Integrated Approaches for Testing and Assessment for Developmental Neurotoxicity

*Organophosphorus flame retardants: A Case Study*

Mamta Behl, Ph.D., DABT

ICCVAM Public Forum
May 21st, 2020
ICCVAM and IATAs

- IATAs integral component of ICCVAM; various applications
  - Skin Sensitization, Endocrine disruption
- Expansion of Biological Space for IATAs
- OECD DNT Expert Group developing a guidance document using IATAs for DNT that can be used for regulatory decision-making
  - Recent Meeting held in April 2020
- NTP developing a DNT IATA case study for the OECD guidance document
  - Efforts could feed into ICCVAM
Today: Flame Retardants as a case example of a DNT IATA for hazard characterization and prioritization
Introduction: Exposure to Flame Retardants

How Toxic Flame Retardants Travel Into You
Introduction: Phased out vs Novel Replacements

- Projected increase in exposure & use of organophosphate flame retardants (OPFRs) following:
  - Voluntary phase-out of polybrominated diphenyl ethers (BDEs)
  - CPSC petition to ban organohalogens in 2017; NAS report generated in response in 2019
- Concerns for DNT in infants and toddlers- car seats; mouthing
- Lack of toxicity data on hazard characterization & risk assessment
  - Regrettable substitutes?
Introduction: Why IATA for OPFRs?

• Projected increase in exposure

• 20-50 compounds in class including commercial and isomeric mixtures

• Cannot test our way through all combinations using traditional animal guideline studies

• Need strategy to prioritize compounds for further in-depth hazard characterization

\[
\begin{align*}
X & \quad Y \\
& \\
& \\
R & = \text{aryl or alkyl}
\end{align*}
\]
Purpose of the IATA

- Screen compounds for prioritization for further testing
- Hazard ID/ characterization
- Timely dissemination of information
### Representative Brominated FRs (BFRs)

<table>
<thead>
<tr>
<th>CAS</th>
<th>Chemical Name</th>
<th>Chemical.ID</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5436-43-1</td>
<td>2,2',4,4'-Tetrabromodiphenyl ether</td>
<td>BDE-47</td>
<td><img src="image" alt="BDE-47 Structure" /></td>
</tr>
<tr>
<td>79-94-7</td>
<td>3,3',5,5'-Tetrabromobisphenol A</td>
<td>TBBPA</td>
<td><img src="image" alt="TBBPA Structure" /></td>
</tr>
</tbody>
</table>

### Organophosphorous FRs (OPFRs) - aliphatic, halogenated

<table>
<thead>
<tr>
<th>CAS</th>
<th>Chemical Name</th>
<th>Chemical.ID</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>13674-87-8</td>
<td>Tris(1,3-dichloro-2-propyl)phosphate</td>
<td>TDCIPP</td>
<td><img src="image" alt="TDCIPP Structure" /></td>
</tr>
<tr>
<td>115-96-8</td>
<td>Tris(2-chloroethyl) phosphate</td>
<td>TCEP</td>
<td><img src="image" alt="TCEP Structure" /></td>
</tr>
</tbody>
</table>

### Organophosphorous FRs (OPFRs) - Aromatic

<table>
<thead>
<tr>
<th>CAS</th>
<th>Chemical Name</th>
<th>Chemical.ID</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-86-6</td>
<td>Triphenyl phosphate</td>
<td>TPHP</td>
<td><img src="image" alt="TPHP Structure" /></td>
</tr>
<tr>
<td>68937-41-7</td>
<td>Phenol, isopropylated, phosphate (3:1)</td>
<td>IPP*</td>
<td><img src="image" alt="IPPI Structure" /></td>
</tr>
<tr>
<td>1241-94-7</td>
<td>2-Ethylhexyl diphenyl phosphate</td>
<td>EHDP*</td>
<td><img src="image" alt="EHDP Structure" /></td>
</tr>
<tr>
<td>1330-78-5</td>
<td>Tricresyl phosphate</td>
<td>TMPP*</td>
<td><img src="image" alt="TMPP Structure" /></td>
</tr>
<tr>
<td>29761-21-5</td>
<td>Isodecyl diphenyl phosphate</td>
<td>IDDP</td>
<td><img src="image" alt="IDDP Structure" /></td>
</tr>
<tr>
<td>56803-37-3</td>
<td>tert-Butylphenyl diphenyl phosphate</td>
<td>BPDP*</td>
<td><img src="image" alt="BPDP Structure" /></td>
</tr>
<tr>
<td>78-30-8</td>
<td>Tri-o-cresyl phosphate</td>
<td>TOCP</td>
<td><img src="image" alt="TOCP Structure" /></td>
</tr>
</tbody>
</table>

*representative isomer in mixture is shown as structure

**BDEs (Phased-out)**

**TBBPA (Extensively used)**

Aliphatic organohalogens (petition to ban)

Aromatic phosphates (novel replacements)
NTPs DNT Screening Battery

2-D in vitro assays + 3-D neurospheres + Zebrafish
Applying our capabilities in deliberate, integrated and complementary ways.
Hypothesis

The replacement aromatic OPFRs are less active (toxic) than the phased-out BDEs and hence are currently being used as substitutes.
Comparison of OPFRs with phased-out compounds (2D + Behavior)

Flame Retardants

<table>
<thead>
<tr>
<th>Compound Description</th>
<th>BHC (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE-153)</td>
<td>[Data]</td>
</tr>
<tr>
<td>2,2',4,4'-Pentabromodiphenyl ether (BDE-99)</td>
<td>[Data]</td>
</tr>
<tr>
<td>2,2',4,4'-Tetrabromodiphenyl ether</td>
<td>[Data]</td>
</tr>
<tr>
<td>2-Ethylhexyl diphenyl phosphate (EHDP)</td>
<td>[Data]</td>
</tr>
<tr>
<td>3,3',5,5'-Tetrabromobisphenol A</td>
<td>[Data]</td>
</tr>
<tr>
<td>Isosceles diphenyl phosphate</td>
<td>[Data]</td>
</tr>
<tr>
<td>Phenyl, isopropylated, phosphate (3:1)</td>
<td>[Data]</td>
</tr>
<tr>
<td>Trioxyl phosphate</td>
<td>[Data]</td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>[Data]</td>
</tr>
<tr>
<td>Tri(2-chloroethyl) phosphate</td>
<td>[Data]</td>
</tr>
<tr>
<td>Triisobutylphenyl diphenyl phosphate</td>
<td>[Data]</td>
</tr>
</tbody>
</table>

Novel replacements show comparable activity to phased-out compounds

Data publicly available on DNT-DIVER
https://sandbox.ntp.niehs.nih.gov/neurotox/
Comparison of OPFRs with phased-out compounds (3D Neurospheres)

3-D models used to capture additional DNT-related biological space

white = no data
grey = no effect

Oligodendrocytes most sensitive end-point
Relevance to Human Exposures

1. Novel substitutes have comparable in vitro activity to older FRs
2. In vitro activity within order of magnitude of in vivo POD (when known)
3. Activity lies within range of human exposure (limited exposure data for novel compounds)

Extensively used (TBBPA), phased-out (BDE47)

Ongoing Efforts at the NTP

• NTP conducting guideline DNT studies on 2 representative compounds to compare findings in the battery with *in vivo* studies

• Evaluating novel short-term behavioral screens that may replace DNT Guideline studies
  – Minimal experimenter interference, automated, social housing
  – Applying principles of artificial intelligence
• IATA being used to prioritize compounds for further *in vivo* testing
  – Collectively discussing what else is required for prediction

• Assumptions of in vitro & alternative animal models
  – Kinetics, metabolism, internal dose, absence of BBB, genetic diversity, gender

• Data analysis pipeline can influence results

• Assumptions in IVIVE modeling
  – Assumptions in clearance; BBB

What is the alternative approach for timely regulation and protection of susceptible populations?
Application of IATA

• Generate data for hazard characterization in a timely manner

Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity


Organophosphate Ester Flame Retardants: Are They a Regrettable Substitution for Polybrominated Diphenyl Ethers?


• Use for prioritizing compounds for further testing
• IATA demonstrates how a battery may be used for prioritization, timely data dissemination, and (depending on the user) decision-making

• Appears that the *in vitro* activity for some of the OPFRs (i.e., TDCIPP and TPHP) is comparable to that of the phased-out BDEs (e.g., BDE-47) and lies within the range of human exposure (TPHP)

• The *in vitro* activity appears to be at levels comparable to the *in vivo* BMCs (PODs) for some compounds (e.g., TDCIPP)
  – Data exist only for select compounds

• Important to consider IATAs to provide rapid and timely relevant information for human health protection especially for sensitive populations
  – Complement time and cost intensive animal studies
The Team

- Jui-Hua Hsieh, NTP
- Fritsche Lab, IUF
- Magdalini Sachana, OECD
- Tim Shafer, USEPA
- Nisha Sipes, NTP

Thanks to other NTP staff and Global Collaborators!
Thank you for your attention!