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
HAAs Other Relevant Data

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: <0.001 - 0.003 cm/hr
  - 1% of total exposure
Rapid with little to no bioaccumulation in tissues

<table>
<thead>
<tr>
<th>HAA</th>
<th>$V_{d_{ss}}$ (mL/kg)</th>
<th>Unbound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCA</td>
<td>618</td>
<td>94</td>
</tr>
<tr>
<td>BCA</td>
<td>881</td>
<td>93</td>
</tr>
<tr>
<td>DBA</td>
<td>400</td>
<td>89</td>
</tr>
<tr>
<td>TCA</td>
<td>782</td>
<td>53</td>
</tr>
<tr>
<td>BDCA</td>
<td>730</td>
<td>49</td>
</tr>
<tr>
<td>CDBA</td>
<td>636</td>
<td>55</td>
</tr>
<tr>
<td>TBA</td>
<td>449</td>
<td>18</td>
</tr>
</tbody>
</table>

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 \(2 \times\) > rat \(5 \times\) > 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
The number and type of halogens influence bioavailability and metabolism

- **Di-HAAs**
  - ↓ Bioavailability vs. Tri-HAAs
  - ↑ First-pass metabolism
  - ↓ $C_{\text{max}}$ vs. Tri-HAAs

- **Tri-HAAs**
  - High bioavailability
  - ↓ Metabolism vs. Di-HAAs
  - Higher $C_{\text{max}}$

- **Br substitution for Cl**
  - ↑ First pass metabolism
  - TBA resembles Di-HAA

*Source: Schultz et al. 1999*
Tri-HAAs are metabolized by P450 reductive dehalogenation

Trihaloacetic acid

P450

Dihaloacetic acid radical

Dihaloacetic acid

unmetabolized

Urine

CO₂ + THM?
Di-HAAs are metabolized via multiple pathways.

**Minor pathway:**
- Monohaloacetic acid radical
- Monohaloacetic acid
- Thiodiacetic acid

**Major pathway:**
- Dihaloacetic acid
- Glyoxyl acid
- Glycolic acid
- Proteins
- Glycine
- Hippuric Acid
- Oxalic acid
- CO₂

**Catalysts:**
- P450
- GSTZ1

The diagram illustrates the metabolic pathways of di-haloacetic acids (Di-HAAs) showing the conversion of dihaloacetic acid into various metabolites, including oxalic acid, CO₂, proteins, glycine, and hippuric acid.
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

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
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

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

All HAAs induce oxidative stress
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
Brominated analogues are more potent

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

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

<table>
<thead>
<tr>
<th>CoCs</th>
<th>Mono-</th>
<th>Di-</th>
<th>Tri-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophilic</td>
<td>All</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Ox stress</td>
<td>All</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Genotoxic</td>
<td>All</td>
<td>All</td>
<td>All (-TCA)</td>
</tr>
<tr>
<td>Hypomethylation</td>
<td>?</td>
<td>DCA</td>
<td>TCA Others?</td>
</tr>
<tr>
<td>GAPDH inhibition</td>
<td>All</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>PDK inhibition</td>
<td>?</td>
<td>DCA</td>
<td>?</td>
</tr>
<tr>
<td>PPARα</td>
<td>?</td>
<td>?</td>
<td>TCA</td>
</tr>
<tr>
<td>Cell transformation</td>
<td>MIA</td>
<td>DBA</td>
<td>?</td>
</tr>
</tbody>
</table>
Questions?
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.