



Water Disinfection Byproduct Pharmacokinetics: Linking Brominated Trihalomethane Exposure to Health Effects

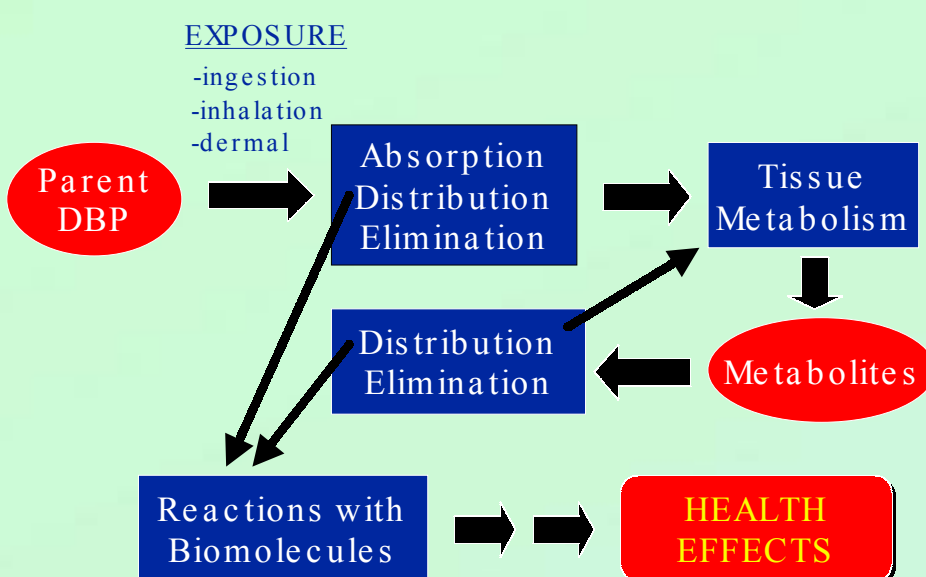
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Key Issues for Brominated THMs

- BrTHMs are among the most prevalent disinfection byproducts (DBPs) in drinking water
- Bromodichloromethane: the most potent carcinogen among the THMs in rodents
 - Drinking water? inhalation?
 - Low doses to humans?
- Concordance of epidemiological and animal research findings (colon cancer and reproductive toxicity)
- Mutagenicity/genotoxicity of BrTHMs: linear risk assessment approach? Dose-response?
- Identification and description of pharmacokinetics and key events/pathways
 - Compare rodents and humans

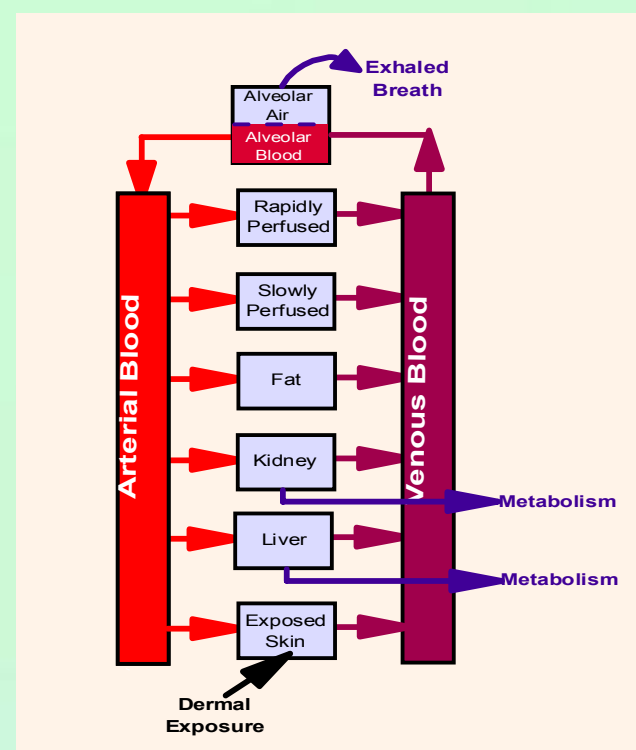
DBP PHARMACOKINETICS



POSTER ABSTRACT

A multi-divisional (ETD, ECD, HSD), cross-agency (EPA, CDC, UNC) effort has generated significant new findings (including human data) on brominated trihalomethane (BrTHM) pharmacokinetics. Results from this work include the development of a physiologically based pharmacokinetic (PBPK) model for bromodichloromethane (BDCM) in rats; determination of human BDCM pharmacokinetics following oral and dermal exposures; discovery and characterization of a unique mutagenic metabolic pathway (glutathione [GSH] conjugation) for the brominated THMs, which is not utilized by chloroform; proof of the capability of intermediates derived from the GSH-conjugation pathway to react with DNA; identification of the types of mutations induced by BrTHMs when metabolized via GSH; and identification and kinetic characterization of human and rodent cytochrome P-450 enzymes that are involved in BDCM metabolism. These findings support the hypothesis that brominated THMs are of greater toxicological significance than chloroform. We have identified metabolizing enzymes that may determine susceptibility to BrTHMs and should be incorporated into future human pharmacokinetic and epidemiological studies. The data will also ultimately be used in the development and calibration of a human PBPK model for BDCM, which will improve dose-response predictions for humans and, consequently, provide an important risk assessment tool for this prevalent DBP.

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING FOR BROMODICHLOROMETHANE



Selected PBPK model parameters for BDCM and chloroform

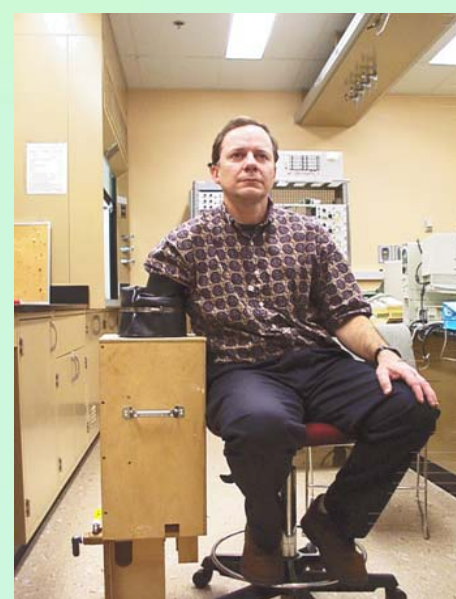
Parameter	BDCM	Chloroform*
<i>Partition Coefficients</i>		
Fat/Air	526	203
Liver/Air	30.6	21.1
<i>Metabolic Constants</i>		
V _{max} (mg/hr/kg)	12.8	6.8
K _m (mg/L)	0.5	0.5

*Corley et al. (1990) Toxicol. Appl. Pharmacol. 103, 512.

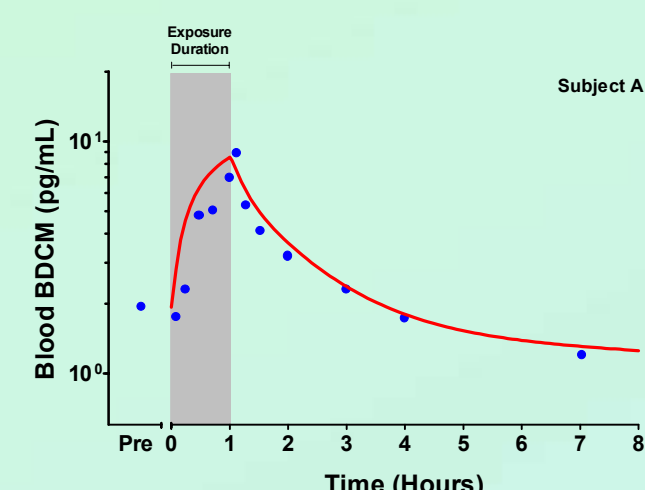
BDCM Rat Model Summary

- Higher partition coefficients demonstrate greater tissue uptake of BDCM compared to chloroform
- BDCM is metabolized to reactive intermediates at a faster rate than chloroform
- The model was able to accurately predict:
 - Blood and tissue concentrations of BDCM after oral and inhalation dosing
 - Metabolite (bromide ion) production after dosing

BROMODICHLOROMETHANE PHARMACOKINETICS IN HUMANS



PBPK modeling of human blood BDCM concentrations during and after dermal exposure



In Vivo Exposure Summary

- Volunteers were exposed either dermally or orally to water containing BDCM at a level normally found in finished drinking water.
- Significantly higher blood concentrations of BDCM were attained with dermal exposure than with oral consumption (40-100-fold difference).
- An initial PBPK modeling effort was able to predict the blood concentration profile.

IN VITRO METABOLISM

Summary of Metabolic Constants for Recombinant Cytochrome P450's

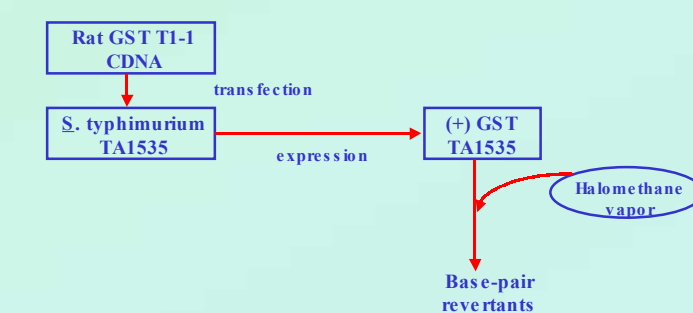
Isoenzyme	Metabolic Parameter ^a			
	Human		Rat	
	K _m	k _{cat}	K _m	k _{cat}
CYP2E1	3.5 (0.5)	2.3 (0.1)	4.6 (0.3)	3.5 (0.1)
CYP1A2	94 (29)	4.6 (0.7)	355 (109)	19.8 (4.4)
CYP2A6	206 (62)	1.4 (0.3)	— ^b	—
CYP3A4	238 (44)	16.6 (1.9)	—	—
CYP2B1	—	—	127 (16)	0.89 (0.06)

^a Units of K_m and k_{cat} are μM and mol BDCM (mol P450 · min)⁻¹ respectively.
^b Enzyme not present.

- The kinetics of CYP2E1-mediated BDCM metabolism are similar in humans and rats.
- We discovered that CYP1A2, CYP3A4, and CYP2A6 also metabolize BDCM.
- BDCM was not metabolized by human CYPs 2B6 and 2D6 or by rat CYPs 3A1 and 2C11.

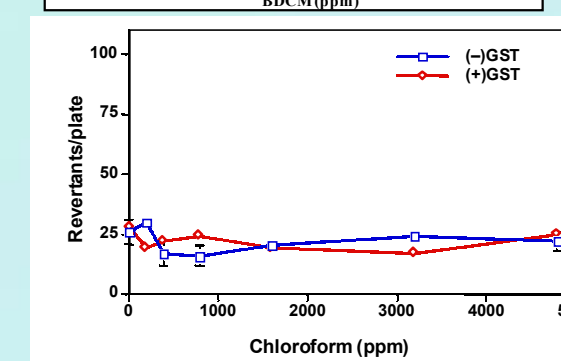
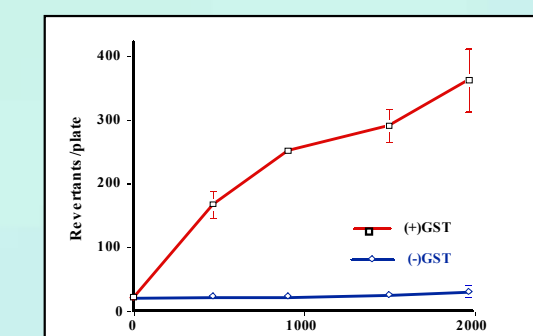
DISCOVERY AND CHARACTERIZATION OF A GENOTOXIC METABOLIC PATHWAY FOR BROMINATED THMs

GSH S-transferase Salmonella TA1535 Mutagenicity Assay System

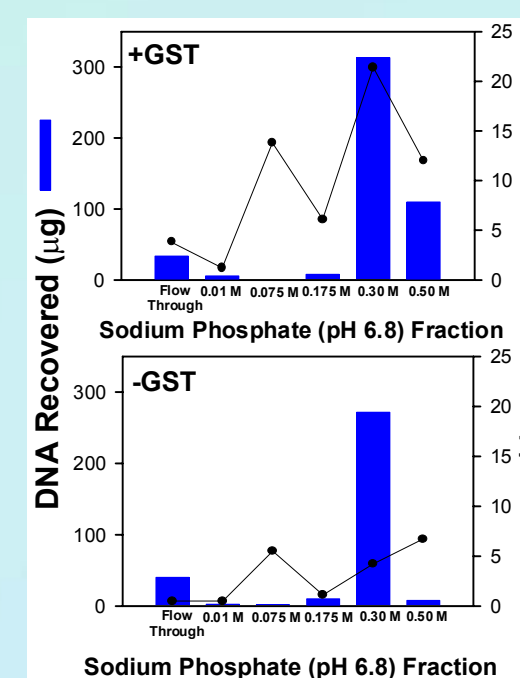


- BrTHMs, but not chloroform, are metabolized to mutagens by the GST-theta enzyme.
- CHBr₂Cl and CHBr₃ are more potent mutagens in this assay than BDCM.
- Relative potency corresponds with ability to induce preneoplastic colon lesions
- These gene mutations are very specific: GC → AT transitions
- The GST T1-1 enzyme required for the pathway is present in humans; it is polymorphically expressed in people
 - Human enzyme expressed in urinary and GI tracts
 - Expression may determine susceptibility

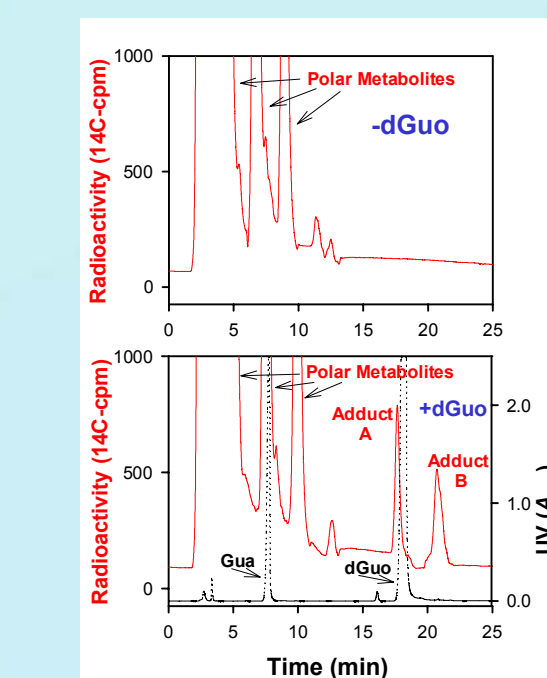
Revertants produced in Salmonella TA1535 (+)GST and (-)GST with BDCM and chloroform



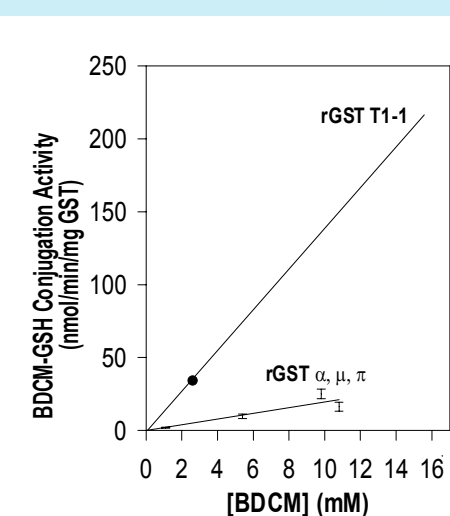
GST T1-1-Dependent DNA Binding by BDCM



GST T1-1-Catalyzed Formation Of Deoxyguanosine Adducts



GSH Conjugation of BDCM Catalyzed by GST T1-1 or GSTs alpha, mu, and pi



- Hydroxyapatite chromatography demonstrated that DNA was covalently modified *in vitro* by GST theta-mediated metabolism of ¹⁴C-BDCM.

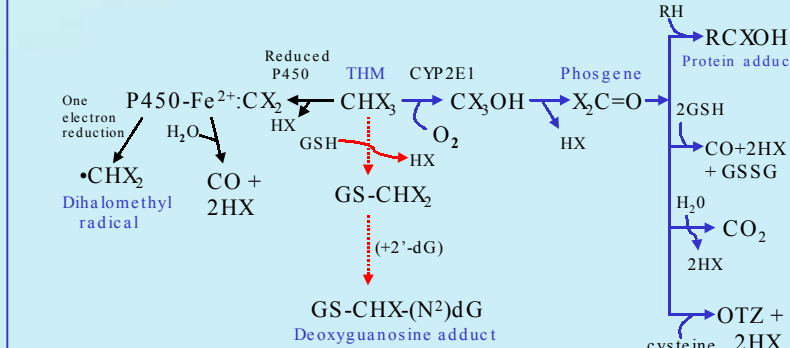
- Formation of adducts A and B was dependent on the presence of dGuo and GSH; adduct B was completely dependent on GST T1-1.

- This graph demonstrates that BDCM conjugation by GSH is GST theta-specific. A mixture of GSTs alpha, mu and pi did not efficiently catalyze the reaction.

SUMMARY

- A PBPK model for BDCM has been developed that will be further parameterized and calibrated for humans using *in vivo* and *in vitro* human pharmacokinetic data.
- Human volunteer studies have demonstrated that much higher blood levels of BDCM occurred following dermal exposures than after drinking water exposures.
- New enzymes have been shown to metabolize BrTHMs. These include CYP1A2, CYP3A4, CYP2A6, and GST T1-1.
- A genotoxic metabolic pathway for BrTHMs has been discovered that is mediated by glutathione S-transferase theta 1-1. This pathway produces intermediates that covalently bind DNA (specifically producing deoxyguanosine adducts), thus leading to gene mutations.
- Recent findings suggest that the metabolites derived from BrTHMs via the GST pathway are more mutagenic than those produced by methylene chloride. The rate of BrTHM-GSH conjugation is, however, less than that of methylene chloride. Additional metabolites have now been identified as products of BrTHM-GSH reactions, including S-formyl-GSH and formate.
- The trihalomethane metabolism scheme can now be modified as shown below to reflect the finding of the new GST pathway.

Trihalomethane Metabolism



IMPACT

- The PBPK model for bromodichloromethane can be used for interspecies, route-to-route, and high-to-low dose extrapolation for risk assessment. The generation of *in vivo* human data provides a unique opportunity to calibrate the model and test the predictive utility of kinetic parameters derived from *in vitro* experiments.
- Pharmacokinetic and mutagenicity findings indicate that the BrTHMs are of greater concern as potential human carcinogens than is chloroform.
- Brominated THMs are activated to mutagens by the GST T1-1 enzyme, which is polymorphically expressed in humans and may therefore be an important determinant of susceptibility to the genotoxic and potential carcinogenic effects of brominated THMs.

Safe Water

Building the scientific foundation for sound environmental decisions

