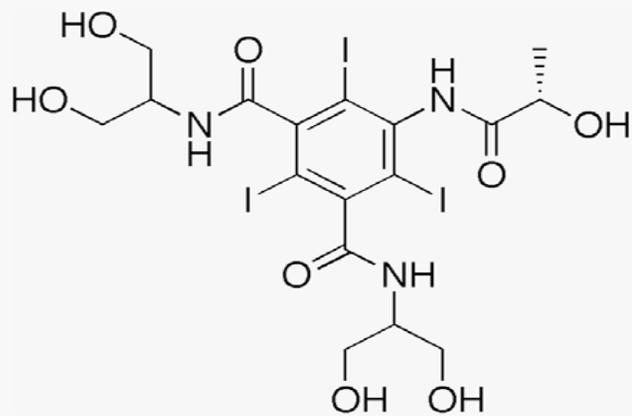
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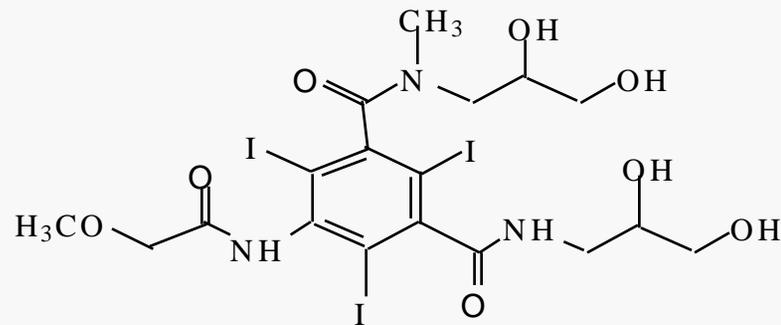
Detection limit = $0.13 \mu\text{g/L}$

What about other sources of iodine?

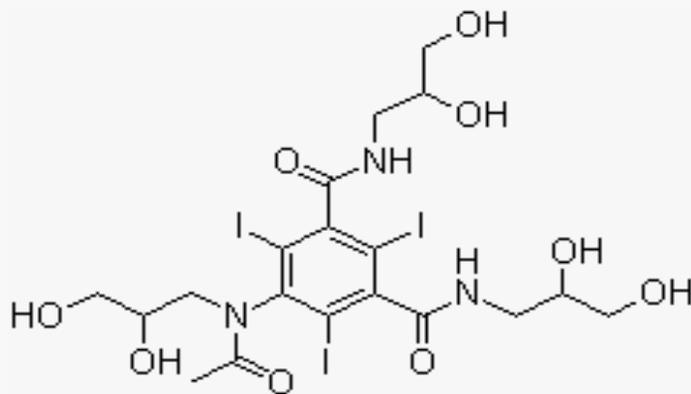
Iodinated X-ray Contrast Media (ICM)



Iopamidol

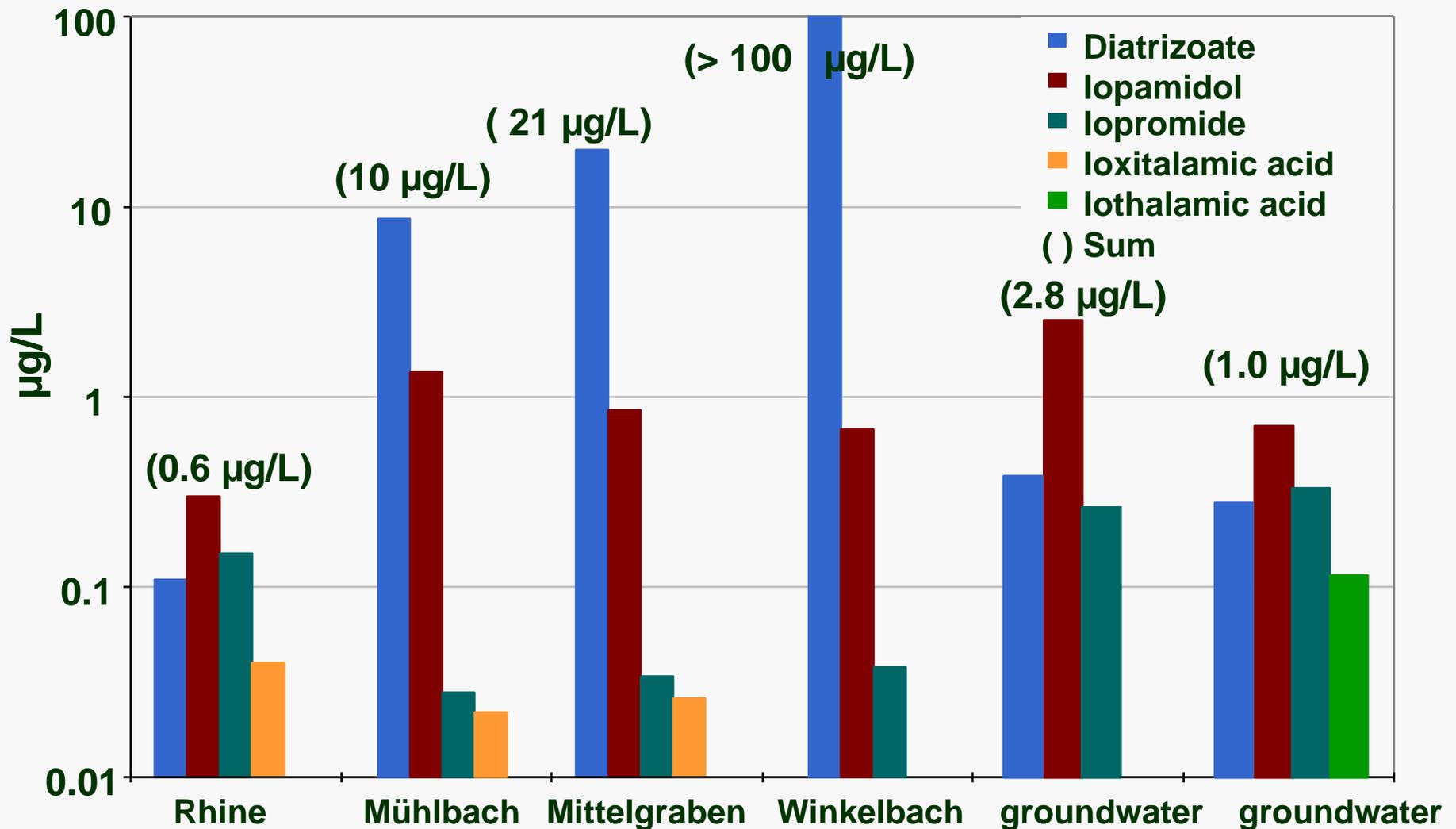


Iopromide



Iohexol

ICM concentrations: rivers, creeks and ground water



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

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

	Iopamidol	Iomeprol	Iopromide	Iohexol	Diatrizoate
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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

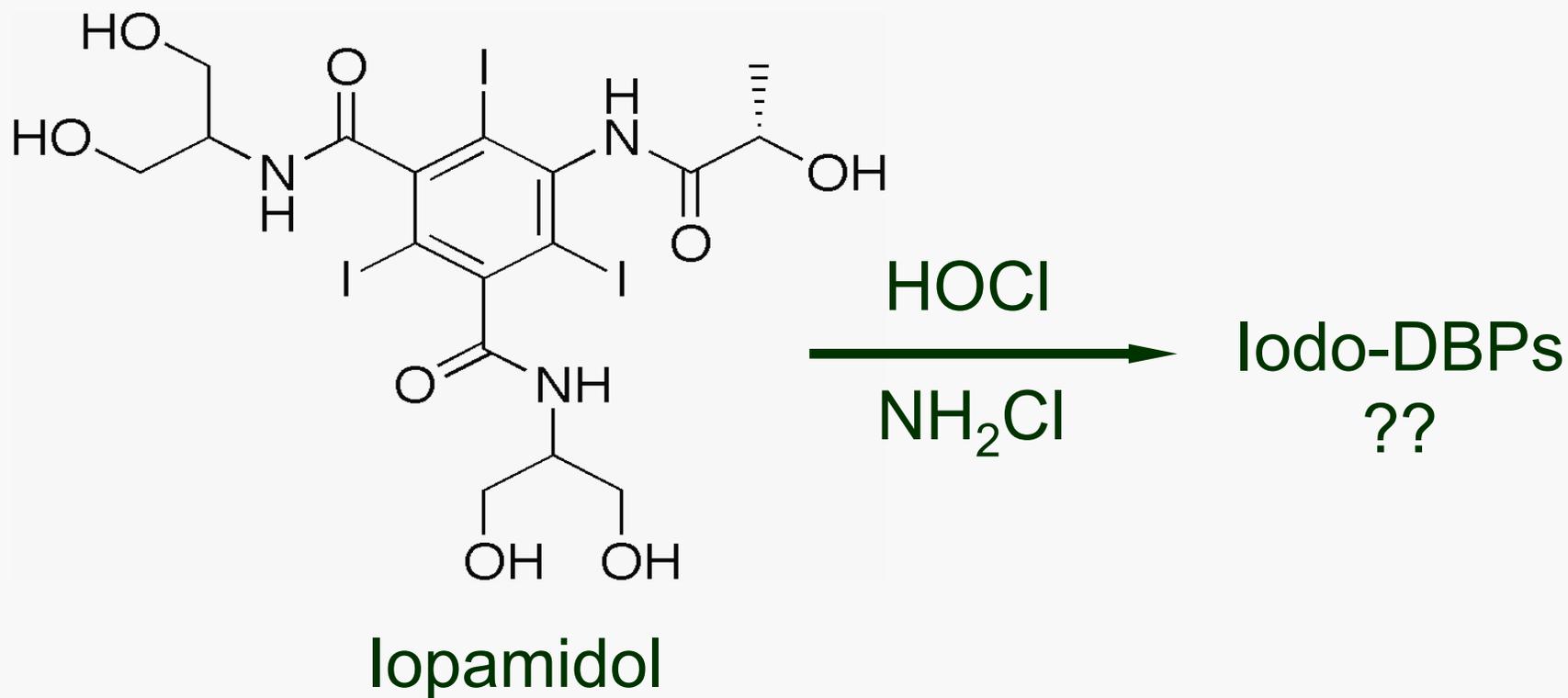
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Plant 11	100	ND	ND	85	ND
Plant 12	280	ND	ND	120	ND
Plant 13	ND	ND	ND	ND	ND
Plant 15	2700	ND	25	ND	ND
Plant 17	ND	ND	ND	ND	ND
Plant 19	ND	ND	ND	ND	ND

Courtesy of Thomas Ternes, Federal Institute of Hydrology, Germany
ICM measured using LC/ESI-MS/MS; DLs = 5-20 ng/L

Do iodinated X-ray contrast media form iodo-DBPs?



Controlled Laboratory Reactions

Experiments

- React ICM with HOCl, NH₂Cl (with and without NOM)
- 3 pHs
- Follow formation of iodo-DBPs
- Identify reaction products and intermediates
- Measure genotoxicity

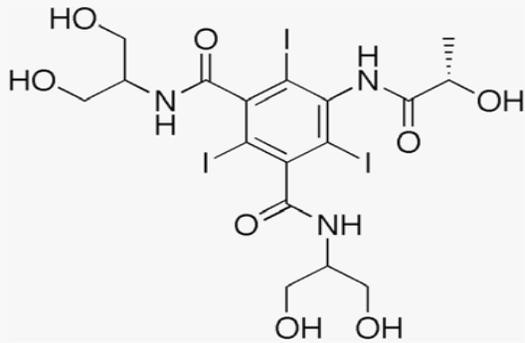
Methods

- Iodo-THMs: GC/EI-MS
- Iodo-Acids: GC/NCI-MS (with derivatization)
- Iopamidol (and other ICM): LC, LC/MS/MS
- Larger MW products and intermediates: LC/MS/MS
- Genotoxicity: Chinese hamster ovary cells, single cell gel electrophoresis



Cristal and Steve

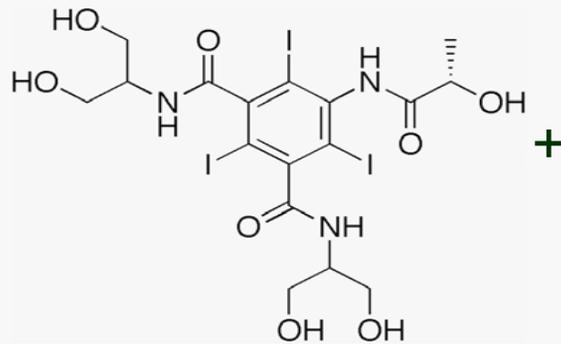
Results



lopamidol

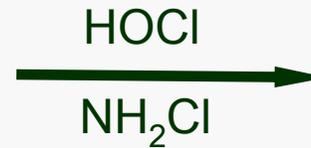


Iodo-DBPs



lopamidol

+ NOM

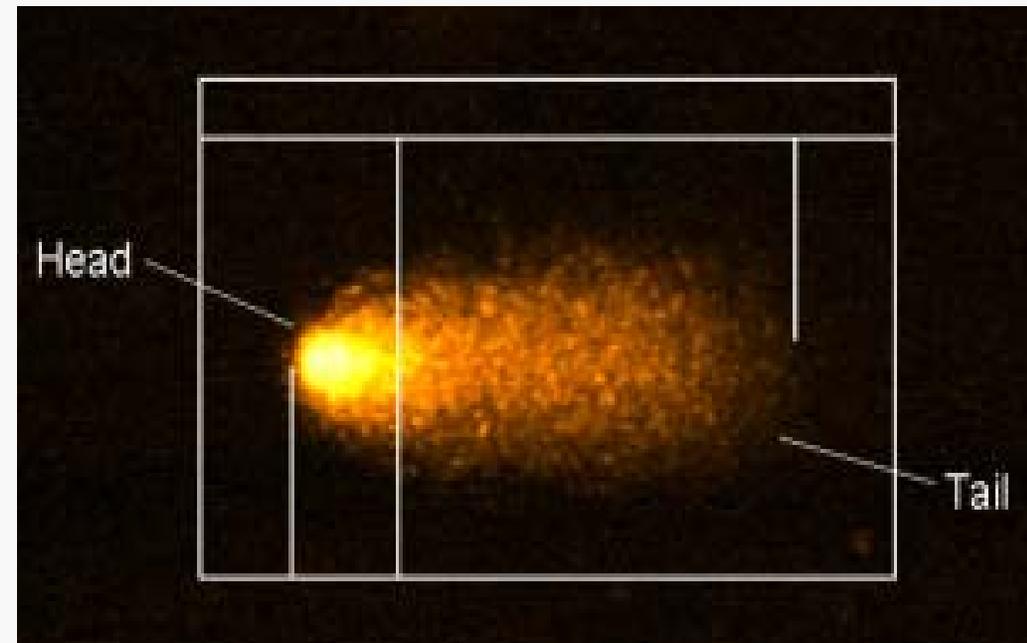
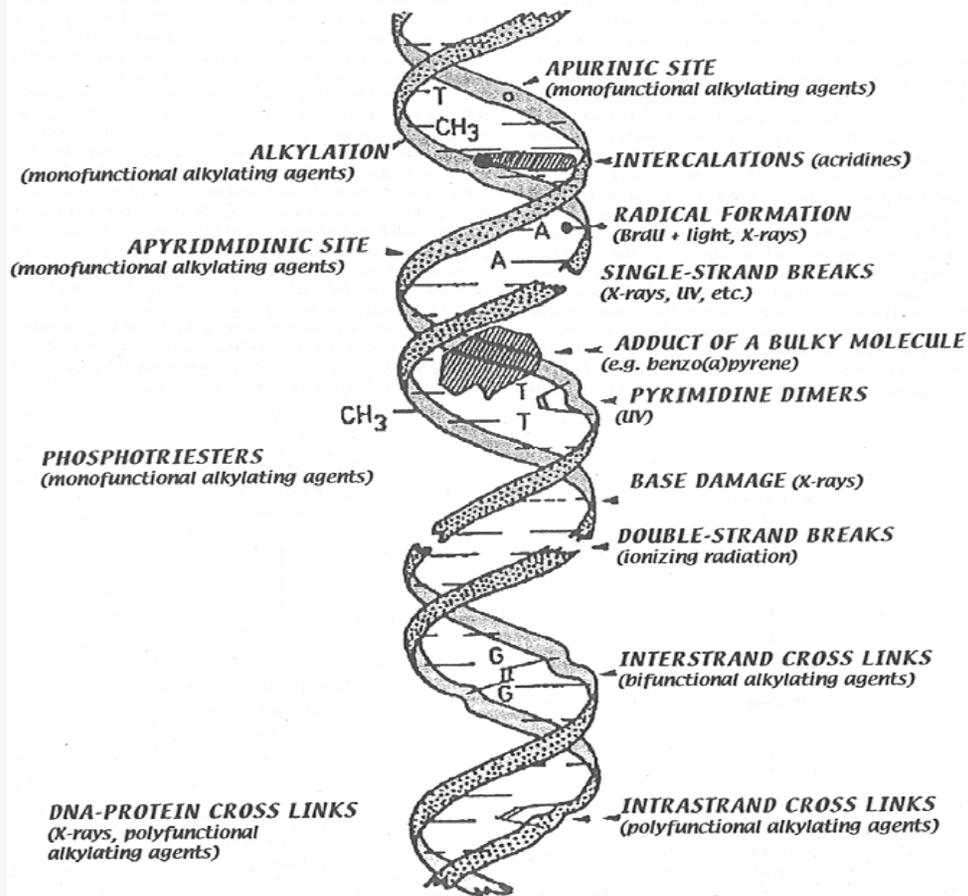


Iodo-DBPs



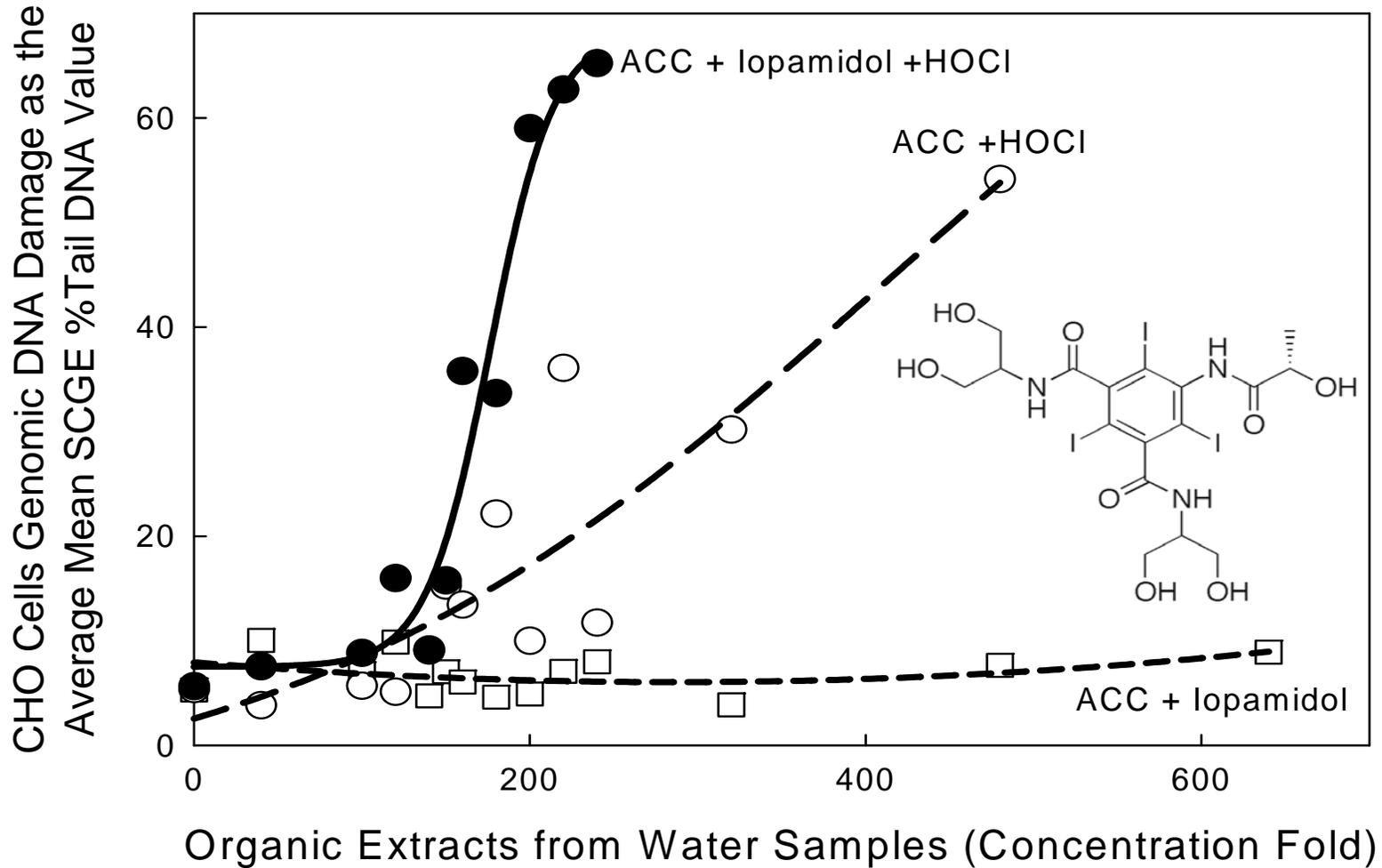
Iodo-THMs
&
Iodo-Acids

Genotoxicity: Comet Assay



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.

Genotoxicity of Chlorinated Waters Containing Iopamidol



Roadmap—Where do we go from here?

- Human health effects not solved yet—need more toxicity studies
- Studies on route of exposure
Have we been looking at the wrong route of exposure?
- DBPs are present as complex mixtures—need toxicity studies addressing this
- What is in the unidentified fraction—anything of concern?
- What about ‘pollutant’ DBPs?
- What about DBPs from alternative disinfectants—do we know everything we need to know before plants switch?
- Chloramination? UV disinfection? Membrane disinfection?
- What about other respiratory/skin effects reported for chloraminated water? Need showering and dermal exposure studies

Serious skin rash issues....



“Before”

Showering with
chloraminated water



Showering with chlorinated
water at the YMCA in another
town

Acknowledgments



Michael Plewa



Jane Ellen Simmons



Tony DeAngelo



David DeMarini

A few fabulous toxicologists who have helped push this field forward....

Also, Mike Narotsky, Sid Hunter, Rex Pegram,

In closing...

**Ever wonder what happens when you have to
scale things up for toxicity testing?**

(Especially when working with Michael Plewa)

The Land of Extraordinarily Large Lab Equipment



Chris

Toxicity?
20 L → 1 mL



Steve



Cristal