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Review of 30 Years of Research on
the Occurrence, Genotoxicity, and
Carcinogenicity of Disinfection By-
products in Drinking Water:
Roadmap for Research

Outline of Water Topics

- Health Effects of Chlorinated Drinking Water
- Regulation of Trihalomethanes and Haloacids to Reduce Adverse Health Effects
- Alternative Disinfection Methods & New Disinfection By-products
- Review of 30 years of DBP research on occurrence, genotoxicity, and carcinogenicity
- Dermal/inhalation exposure, genotype
- Research needs

Health Benefits of Treated Drinking Water

Reduction of Water-borne
Microbial Diseases

Adverse Health Effects Associated with Chlorinated Drinking Water

- **Cancer**
 - Colorectal
 - Bladder
- **Reproductive Effects**
 - Spontaneous abortion
 - Still birth
 - Low-birth weight
 - Neural tube defects

Regulation of Trihalomethanes in the U.S.

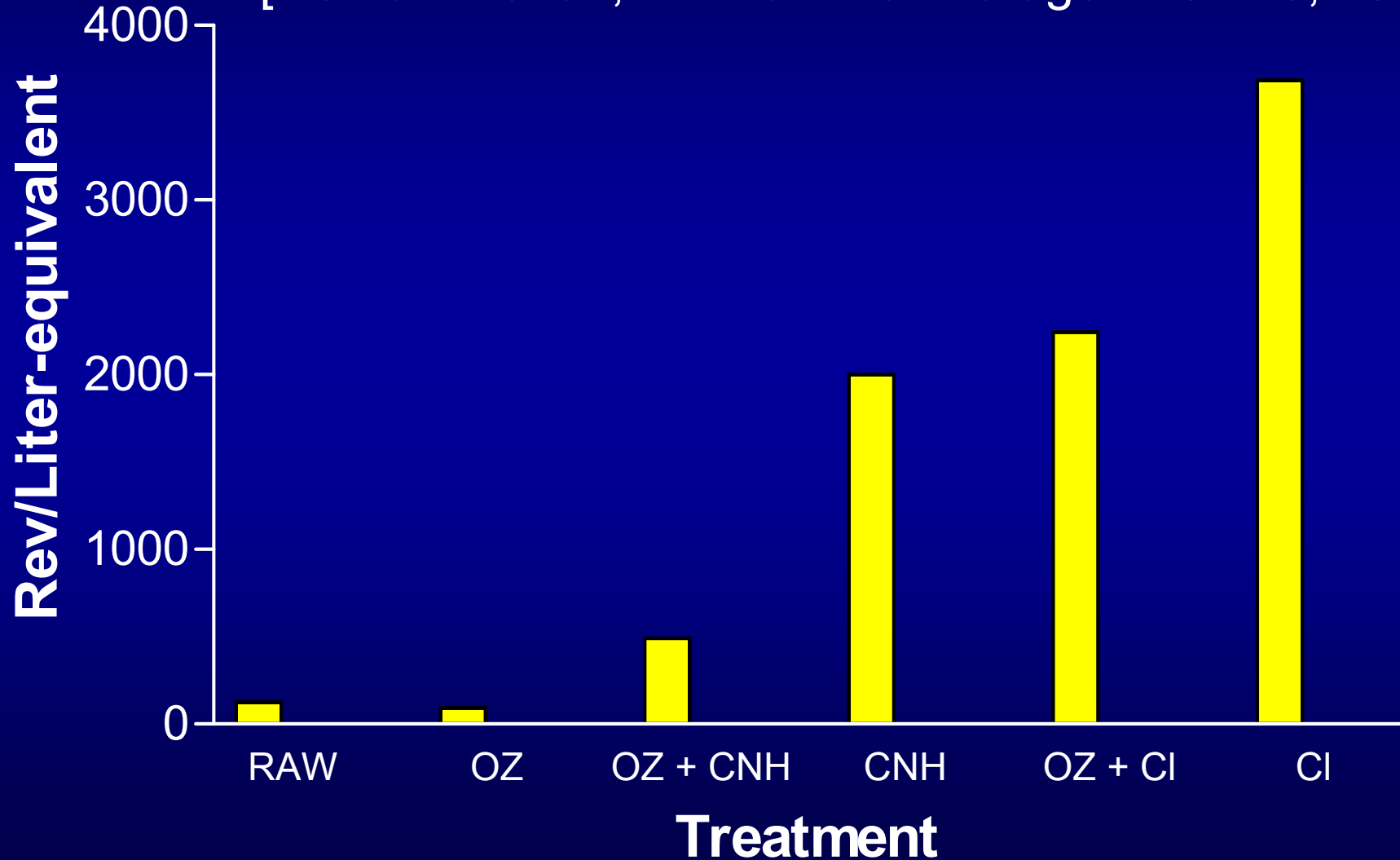
- Trihalomethanes (THMs) are regulated at 80 $\mu\text{g}/\text{L}$, and Stage-2 Rule changes monitoring of THMs to better control for risk of developmental/reproductive effects from short-term exposures.
- In the 1990s, alternative disinfection methods (ozonation or chloramination) were promoted to replace chlorination to reduce the levels of THMs.
- An EPA survey in 2004 showed that the new methods reduce levels of THMs, but they promote formation of new compounds, such as iodo-THMs, iodo-acids, and bromonitromethanes.

Mutagenicity and Mutation Spectra of Drinking Water Made by Different Methods

- XAD/ethyl acetate extracts of Mississippi River water near New Orleans, Louisiana, and the Ohio River.
- Water prepared by ozonation, chloramination, chlorination, ozonation + chloramination, or ozonation + chlorination
- Evaluated for ability to induce mutations in *Salmonella* strain TA100, which detects base substitution mutations. (Essentially all chlorinated drinking water is mutagenic in this strain, and nearly all of the 100 IARC-declared human carcinogens are mutagenic in this strain.)

Mutagenicity of Organic Extracts of Drinking Water in TA100 -S9

[DeMarini et al., Environ Mol Mutagen 26:270, 1995]



Occurrence, Genotoxicity, and
Carcinogenicity of Regulated and
Emerging Disinfection By-products in
Drinking Water: A Review and
Roadmap for Research

SD Richardson, MJ Plewa, ED Wagner,
R Schoeny, DM DeMarini

Mutation Research 636:178-242, 2007

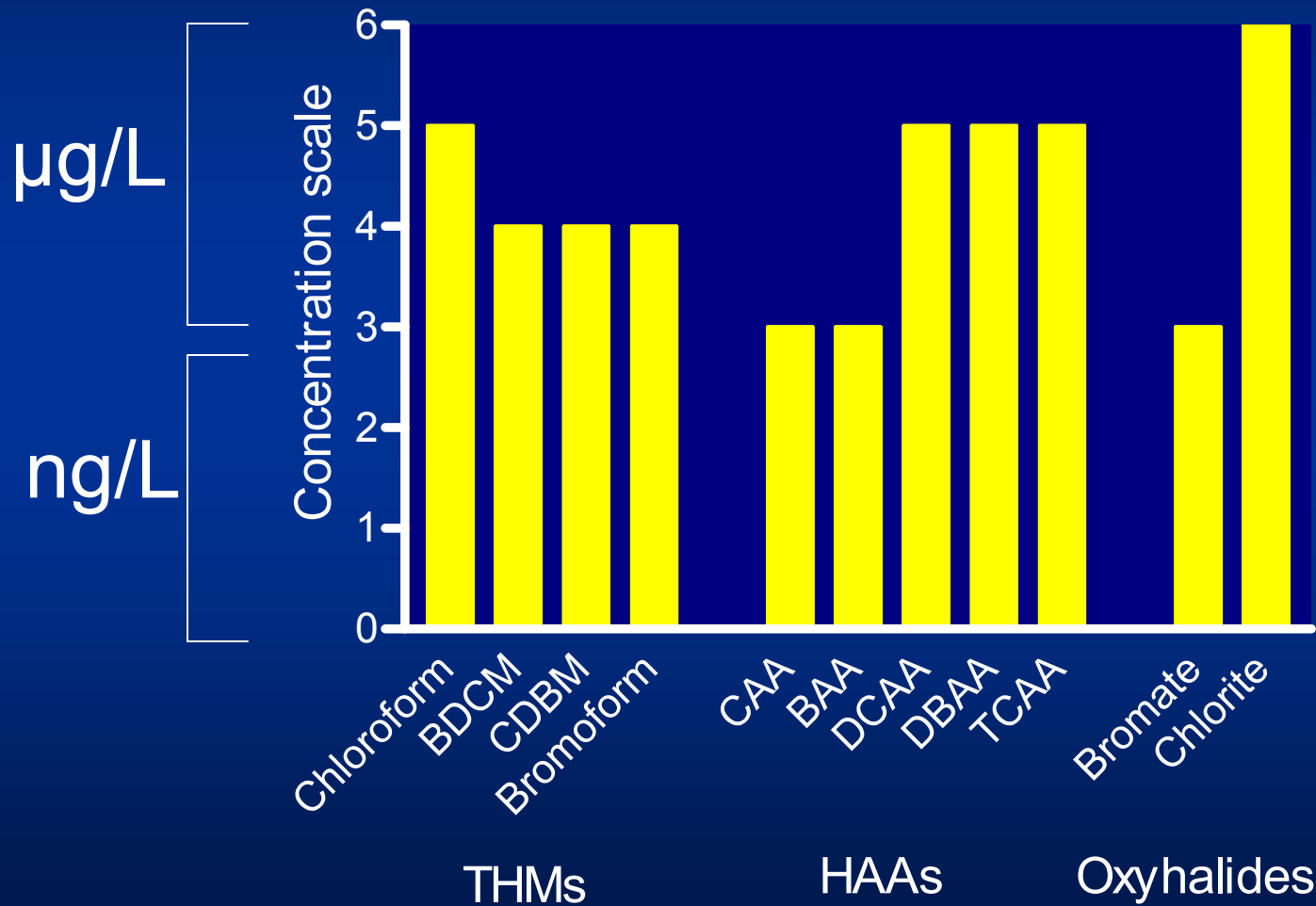
The Regulated DBPs

- Occurrence
- Genotoxicity
- Carcinogenicity

Concentrations of Regulated DBPs

[EPA/600/R02/068 (2002)]

[SW Kranser et al., Environ Sci Technol 40:7175, 2006]



Genotoxicity of Regulated DBPs

DBP	Gene Mutat.	Chrom. Mutat.	DNA Damage
Chloroform	-	-	+
BDCM	+	-	+
CDBM	+	+	-
Bromoform	+	+	+
CAA	+		-
BAA	+		+
DCAA	+	+	-
DBAA	+		+
TCAA	-		-
Bromate	+	+	+
Chlorite			

Carcinogenicity of Regulated DBPs

DBP	Mouse	Rat
Chloroform	+, -	+, +, +
BDCM	+, -, -	+, +, -
CDBM	+	-
Bromoform	-	+
CAA	-	-, -
BAA		
DCAA	+	+
DBAA	+	+
TCAA	+	-
Bromate	+	+
Chlorite	-, -	-

Features of IARC-Declared Human Carcinogens

[RW Tennant, Mutat Res 286:111, 1993]

[MD Shelby, E Zeiger, Mutat Res 234:257, 1990]

- Are trans-species carcinogens, e.g, they cause tumors in both rats and mice.
- Frequently cause tumors at multiple organs.
- Are generally mutagenic across a variety of genotoxic endpoints, e.g., gene and chromosomal mutation.

5 Regulated DBPs with Features of Human Carcinogens

DBP	Occur	Gene Tox G/C/D	Carcinogenicity ^a		
			Mouse	Rat	Human
Chloroform	5	-/-/+	+, -	+, +, +	2B
DCAA	5	+/+/-	+	+	2B/B2
DBAA	5	+/ /+	+	+	
BDCM	4	+/ /+	+, -, -	+, +, -	2B/B2
Bromate	3	+/+ /+	+, -	+, +, -	2B/B2

^aIARC 2B = possible; EPA B2 = probable human carcinogen.

The Unregulated DBPs

- Occurrence
- Genotoxicity
- Carcinogenicity

76 Unregulated DBPs at ng- μ g/L

- 9 Halonitromethanes
- 5 Iodo-acids
- 4 Other halo-acids
- 9 Iodo- & other THMs
- 12 MX compounds
- 13 Haloamides
- 10 Haloacetonitriles
- 1 Tribromopyrrole
- 5 Nitrosamines
- 7 Aldehydes
- 1 Chlorate

25 of the 76 Unregulated DBPs Occur at ng/L Levels

- Of these 25, 5 are nitrosamines, all of which are mutagenic and carcinogenic, and MX is carcinogenic.
- Of the remaining 20, all but 2 are mutagenic (the 2 have not been tested).
- Thus, 23/25 are mutagenic, and 6/25 are carcinogenic. Thus, some of the unregulated DBPs have been tested for carcinogenicity.

51 of the 76 Unregulated DBPs Occur at $\mu\text{g/L}$ levels

- 44 occur at sub- to low- $\mu\text{g/L}$
- 6 occur at low- $\mu\text{g/L}$ (chloropicrin, BCAA, BDCAA, DBCAA, TBAA, chloral hydrate)
- Chlorate occurs at high- $\mu\text{g/L}$

Carcinogenicity of Unregulated DBPs

DBP	Occur	Mouse	Rat
Chlorate	6	-	+
BDCAA	4	+	on test
BCAA	4	+	+
DBCAA	4	+	-, -
Chloral hydrate	4	+, +, +, +	-, -
Acetaldehyde	3		+
Chloroacetaldehyde	3	+	
Dibromoacetonitrile	3	on test	on test
Formaldehyde	3	+	+, +, -
MX	2		+
Nitrosamines	2	+	+

Unregulated DBPs with Some Features of Human Carcinogens

DBP	Occur	Gene Tox	Carcinogenicity		
		G/C/D	Mouse	Rat	Human
BCAA	4		+	+	
Formaldehyde	3	+ / + / +	+	+, -	1
Acetaldehyde	3	+ / + / +		+	2B
MX	2	+ / + / +		+	2B
Nitrosamines	2	+ / + / +	+	+	1/B2

Other Issues

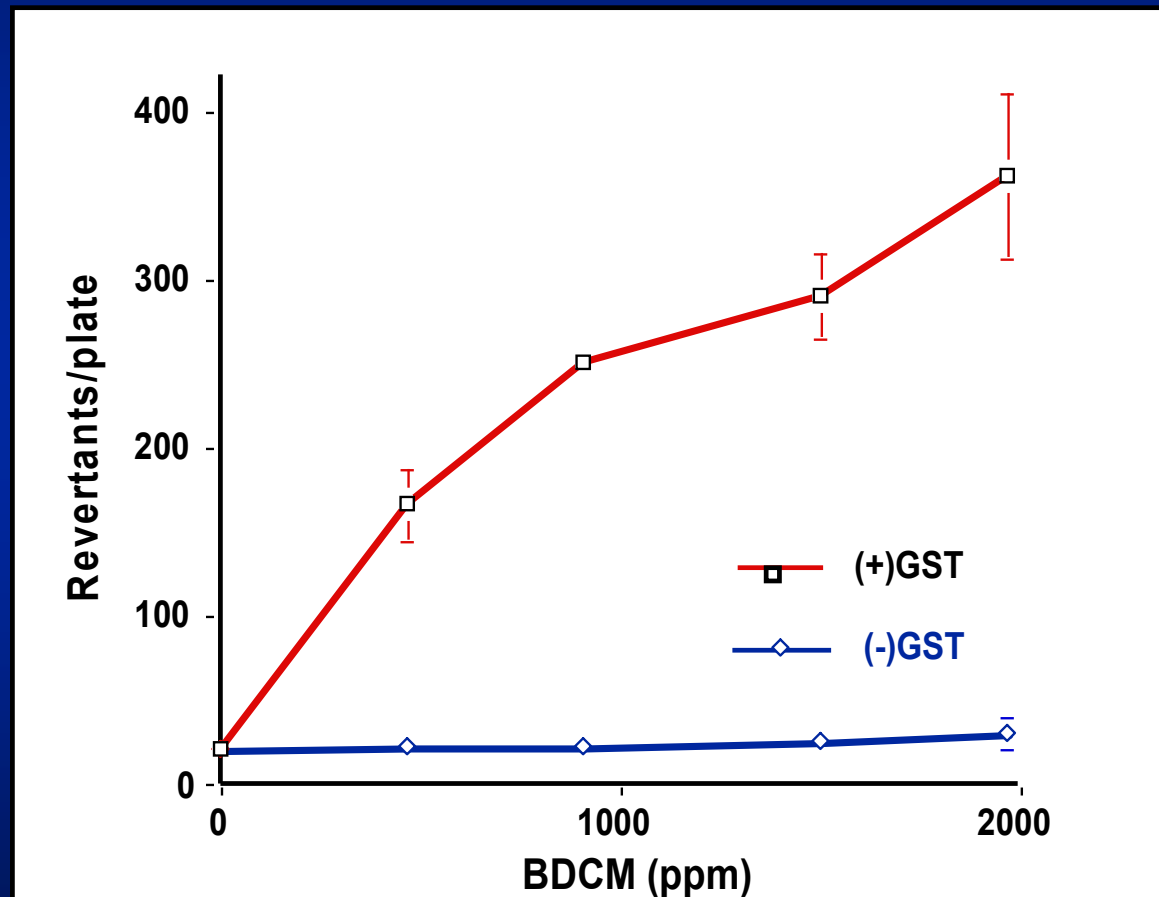
- **Systematic toxicity studies** of as many congeners of DBPs in each chemical class in the same assay(s).
- **Route of exposure studies:** mechanistic and epi studies showing importance of dermal or inhalation exposure for bladder cancer, role of genotype, and swimming as a model for dermal/inhalation exposure

Activation of Halomethanes,
but Not Chloroform, to
Mutagens by *GSTT1-1*

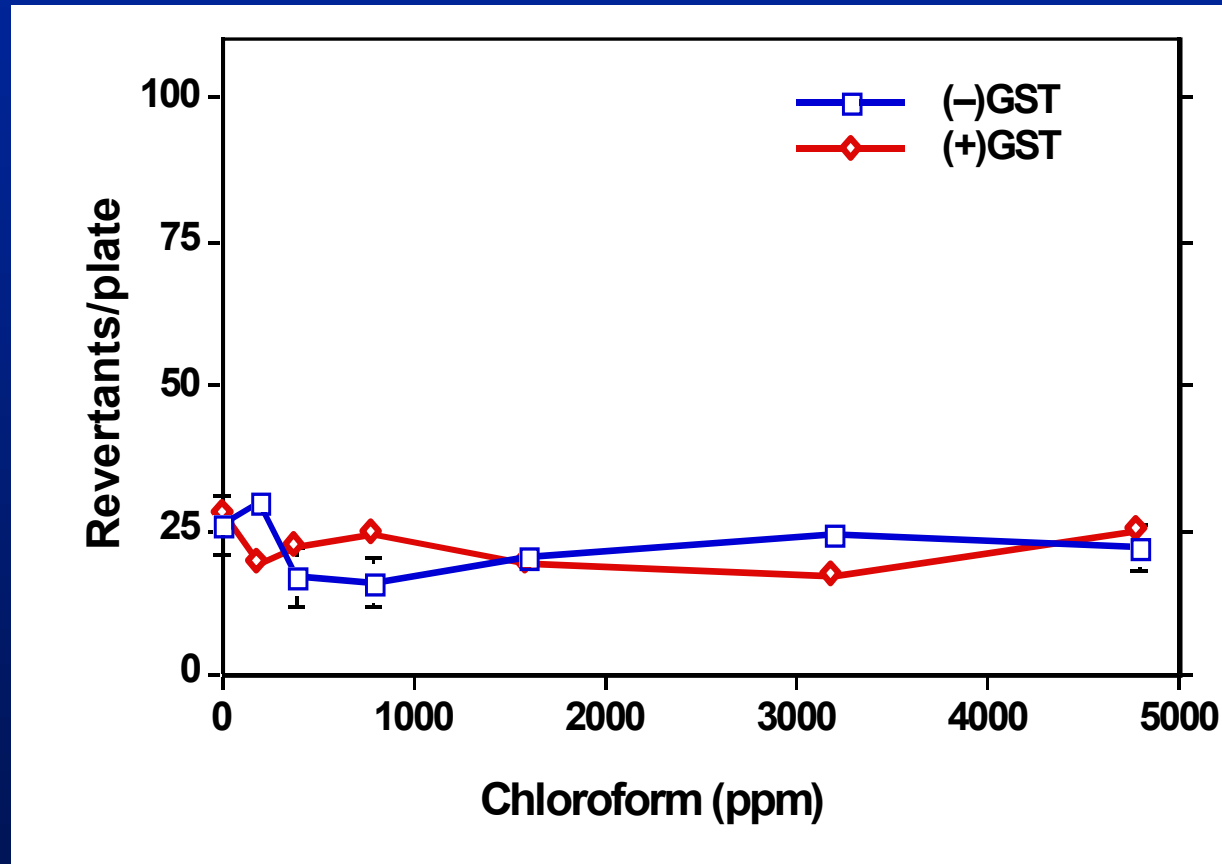
RA Pegram et al.

Toxicol Appl Pharmacol 144:183, 1997

Activation of Bromodichloromethane in Transgenic Strain of *Salmonella* TA1538 Containing *GSTT1-1*



Revertants Produced in Salmonella TA1535 (+)GST and (-)GST with Chloroform



Are Mutations Induced by Drinking Water Found in Drinking Water-Associated Tumors?

- No molecular epidemiology study has been done in which such tumors have been collected and mutations analyzed.
- Tumors associated with consumption of drinking water are bladder and colon cancer.
- Our data would predict a predominance of GC > TA mutations (MX); however, in tissues in which *GSTT1-1* is expressed, GC > AT might be predominant (halomethanes), and a guanine-Br-THM DNA adduct has been demonstrated in rodents (Ross & Pegram, *Toxicol Appl Pharmacol* 195:166, 2004).

First Pharmacokinetic Study of a DBP in Humans

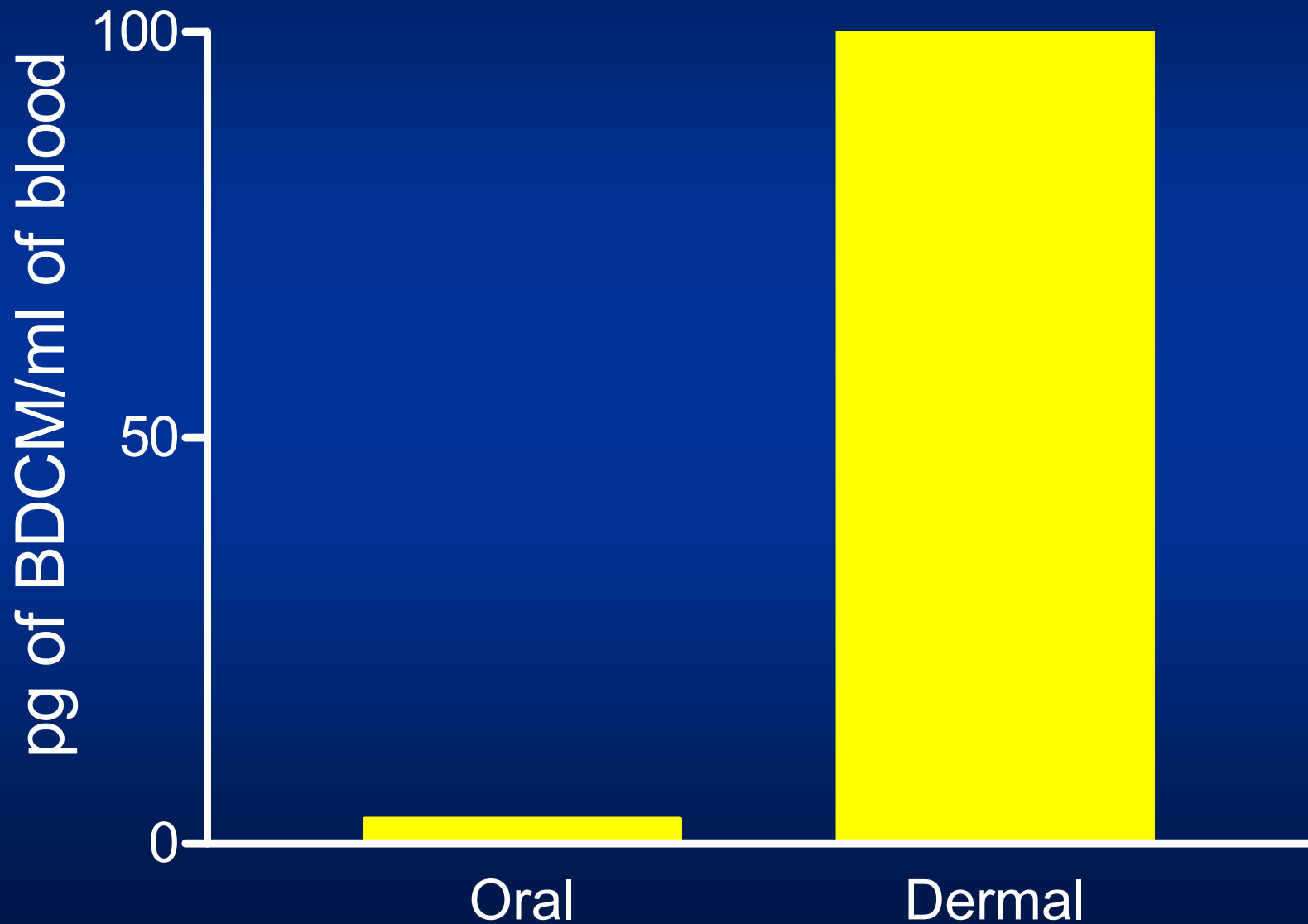
Disposition of Bromodichloromethane in
Humans Following Oral and Dermal
Exposure

TL Leavens et al., Toxicol Sci 99:432, 2007



Subject's arm was in water for 1 h with bromodichloromethane (~50 ppm or 50 ng/kg), or subject drank water giving 50 ng/kg exposure. **BDCM was metabolized much faster by the oral route than dermal route of exposure.**

Peak Concentration in Blood of Bromodichloromethane at 4 h



First Cancer Epidemiology Study of Drinking Water That Assessed Route of Exposure

Bladder Cancer and Exposure to Water
Disinfection By-products Through Ingestion,
Bathing, Showering and Swimming in Pools

[CM Villanueva et al.
Am J Epidemiol 165:148, 2007]

Dermal/Inhalation Exposure to Drinking Water Linked to Bladder Cancer

- A 2-fold increased risk for bladder cancer was associated with swimming or showering/bathing with chlorinated water.
- This 2-fold increased risk for bladder cancer was found regardless of whether the people drank chlorinated or unchlorinated (bottled) water. Thus, dermal exposure may be important.
- Subjects who had *GSTT1-1* were most at risk for bladder cancer.

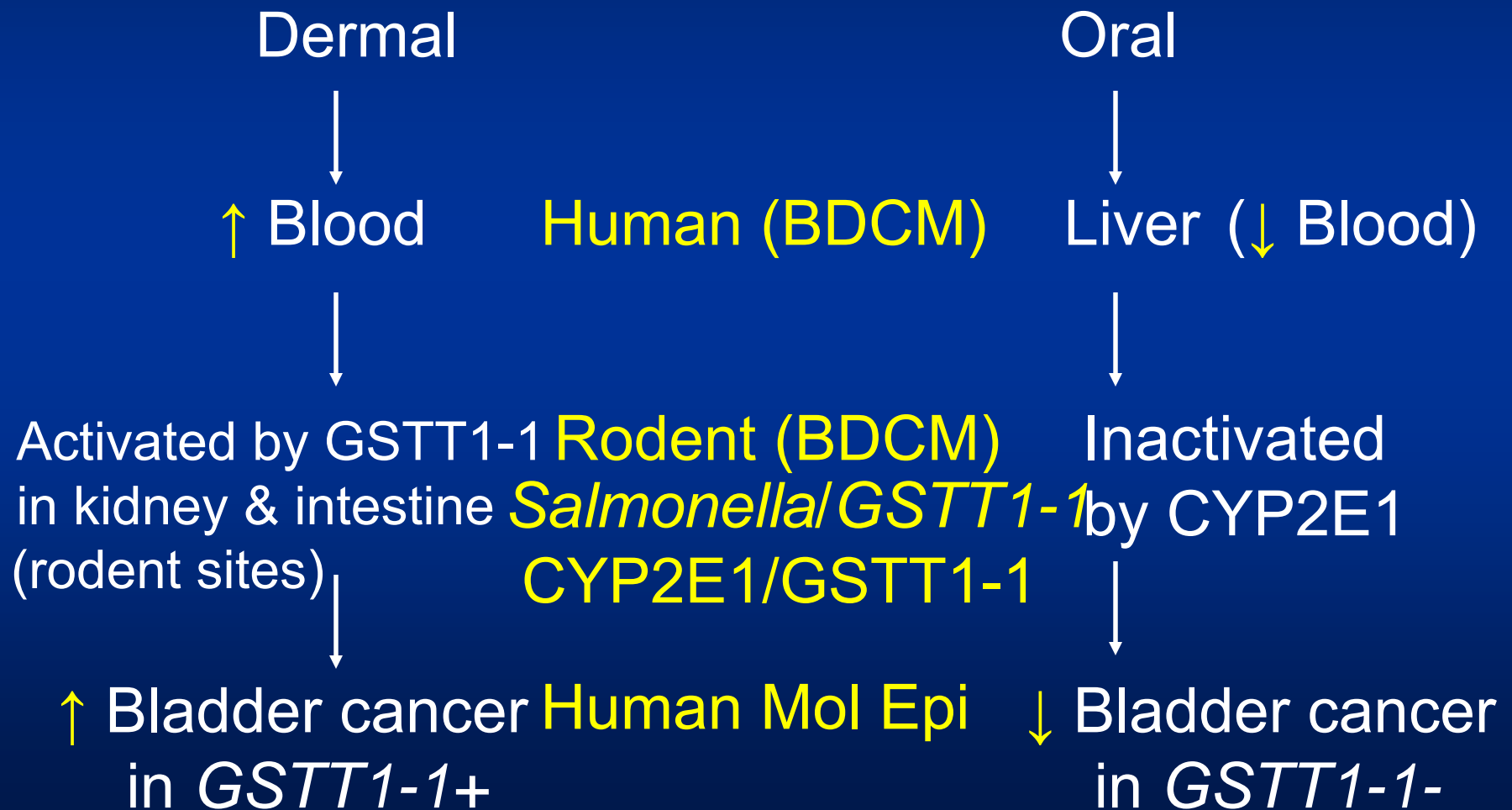
Review of Chemistry and Health Effects of Swimming Pool Water

[C Zwiener et al., ES&T 41:363, 2007]

- Swimming may be a model system for analyzing effects of dermal/inhalation exposure to DPBs.
- Swimmers have 1.6-fold increased risk of bladder cancer.
- Molecular epidemiology study of swimmers is currently underway in Barcelona, Spain; pool water is mutagenic

Model for Br-THM-Induced Bladder Cancer

[MK Ross & RA Pegram, Tox Appl Pharmacol 195:166, 2004]



Research Needs

1. Prioritize DBPs for testing

- 6 regulated DBPs still have significant genotoxicity data gaps, and 1 has never been tested for carcinogenicity.
- 5 **unregulated** DBPs at $\mu\text{g/L}$ levels have data gaps but already exhibit some toxicological features of human carcinogens.
- 14 **unregulated** DBPs at $\mu\text{g/L}$ levels but have no toxicology data, but two are carcinogens.
- 29 **unregulated** DBPs at $\mu\text{g/L}$ levels are genotoxic.

Considerations for 11 Regulated DBPs

- 2 DBPs (chloroacetic acid and chlorite) are not carcinogenic (each in 2 species)
- Chlorite is not carcinogenic in 3 rodent assays but has never been tested for genotoxicity
- 1 DBP (bromoacetic acid) has never been tested for carcinogenicity
- Chloroform and trichloroacetic acid are not genotoxic; both are carcinogenic

2. Systematic generation of quantitative genotoxicity data

- ~70 DBPs studied systematically and quantitatively for DNA damage (comet assay) in CHO cells (M. Plewa, U. of IL)
- ~20 DBPs studied similarly in Salmonella (D. DeMarini, EPA)

3. Studies on route of exposure & genotype

- Rodent studies (dermal-bladder/colon)
- Human studies (experimental & epi)

4. Chemical identification of unknown fraction of drinking water

- >50% of total organic halide (TOX) and assimilable organic carbon (AOC) have not been identified as specific compounds. Savitz et al. (2006) epi study on chloraminated water showed correlation between TOX and pregnancy loss.
- Additional analytical studies should be conducted in concert with toxicological studies.

5. Evaluate DBPs from alternative disinfection methods

- chloramine, ozone, and chlorine dioxide
- UV and membrane technologies

6. Evaluate use of bioassays for assessing source-water and drinking water quality

- Salmonella assay used already in a region of Brazil for source-water assessment and regulation
- Salmonella assay literature on drinking water mutagenicity is extensive but not linked well to chemical analysis.

7. Complex mixture studies

- >600 identified DBPs, many are toxic, exposed via oral, dermal, and inhalation.
- This complexity is not reflected in any of the toxicology studies of individual DBPs.
- Need for bioassay-directed fractionation of water extracts for genotoxicity, from different source waters and treatment methods (4-lab).

8. Review of reproductive and developmental studies

- Merge with current review to permit a sound, scientific assessment of current regulations and directions for future research.

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By water everything lives.

The Koran

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