Predicting acute toxicity hazard in the absence of experimental data: Case studies from the alternatives assessment paradigm

Jay Tunkel

SRC, Inc. Syracuse, New York, USA
Who Are These Guys?

- Founded in 1957 as Syracuse University Research Corporation as a not-for-profit organization by the NY State Board of Regents
- Became Syracuse Research Corporation in 1976, and SRC, Inc. in 2009
EPA
- New Chemicals program (>25,000 PMNs assessed)
- Safer Choice/DfE (supported AAs, SPLP, and SCIL since their inception)
- IRIS

Other Government
- NLM/NIH – HSDB
- CDC/ATSDR Toxicological profiles
- OSHA – PELs
- State Agencies
- DoD
The Chemical Hazard Assessment Group

QSAR

Databases

New Chemicals

Toxicology

Safer Chemicals

Existing Chemicals
All chemical information presented herein is available in the public domain.

Views expressed are those of the author alone.
# PBT Characteristics

<table>
<thead>
<tr>
<th>Persistence</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life</td>
<td>&lt;60 days</td>
<td>60-180 days</td>
<td>&gt;180 days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bioaccumulation</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCF</td>
<td>&gt;1,000</td>
<td>1,000-5,000</td>
<td>&gt;5,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aquatic Toxicity</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>&gt;100 mg/L</td>
<td>1-100 mg/L</td>
<td>&lt;1 mg/L</td>
</tr>
<tr>
<td>Chronic</td>
<td>&gt;10 mg/L</td>
<td>0.1-10 mg/L</td>
<td>&lt;0.1 mg/L</td>
</tr>
</tbody>
</table>

**QSAR?**

- ✓
Hierarchy for Predicting HH Hazard

- **Analog**
  - Data available on closely related compounds

- **Read Across**
  - Data available for multiple analogs

- **Chemical class**
  - Local effects
  - Functional groups
  - Mechanistic basis

- **Also considered...**
  - Metabolites
  - Reaction products (e.g., hydrolysis)
### Predicting Concern, Includes LOW Toxicity

<table>
<thead>
<tr>
<th>Company</th>
<th>Chemical</th>
<th>% in Formulation</th>
<th>Human Health Effects</th>
<th>Ecotoxicity</th>
<th>Environmental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cancer Hazard</td>
<td>Skin Sensitizer</td>
<td>Reproductive</td>
</tr>
<tr>
<td>Albemarle</td>
<td>ANTIBLAZE 180 and ANTIBLAZE 195</td>
<td>95%</td>
<td>M</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Tris(1,3-dichloro-2-propyl)Phosphate CAS # 13674-87-8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albemarle</td>
<td>ANTIBLAZE 182 and ANTIBLAZE 205</td>
<td></td>
<td>M</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Proprietary A Chloroalkyl phosphate (1)</td>
<td></td>
<td>M</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Proprietary B Aryl phosphate</td>
<td></td>
<td>L</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Triphenyl Phosphate CAS # 115-86-6</td>
<td></td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Albemarle</td>
<td>ANTIBLAZE V500</td>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Proprietary C Chloroalkyl phosphate (2)</td>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Proprietary B Aryl phosphate</td>
<td></td>
<td>L</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Triphenyl Phosphate CAS # 115-86-6</td>
<td></td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Route</td>
<td>VH</td>
<td>H</td>
<td>M</td>
<td>L (P/F)</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Oral, LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg bw)</td>
<td>≤50</td>
<td>&gt;50-300</td>
<td>&gt;300-2,000</td>
<td>&gt;2,000</td>
<td></td>
</tr>
<tr>
<td>Dermal, LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg bw)</td>
<td>≤200</td>
<td>&gt;200-1,000</td>
<td>&gt;1,000-2,000</td>
<td>&gt;2,000</td>
<td></td>
</tr>
<tr>
<td>Inhalation, LC&lt;sub&gt;50&lt;/sub&gt; (vapor/gas; mg/L)</td>
<td>≤2</td>
<td>&gt;2-10</td>
<td>&gt;10-20</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>Inhalation, LC&lt;sub&gt;50&lt;/sub&gt; (dust/mist/fumes; mg/L/d)</td>
<td>≤0.5</td>
<td>&gt;0.5-1</td>
<td>&gt;1.0-5</td>
<td>&gt;5</td>
<td></td>
</tr>
</tbody>
</table>
We Are Modelers

EPISuite™

EPI Suite - Welcome Screen

DEXAMETHASONE
Pregna-1, 4-diene-3, 20-dione,
9-fluoro-11,17,21-trihydroxy-16-
methyl- (11.beta,16.alpha)-
• C22H29F3O3

CAS RN: 50-02-2
SMILES:
C1=O=C/C2CCC3C4C(C)(C)O(C)(C)
(=O)(O)(C)C4(C)(C)O=C/C2=C(C)(O)C="C1

log Kow = 1.72 log BCF = 0.80

ECOSAR

Defense Environment Intelligence
On the Way to Work....
International QSAR Workshop

The 13th International Workshop on QSARs in the Environmental Sciences

June 8th - 12th, 2008  ·  Syracuse, New York, USA

QSAR 2016  ~  June 13th-17th  ~  Miami Beach
What About the Dis-Organics?

- Organic: 60%
- Polymer: 30%
- Inorganic: 5%
- Organometallic: 5%
Don’t forget the importance of Physical/Chemical properties!

Mechanistic understanding & verification of:
- Functional group(s)
- Mechanism
- Metabolites
- Electronic affects
- Steric demands
- Initiating event (AOP)
- Mixtures
Analog Identification Methodology (AIM)

Product developed to address stakeholder comments from EPA’s Sustainable Futures Initiative

Free download from: www.epa.gov/oppt/sf/tools/aim.htm
AIM Methodology

Pass 1

Pass 2
AIM Results

Analog Report
For

CAS / ID: 111762
Name: Ethanol, 2-butoxy-
SMILE: O(CCCC)CCO
Options: None
Date: Jul 24, 2015 4:22 PM
78 AIM Results Found

Exact Chemical Match

2-BUTOXYETHANOL [111-76-2]
O(CCCC)CCO

Toxicity Data Available for this Compound
- RTECS
- HPV Challenge
  - OECD HPV
    * May also be located at: OECD
- ECOTOX
- TSCATS II
- ACToR
- TSCATS
- IRIS
## AIM Example

<table>
<thead>
<tr>
<th></th>
<th>Sandalore</th>
<th>Ebanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td>Sandalore</td>
<td>Ebanol</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td><img src="image1" alt="Structure of Sandalore" /></td>
<td><img src="image2" alt="Structure of Ebanol" /></td>
</tr>
<tr>
<td><strong>CAS No.</strong></td>
<td>65113-99-7</td>
<td>67801-20-1</td>
</tr>
<tr>
<td><strong>Acute toxicity</strong></td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Similarity = 91%**
ChemACE

- Chemical Assessment Clustering Engine
- Useful tool for building potential fragrance clusters
- Designed for non-experts
- Clusters chemicals from a user-supplied list based on common fragments
- Methodology defines clusters where members are analogs of each other

Free download at: www.epa.gov/oppt/sf/tools/chemace.htm
### Clustering (ChemACE)

<table>
<thead>
<tr>
<th>Cluster:</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ID</strong></td>
<td>107982</td>
</tr>
<tr>
<td><strong>SMILES</strong></td>
<td>O(CC(O)C)C</td>
</tr>
<tr>
<td><strong>Name</strong></td>
<td>1-Methoxy-2-Propanol</td>
</tr>
<tr>
<td><strong>Best MOA</strong></td>
<td>None</td>
</tr>
</tbody>
</table>
Case Studies

Organics
Read Across – Norobornyl Fragrances

<table>
<thead>
<tr>
<th>P; B; T</th>
<th>VL; L; M</th>
<th>L; M; M</th>
<th>L; M; H</th>
<th>VL; M; VH</th>
<th>L; M; H</th>
<th>L; L; M</th>
<th>L; M; H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Fail</td>
<td>Fail (Analog)</td>
<td>Fail (Analog)</td>
<td>Fail (Analog)</td>
<td>Fail (Analog)</td>
<td>Fail (Analog)</td>
<td>Fail (Analog)</td>
</tr>
</tbody>
</table>

- Rat (inhalation), LD<sub>50</sub> = 0.5 mg/L (ECHA)
- Rat (inhalation-dust), 2-hr, LD<sub>50</sub> > 10 mg/L (ECHA)
- Mouse (inhalation), 3-hr, LD<sub>L0</sub> = 0.4 mg/L (ChemID)
## Cyanide Formation from Nitriles

<table>
<thead>
<tr>
<th>Name</th>
<th>Benzyl cyanide</th>
<th>Geranyl nitrile</th>
<th>Citronellyl nitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td><img src="image" alt="benzyl cyanide structure" /></td>
<td><img src="image" alt="geranyl nitrile structure" /></td>
<td><img src="image" alt="citronellyl nitrile structure" /></td>
</tr>
<tr>
<td><strong>Rat Acute LD$_{50}$ (mg/kg)</strong></td>
<td>270</td>
<td>3,100</td>
<td>5,300</td>
</tr>
<tr>
<td><strong>Rabbit Acute LD$_{50}$ (mg/kg)</strong></td>
<td>270</td>
<td>4,300</td>
<td>No data</td>
</tr>
</tbody>
</table>

Structural alert for nitriles when stabilized

- Benzyl cyanide
- Geranyl nitrile
- Citronellyl nitrile
BPA In Thermal Paper

1) Heat (melt)
2) Transfer H⁺
3) Cool (solidify)
### Limited Data – Read Across from BPA

Endpoints in colored text (VL, L, M, H, and VH) were assigned based on empirical data. Endpoints in black italics (VL, L, M, H, and VH) were assigned using values from estimation software and professional judgment. Based on analogy to experimental data for a structurally similar compound.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Chemical (for TSCA inventory name and relevant trade names see the individual profiles in Section 4.8)</th>
<th>CASRN</th>
<th>Acute Toxicty</th>
<th>Carcinogenicity</th>
<th>Genotoxicity</th>
<th>Reproductive</th>
<th>Developmental</th>
<th>Neurological</th>
<th>Repated Dose</th>
<th>Skin Sensitization</th>
<th>Respiratory Sensitization</th>
<th>Eye Irritation</th>
<th>Dermal Irritation</th>
<th>Acute</th>
<th>Chronic</th>
<th>Persistence</th>
<th>Bioaccumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bisphenol A</td>
<td>80-05-7</td>
<td>L</td>
<td>M</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>VH</td>
<td>M</td>
<td>VL</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bisphenol F</td>
<td>620-92-8</td>
<td>L</td>
<td>M</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>M</td>
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<td>M</td>
<td>VH</td>
<td>M</td>
<td>H</td>
<td>L</td>
<td></td>
<td>L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bisphenol C</td>
<td>79-97-0</td>
<td>L_i</td>
<td>M</td>
<td>M_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>H_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>H</td>
<td></td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BisOPP-A</td>
<td>24038-68-4</td>
<td>L_i</td>
<td>M</td>
<td>M_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>L</td>
<td>H_i</td>
<td>H_i</td>
<td>M</td>
<td></td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bisphenol AP</td>
<td>1571-75-1</td>
<td>L_i</td>
<td>M</td>
<td>M_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>H_i</td>
<td>H_i</td>
<td>H_i</td>
<td>M</td>
<td></td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Substituted phenolic compound, PROPRIETARY #1</td>
<td></td>
<td>L_i</td>
<td>M</td>
<td>M_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>H_i</td>
<td>H_i</td>
<td>H_i</td>
<td>M</td>
<td></td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Substituted phenolic compound, PROPRIETARY #2</td>
<td></td>
<td>L_i</td>
<td>M</td>
<td>M_i</td>
<td>H_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>M_i</td>
<td>H_i</td>
<td>H_i</td>
<td>H_i</td>
<td>H</td>
<td></td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>
Flame Retardant Alternatives

<table>
<thead>
<tr>
<th>CASRN</th>
<th>Chemical</th>
<th>Molecular Weight</th>
<th>State</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-96-8</td>
<td>Tris (2-chloroethyl) phosphate</td>
<td>285.49</td>
<td>Liquid</td>
<td>46.4 mg/kg Rat</td>
</tr>
<tr>
<td>13674-84-5</td>
<td>Tris (2-chloro-1-methylethyl) phosphate</td>
<td>327.57</td>
<td>Liquid</td>
<td>930 mg/kg Rat</td>
</tr>
<tr>
<td>13674-87-8</td>
<td>Tris(1,3-dichloro-2-propyl)phosphate</td>
<td>430.91</td>
<td>Liquid</td>
<td>&gt;2000 mg/kg Rat</td>
</tr>
</tbody>
</table>
Melamine (TBBPA AA)

Rat LD$_{50}$ > 3,160 mg/kg

Rat LD$_{50}$ > 5,000 mg/kg

Super-strong hydrogen Bonds (pH dependent)

Forms insoluble crystals in kidneys

Can QSARs be expected to provide reasonable results for these exceptions?
Phase I clinical testing, all subjects experienced acute renal failure (Mike Bolger Webinar)

“...physicochemical properties can serve a critical role in alternatives assessment processes…” (NAS 2014; Tickner, et al. 2015)
AOP Development

ADME

Molecular Initiating Events

Absorption of 2-butoxyethanol

Metabolism to butoxyacetic acid

Erythrocyte uptake of butoxyacetic acid

↑ Affinity for K⁺ on intracellular side of Na⁺/K⁺ ATPase pump (Skou and Esman 1980)

↑ [H⁺]₁ (Louisse et al. 2010)

Activation of Ca²⁺/H⁺ antiporter

Chelation of extracellular Ca²⁺ (Starek and Nowak, 2006)

Erythrocyte response

Intravascular response

Extravascular response

Compensatory hematopoiesis; hemoglobinuria; hemosiderin deposition in liver

↑ [Na⁺]₁ (Udden 2002)

Water influx, swelling (Udden 2002)

Osmotic lysis (Udden et al., 2005)

↓ deformability (Udden 2002)

Activation of Gardos channel (Ca²⁺/K⁺ antiporter)

Externalization of membrane phosphatidylserine (Tamura et al., 2002)

RBC adhesion to endothelial cells, generation of thrombin (Koshkanyev et al. 2003)

Intra-vascular occlusion and infarctions (unpublished data, as cited in Ghayum 1990)

Disseminated thrombosis

Bold outline: potential molecular initiating event
AOP - Oxygen is Important

\[ \text{Metal cation} \rightarrow \text{Complex} \]

1. \( RCO\_2OH \) + Metal cation \( \rightarrow \) \( RCO\_2M \_OR\_2 \)
2. \( RCO\_2OH \) + Metal cation \( \rightarrow \) \( \text{Complex} \)
Case Studies

Inorganics, Organometallics and Polymers
Inorganics and Organometallics

**Chemicals**
- Dicopper chloride trihydroxide
- Copper sulfate pentahydrate
- Dicopper oxide

**LD₅₀**
- 299-2,006 mg/kg; in rat and mice

**Safer Choice (DfE)**
- Fail

Porphyrrins (includes phthalocyanines) – 6 members total, data on 2

- Chlorophyll
- C.I. Pigment Green 7 2,000 mg/kg
- Direct Blue 86 >5,000 mg/kg
AOP Development

**ADME**
- Absorption of 2-butoxyethanol
  - Metabolism to butoxyacetic acid
    - Erythrocyte uptake of butoxyacetic acid

**Molecular Initiating Events**
- ↑ Affinity for K⁺ on intracellular side of Na⁺/K⁺ ATPase pump (Skou and Esman 1980)
  - ↑ [H⁺]ᵢ (Louisse et al. 2010)
  - Activation of Ca²⁺/mH⁺ antiporter
    - Chelation of extracellular Ca²⁺ (Starek and Nowak, 2006)
  - ↑ [Ca²⁺]ᵢ (Udden et al. 2005)

**Erythrocyte Response**
- Water influx, swelling (Udden 2002)
- ↑ Activity of Na⁺/K⁺ ATPase pump
- Activation of Gardos channel (Ca²⁺/K⁺ antiporter)
- ↓ ATP (Ghanayem et al. 1992; Starek, 2008)
- Externalization of membrane phosphatidyl serine (Tamara et al. 2002)

**Intravascular Response**
- Osmotic lysis (Udden et al. 2005)
- ↓ deformability (Udden 2002)
- RBC adhesion to endothelial cells, generation of thrombin (Koshkarev et al. 2003)
- Intra-vascular occlusion and infarctions (unpublished data, as cited in Ghanayem 1996)

**Extravascular Response**
- Compensatory hematopoiesis; hemoglobinuria; hemosiderin deposition in liver

**Bold outline:** potential molecular initiating event
AOP - Oxygen is Important

\[
\begin{align*}
\text{R-O-CH}_2-\text{COOH} + \text{Metal cation} & \rightarrow \text{R-O-CH}_2-\text{COOM} + \text{COOH} \\
\text{R-CH}_2-\text{CH(OH)-COOH} + \text{Metal cation} & \rightarrow \text{R-CH}_2-\text{CH(OH)-COOM} + \text{COOH}
\end{align*}
\]
Predicting Polymer Toxicity

**Category 1**
- Low molecular weight polymers
- $\text{MW}_n < 1,000$

**Category 2**
- Polymers with high & low MW components
- $\text{MW}_n > 1,000$ and $\geq 25\%$ with $\text{MW} < 1,000$; $\geq 10\%$ with $\text{MW} < 500$

**Category 3**
- High molecular weight polymers
- $\text{MW}_n > 1,000$

Polymers with potential toxicity are:
- Swellable polymers
- Pendant functional groups of concern (e.g., epoxides)

http://www.epa.gov/oppt/newchems/pubs/hmwtpoly.htm
Polymer Flame Retardant Alternatives
Thank You! Questions?

Tunkel@srcinc.com