

Other Relevant Data



Stan Atwood, MS, DABT

Integrated Laboratory Systems, Inc.

Contractor supporting the Office of the Report on Carcinogens

National Institute of Environmental Health Sciences

July 24, 2017



Outline

Disposition & Toxicokinetics

- ADME
- Clearance

Mechanistic Data

- Characteristics of carcinogens
- Potential modes of action and key events



Ingestion is the predominant exposure pathway

- **Ingestion**

- Rapid and extensive
- 94% of total exposure

- **Inhalation**

- Vapor pressure: 0.0003 – 0.18 mm Hg
- 5% of total exposure

- **Dermal**

- Permeability: <math><0.001 - 0.003\text{ cm/hr}</math>
- 1% of total exposure





Rapid with little to no bioaccumulation in tissues

HAA	Vd _{ss} (mL/kg)	Unbound (%)
DCA	618	94
BCA	881	93
DBA	400	89
TCA	782	53
BDCA	730	49
CDBA	636	55
TBA	449	18

Source: Schultz *et al.* 1999

Tissue:blood partition coefficients are ~ 1 and indicate uniform distribution



Metabolism is similar in humans and rodents

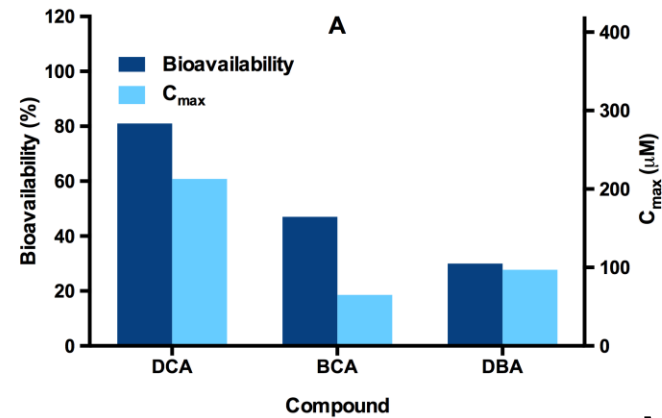
- Known metabolic pathways/metabolites are similar
 - Involves both microsomal and cytosolic enzymes
 - All pathways have not been completely described
- Extent and rate of metabolism is variable
 - Rate: mice 2X > rat 5X > human (DCA)
 - Extent varies with the number and type of halogen
 - Di-HAAs > Tri-HAAs
 - brominated > chlorinated forms
 - Other factors
 - Dose & age
 - Mixtures/pre-exposure to HAAs



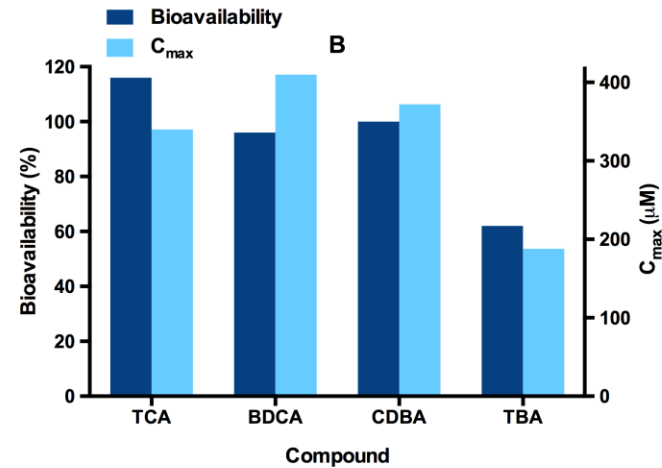
Bioavailability, First Pass Metabolism & C_{max}

The number and type of halogens influence bioavailability and metabolism

- Di-HAAs
 - ↓ Bioavailability vs. Tri-HAAs
 - ↑ First-pass metabolism
 - ↓ C_{max} vs. Tri-HAAs
- Tri-HAAs
 - High bioavailability
 - ↓ Metabolism vs. Di-HAAs
 - Higher C_{max}
- Br substitution for Cl
 - ↑ First pass metabolism
 - TBA resembles Di-HAA



Male Rats

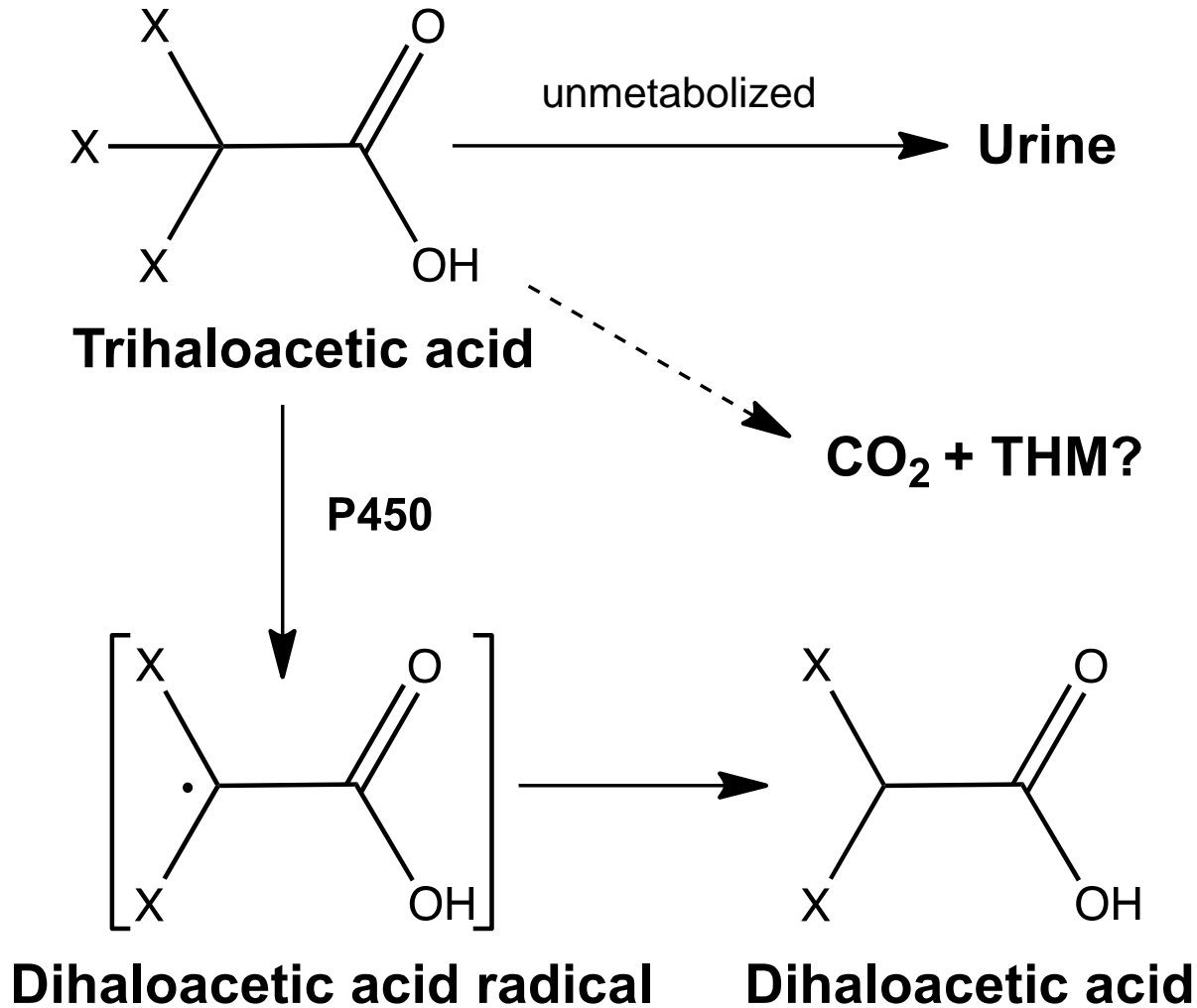


Source: Schultz et al. 1999



Tri-HAA Metabolism and Excretion

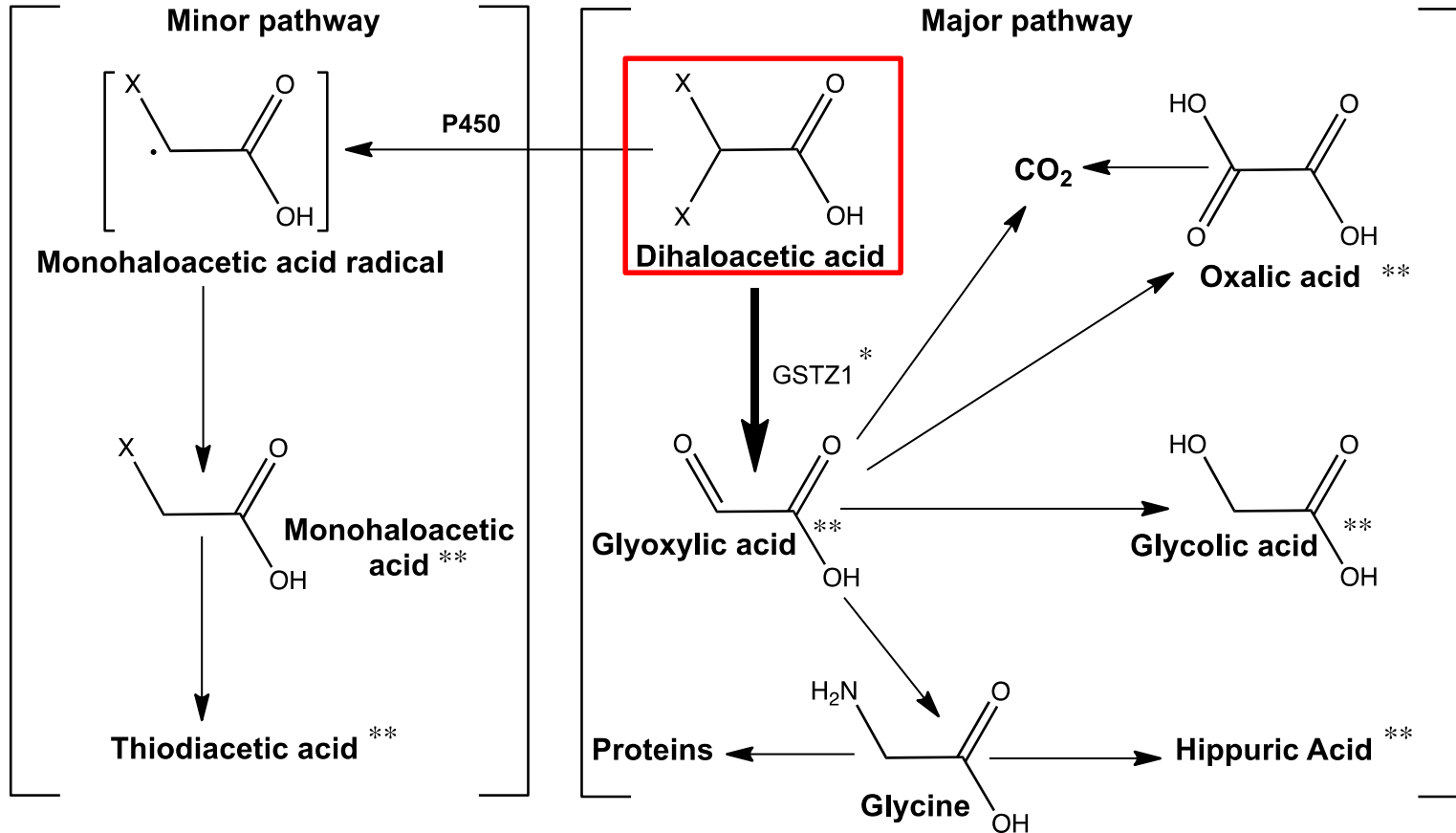
Tri-HAAs are metabolized by P450 reductive dehalogenation





Di-HAA Metabolism and Excretion

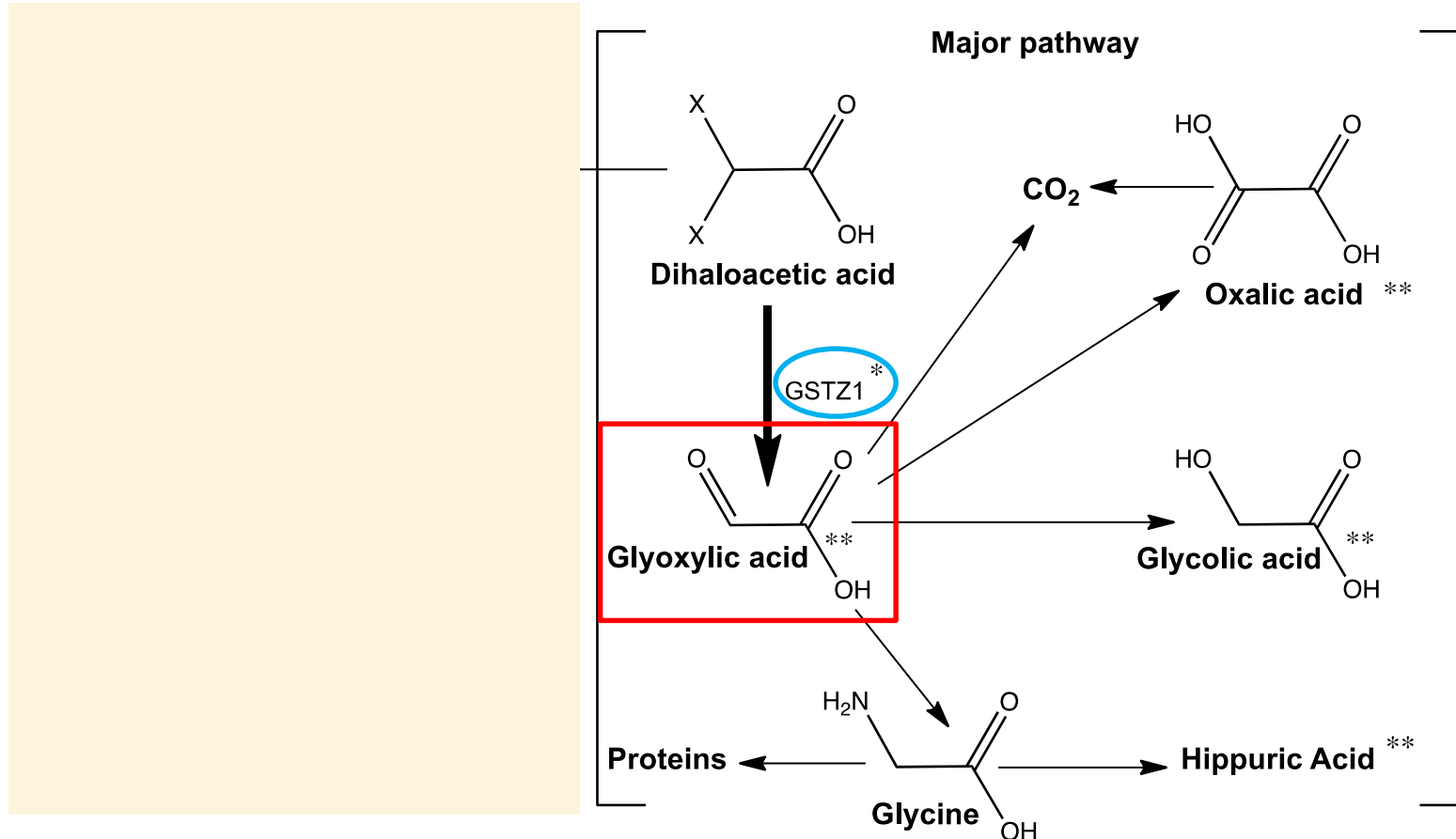
Di-HAAs are metabolized via multiple pathways





Di-HAA Metabolism and Excretion

Major pathway: cytosolic GST- ζ to glyoxylic acid



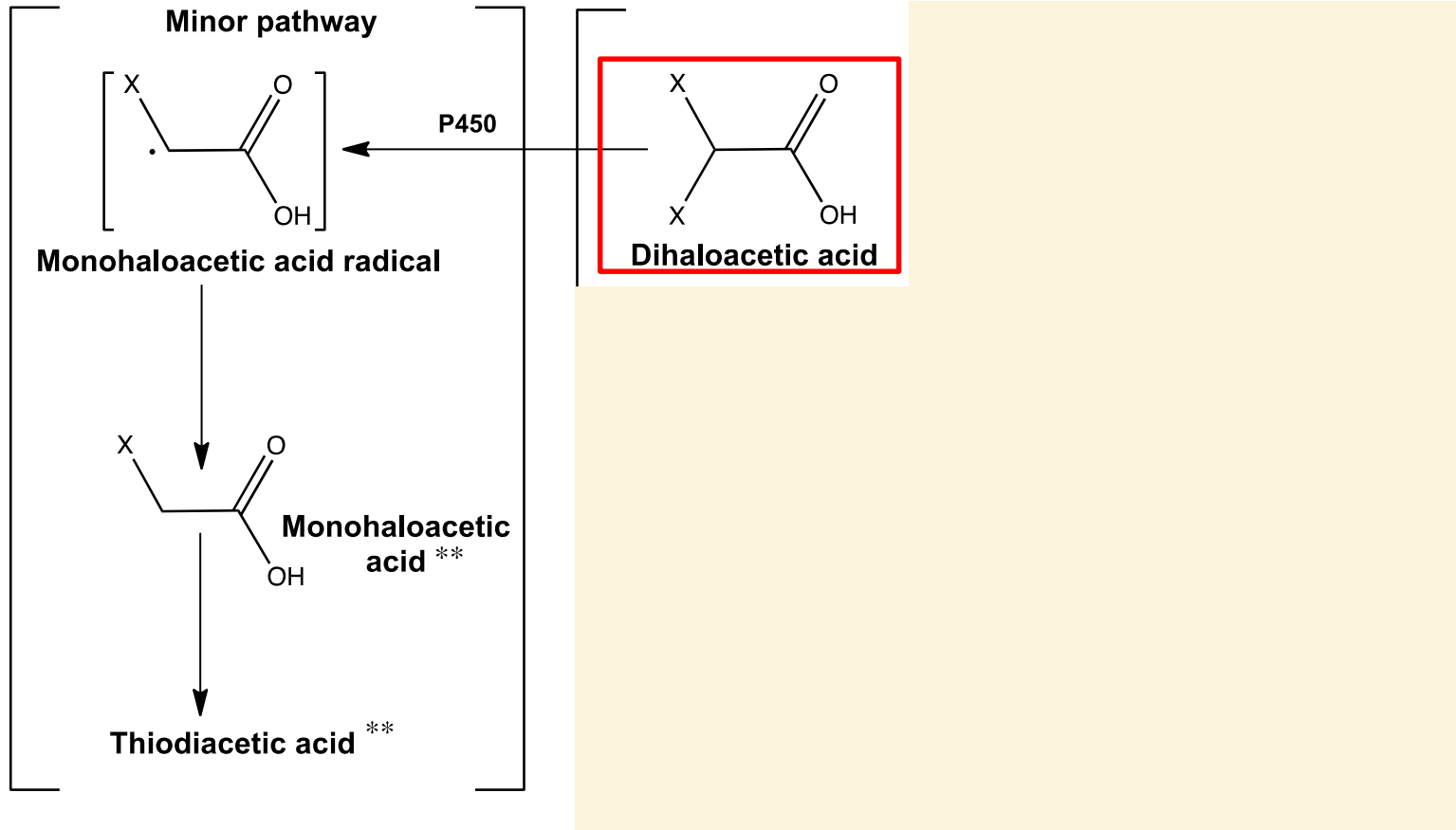
* Di-HAAs are irreversible inhibitors of GST- ζ (also known as maleylacetoacetate isomerase [MAAI]) and catalyzes the penultimate step in the tyrosine catabolism pathway.

** Urinary metabolites



Di-HAA Metabolism and Excretion

Di-HAAs: Minor pathway P450 reductive dehalogenation



** Urinary metabolites



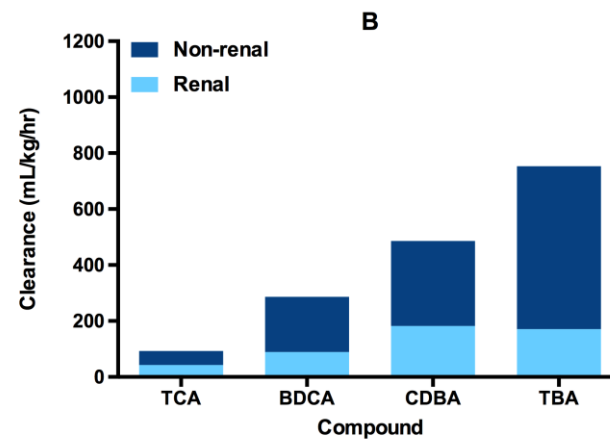
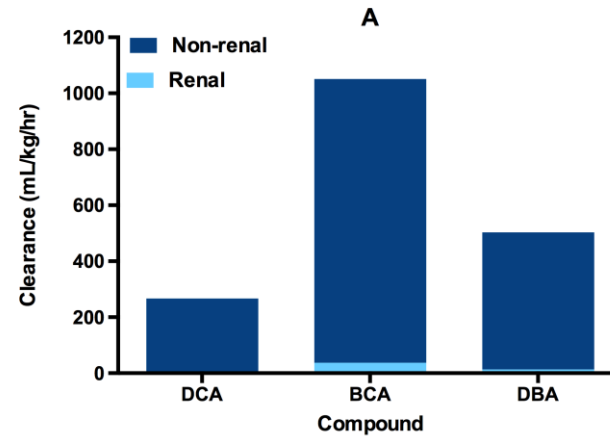
Number and type of halogens affect clearance

- Di-HAAs

- Metabolism (non-renal) clearance is dominant
- Br substitution for Cl increases non-renal clearance vs. DCA
- Unknown GSTs

- Tri-HAAs

- Both renal and non-renal clearance are important
- Br substitution for Cl increases both renal and non-renal clearance



Male Rats



Summary

- Oral route most important exposure pathway
- Do not accumulate in tissues
- Metabolism is not fully understood
- Metabolism and clearance influenced by halogen substitution pattern
 - **TCA**: Low metabolism and moderate renal clearance
 - **Br-Tri-HAAs**: Moderate-high metabolism and renal clearance
 - **Di-HAAs**: High metabolism and low renal clearance



HAA's Disposition and Toxicokinetics

A photograph of a hand filling a glass with water from a faucet. The faucet is a modern, polished chrome design with a spherical aerator. The water is being poured into a clear glass, creating bubbles. The background is a white tiled wall. The word "Questions?" is overlaid in the center of the image.

Questions?



No clearly defined mechanisms of carcinogenicity

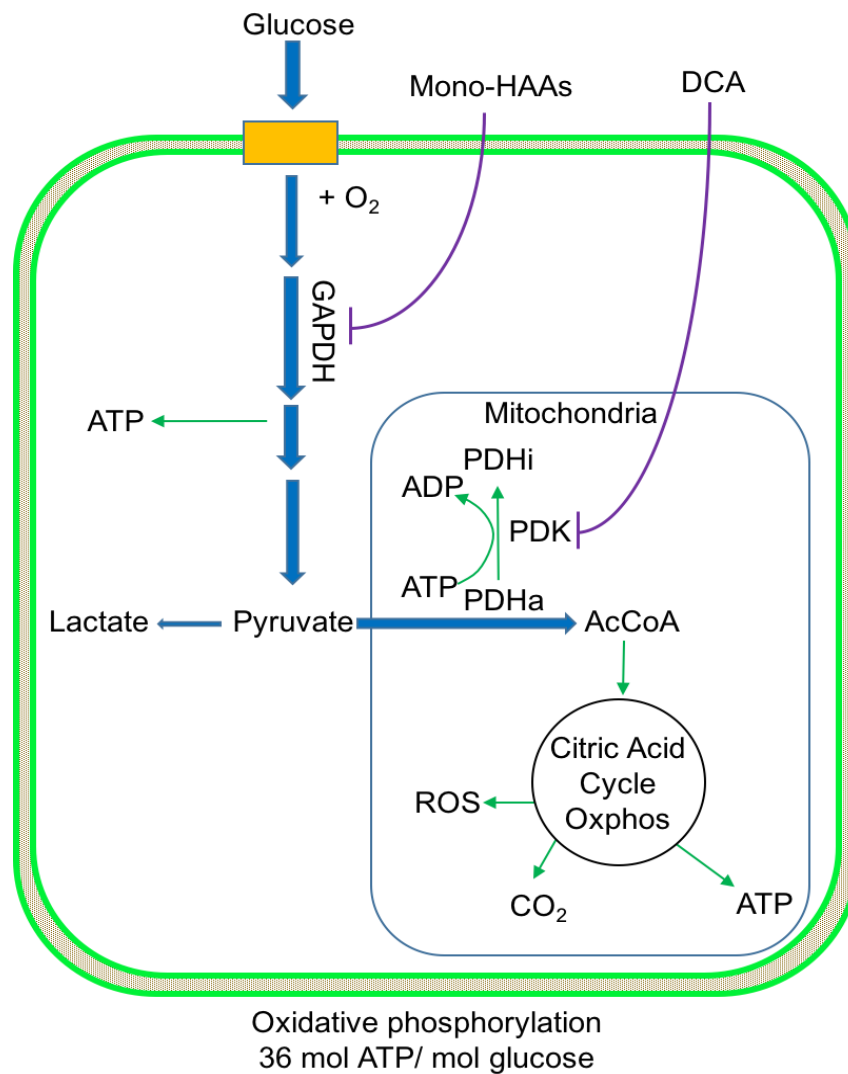
- Informed by characteristics of carcinogens (CoC)*
 - Act as an electrophile
 - Induce oxidative stress
 - Induce genotoxic effects
 - Induce epigenetic effects
 - Modulate receptor-mediated effects
 - Alter cell proliferation, death, nutrient supply
 - Cause cell immortalization
- Evaluate trends for each CoC
 - Number of halogens
 - Types of halogens

* Smith *et al.* 2016



All HAAs are relatively soft electrophiles

- E_{LUMO} indicates electrophilic nature
- Binds to proteins
 - GAPDH
 - PDK
 - GST- ζ
- $E_{LUMO} + pK_a$ correlates with cytotoxicity, oxidative stress, genotoxicity
- GAPDH inhibition rate correlates with E_{LUMO} , cytotoxicity and genotoxicity of mono-HAAs





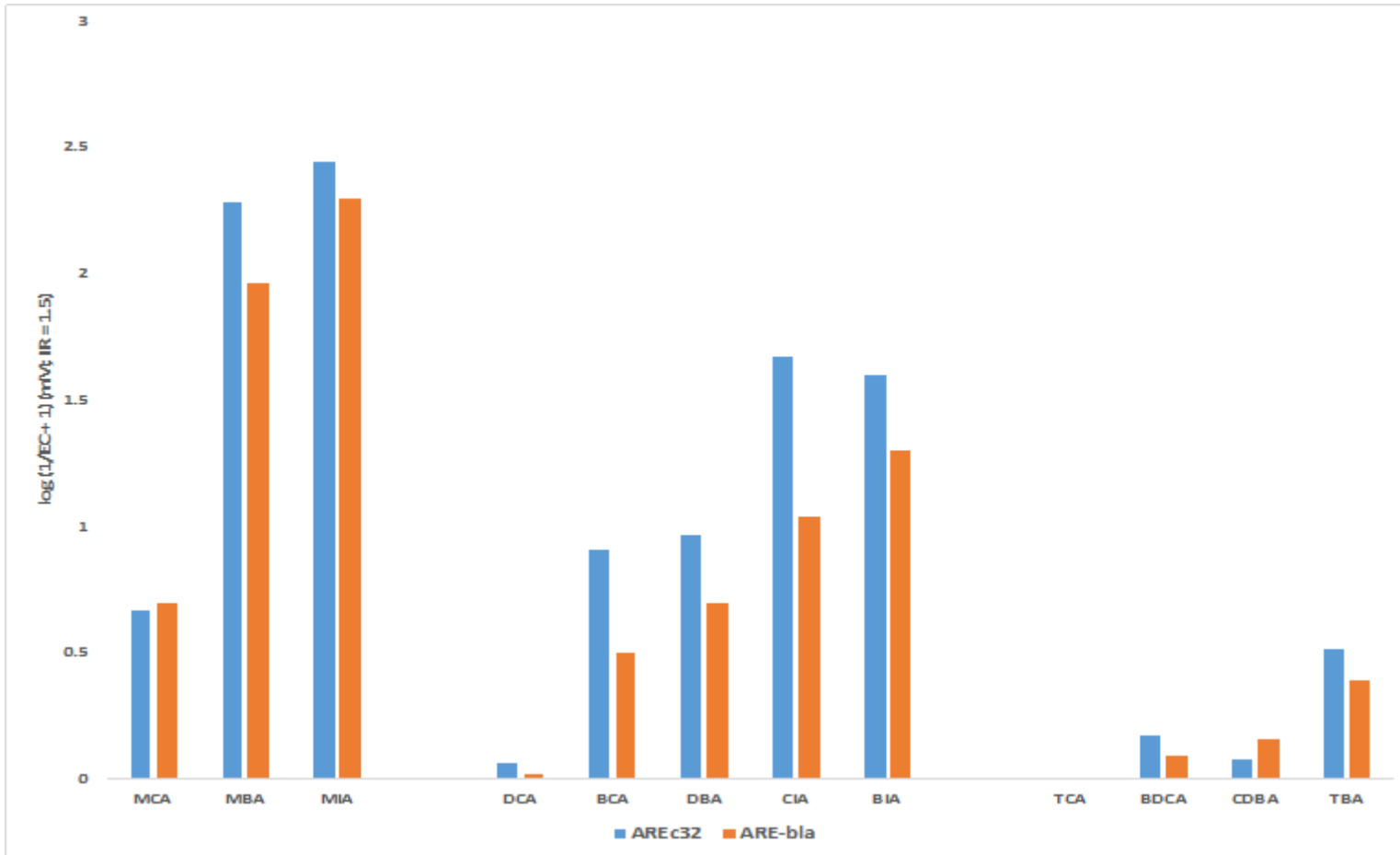
All HAAs induce oxidative stress

- Oxidative stress may be generated by multiple pathways
 - Metabolism via reductive dehalogenation
 - Disruption of energy metabolism, mitochondrial stress
 - GST- ζ inhibition
 - Activate Nrf2/ARE pathway
 - Oxidative damage DNA
 - Lipid peroxidation
 - PPAR α activation
- Positive correlation with genotoxicity
 - Treatment with antioxidants reduced genotoxicity



Oxidative Stress: Nrf2/ARE assay

Potency decreases with the number of halogens



Source: Stalter *et al.* 2016

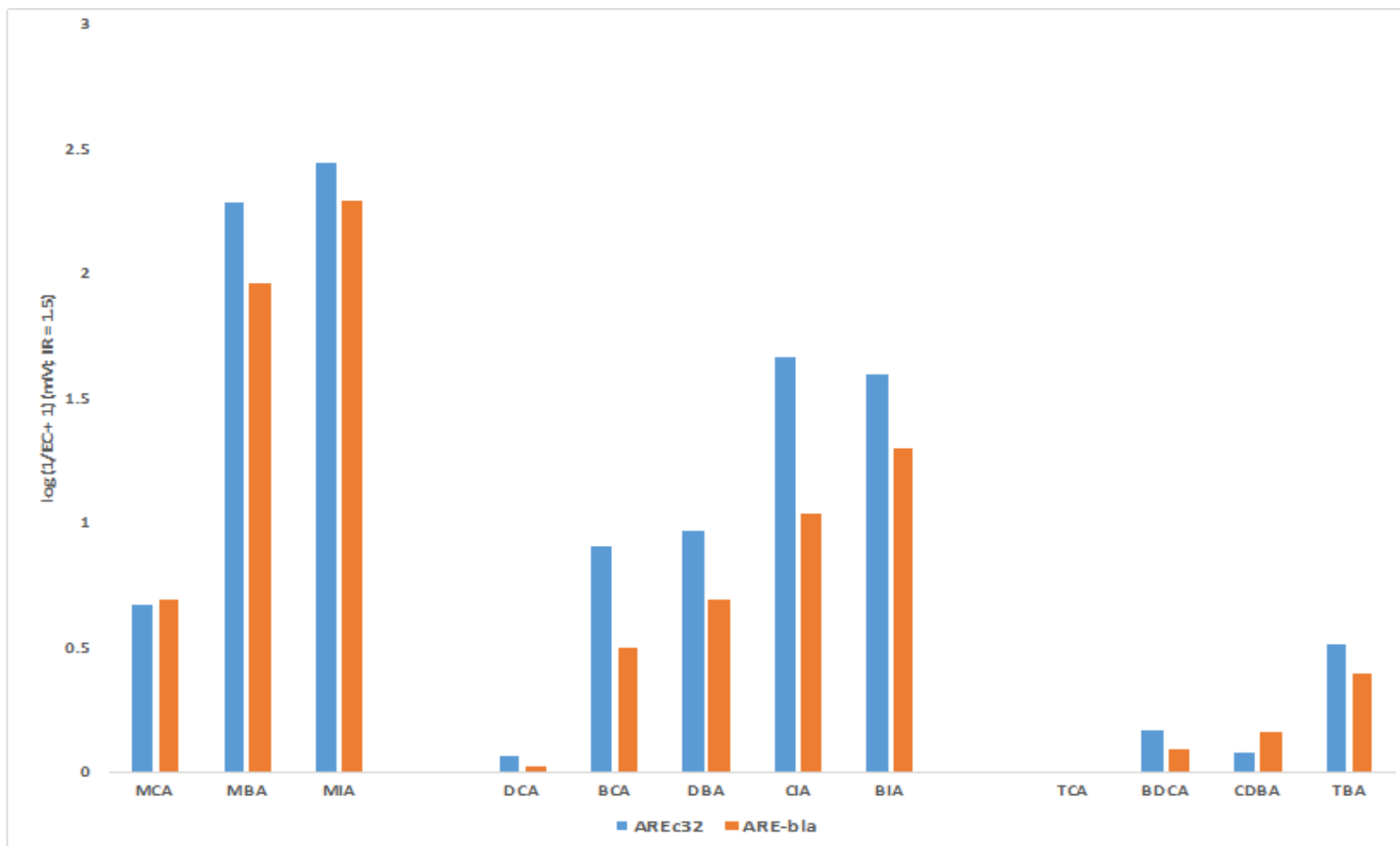
AREc32 = MCF-7 breast cancer cell line

ARE-bla = HepG2 hepatocellular carcinoma cell line



Oxidative Stress: Nrf2/ARE assay

Potency increases with the type of halogen: I > Br >> Cl



Source: Stalter *et al.* 2016

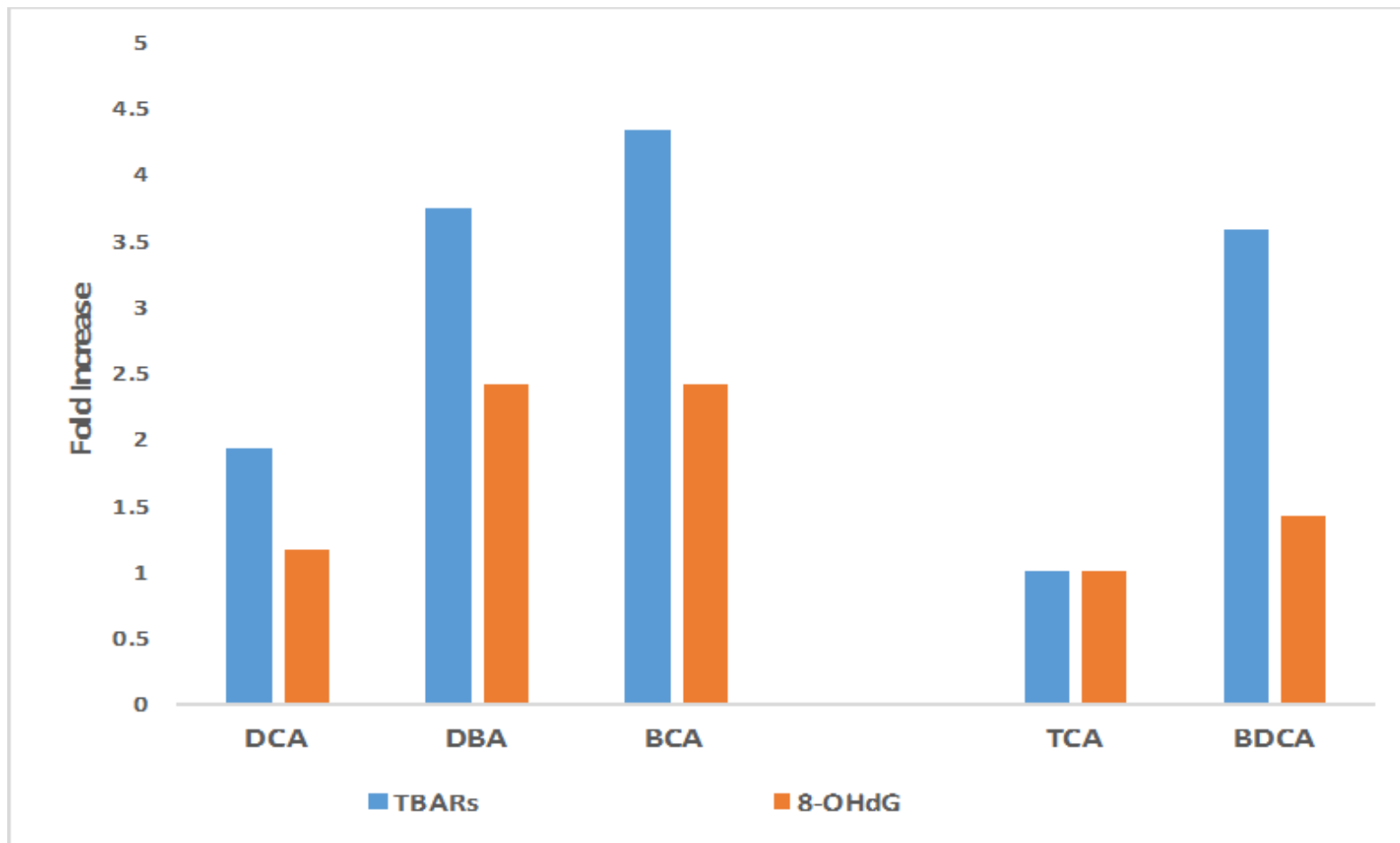
AREc32 = MCF-7 breast cancer cell line

ARE-bla = HepG2 hepatocellular carcinoma cell line



Oxidative Damage: *In Vivo* Mouse Liver

Brominated analogues are more potent



Sources: Larson and Bull 1992, Austin et al. 1996



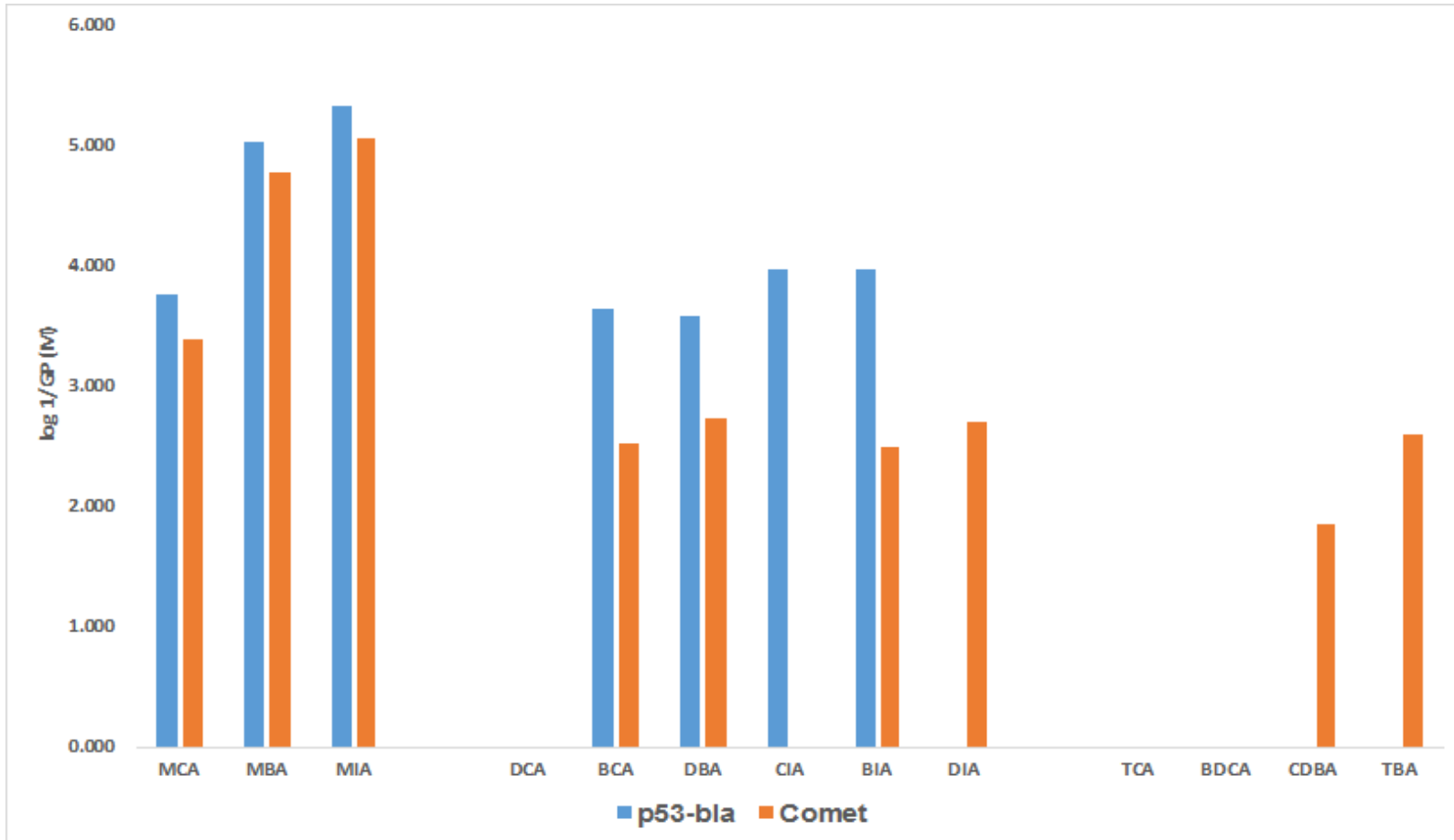
Most HAAs induce genotoxic/mutagenic effects

- Strongest evidence is from *in vitro* studies
 - Induces mutations in bacteria: generally + in TA100 w/o metabolic activation
 - Induces mutations and DNA damage in human and rodent cells
- Some evidence that HAAs can cause DNA or chromosome damage *in vivo*
 - limited data
 - mixed results
- DNA damage associated with oxidative stress



DNA Damage in Mammalian Cells

Potency decreases with the number of halogens



Sources: Stalter et al. 2016, Plewa et al. 2010
p53-bla = HCT-116 human colon carcinoma cell line
Comet assay = CHO cells



Other potential modes of action

- Hypomethylation (DCA, DBA, TCA)
 - Liver DNA
 - Promoter regions of *c-jun*, *c-myc*, IGF-II
 - Correlated with carcinogenicity and tumor promoting activity
- Alters energy metabolism
 - GAPDH inhibition (Mono-HAAs)
 - PDK inhibition (DCA)
- PPAR α activation (TCA)
- Cell transformation (MIA, DBA)



Summary

- Mechanisms are unclear
- Associated with many of the characteristics of carcinogens
- General trends observed for several relevant endpoints
 - I > Br >> Cl
 - Mono > Di > Tri

HAAs associated with various characteristics of carcinogens

CoCs	Mono-	Di-	Tri-
Electrophilic	All	All	All
Ox stress	All	All	All
Genotoxic	All	All	All (-TCA)
Hypomethylation	?	DCA DBA Others?	TCA Others?
GAPDH inhibition	All	?	?
PDK inhibition	?	DCA Others?	?
PPAR α	?	?	TCA
Cell transformation	MIA	DBA	?



HAA's Mechanistic and Other Relevant Data



Questions?



HAAs Mechanistic and Other Relevant Data

Reviewer Questions

- Comment on whether the information on Disposition and Toxicokinetics is clear, technically correct, and objectively presented.
- Comment on whether the information on Mechanistic and Other Relevant Data is clear, technically correct, and objectively presented.
- Comment on and provide any scientific criticisms of NTP's assessment of the mechanistic data for haloacetic acids found as water disinfection by-products.
- Identify any information that should be added or deleted.