Other Relevant Data

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HAAs Other Relevant Data

Outline

Disposition & Toxicokinetics	• ADME • Clearance
Mechanistic Data	• Characteristics of carcinogens • Potential modes of action and key events

HAAs Absorption

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: <0.001 - 0.003 cm/hr
  - 1% of total exposure

HAAs Distribution

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

HAAs Metabolism

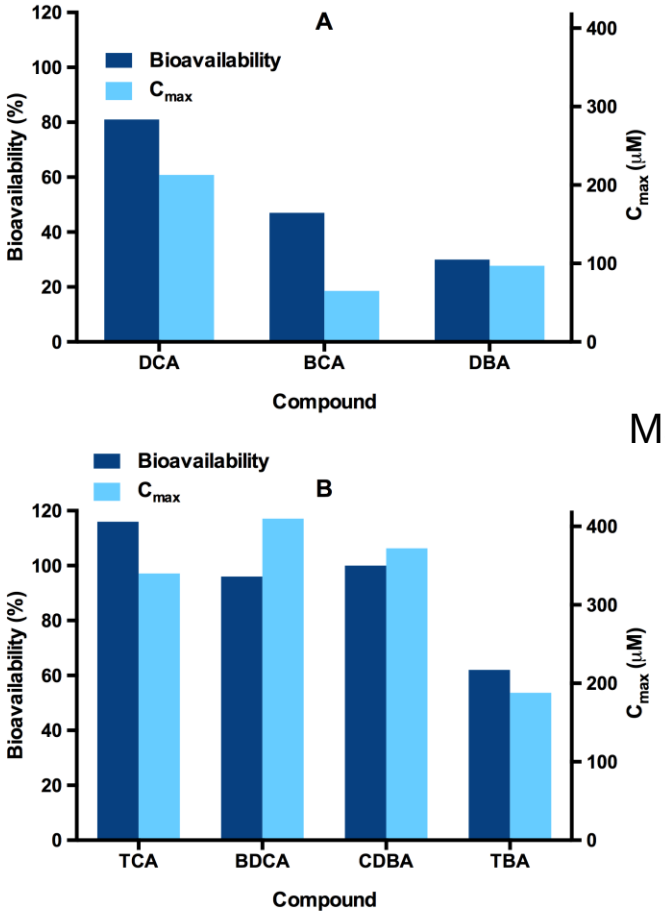
Metabolism is similar in humans and rodents

- 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 2× > rat 5× > 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 & Cmax

The number and type of halogens influence bioavailability and metabolism

- Di-HAAs
  - ↓ Bioavailabilty 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

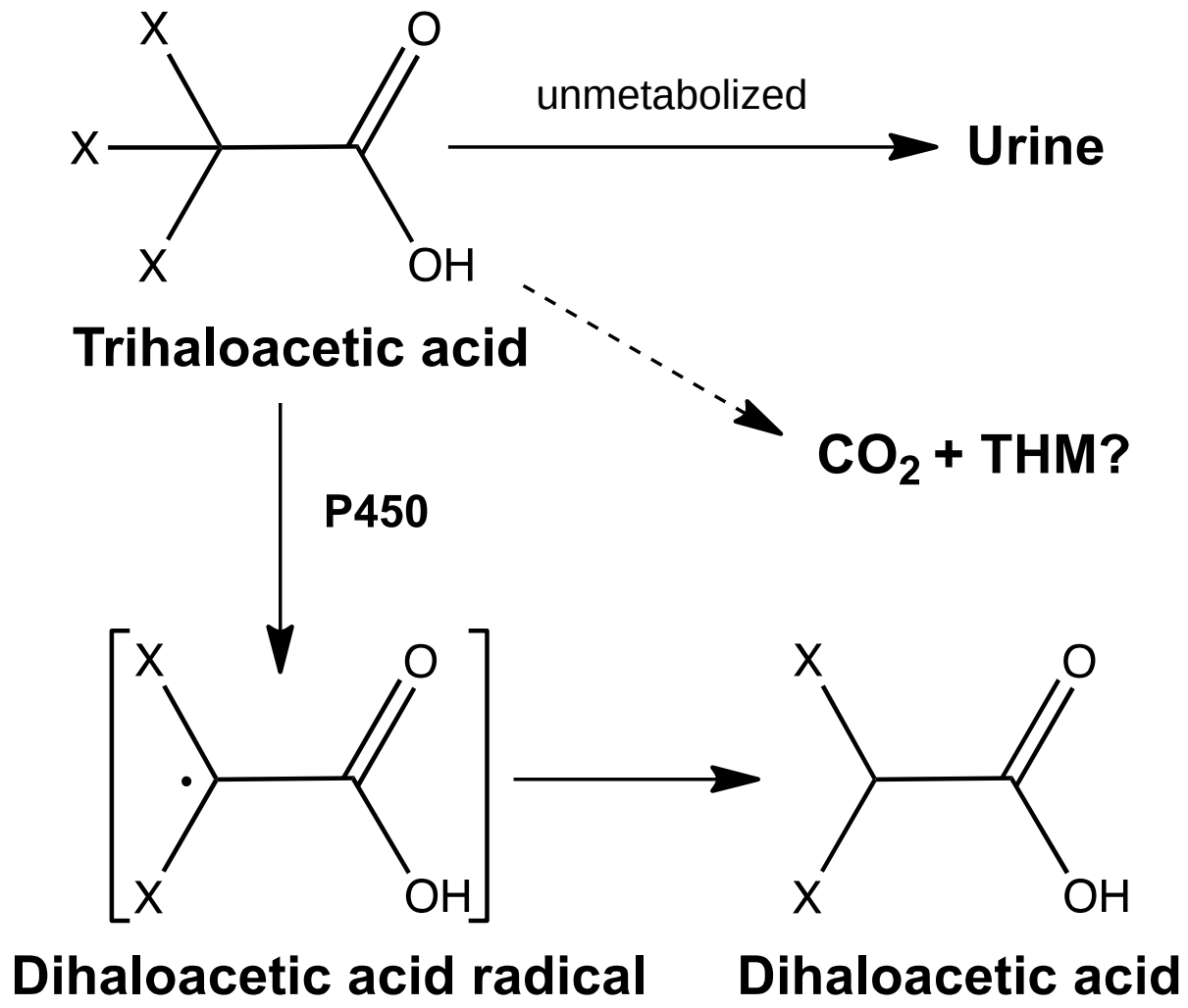


Male Rats

Source: Schultz et al. 1999

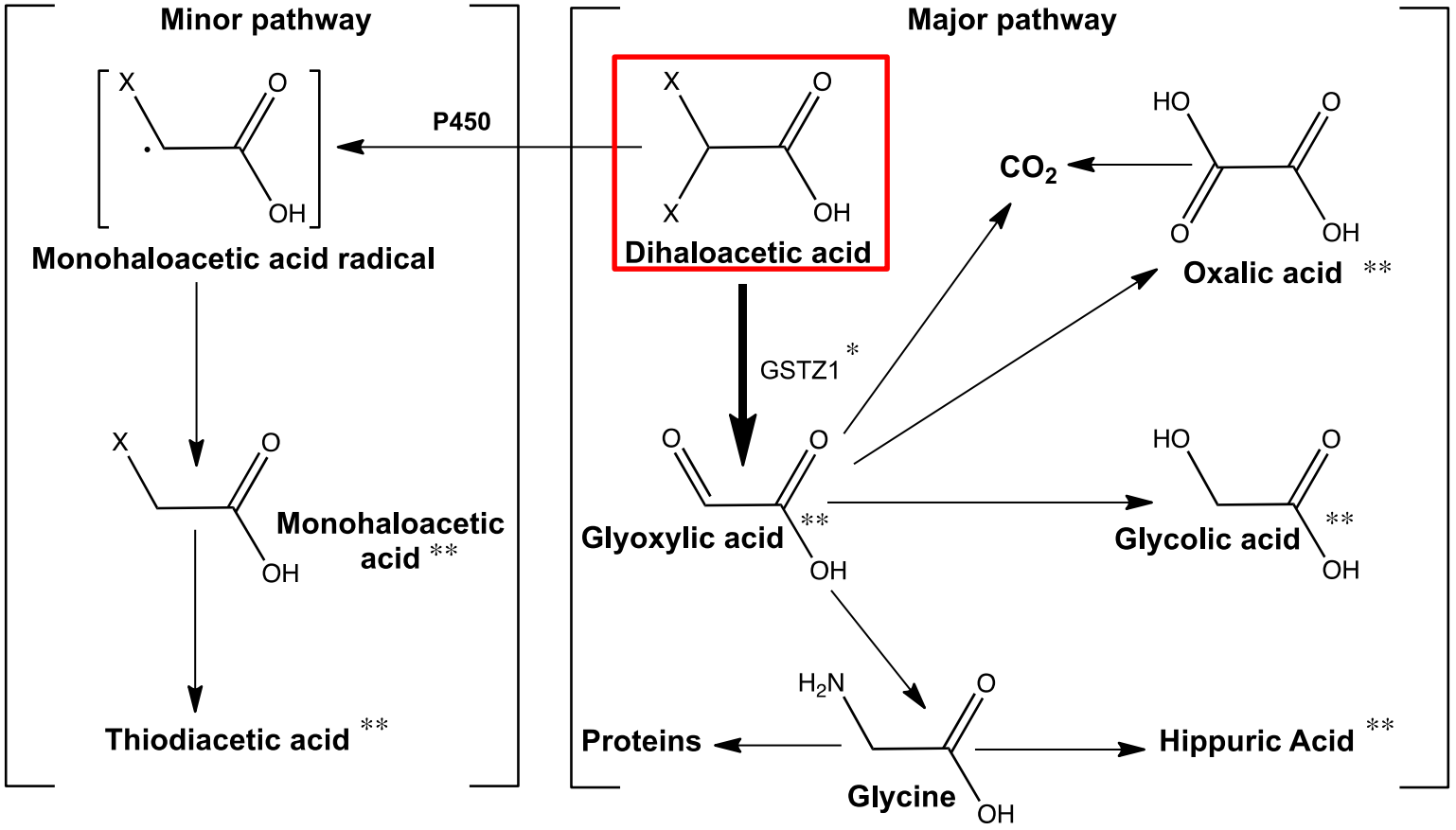
Tri-HAA Metabolism and Excretion

Tri-HAAs are metabolized by P450 reductive dehalogenation



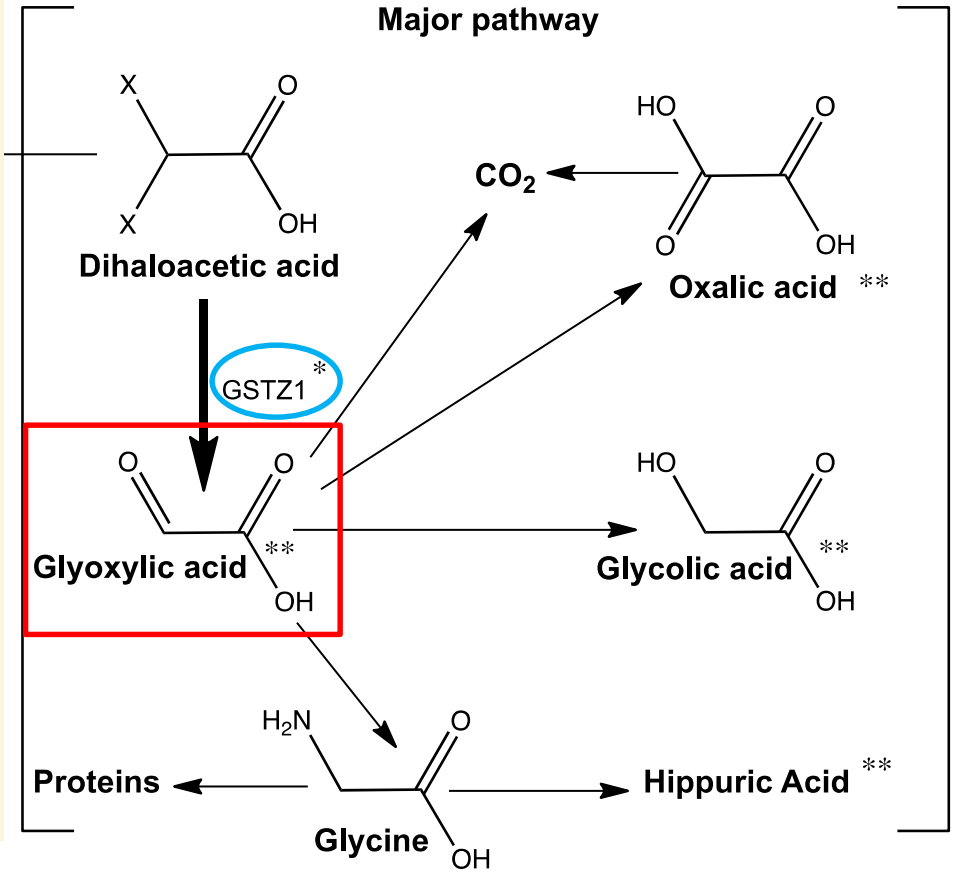
Di-HAA Metabolism and Excretion

Di-HAAs are metabolized via multiple pathways



Di-HAA Metabolism and Excretion

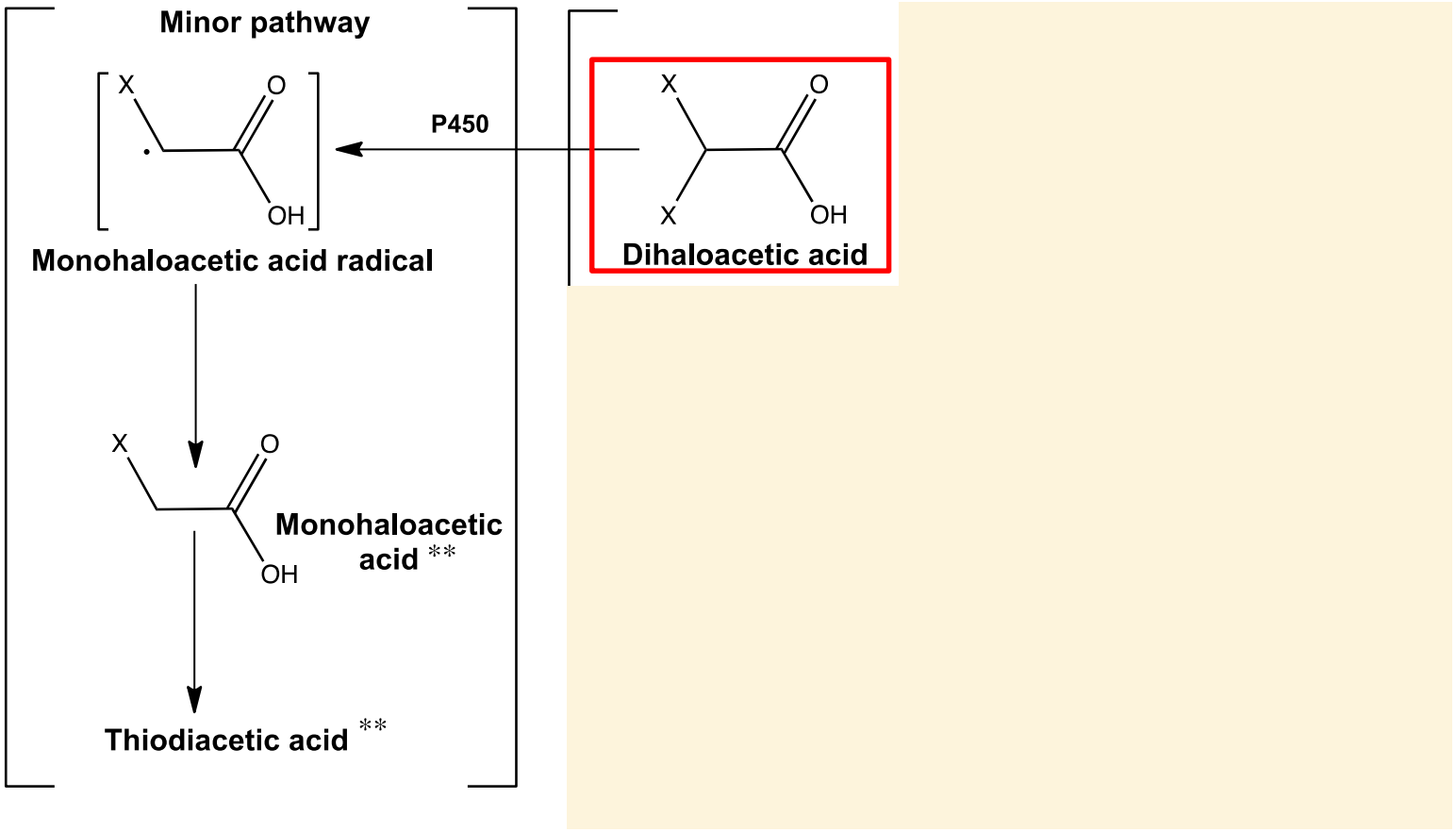
Major pathway: cytosolic GST-ζ to glyoxylic acid



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

Di-HAA Metabolism and Excretion

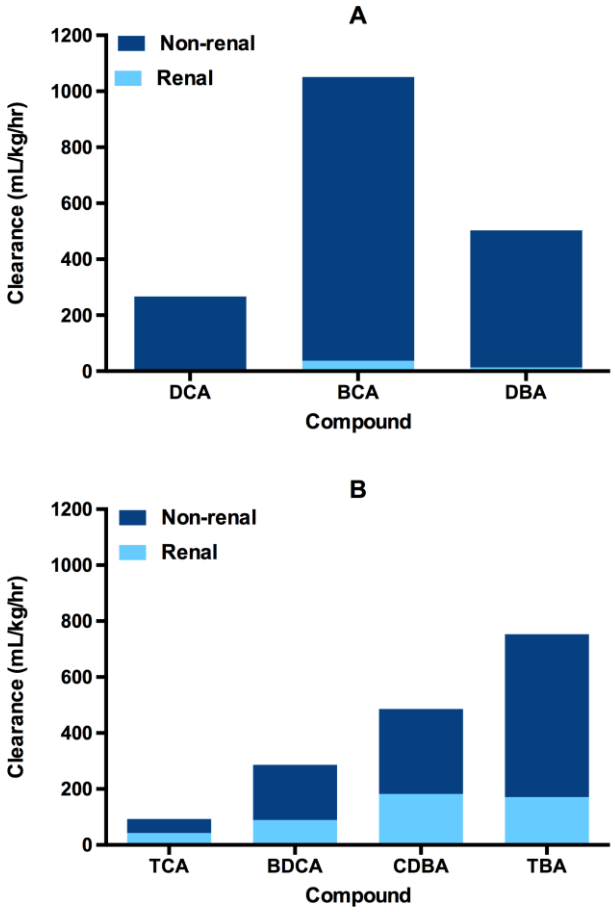
Di-HAAs: Minor pathway P450 reductive dehalogenation



** 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

Source: Schultz *et al.* 1999

HAAs Disposition and Toxicokinetics

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

HAAs Disposition and Toxicokinetics

Questions?

HAAs Mechanistic Data: Approach

No clearly defined mechanisms of carcinogenicity

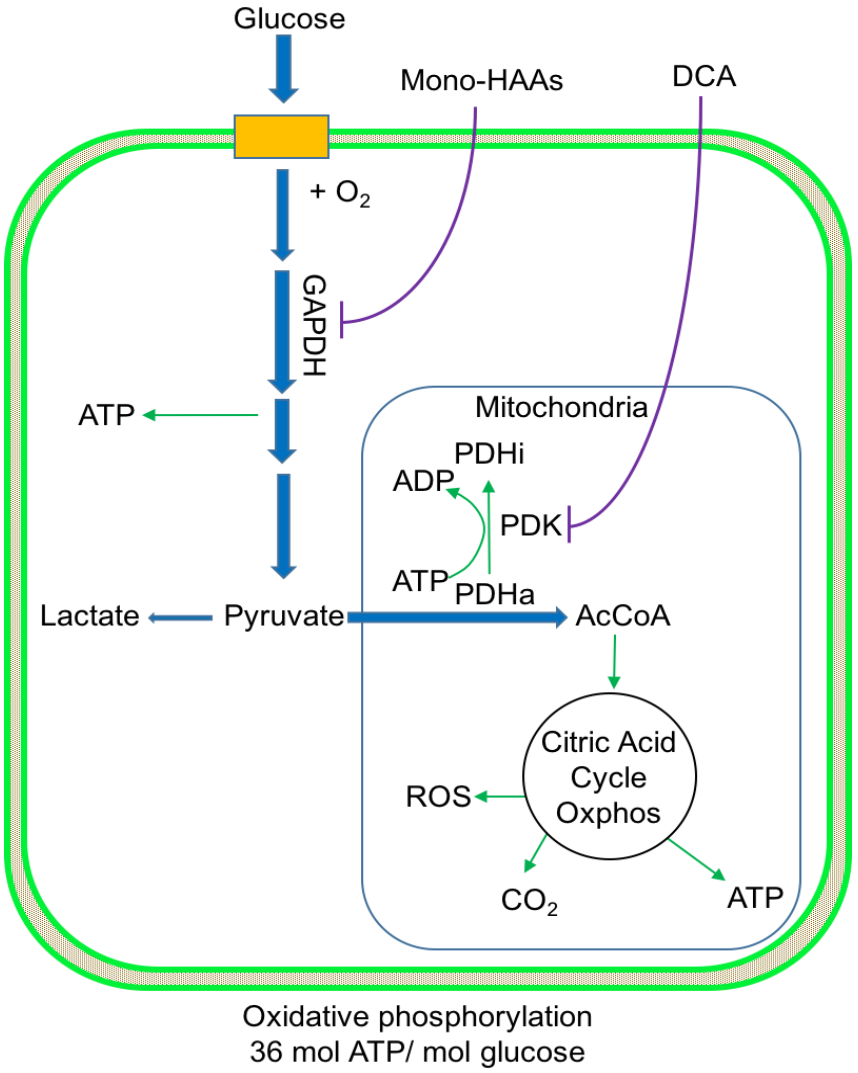
- Informed by characteristics of carcinogens (CoC)*
  - Act as an electrophile
  - Induce oxidative stress
  - Induce genotoxic effects
  - Induce epigenetic effects
  - Modulate receptor-mediated effects
  - Alter cell proliferation, death, nutrient supply
  - Cause cell immortalization
- Evaluate trends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

* Smith *et al.* 2016

Electrophilic Properties

All HAAs are relatively soft electrophiles

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

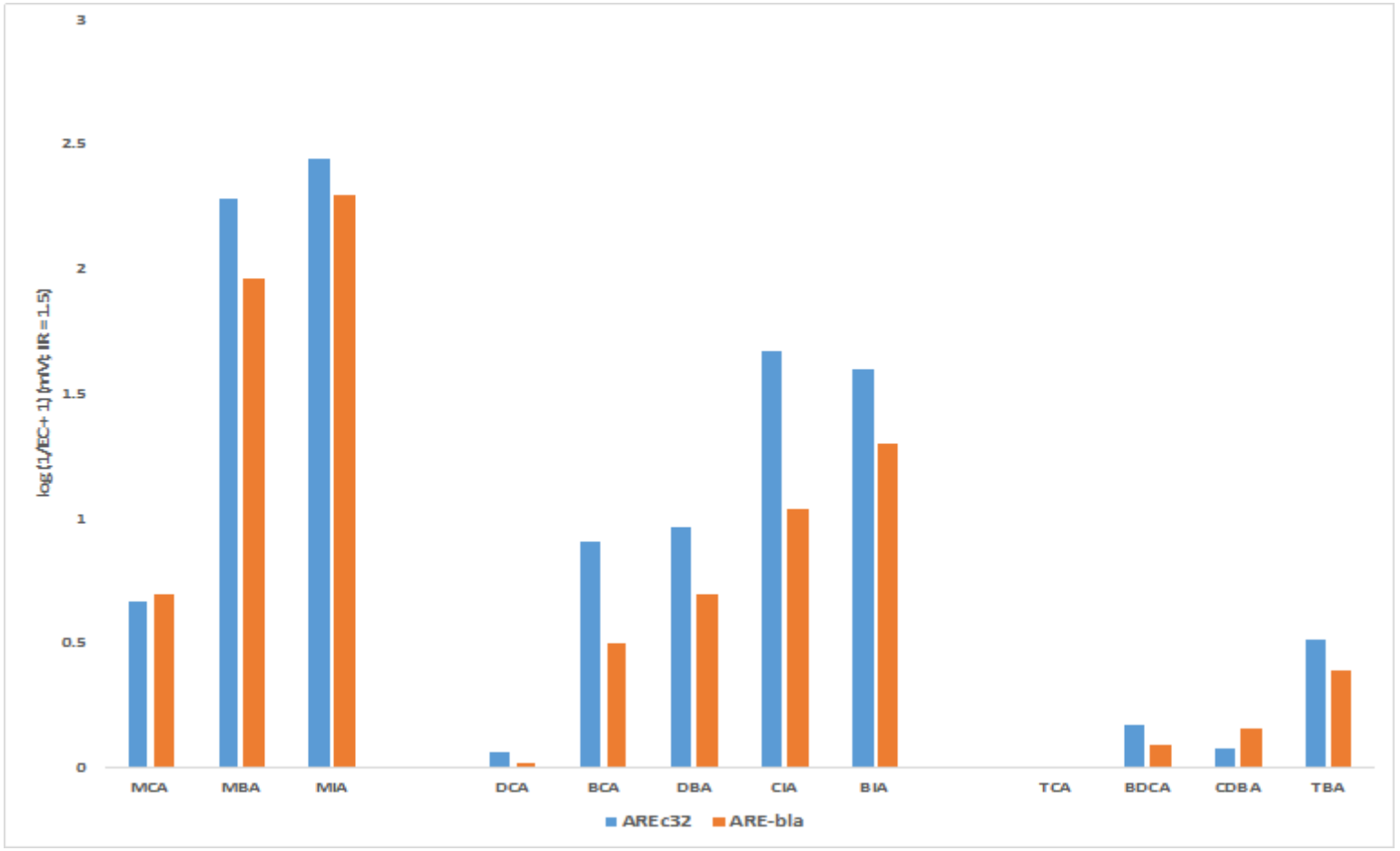


All HAAs induce oxidative stress

- 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
  - PPARα activation
- Positive correlation with genotoxicity
  - Treatment with antioxidants reduced genotoxicity

Oxidative Stress: Nrf2/ARE assay

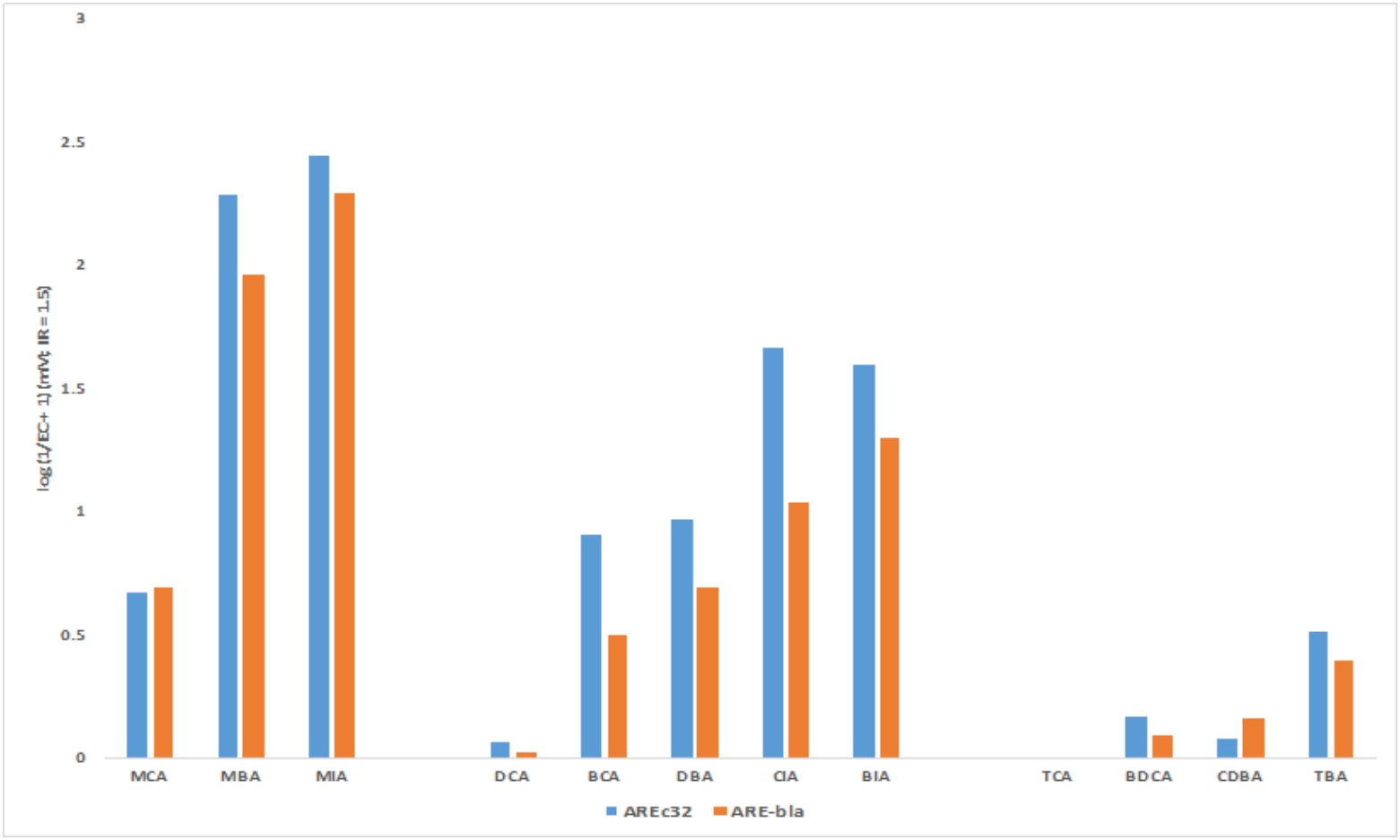
Potency decreases with the number of halogens



Source: Stalter *et al.* 2016
AREc32 = MCF-7 breast cancer cell line
ARE-bla = HepG2 hepatocellular carcinoma cell line

Oxidative Stress: Nrf2/ARE assay

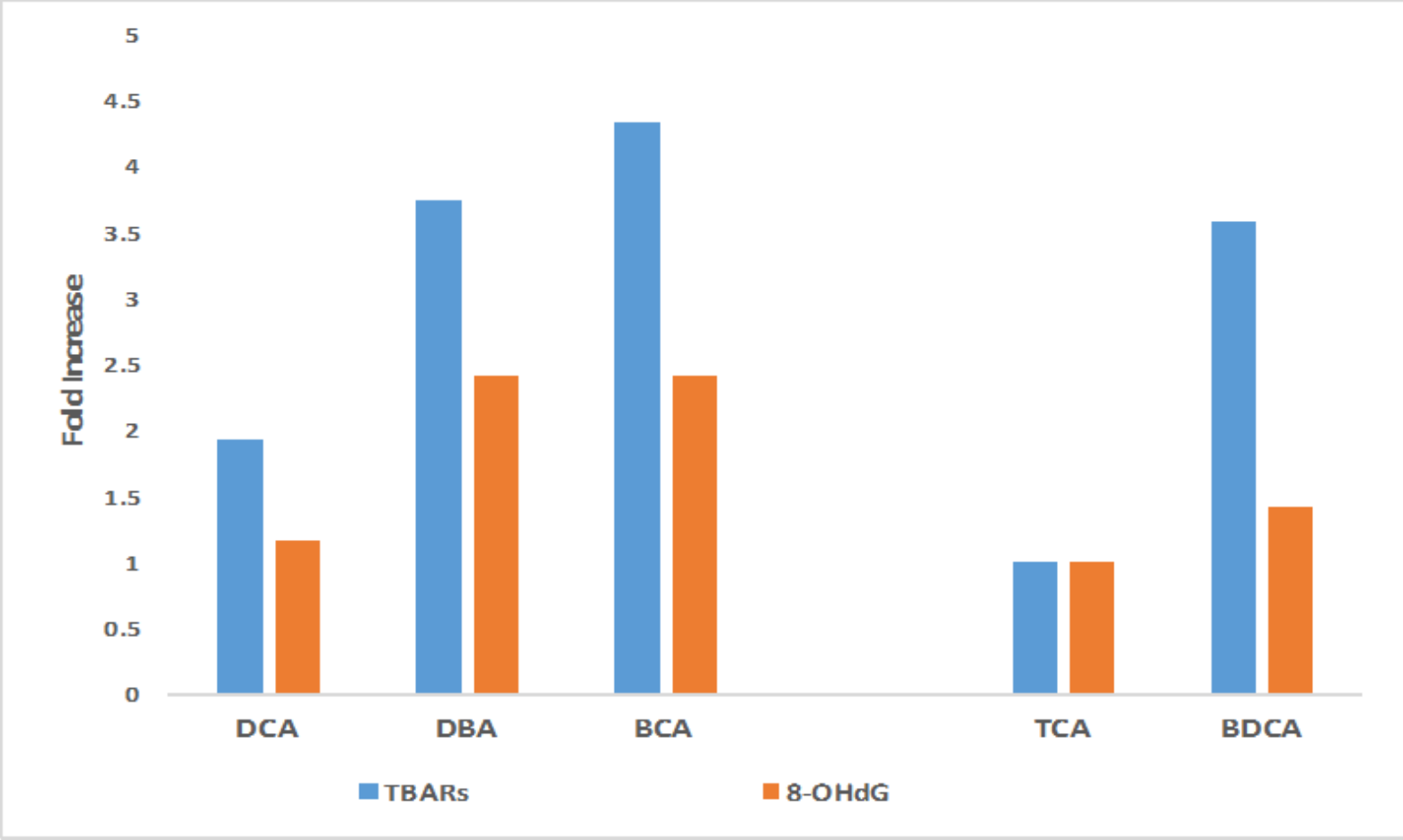
Potency increases with the type of halogen: I > Br >>Cl



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



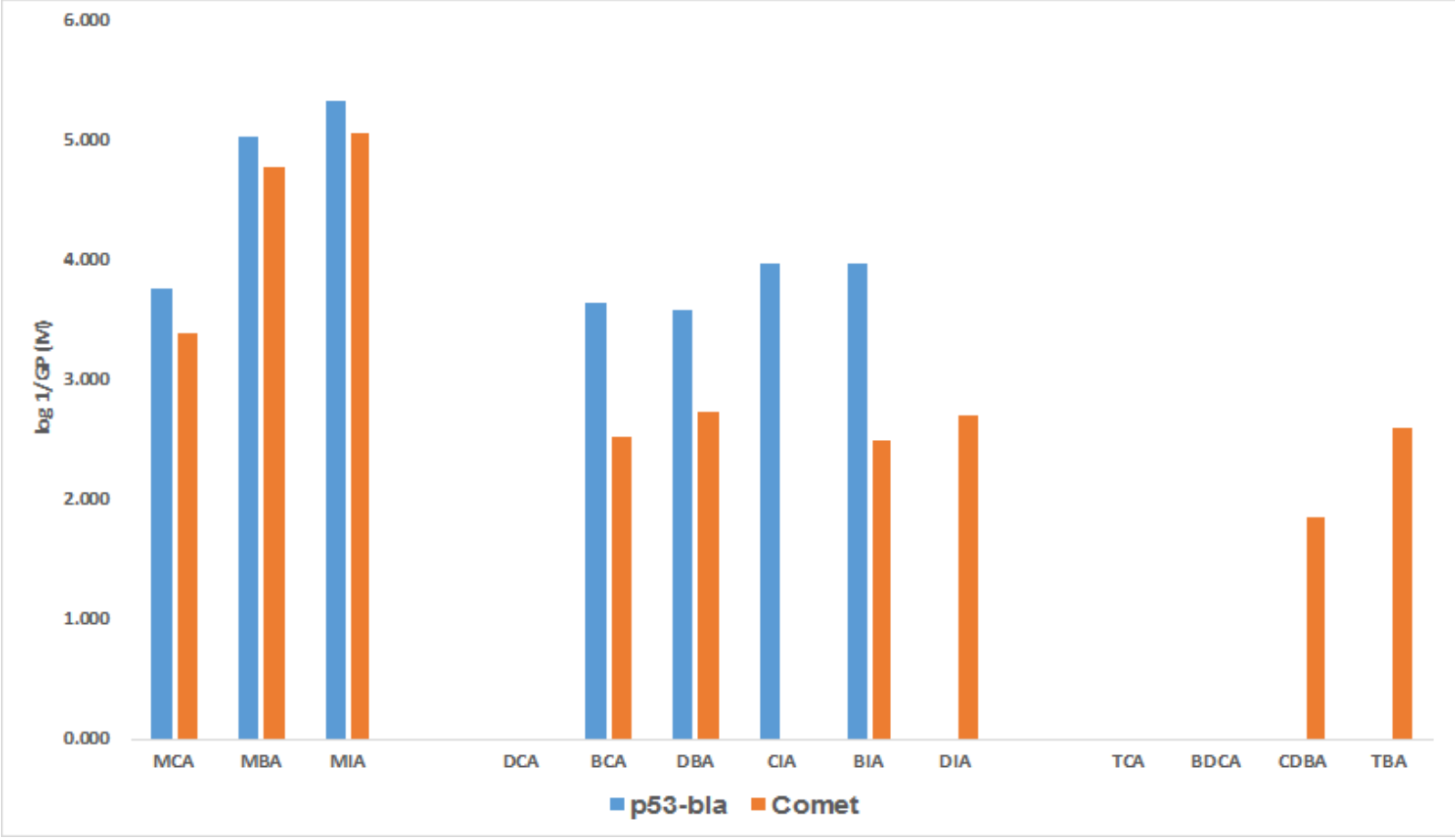
Sources: Larson and Bull 1992, Austin et al. 1996

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)
- PPARα activation (TCA)
- Cell transformation (MIA, DBA)

HAAs Mechanistic Data

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)
Hypomethyl-ation	?	DCA DBA Others?	TCA Others?
GAPDH inhibition	All	?	?
PDK inhibition	?	DCA Others?	?
PPARα	?	?	TCA
Cell trans-formation	MIA	DBA	?

HAAs 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.