Welcome to the CLU-IN Internet Seminar

Early-life Exposures - Long-term Health Consequences: Part 1 Brominated Flame Retardants

Sponsored by: NIEHS Superfund Research Program

Delivered: February 3, 2012, 1:00 PM - 3:00 PM, EST (18:00-20:00 GMT)

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Moderator:

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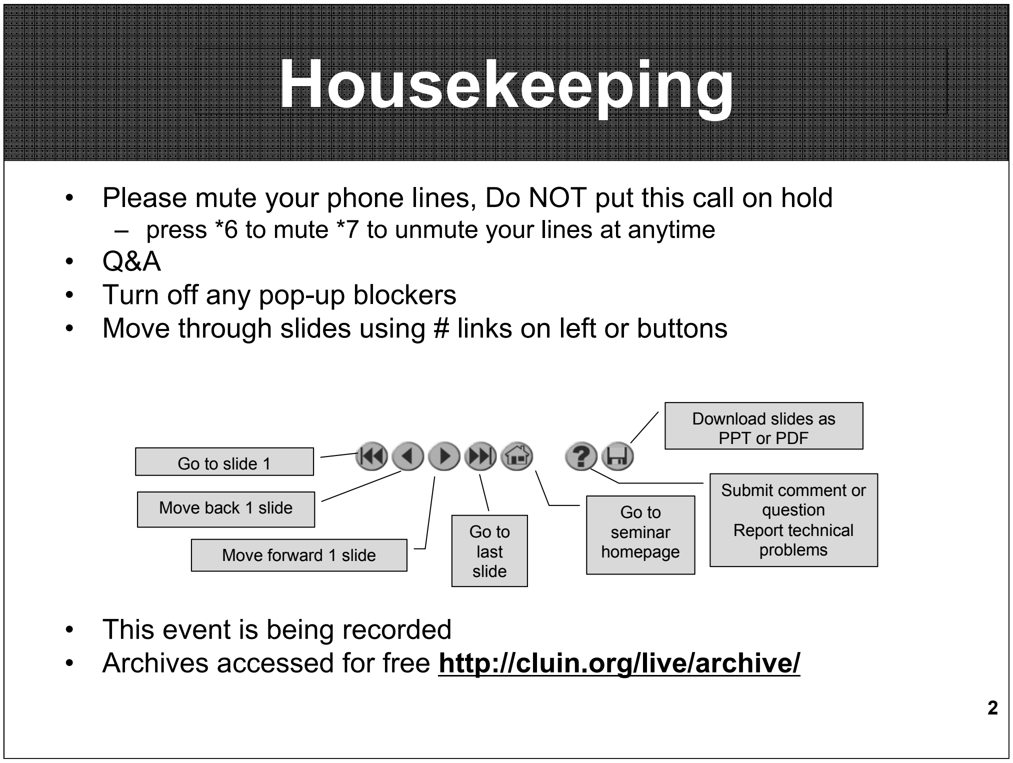


Although I'm sure that some of you have these rules memorized from previous CLU-IN events, let's run through them quickly for our new participants.

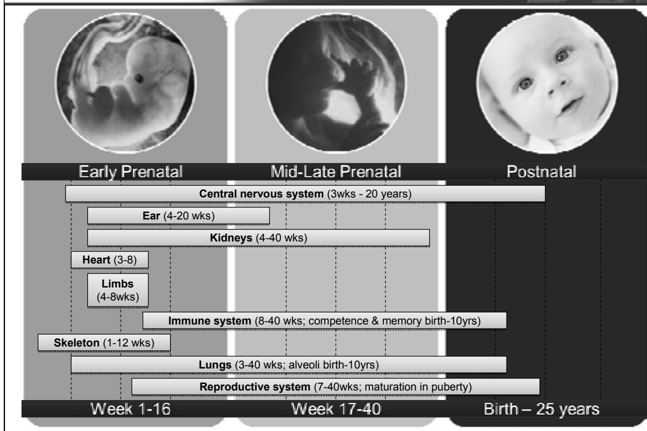
Please mute your phone lines during the seminar to minimize disruption and background noise. If you do not have a mute button, press *6 to mute #6 to unmute your lines at anytime. Also, please do NOT put this call on hold as this may bring delightful, but unwanted background music over the lines and interupt the seminar.

You should note that throughout the seminar, we will ask for your feedback. You do not need to wait for Q&A breaks to ask questions or provide comments. To submit comments/questions and report technical problems, please use the ? Icon at the top of your screen. You can move forward/backward in the slides by using the single arrow buttons (left moves back 1 slide, right moves advances 1 slide). The double arrowed buttons will take you to 1st and last slides respectively. You may also advance to any slide using the numbered links that appear on the left side of your screen. The button with a house icon will take you back to main seminar page which displays our agenda, speaker

Early Life Exposures and Brominated Flame Retardants

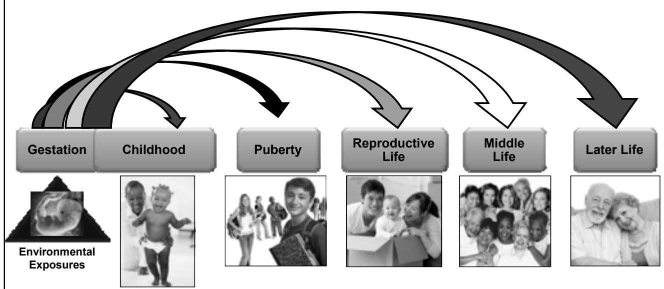
Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S
Director
National Institute of Environmental Health Sciences
National Toxicology Program

Risk eLearning Web Seminar
Friday, February 3, 2012

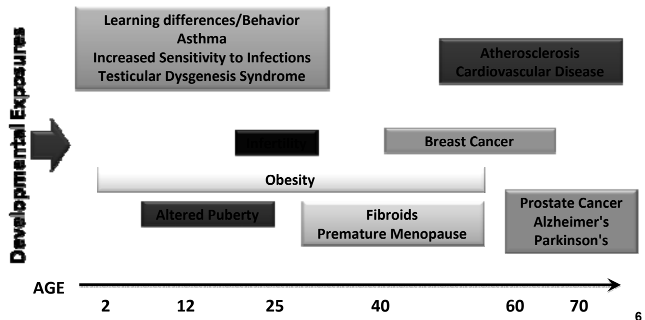


Source: Altshuler, K; Berg, M et al. *Critical Periods in Development*, OCHP Paper Series on Children's Health and the Environment, February 2003.

Developmental Origins of Disease:
Developmental Stressors Lead to Disease Throughout Life



Diseases over the Lifespan from Developmental Exposures



PBDEs have had a lot of publicity:

found in breast milk, potential human thyroid hormone disruptor and developmental neurotoxicant.

USA TODAY

CBS

TODAY

BFRs do not bind chemically to polymers in textiles or plastics, they can leach out or evaporate from flame retarded products.

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Halogenated Fire Retardants
(contain bromine or chlorine and carbon)

Uses (in order, by volume in the U. S.)

1. Electronics
2. Insulation in Buildings
3. Polyurethane foam
4. Wire and cable

Polybrominated Diphenyl Ethers

- Prenatal BDE-99 increased mouse birth weight
- Pre- and post- natal exposure to BDE-47 increased rat body weights from birth to puberty (when the study ended)
- Postnatal BDE-47 study, mice exposed 10 days after birth had increased body weights from postnatal day 47 until 4 months of age, when the study ended
- Developing shrimp exposed to BDE-47 had increased cholesterol

Lilienthal 2006, Suvorov 2009, Gee 2008, Key 2008, van der Ven 2008

Polybrominated Diphenyl Ethers

- **Cryptorchidism**
  - Main et al, 2007
- **Reproductive Hormone Effects**
  - Meeker et al, 2009: Decrease in Androgens and LH; Increase in FSH and Inhibin
  - Meijer et al, 2008: Decrease in Testosterone
- **Reproductive Effects**
  - Eskenazi et al, 2009: Low Birth Weight & Altered Behaviors
  - Harley et al, 2010: Increased time to pregnancy
- **Neurological Effects**
  - Herbstman et al, 2010: Decreased IQ
- **Decreased Sperm Quality**
  - Akutse et al, 2008
- **Diabetes**
  - Lim et al, 2008
  - Turyk et al, 2009 (only in hypothyroid subjects)
- **Thyroid Homeostasis**
  - Stapleton et al, 2011: T4 elevated during pregnancy
  - Chevrier et al, 2010: TSH elevated in pregnancy
  - Meeker et al, 2009: elevated T4 & TBG
  - Dallaire et al, 2009: Elevated T3 from BDE47
  - Eskenzai et al, 2009: Low TSH

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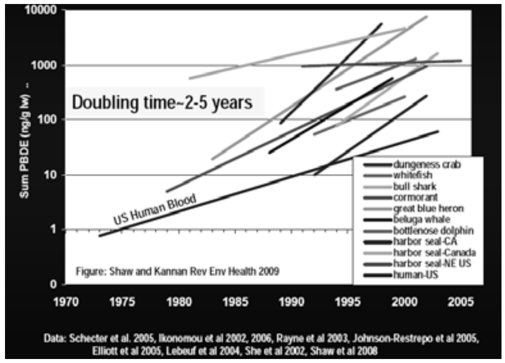
PBDEs Increasing in North America 1970 - 2005



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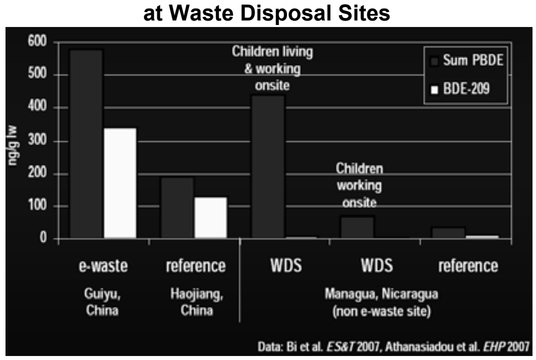
Highest PBDE Levels in Blood of Humans at Waste Disposal Sites



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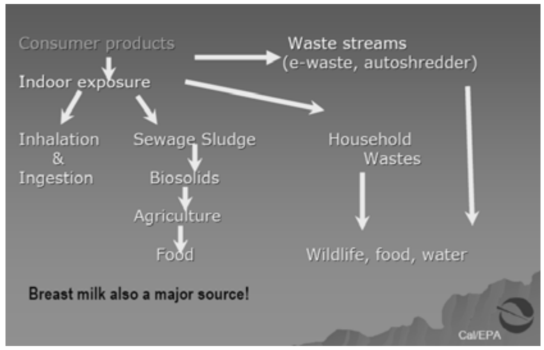
Major Flame Retardant Exposure Pathways



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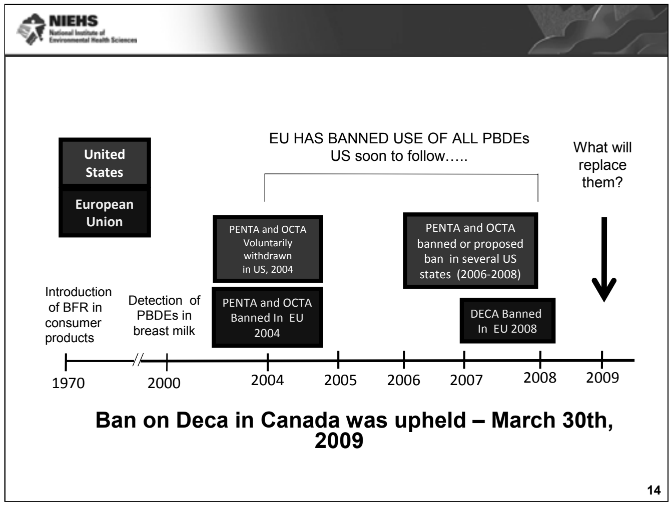
NIEHS
National Institute of Environmental Health Sciences
United States
European Union
EU HAS BANNED USE OF ALL PBDEs
US soon to follow…..
What will replace them?
PENTA and OCTA Voluntarily withdrawn in US, 2004
PENTA and OCTA banned or proposed ban in several US states (2006-2008)
Introduction of BFR in consumer products
Detection of PBDEs in breast milk
PENTA and OCTA Banned In EU 2004
DECA Banned In EU 2008
1970
2000
2004
2005
2006
2007
2008
2009
Ban on Deca in Canada was upheld – March 30th, 2009
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Regulation of BFRs

- TBBPA – not regulated
- HBCD banned in Norway & EU
- "SVHC"Nominated as a "POP" in November 2009
- PBDEs –
  - Penta and Octa targeted for elimination under Stockholm Convention, May 9, 2009
  - Deca – EPA (March, 2010) announced voluntarily US phase-out by 2013

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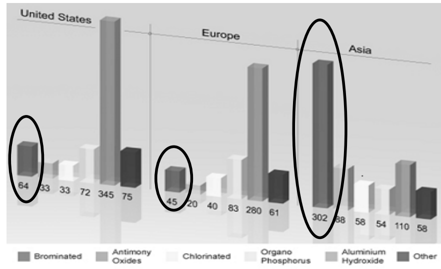
Asian Market = BFR Global Concern

Between 2005-2008 uses of BFRs has increased from 139,000 to 246,000 tonnes over 3 years (mostly in Asia).



From: Stephan Posner 2010

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NIEHS National Institute of Environmental Health Sciences

Considerations for Flame Retardant Alternatives

- Alternative chemicals other than BFRs or other classes of FRs
- Minimize potential for hazard and exposure
- Low persistence and bioaccumulation, for breakdown products as well as parent chemicals
- Low toxicity, less potential for harm when exposure occurs
- Low exposure, less potential for release

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Other Considerations for Flame Retardant Alternatives

- Aesthetic and performance considerations: appearance, durability, fire safety
- Process equipment cost
- Alternative technologies, barriers, surface treatments, graphite-impregnated foams
- The main consideration: **Minimize risk to human health and the environment!**

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Thank you!

NIEHS Strategic Plan Website
http://www.niehs.nih.gov/strategicplan

Today's Webinar:
Polybrominated Diphenyl Ethers - Exposures and Toxicity

- Heather Stapleton Assistant Professor, Duke University, Nicholas School of the Environment
  - "Early Life Exposure to Flame Retardant Chemicals in Indoor Environments and Impacts on Thyroid Hormone Regulation"
- Prasada Rao S. Kodavanti, Neurotoxicology Branch, Toxicity Assessment Division, NHEERL, ORD, US Environmental Protection Agency (US EPA)
  - "Neurobehavioral, Hormonal, and Reproductive Effects following Developmental Exposure to a Commercial PBDE Mixture, DE-71"

Early Life Exposure to Flame Retardant Chemicals in Indoor Environments and Impacts on Thyroid Hormone Regulation

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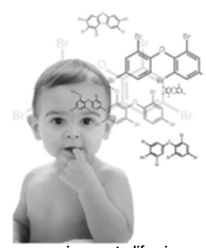

NICHOLAS SCHOOL OF THE ENVIRONMENT
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Outline

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 Pregnancy Cohort: Associations with Thyroid Hormones and Birth Outcomes
   b. Toddlers Exposure to PBDEs in Indoor Environments: Exposure Pathways and Associations with SES

3. **Health Affects Related to PBDE Exposures**
   a. Toxic Mechanisms reported from *in vitro* and animal studies, effects on thyroid regulation
   b. Human health effects and neurodevelopment problems in children

4. **Conclusions/ Discussion**

What is a Flame Retardant?

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*

Statistics:

- Every year in the U.S. there are over a million fires reported
- Direct losses account for
  - billions in damages

Regulations That Govern the Use of FRs

<u>Furniture</u>:

- California Technical Bulletin 117
- California Technical Bulletin 603
- Federal Mattress Flammability Standard (CFR 1633)

<u>Electronics:</u>

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

<u>Textiles:</u>

- Children's Sleepwear (CPSC)
- Seats and Drapes in Public Buildings (NFPA 701, CA TB 133)
- Camping Equipment (CPAI-84)

<u>Building and Construction:</u> (variable)

What is TB 117?

- Promulgated by California Bureau of Home Furnishing and Thermal Insulation, within the Department of Consumer Affairs
- Requires 12-second open flame testing for polyurethane inside furniture
- Has required the use of large quantities of halogenated flame retardants (FR)
- CA standard affected furniture composition throughout the U.S.

What Type of Products are Treated with Flame Retardants in Your Home?

PBDE Commercial Mixtures

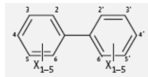
Congener (# of Br atoms)	% of Mixture	Product Applications
PentaBDE Commercial Mixture (DE-71; Phased out 2004)		
BDE 47 (4)	38.2	
BDE 85 (5)	2.96	
BDE 99 (5)	48.6	
BDE 100 (5)	13.1	
BDE 153 (6)	5.44	
BDE 154 (6)	4.54	
OctaBDE Commercial Mixture (DE-79; Phased out 2004)		
BDE 153 (6)	8.66	
BDE 154 (6)	2.68	
BDE 183 (7)	42.0	
BDE 196 (8)	10.5	
BDE 197 (8)	22.2	
BDE 207 (9)	11.5	
DecaBDE Commercial Mixture (Saytex 102E)		
BDE 206 (9)	2.19	
BDE 207 (9)	0.24	
BDE 208 (9)	0.06	
BDE 209 (10)	96.8	



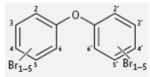



(La Guardia et al 2006)

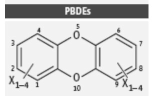
Toxic Effects from PBDEs



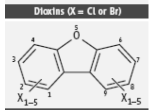
PCBs (X = Cl) and PBBs (X = Br)



PBDEs



Dioxins (X = Cl or Br)



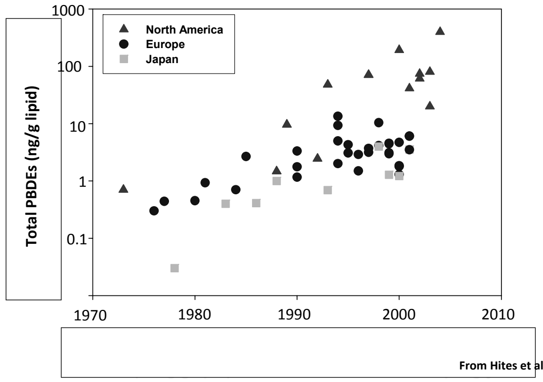
Furans (X = Cl or Br)

- PBDEs have chemical structures which are very similar to known cancer causing and toxic compounds: **PCBs, dioxins, furans, etc**.
- Laboratory studies now demonstrate that PBDEs have very similar toxic effects as these legacy contaminants.

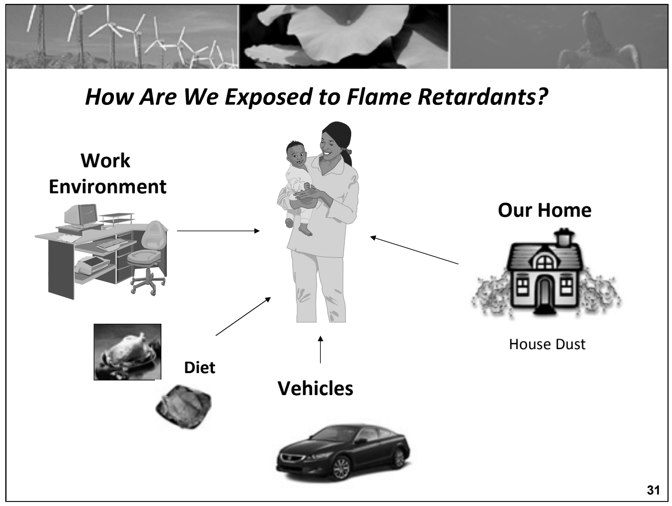
Major Concerns about PBDEs

- Rapidly accumulating in humans and environment
- Hormonal disruption
  - Animal exposure studies have observed decreases in thyroid hormone levels (Zhou et al., 2001; Tomy et al. 2004)
  - Associations between PBDEs and thyroid hormones (Turyk et al., 2008; Chevrier et al., 2010) and reduced fecundability (Harley et al., 2010) in human population
- Developmental effects
  - Associations between cryptorchidism and PBDEs in male infants (Main et al., 2007);
  - Associations between PBDE exposure at birth and neurodevelopment measures in children (Roze et al., 2009; Herbstman et al., 2010);
- Cancer?
  - Structures similar to known carcinogens (PCBs, PBBs)

PBDEs in Human Samples From Around the World



From Hites et al., 2005


How Are We Exposed to Flame Retardants?
Work Environment
Our Home
House Dust
Diet
Vehicles
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Previous Studies on PBDE Exposure

- Exposure models had suggested that *infants* would receive the highest exposure among various age classes due to breast milk ingestion (Jones-Otazo et al., 2005; Schecter et al. 2003)

- 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

Research | Children's Health

Environ. Health Perspect. 2011

Associations between Polybrominated Diphenyl Ether (PBDE) Flame Retardants, Phenolic Metabolites, and Thyroid Hormones during Pregnancy

Heather M. Stapleton,[1] Sarah Eagle,[1] Rebecca Anthopolos,[1] Amy Wolkin,[2] and Marie Lynn Miranda[1,3]

[1]Nicholas School of the Environment, Duke University, Durham, North Carolina, USA; [2]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [3]Department of Pediatrics, Duke University, Durham, North Carolina, USA

Objectives of Study

- To measure the levels of PBDEs and their phenolic metabolites in serum collected from pregnant women during 3rd trimester;
- To determine if there are any significant associations between serum PBDE levels and thyroid hormone levels in pregnant women;
- To examine associations between PBDE levels and birth outcomes.

Methods

- Pregnant women attending the Lincoln Community Health Center (Durham, NC, USA), who are part of a larger cohort of women currently enrolled in a pregnancy outcomes study, were approached and asked to participate in this study. (>34 weeks gestation)

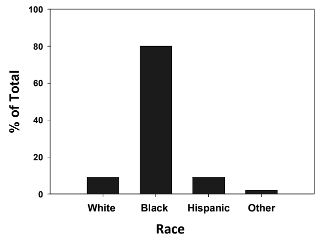
- Two tubes of blood were collected during a routine blood draw (thyroid hormones and PBDEs).

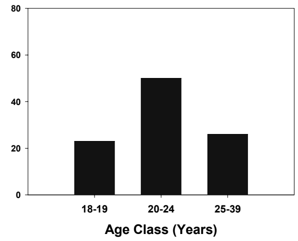
- Thyroid hormones analyzed by Duke University Hospital Clinical Laboratory for:
  Thyroid Stimulating Hormone (TSH); Thyroxine (T4) (free and total) and Triiodothyronine (T3) (free and total)

- Serum analyzed for PBDEs and phenolic metabolites using mass spectrometry

Population Demographics

(n=137)





Individuals recruited between September 2008- June 2010

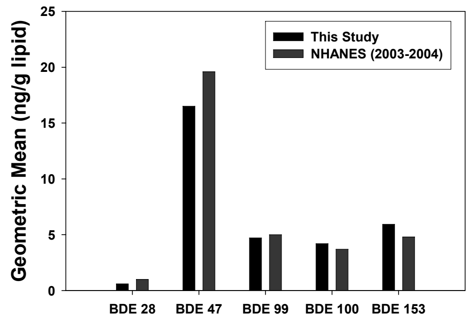
PBDEs

Concentrations in ng/g lipid (n=137)

Congener	Detection Frequency	MDL	Min	Max	Geometric Mean	95th Percentile
BDE 28	38.7	1.2-3.0	<1.2	16.9	N/A	6.00
BDE 47	94.9	2.0-4.5	<2.0	297.5	16.5	114.4
BDE 99	64.2	2.0-4.5	<2.0	249.1	4.72	49.8
BDE 100	89.1	1.2	<1.2	107.5	4.19	25.9
BDE 85,100	16.1	1.2	<1.2	10.5	N/A	4.58
BDE 153	96.4	1.2	<1.2	67.6	5.93	32.3
BDE 154	48.2	1.2	<1.2	52.9	N/A	7.59
ΣPBDEs				694	36.6	228

****BDE 209 quantified but not reported here. Blank levels were too high for accurate quantification**

Comparison of Geometric Mean Values



* Sjodin et al., 2008- 2032 total samples – data presented are from females only

PBDE Metabolites/Alt BFRs

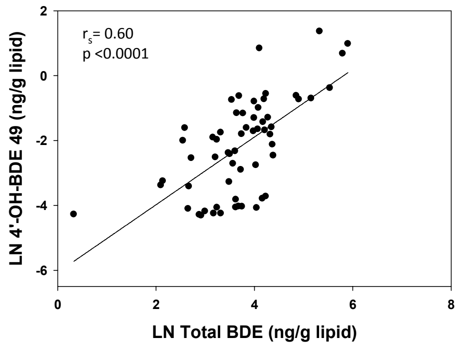
A sub-set of the serum extracts (n=57) were quantified for 2,4,6-tribromophenol (246-TBP) and the following OH-BDE standards:

6-OH-BDE 47, 4'-OH-BDE 49, 6'-OH-BDE 49, 6-OH-BDE 99

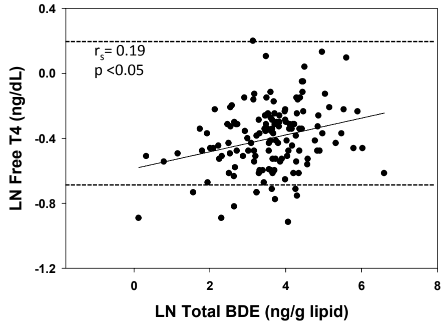
Results (ng/g lipid)

Analyte	Detection Frequency	MDL	Min	Max	Geometric Mean	95th Percentile
246 – TBP	38.2	1.4-2.5	<1.4	150.7	N/A	119.7
4'-OH-BDE 49	71.9	0.03	<0.03	3.92	0.11	2.32
6-OH-BDE 47	66.7	0.03	<0.03	10.8	0.17	5.82

Correlation Between PBDEs and OH-BDEs



Association Between PBDEs in Pregnant Women and Thyroxine (T4)



**Same trend observed for total T4 (r_s = 0.20; $p<0.05$)

Multiple Linear Regression Models for Thyroid Hormones

(Controlling for Maternal Characteristics)

	Explanatory Variables									
	LN BDE 47		LN BDE 99		LN BDE 100		LN BDE 153		LN ∑ BDE	
Thyroid Hormone	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI
TT4	0.42*	0.05, 0.78	0.32*	0.02, 0.63	0.41*	0.003, 0.82	0.12	-0.35, 0.58	0.50*	0.06, 0.94
LN FT4	0.05**	0.01, 0.08	0.02	-.009, 0.05	0.02	-0.02, 0.06	0.05*	0.006, 0.09	0.05*	0.01, 0.09
LN TSH	0.07	-0.02, 0.16	0.04	-0.04, 0.11	0.01	-0.09, 0.11	0.03	-0.08, 0.14	0.06	-0.04, 0.17
LN TT3	0.04	-0.01, 0.08	0.01	-0.03, 0.05	0.001	-0.05, 0.05	0.01	-0.04, 0.07	0.02	-0.03, 0.08
LN FT3	0.01	-0.01, 0.03	0.003	-0.01, 0.02	0.0004	-0.02, 0.02	-0.02	-0.04, 0.01	0.01	-0.02, 0.04

**P<.01 *P<.05

CI Confidence Interval

These models report the individual BDE congener-thyroid hormone association after controlling for smoking status, maternal race, age, gestational age at blood draw, and parity.

- **Significant associations with T4, but no significant associations with TSH or T3**

- **No significant associations noted between thyroid hormones and phenolic metabolites; however, a negative relationship between TT3 and OH-BDE 49 was suggestive (p = 0.08).**

Observed Relationships between Thyroid Hormones and PBDEs

Cohort	↑TSH	↑FT3/TT3	↑FT4/TT4
Human Studies			
USA (n = 297) Herbstman et al., 2008	No effect	No effect	↑BDE 100/BDE 153
USA (n =405) Turyk et al., 2008	↓BDE 47	No effect	↑∑BDEs
USA (n=270) Chevrier et al., 2010	↓PBDEs	NM	No effect
USA (n=137) Stapleton et al., 2011	No effect	↓ OH-BDE 49	↑∑BDEs
USA (n=25) Zota et al., 2011	↑PBDEs/OH-BDEs	NM	No effect
Animal Studies			
Rats Zhou et al., 2001	No effect	No effect	↓PBDEs
American Kestrels Fernie et al., 2005	NM	No effect	↓PBDEs
Tomy et al., 2004 Juvenile Lake trout	NM	No effect	↓PBDEs

NM- not measured

Are Serum PBDEs in Pregnant Women Associated with Negative Birth Outcomes?

- **Preliminary analyses indicate that serum PBDEs are negatively associated with infant head circumference in both unadjusted and adjust models;**

- **No significant associations observed with birth weight or length, although all relationships are negative;**

- **Harley et al (2011) observed a negative relationship between serum PBDEs and birth weight in CHAMACOS cohort, no relationship with head circumference**

Part II: 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**

http://www.theage.com.au/articles/2006/05/02/1146335739915.html

Serum PBDEs in US Toddlers: Associations with Hand Wipes, House Dust and Socioeconomic Variables

(Stapleton et al. 2012, In Review)

Research Hypotheses:

1. **Children residing in the US between the ages of 1-3 yrs of age are receiving the highest exposure to PBDEs in the world, due to dust exposure and subsequent hand-to-mouth activities;**

2. **Dust is the primary source of exposure to young children; not breast milk or diet;**

3. **PBDE exposure are higher in minorities and families with lower income;**

Methods

Recruitment:

- Targeted families with children between the ages of 12 – 36 months; residents residing in central North Carolina;
- 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

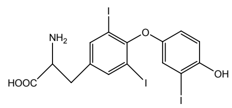
Summary of Toddlers Exposure Data

- PBDEs present in all toddler serum samples;
- Significant associations observed between PBDEs in serum and PBDE residues on hand wipes;
- Toddlers exposure to PBDEs is associated with hand-to-mouth behavior, SES, breast milk ingestion and age;
- Are PBDEs an environmental justice issue?

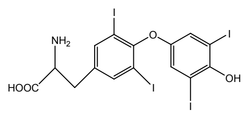
What are the consequences of this early life exposure??

PBDEs are Thyroid Hormone Mimics

Thyroid Hormones

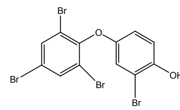


Triiodothyronine (T3)

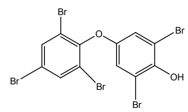


Thyroxine (T4)

PBDE Oxidative Metabolites

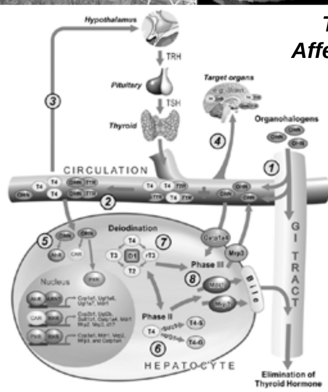


T3-like OH-BDE



T4-like OH-BDE

Toxic Modes of Action Affecting Thyroid Regulation



(From Kodavanti and Curras-Collazo 2010)

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;

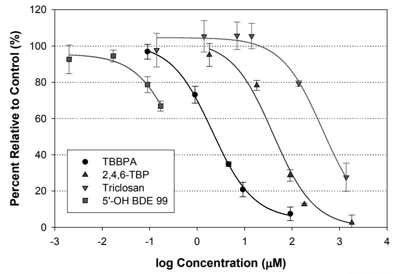
5. Upregulation of xenobiotic metabolizing enzymes (XMEs) (Szabo et al 2009)

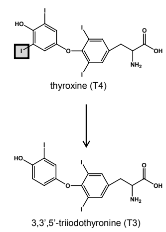
6. XMEs conjugate T4; increased or decreased clearance of THs (Butt et al., in Progress);

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

Inhibition of Thyroxine Deiodination by Flame Retardants

(Butt et al., 2011)



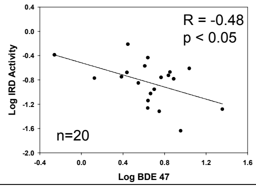


Mean ± 1 std. deviation (n=3)

In Vitro Experiments Conducted with Pooled Human Liver Microsomal Samples

Do PBDEs/OH-BDEs Inhibit DI Activity In Vivo?

- Fathead minnows exposed to DecaBDE (10 µg/g) for 28 days experienced a 74% decrease in DI activity relative to controls (Noyes et al. 2011);
- Type 3 deiodinase is essential in buffering thyroid hormones between the mother and fetus during pregnancy. Type 3 DI knock-out mice were shown to have significant fetal growth restrictions (Hernandez et al., 2006,2007`.



- **Analysis of 20 anonymous placental tissues for PBDEs and DI Activity**
- **Negative correlation between BDE 47 and IRD activity observed**

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)

If PBDEs are now phased out….does the problem go away????

Environmental Science & Technology

ARTICLE

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Identification of Flame Retardants in Polyurethane Foam Collected from Baby Products

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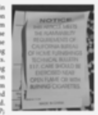
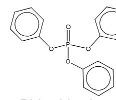
Supporting Information

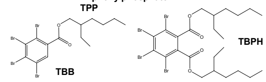
ABSTRACT: With the phase-out of PentaBDE in 2004, alternative flame retardants are being used in polyurethane foam to meet flammability standards. However, insufficient information is available on the identity of the flame retardants currently in use. Baby products containing polyurethane foam must meet California state furniture flammability standards, which likely affects the use of flame retardants in baby products throughout the U.S. However, it is unclear which products contain flame retardants and at what concentrations. In this study we surveyed baby products containing polyurethane foam to investigate how often flame retardants were used in these products. Information on when the products were purchased and whether they contained a label indicating that the product meets requirements for a California flammability standard were recorded. When possible, we identified the flame retardants being used and their concentrations in the foam. Foam samples collected from 101 commonly used baby products were analyzed. Eighty samples contained an identifiable flame retardant additive, and all but one of these was either chlorinated or brominated. The most common flame retardant detected was tris(1,3-dichloroisopropyl) phosphate (TDCPP; detection frequency 36%), followed by components typically found in the Firemaster550 commercial mixture (detection frequency 17%). Five samples contained PBDE congeners commonly associated with PentaBDE, suggesting products with PentaBDE are still



New Use Flame Retardants Detected in Furniture and in House Dust



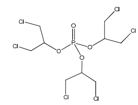
Triphenylphosphate
TPP



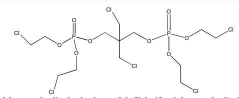
TBB

TBPH

Firemaster® 550/600



Tris (1,3-dichloroisopropyl)phosphate
TDCPP



2,2-bis(chloroethyl)triethylene bis[bis(2-chloroethyl)phosphate]
"V6"

Conclusions

- Exposure to PBDEs occurs during early development;
- PBDEs are significantly associated with circulating thyroid hormone levels during pregnancy;
- Maternal PBDE levels are associated with deficits in birth outcomes (e.g. birth weight and head circumference)
- Children have higher body burdens than adults and toddlers may represent the age class with the highest exposure to PBDEs;
- PBDEs on hand wipes are a better predictor of serum PBDE levels in toddlers compared to house dust;
- PBDE exposure may be an environmental justice issue;
- PBDEs affect thyroid hormone regulation via multiple mechanisms which may be influencing growth and neurodevelopment;
- New flame retardants on the market need to be studied to understand whether any human health concerns are warranted.

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- Beth Patterson, recruiters, and the study participants

Neurobehavioral, Hormonal, and Reproductive Effects Following Developmental Exposure to a Commercial Mixture, DE-71

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NHEERL/ORD
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Co-authors:

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NIEHS Superfund Webinar – February 3, 2012

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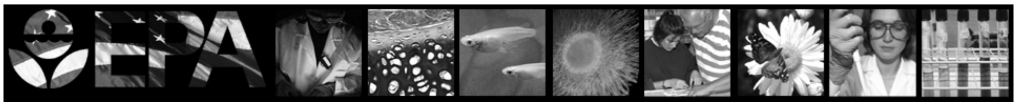
United States Environmental Protection Agency

OUTLINE OF TALK

- What are Brominated Flame Retardants?
  - Benefits, market demand, and use
- Types of BFRs
  - TBBPA
  - HBCD
  - PBDEs – Environmental contamination
    - Human exposure
    - Structural similarities with PCBs
    - Similarities in health effects with PCBs
- Developmental effects of a commercial PBDE mixture
    - Neurobehavioral effects
    - Hormonal effects
    - Reproductive effects



Benefits of BFRs
(as per industry/BSEF)

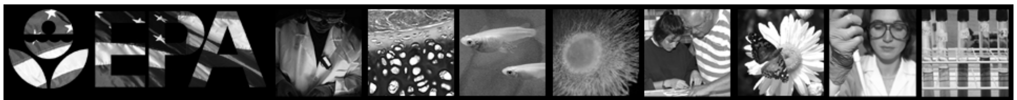
Fire regulations require a high degree of protection
(Fires kill 3000 people, injure more than 20,000 people, and result in property damages exceeding $11 million in US alone)

Flame retardants save lives and property

$ 2 billion/year industry; 300 million kg/year; US usage – 1/3

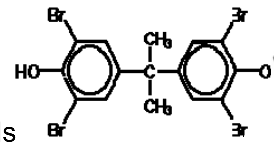
Cost-effective

BFRs prevent the spread of fires or delay the time of flashover, enhancing the time people have to escape

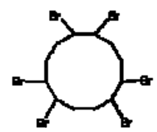


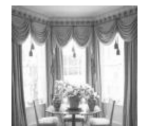
BFRs: Family of 75 substances with different properties

- TBBPA
  (Tetrabromobisphenol A)
  Reactive (90%) & Additive (10%)
  - Primary use – Electronics/circuit boards

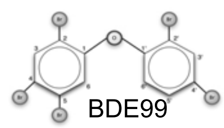


Hexabromocylododecane (HBCD)
  Additive
    Used in Electronics; Textile Backings
    Thermal Insulation in Buildings





Polybrominated diphenyl ethers
  Additive, Used in cushions, Sofas etc



PBDEs: High Production Volume Chemicals (Common name: Bromkal, Tardex, Saytex)

3 commercial mixtures (Penta and Octa no longer made)

- **Penta-BDE** ***(used in foam; 40% tetra, 45% penta, 6% hexa)***
  - **18.3 million pounds per year in the Americas**
  - **98 % of world use is in the Americas**
  - **All congeners highly bioaccumulative**
  - **86 to 99% of congeners found in human tissues**
- **Octa-BDE** ***(plastics, textiles; 10% hexa, 40% hepta, 30% octa, 20% nona)***
  - **3.0 million pounds per year in the Americas**
- **Deca-BDE** ***(plastics, textiles; 98% deca and 2% nona)***
  - **53.6 million pounds per year in the Americas**

"They're everywhere"

<u>PBDEs are now ubiquitous environmental contaminants</u>:

- –**Indoor and outdoor Air**
- –**House and office dust**
- –**Rivers and lakes and sediments**
- –**Sewage sludge**
- –**Remote Arctic regions (i.e., long-range transport)**
- –**Food**
- –**Biota (terrestrial & marine mammals, fish, humans)**

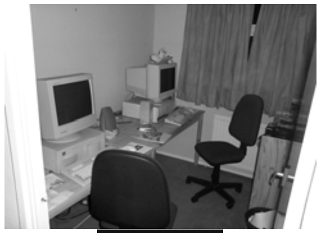
PBDE Point Sources

Chemical Plants

Wastewater Treatment Plants

Landfills

PBDE Non-Point Sources



Plastics

Furnishing Foam

Electrical Circuitry

Furnishing Foam

Human Exposure

Breastmilk

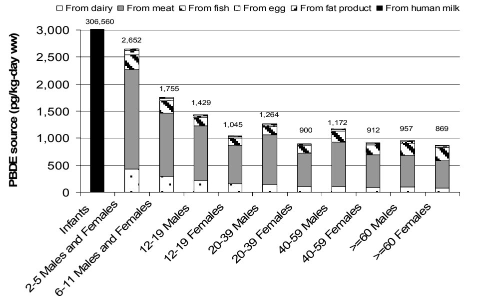
Maternal transfer to fetus

Diet (esp., fish)

Indoor, house & office dust, outdoor air

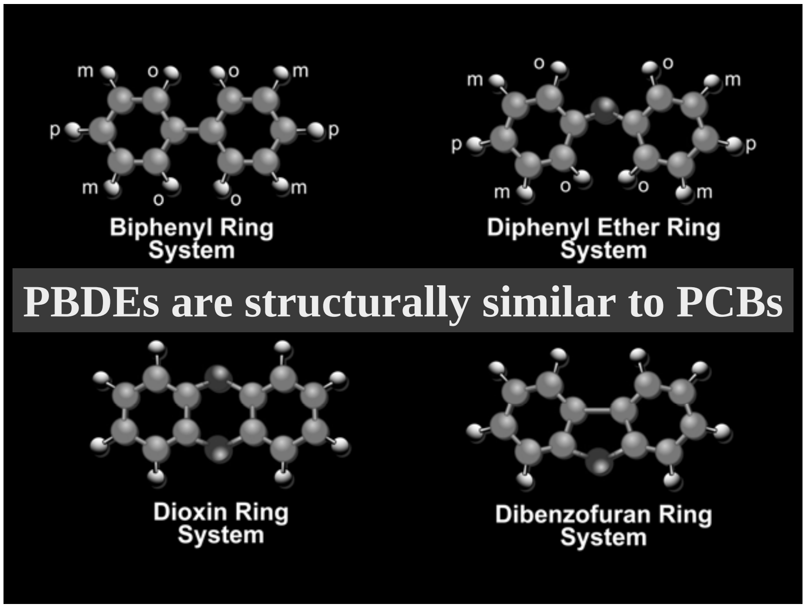
Occupation

PBDE Dietary Intake of U.S. Population by Age and Food Group (Schecter et al., 2006)



Why do we care about Polybrominated diphenyl ethers?

- Persistent, bioaccumulative, and structurally similar to PCBs, DDT, and other POPs.
- Levels are rapidly increasing in the environment and biological samples
- Effects seen in animals are similar to those seen with PCBs


m
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Biphenyl Ring System
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o
m
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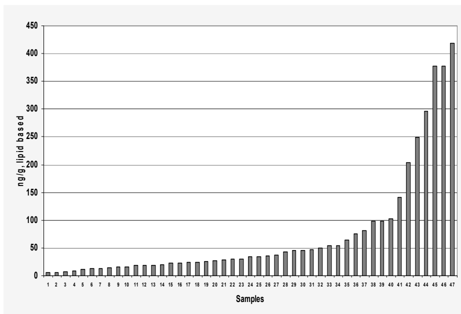
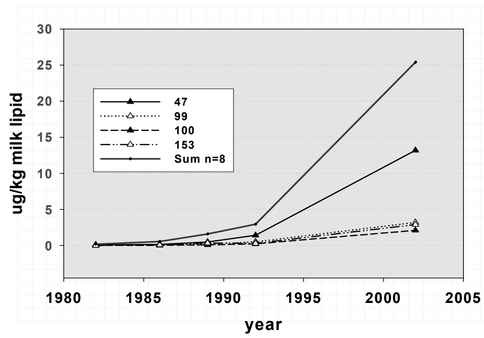
p
p
m
o
o
m
Diphenyl Ether Ring System
PBDEs are structurally similar to PCBs
Dioxin Ring System
Dibenzofuran Ring System

Levels are increasing in the biological samples

Total PBDEs in 47 human milks from Texas, 2002 (ppb lipid)
[Mean – 73.9; Median – 34.0 (6.2-418.8)]
(Schecter et al., SOT 2003)



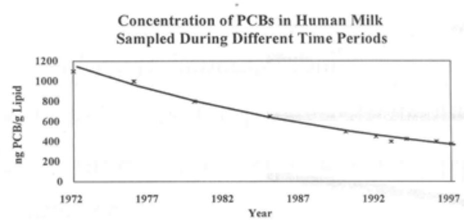
Time Trends of PBDEs in Canadian Breast Milk (Ryan and Patry, 2002)



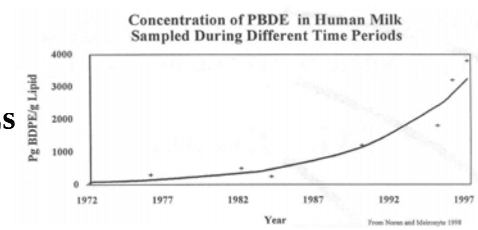
Are PBDE levels approaching to those of PCBs

PBDEs are increasing while PCBs and other POPs are decreasing in Human Milk in Sweden (Norén and Mieronyté, 1997)

PCBs



PBDEs



Are PBDE levels approaching to those of PCBs

Comparison of approximate PBDE adipose levels to PCB adipose levels among Californians

PBDE	PCB	Difference (PCB/PBDE)
PBDE-47 (33 ng/g)	PCB-153 (170 ng/g)	5-fold
sum PBDE (86 ng/g)	sum PCB (690 ng/g)	8-fold

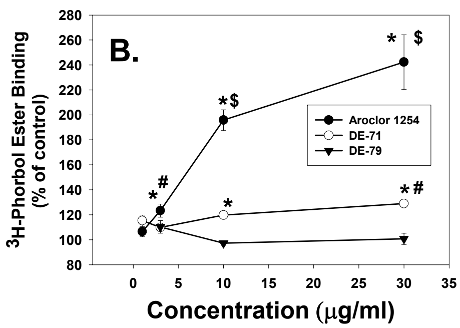
. . . and PBDE levels are increasing

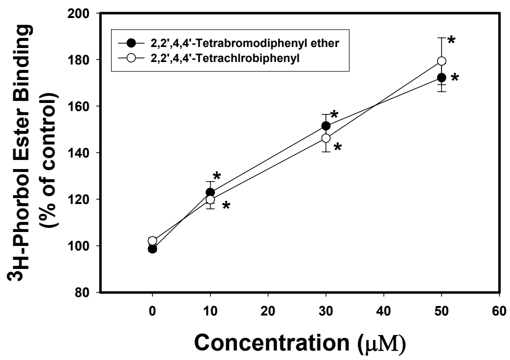
Developmental Neurotoxicity of PBDEs, similar to PCBs

- **Both mice and rats**
  - **Mice very sensitive (clear effects at 0.8 mg BDE-99/kg) in infantile period**
- **Sensory and Cognitive Effects**
- **Mechanism Unknown**
  - **Depression in serum T4**
  - **Effects on Intracellular signaling**
  - **Effects on neurotransmitters**

Neurochemical effects of PBDEs, similar to PCBs

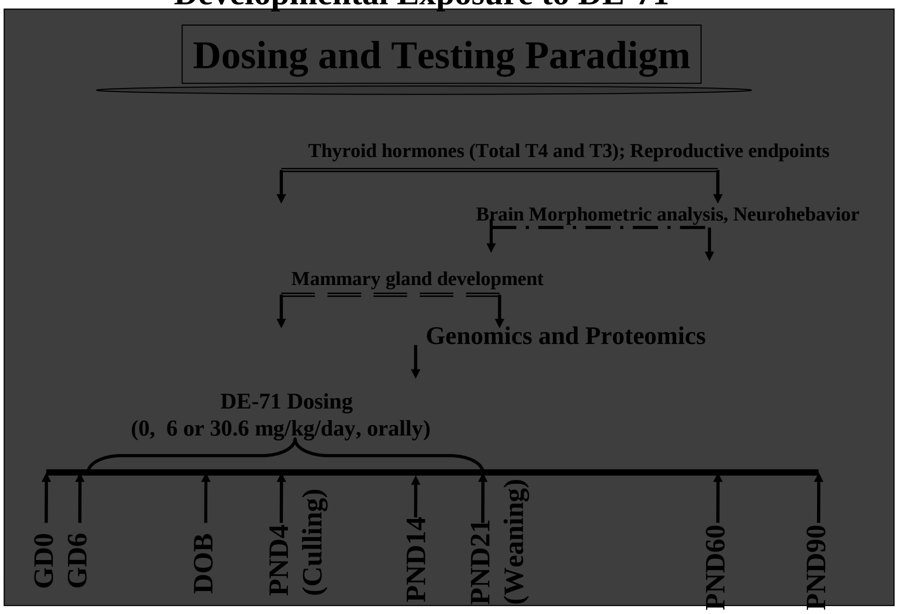
(Kodavanti and Ward, 2004)





PBDE 47 and PCB 47 are equally efficacious on a molar basis.

Developmental Exposure to DE-71



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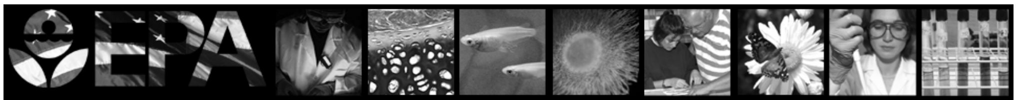
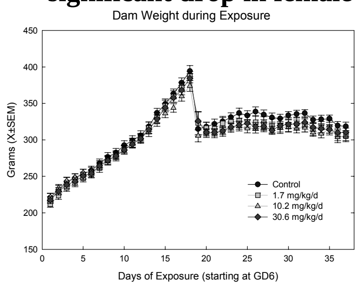
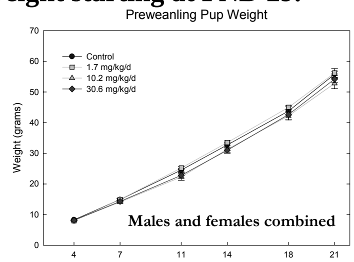
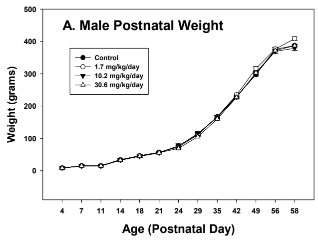
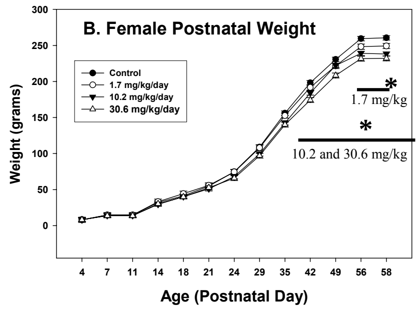


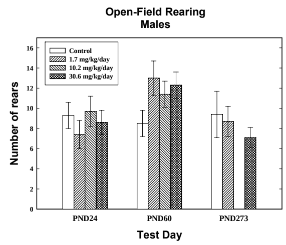
No effect on Dam weight or preweaning pup weight. However, there is a significant drop in female offspring weight starting at PND 29.

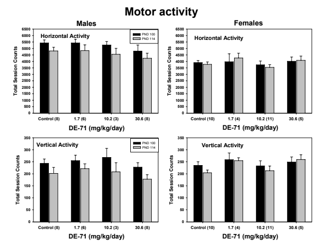




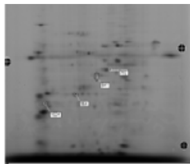








No significant effect on Neurobehavior except dose-by-age interaction in the number of rears in open field test.



Gel # 76276,

Samples:
This gel compares samples 2M control (PND 14) vs D7M (DE-71, 30.6mg/Kg)(PND 14).

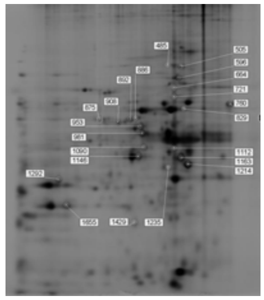
Tissue:
CEREBELLUM.

Protein Table   T-test and Av.Ratio: Treated / Control

Pos.	Master No.	Appearance	T-test	Av. Ratio	1-ANOVA
1	1024	6 (9)	0.048	1.57	0.048
2	964	9 (9)	0.044	1.46	0.044
3	841	9 (9)	0.046	1.62	0.046
4	792	9 (9)	0.044	1.34	0.044

Sample (Band/Spot On Gel)	Protein Name	Species	Database Accession ID[1]	MW (Da)	MS & MS/MS Score[2]	Peptide Sequenced (Ion Score)[3]	PID[4]
03-792	Ratsg1.- Rattus norvegicus (Rat).	Rattus norvegicus	Q8VHV7_RAT	40580.1	187	139	☑
03-792	Heterogeneous nuclear ribonucleoprotein H2.- Rattus norvegicus (Rat).	Rattus norvegicus	Q6AY09_RAT	49262.3	184	133	☐
03-792	Hnrph1 protein.- Rattus norvegicus (Rat).	Rattus norvegicus	Q499R8_RAT	20567.3	152	113	☐
04-841	BC078896 NID: - Rattus norvegicus	Rattus norvegicus	AAH78896	47098.2	551	406	☑
05-964	Aldolase C, fructose-biphosphate.- Rattus norvegicus (Rat).	Rattus norvegicus	Q54AI4_RAT	39259.2	1200	920	☑
06-1024	AF463524 NID: - Mus musculus	Mus musculus	AAN76992	37063.2	285	209	☑
06-1024	Albumin.- Rattus norvegicus (Rat).	Rattus norvegicus	Q5U3X3_RAT	68714.1	47	37	☐

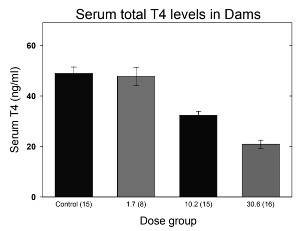
Proteins in Cerebellum with significant changes following developmental exposure to DE-71 at PND 14. Four proteins were affected by chemical exposure.

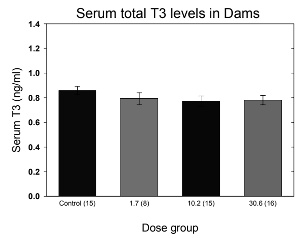


SPOT	NAME	LINK
1 = 485	Heat shock protein 105:	http://www.pir.uniprot.org/cgi-bin/upEntry?id=Q66HA8
2 = 505	Heat shock 70 kDa protein 4:	http://www.pir.uniprot.org/cgi-bin/upEntry?id=HSP74_RAT
3 = 596	Transitional Endoplasmic Reticullum ATPase	http://www.pir.uniprot.org/cgi-bin/upEntry?id=TERA_RAT
4 = 664	Eucariotic Translation Initiation Factor 4B	http://www.pir.uniprot.org/cgi-bin/upEntry?id=Q5RKG9_RAT
5 = 721	NADH dehydrogenase (Ubiquinone) Fe-S protein 1,	http://www.pir.uniprot.org/cgi-bin/upEntry?id=NDUS1_RAT
6 = 829	Neurofilament triplet L protein (68 kDa neurofilament	http://www.pir.uniprot.org/cgi-bin/upEntry?id=NFL_RAT
	dnaK-type molecular chaperone hsp72-ps1 - rat	http://www.ncbi.nlm.nih.gov/entrez/Op8gQvLmg8C21BQH7n8zCcu
7 = 760	myristylated alanine-rich protein kinase C substrate,	http://www.pir.uniprot.org/cgi-bin/upEntry?id=MARCS_RAT
8 = 875	Dihydropyrimidinase related protein 5	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DPYL5_RAT
9 = 908	Stress-induced phosphoprotein 1	http://www.pir.uniprot.org/cgi-bin/upEntry?id=STIP1_RAT
	Dihydropyrimidinase related protein 5	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DPYL5_RAT
10 = 892	Dihydropyrimidinase related protein 2	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DPYL2_RAT
11 = 886	Dihydropyrimidinase related protein 2	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DPYL2_RAT
12 = 953	Dihydropyrimidinase related protein 2	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DPYL3_RAT
13 = 981	Protein disulfide isomerase A3 precursor	http://www.pir.uniprot.org/cgi-bin/upEntry?id=PDIA3_RAT
14 = 1146	Alpha-enolase (EC 4.2.1.11) (2-phospho-D-glycerate	http://www.pir.uniprot.org/cgi-bin/upEntry?id=ENOA_RAT
15 = 1292	Phosphoglycerate kinase 1.- Rattus norvegicus (Rat).	http://www.pir.uniprot.org/cgi-bin/upEntry?id=PGK1_RAT
	fructose-bisphosphate aldolase (EC 4.1.2.13) A - rat	http://www.pir.uniprot.org/cgi-bin/upEntry?id=ALDOA_RAT
16 = 1655	Glyceraldehyde-3-phosphate dehydrogenase (EC	http://www.pir.uniprot.org/cgi-bin/upEntry?id=G3P_RAT
17 = 1112	glial fibrillary acidic protein, astrocyte - rat	http://www.pir.uniprot.org/cgi-bin/upEntry?id=GFAP_RAT
	f1-atpase beta chain beta chain (EC 3.6.1.34), chain B -	http://www.ebi.ac.uk/interpro/potm/2005_12/Page4.htm
18 = 1163	Dynactin 2.- Rattus norvegicus (Rat).	http://www.pir.uniprot.org/cgi-bin/upEntry?id=DCTN2_RAT
19 = 1214	phosphopyruvate hydratase (EC 4.2.1.11) gamma - rat	http://www.pir.uniprot.org/cgi-bin/upEntry?id=ENOG_RAT
20 = 1235	Creatine Kinase B type	http://www.pir.uniprot.org/cgi-bin/upEntry?id=KCRB_RAT
21 = 1429	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37)	http://www.pir.uniprot.org/cgi-bin/upEntry?id=MDHC_RAT

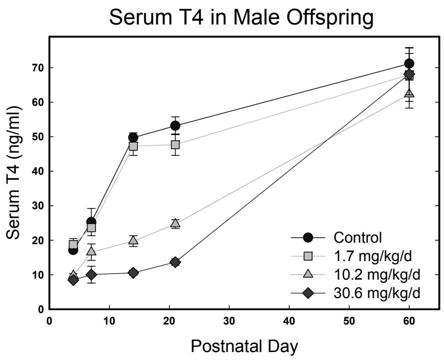
Proteins in Hippocampus with significant changes following developmental exposure to DE-71 at PND 14. Fifty two proteins were affected, but only few shown in the table. These proteins belong to energy metabolism, calcium signaling and growth of the nervous system.

Decreased Thyroxine in Dams PND22

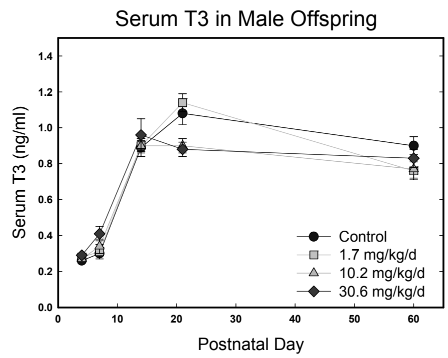


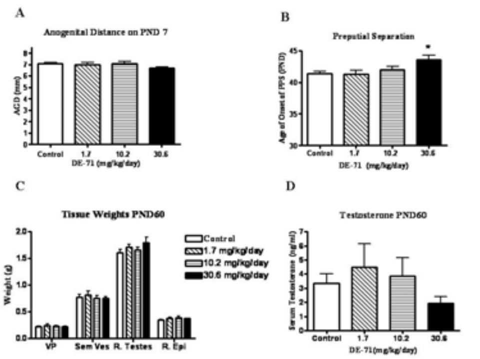


Decreased Thyroxine in Pups

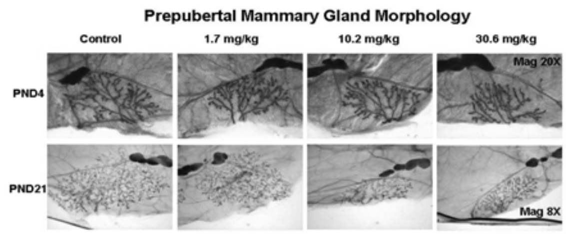


Females similarly affected

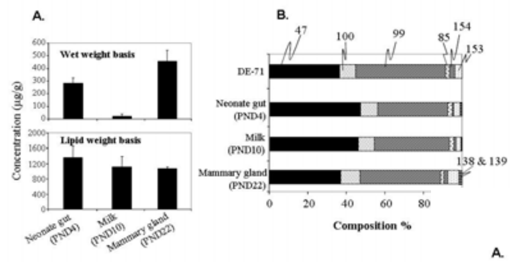




DE-71 affected anogenital distance and preputial separation in male pups. Rep Tissue weights and serum testosterone conc were not altered.



DE-71 affected mammary gland development significantly at PND 21.



PBDE concentrations were comparable among various brain regions.
PBDE 47 is a predominant congener followed by PBDE 99 and 100.

Congener-specific analysis of PBDEs indicated accumulation in all tissues examined. Highest conc were found in fat including milk whereas blood has the low conc on a wet wt basis.

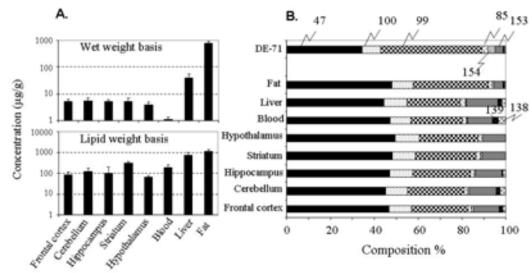


Figure 12.

Summary

Developmental exposure to DE-71

- No dramatic effect on Neurobehavior, but proteins related to energy metabolism, calcium signaling and growth of the nervous system were affected.
- Caused severe hypothyroxinemia in dams and offspring
- Affected male reproductive system (anogenital distance, preputial separation)
- Affected mammary gland development in females
- Highest conc were found in fat including milk. PBDE conc were comparable among brain regions, but still lower than liver and fat.

Potential Risk Assessment of PBDEs

(Proposed by Dr. Deb Rice)

Since there is not sufficient pharmacokinetic data for extrapolation from rodents to humans, one approach could be to compare current levels of PBDEs in humans with the levels of PCBs that are known to produce adverse human health effects including developmental neurotoxicity which is considered to be one of the most sensitive endpoints.

Studies from Netherlands & Germany documented adverse effects associated with cognition when breast milk levels of PCBs were in the range of 263-1615 ng/g (median = 690 ng/g).

In North America (TX and NC), PBDEs in breast milk were reported to be in the range of 6-1078 ng/g with a median of 34-58 ng/g, which is ten times less than those of PCBs.
In North America (NY), cord blood conc varied from 1 to 955 ng/g with a median of 19 ng/kg

However, the top 5% of population have levels similar to PCBs and this may pose a risk.

Since the effects of PCBs and PBDEs are mostly additive and some times synergistic, the levels of PBDEs at current level may be producing adverse health effects.

Additional research is needed to better assess the risk associated with exposure to these persistent chemicals.

Thank you

for your attention

Upcoming Webinars

- **Session II: Metals and Metal Mixtures**
  - March 28th, 1:00 – 3:00 PM ET
  - **Robert Wright (Harvard School of Public Health):** Neurodevelopmental consequences of mixed metal exposures (Pb, As, Mn), comparing different developmental windows.
  - **Rebecca Fry (University of North Carolina):** Prenatal exposure to cadmium, poor birth outcomes, and inflammatory mechanisms.
- **Session III: PCE and Phthalates**
  - April 2nd, 1:00 – 3:00 PM ET
  - **Ann Aschengrau (Boston University School of Public Health):** Early life exposure to PCE-contaminated drinking water and later-life neurotoxic effects.
  - **Rita Lock-Caruso and John Meeker (University of Michigan School of Public Health):** Phthalate exposure and preterm birth in Puerto Rico: environmental, genetic, demographic, and behavioral factors.

Other SRP Early-Life Exposure Researchers

- Camenisch, Todd. P42ES004940, University of Arizona, *Project: "As Effects On Cardiovascular Development and Disease"*
- Corley, Richard. P42ES016465, Oregon State University, *Project: "Cross-Species Comparison of Transplacental Dosimetry PAHs"*
- Furlong, Clement. P42ES004696, University of Washington, *Project: "Biomarkers of Susceptibility to Environmentally-Induced Diseases"*
- Karagas, Margaret. P42ES007373, Dartmouth College, *Project: "Epidemiology, Biomarkers and Exposure Assessment of Metals"*
- Lantz, Robert. P42ES004940, University of Arizona, *Project: "Pulmonary Response to Toxicants in Susceptible Population"*
- Lasley, Bill. P42ES00004699, University of California-Davis, *Project: "Assessing Adverse Effects of Environmental Hazards on Reproductive Health"*
- Sharma, Surendra. P42ES013660, Brown University, *Project: "Genetic Stress and Toxicant-induced Pregnancy Disruption"*
- Slotkin, Theodore. P42ES10356, Duke University, *Project: "Developmental Neurotoxicants: Sensitization, Consequences, and Mechanisms"*
- Smith, Allan. P42ES004705, University of California-Berkeley, *Project: "Arsenic Biomarker Epidemiology"*

http://tools.niehs.nih.gov/srp/search/index.cfm

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Thank you!

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