Children’s Exposure to Flame Retardant Chemicals (Old and New) in Indoor Environments

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Outline

1. Introduction and Background
   a. What is a flame retardant (FR) and how do they work?
   b. What regulations govern the use of FRs in products?
   c. What type of products contain FRs?
   d. What type of FRs are used in consumer products?

2. Early Exposure to PBDEs
   a. Serum PBDEs in a U.S. Pregnancy Cohort: Associations with Thyroid Hormones and Birth Outcomes (Stapleton et al. 2011; Miranda et al. In review)
   b. U.S. Toddlers Exposure to PBDEs in Indoor Environments: Exposure Pathways and Associations with SES

3. Effects of PBDE Exposure on Thyroid Hormone Regulation
   a. Toxic Mechanisms reported from in vitro and animal studies
   b. Human health studies

4. PBDE Replacement Chemicals: Are they Better or Worse????
   a. Levels of Alt FRs in Indoor Dust
   b. Prenatal Exposure to FM 550 in Rats: Signs of Endocrine Disruption?
Definition:
“A substance added or a treatment applied to a material in order to suppress, significantly reduce or delay the combustion of the material”  
EHC:192, WHO 1997

Regulations That Govern the Use of FRs

U.S. Residential Furniture:
• California Technical Bulletin 117

Electronics:
• Underwriters Laboratory Certifications for Insurance purposes (e.g. UL 746 and -94 V-2 – E&E)

Textiles:
• Children’s Sleepwear (CPSC)
• Seats and Drapes in Public Buildings (NFPA 701, CA TB 133)
What Type of Products are Treated with Flame Retardants in Your Home?

- Sleeping Positioners
- Nursing Pillow
# PBDE Commercial Mixtures

<table>
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<tr>
<th>Congener (# of Br atoms)</th>
<th>% of Mixture</th>
<th>Product Applications</th>
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<td><strong>PentaBDE Commercial Mixture (DE-71; Phased out 2004)</strong></td>
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<td>BDE 209 (10)</td>
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</table>

(La Guardia et al 2006)
PBDE Nomenclature

PentaBDE Congeners:

2,2',4,4'-tetrabromodiphenyl ether
BDE-47

2,2',4,4',5-pentabromodiphenyl ether
BDE-99

2,2',4,4',5,5'-hexabromodiphenyl ether
BDE-153

DecaBDE:

BDE 209
PBDEs in Human Samples From Around the World

Total PBDE concentrations in human blood, milk and tissue (in ng/g lipid) shown as a function of sampling year.


Total PBDE conc. (ppb lipid)

0.01 0.1 1 10 100 1000

North America
Europe
Japan

From Hites et al., 2005
Previous Studies on PBDE Exposure

- Exposure models suggested that *infants* would be receiving the highest exposure due to breast milk ingestion (Jones-Otazo et al., 2005; Schecter et al. 2003)

- Exposure studies in US *adults* have observed significant associations with both diet (Wu et al., 2007; Fraser et al., 2010) and dust (Johnson et al., 2010)

- Fewer studies on children’s exposure:
  - Rose et al. (2010) reported levels in 2-5 year old children in California and found concentrations 2-50X higher than adults
  - Windham et al. (2010) measured PBDEs in 6 to 8 year old girls from California and Ohio; significantly higher concentrations in CA vs Ohio; higher in blacks compared to whites

- Quiros-Alcala et al. (2011) measured PBDEs in dust from low-income households; concentrations were among highest measured

- Zota et al. (2010) wrote perspective article on PBDEs and socio-economic disparities
Children’s Exposure to Flame Retardants

- Children are spending more time indoors
- Indoor environments are often more polluted than outdoor environments (PBDEs in Dust >> PBDEs in Soils)
- Children have a high number of hand-to-mouth contacts
- Children are physically in contact with many FR treated products

Serum PBDEs in US Toddlers: Associations with Hand Wipes, House Dust and Socioeconomic Variables
(Stapleton et al., 2012; Environmental Health Perspectives)

Research Questions:

1. How much exposure are children receiving from exposure to indoor dust?

2. Can we predict exposure by measuring residues on children’s hands?

3. Are PBDE exposures higher in families of lower socio-economic status (SES)?
Methods

Recruitment:

- Targeted families with children between the ages of 12 – 36 months; residents residing in central North Carolina (USA);
- Recruited at the North Roxboro Duke Pediatrics Health Clinic, or by letters;
- Recruited Between May 2009 – September 2010
- All families signed informed consent

Sample Collection:

- Blood sample (venipuncture)
- Hand wipe sample (Investigator Collected)
- House dust sample (Investigator Collected)
- Researcher administered questionnaire

Sample Analysis:

- Serum analyzed for PBDEs (CDC)
- Hand wipes and house dust analyzed for PBDEs and new flame retardants in our laboratory using mass spectrometry
## Toddler Cohort Characteristics
(N=83)

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<th>Characteristic</th>
<th>Number</th>
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<td>25-36 mo</td>
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<td>Non-Hispanic White</td>
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<td>Graduate Degree</td>
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</table>

| Father’s Education Level * |      |    |
| Some High School         | 10    | 14 |
| Completed High School    | 11    | 15 |
| Some College             | 7     | 9  |
| Completed College        | 18    | 24 |
| Graduate Degree          | 28    | 38 |

| Breast Feeding Duration |      |    |
| Less than 1 month       | 20    | 27 |
| 1 to 6 months           | 21    | 28 |
| 6 months or more        | 34    | 45 |

* N=73 (Information on Father’s Education Level was not reported or unavailable in a few cases).
A Comparison of Serum PBDEs by Gender

- $\sum$BDEs higher in boys relative to girls
- $\sum$BDE not statistically significant but suggestive ($p=0.086$)
- BDE 100 was significantly different between groups ($p<0.05$)
A Comparison of Serum PBDEs by Age

Kruskal-Wallis Test
$\sum$BDE, $p=0.59$; BDE 153, $p<0.05$
The Influence of Breastfeeding on Serum PBDEs

*No trend observed for BDE 47, 99, 100 or $\sum$BDE
Potential Dust Exposure Paradigm

Home Environment → Personal Exposure → Internal Dose

House Dust → Handwipes → Serum

What are the associations between these three “compartments”?
Potential PBDE Exposure Paradigm

Home Environment → Personal Exposure → Internal Dose

- House Dust → Handwipes → Serum
  - $r = 0.32 \ (p<0.01)$
  - $r = 0.59 \ (p<0.0001)$
  - $r = 0.34 \ (p<0.01)$
**PBDE Residues on Your Hands Are Related to What’s in Your Body**

![Graph showing the correlation between PBDE residues on handwipe and in serum.](image)

- Sum BDE Serum (ng/g lipid)
- Sum BDE on Handwipe (ng)

$r_s = 0.57$ (p<0.001)

$r_p = 0.59$ (p<0.0001)
Predictors of Serum PBDE: A Multivariate Analysis

- ΣBDEs 47,99,100:
  - Age (60% increase/year; p=0.05)
  - Paternal Education (2X higher when father had less than college degree; p<0.01); race displayed same trend and both variables were highly correlated;
  - Handwipe levels (3.66X higher in third tertile compared to lowest tertile; p<0.01)

- BDE 153:
  - Age (70% increase/year; p<0.05)
  - Breastfeeding Duration (7%/month; p<0.001)
  - Handwipe Levels (2.44X higher in third tertile compared to lowest; p<0.01)

* Education was categorized by either having a completed college degree (AD, BD, GD) or less than.
What are the consequences of this early life exposure??
PBDEs are Thyroid Hormone Mimics

**Thyroid Hormones**

- Triiodothyronine (T3)
- Thyroxine (T4)

**PBDE Oxidative Metabolites**

- T3-like OH-BDE
- T4-like OH-BDE
Toxic Modes of Action Affecting Thyroid Regulation

2. PBDE metabolites displace T4 from serum transporters (Meerts et al., 2000);

4. Transporters deliver PBDEs or metabolites to brain where agonism/antagonism with nuclear receptors may occur;

5. Upregulation of xenobiotic metabolizing enzymes (XMEs) (Szabo et al. 2009)

6. XMEs conjugate T4; increased or decreased clearance of THs (Butt et al., in Progress);

7. Disruption of Deiodinase Activity by PBDE metabolites (Butt et al., 2011)
Deiodinase Reactions

3,3',5-triiodothyronine (T3)

3,3',5'-triiodothyronine (rT3)

thyroxine (T4)

3,3'-diiodothyronine (T2)

3,3',5'-triiodothyronine (T3)

DI I: outer & inner ring
DI II: outer ring
DI III: inner ring
Inhibition of Thyroxine Deiodination by Flame Retardants
(Butt et al., 2011)

In Vitro Experiments Conducted with Pooled Human Liver Microsomal Samples

Mean ± 1 std. deviation (n=3)
Neurodevelopmental Effects Observed in Animal Studies

- PBDEs shown to affect development of fetal human neural progenitor cells *in vitro* which was mediated by thyroid hormone signaling (Schreiber et al. 2010)

- Studies conducted in rodent models observed significant alterations in spontaneous behavior and habituation, deficits in learning and memory, and changes in cholinergic nicotinic receptors, primarily occurring when exposure occurs during “rapid brain growth” (Eriksson et al., 2001, 2002; Viberg et al., 2003, 2006, 2007).

- Mice exposed to BDE 209 during rapid brain growth were observed to have altered expression of CAMKII, GAP-43 and BDNF in different regions of the brain (Viberg et al., 2007).
Neurodevelopmental Deficits Associated with PBDEs in Children
(Herbstman et al. 2010)

- PBDE levels in cord blood at birth were negatively associated with:
  - Mental Developmental Index at 24 months of age (BDEs 47, 99, and 100, univariate and adjusted models);
  - Full and Verbal IQ at 48 months (BDE 47 and 100, adjusted models);
  - Full and Performance IQ at 72 months (BDE 100 and 153; univariate and adjusted models)
The Case of the Chemical Conveyor Belt.....

When one flame retardant is banned, another chemical moves in to take it's place, and less is known about the replacement chemical...
Flame Retardants in Consumer Products

• Identification of FRs in Baby Products (Stapleton et al 2011)
  – Screened 102 baby products (car seats, nursing pillows, sleep positioners, etc)
  – 80% contained FR
  – “Chlor Tris”, and FM 550 most common FRs identified

• Identification of FRs in Residential Furniture (Stapleton et al. In Draft)
  – Screened 101 different samples from couches
  – 86% contained FR
  – “Chlor Tris”, and FM 550 most common FRs identified
# Firemaster® 550 – 4 Components
*(Stapleton et al. 2008)*

## Section II - Composition/Information on Ingredients

<table>
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<th>Ingredient Name</th>
<th>CAS No.</th>
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<th>Exposure Limits</th>
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<td>Y (Hazardous)</td>
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<td></td>
<td>Not established (ACGIH TLV TWA)</td>
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<td><em>2. Triphenyl phosphate</em></td>
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<td>Not established (ACGIH TLV CEIL)</td>
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*Indented chemicals are components of previous ingredient.

Additional Information:

Additional exposure limits for triphenyl phosphate:
NIOSH REL TLV = 3 mg/m³
**EPA Issues Consent Order on FM 550**

- EPA required further testing on FM 550 components

- **Oral two generation reproduction and fertility study in rats** – Observed significant effects on body weight in dams and pups; F2 pups had significant effects on reproductive traits and thyroid at 50 mg/kg/day
  - No Observable Adverse Effect Level (NOAEL) = 50 mg/kg/day

- **Prenatal developmental toxicity in rats** – Observed significant effects on body weight in dams and pups: pups also displayed fused cervical vertebral neural arches and incomplete ossification of skull bones at 100 and 300 mg/kg/day
  - NOAEL = 50 mg/kg/day

- **Migration Study from foam** simulating wet transfer of the mixture to skin via dermal contact with upholstery. Data demonstrated that the substance is not absorbed via dermal contact with foam.
Pregnant Wistar Rats (~350 g) exposed from GD6 to PND 21

Exposed to 20 uL of each treatment on cookie once per day; water and food ad libitum

Treatment Groups (n=3 dams/treatment):

A – Control

B – High Dose (3.01 mg/kg/day)

C – Low Dose (301 ug/kg/day)

Monitor uptake of TBB and TBPH in dams/pups

Examine effects on pup growth, thyroid levels, reproductive development and behavior
**FM550 Rat Exposure Design**

**Dams**: collected serum, liver, brain, fat, and muscle on PND 21

**Pups**: collected serum (limited), liver, brain, fat, and muscle from pups on PND 21, and 7 months of age

- **Control**: n=3
- **Low Dose**: n=3
- **High Dose**: n=3

- **Pregnant Dams**
  - **Control**: n=3
  - **Low Dose**: n=3
  - **High Dose**: n=3

  **High Dose**: **one dam lost litter in high dose**
Dam Hepatic Tissue on PND 21:

**Parent Compounds**

- TBB
  - Control: <MDL
  - Low Dose: <MDL
  - High Dose: 1800 ng/g ww

- TBPH
  - Control: <MDL
  - Low Dose: 600 ng/g ww
  - High Dose: 1000 ng/g ww

**Metabolites**

- TBBA
  - Control: <MDL
  - Low Dose: 1000 ng/g ww
  - High Dose: 1600 ng/g ww

- Hepatic TBMEHP: <MDL
**Thyroid Hormones in Dam Serum PND 21**

Bars represent mean ± 1 standard error
Data were analyzed using ANOVA and Dunnett’s post-hoc when appropriate
* Indicates significant difference from control of p < 0.05
n=3 unless otherwise noted
**Thyroid Hormones in Pup Serum: PND 21**

<table>
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<tr>
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<th>Total T4 (ng/mL)</th>
<th>Total T3 (ng/mL)</th>
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<td>6.2</td>
<td>0.3</td>
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<tr>
<td>Low Dose</td>
<td>4.5</td>
<td>0.2</td>
</tr>
<tr>
<td>High Dose</td>
<td>3.0</td>
<td>0.1</td>
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Bars represent mean ± 1 standard error. Data were analyzed using ANOVA and Dunnett’s post-hoc when appropriate. *Indicates significant difference from control of p < 0.05. n=3 unless otherwise noted.
Pup Body Weight

*Males in high dose significantly heavier than controls at all time points*

*Females in high dose significantly heavier than controls starting at PND 21*

Bars represent mean ± 1 standard error
* Indicates significant difference from control of p < 0.05
n=3 unless otherwise noted
**Female Glucose Challenge Data**
*(4 Months of Age)*

**Female Glucose Challenge Data**
*(4 Months of Age)*

![Graph showing blood glucose levels over time for vehicle, low dose, and high dose groups.](image)

- **Blood Glucose (mg/dl)**
  - 0
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300
  - 350

- **Time (Seconds)**
  - 0
  - 20
  - 40
  - 60
  - 80
  - 100
  - 120
  - 140

- **Vehicle**
- **Low Dose**
- **High Dose**

* (both doses)

**p < 0.01; * p < 0.05**

![Area Under the Curve (AUC) comparison chart.](image)

- **Control**
- **Low Dose**
- **High Dose**

**Females**

**p = 0.07**

**Females**

**Vehicle**
**Low Dose**
**High Dose**

**Area Under the Curve (AUC)**

**Control**
**Low Dose**
**High Dose**
Timing of Female Pubertal Onset

** p<0.01; * p<0.05

Age at Vaginal Opening

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<tr>
<td>36</td>
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** p<0.01; * p<0.05
Conclusions:

- Toddlers are receiving elevated exposures to FRs in house dust, likely due to hand to mouth contacts;

- Age, breast milk ingestion, SES and PBDE residues on the hands are significant predictors of serum PBDE levels in toddlers (explaining 40% of serum levels);

- Non-PBDE flame retardants are now used in furniture products and are accumulating in indoor dust at levels similar to PBDEs;

- Prenatal exposure to FM 550 in rats led to significant weight gain, changes in glucose metabolism, early puberty, and increased anxiety (based on behavioral tests; data not shown)

- What is the true NOAEL for FM 550? Should FM 550 or its components be considered an endocrine disruptor or chemical obesogen?
Acknowledgements

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• Dr. Thomas F. Webster (Boston University); Dr. Andreas Sjödin (Centers for Disease Control and Prevention); Beth Patterson, recruiters, and the study participants