Advances in Research Tools & Methods for Use in Observational and Longitudinal Studies

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Contributing Organizations: ORD, NERL, NHEERL, NCER Grantees

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LTG 3 Poster 05

Science Questions

- Which tools are required to measure environmental exposures in susceptible populations?
- Can surrogate analyses provide realistic information on children's environmental exposures?
- How can biomarkers of exposure and effect be implemented in longitudinal study designs?

Research Goals

Goals for this research program:

(1) Develop tools and methods for biomarkers and environmental measures of toxicant exposure or effects

(2) Focus on biospecimens obtainable with minimal invasiveness from human subjects, including children, infants and pregnant/lactating women

(3) Implement/validate biomarker use in longitudinal research studies of susceptible populations.

Findings and Conclusions

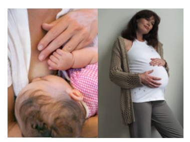
Our cross-ORD and inter-Agency research efforts have:

- Created methods for the collection, preservation and storage of biological samples, as well as indoor and dietary exposure samples.
- Developed analytical assays to compare endogenous and exogenous constituents of breast milk, in contrast to that in blood, saliva, and urine of the same woman.
- Developed multi-media and -residue methods (including pesticides, brominated flame retardants [BFRs], phthalates, perfluoroalkyl acids [PFAAs]) to determine total environmental exposures from water, house dust, indoor air, foods and life stage-specific activities. Integrated across-Agency research highlighting BFR methods for analysis of biospecimens in the MAMA study and analytical outcomes from a representative chromatogram in NERL are shown. PBDEs at high concentrations in breast milk.
- Developed methods to measure pesticide metabolite concentrations in biological samples in a variety of studies and evaluated the use of surrogate samples for estimates of infant exposure *in utero*, as well as early postnatal periods.
- Developed databases to facilitate searching the peer-reviewed literature for child-specific biomarkers (shown at left) and for describing time-activity patterns of susceptible populations. CHAD provides integrated time-activity information for model inputs (shown below).
- Created non-invasive methods to accurately collect and estimate children's indoor exposure to pesticides. Analytical methods were used to quantitatively measure dietary pesticide intake (composite diets).
- Validated assays were developed to measure chemicals in urine, serum, amniotic fluid (cord blood), saliva, and milk (e.g., phenols, PFAAs, parabens, phthalates, BFRs, PCBs, POPs, perchlorates, and pesticides) of susceptible populations. The Methods Advancement for Milk Analysis (MAMA, US EPA) study and the CHAMACOS study (UC-Berkeley) collected biological fluids from lactating women.
- Non-invasive sampling methods in infants, reflective of *in utero* exposures, were developed to demonstrate the usefulness of surrogate DNA for assessing metabolic and detoxification genetic polymorphisms (fingernails, meconium, hair, etc).
- Demonstrated a measureable decrease in meconium-derived chlorpyrifos following the deregistration of this compound from products for residential pest control.
- The validation/testing of identified biomarkers, such as TCPy, in longitudinal children's studies has just begun. We have on-going studies at Duke University (autism) and the University of Washington/UC Berkeley (neurodegenerative disease in farming children), as well as other studies described in LTG-1 and LTG-2.

Methods/Approach

Our Research Focus

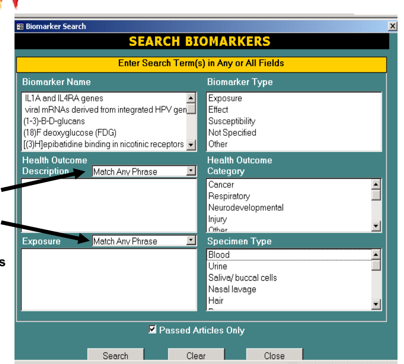
Infants and Children



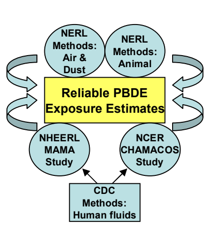
Pregnant and/or Lactating Populations

Children's Health Biomarkers Database

http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=85844

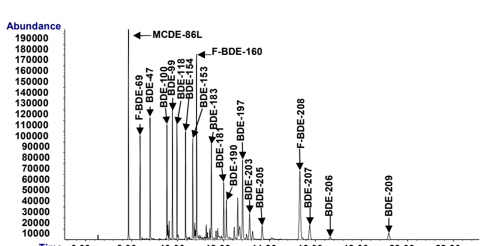


PBDE's: Multi-targeted Methods

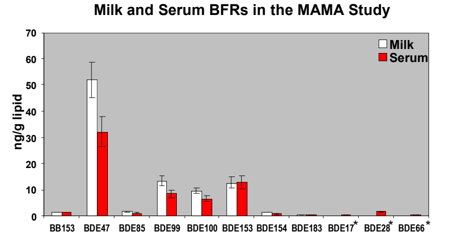


PBDEs Detected in MAMA Study (milk/serum)

Compound	% Detectable
BDE47 (penta)	100/100
BDE85 (penta)	85/100
BDE99 (penta)	98/100
BDE100 (penta)	98/100
BDE153 (hexa)	88/100
BDE154 (hexa)	94/100
BDE183 (hepta)	96/100
BDE17 (tri), BDE28, BDE66	n.m./100
BB153 (POP)	98/100

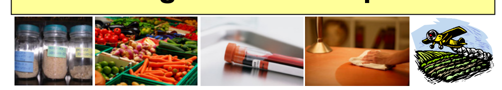


Milk and Serum BFRs in the MAMA Study



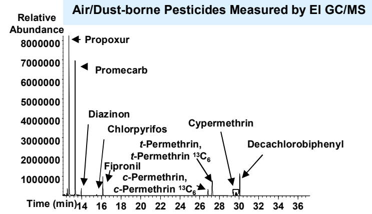
Lipid-adjusted milk concentrations equal to or higher than adjusted serum levels for all congeners measured in milk. No time-related difference in BFRs was detected.

Measuring Pesticide Exposures



Dietary Body Fluids Dust & Air

Air/Dust-borne Pesticides Measured by EI GC/MS



Data Collection

Where are Pesticides Detected?

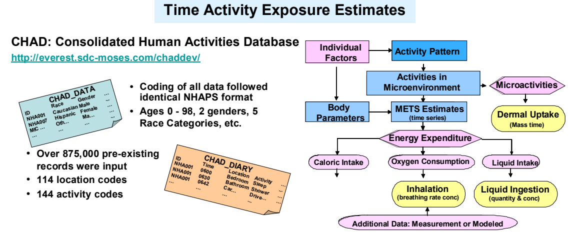
Study Name	Study Institution	Assays for Which Media?
MAMA Study	NHEERL; US EPA	Maternal milk, serum, urine, saliva
CHAMACOS	NCER; UC Berkeley	Breast milk, urine (mother & child)
CCCEH	NCER; Columbia Univ.	Maternal plasma, urine, & cord blood
Numerous PIs	NERL; US EPA	Indoor air, dust, composite diet, contact exposures, daycares
Mothers & Newborns Study	NCER; Columbia & Mt. Sinai Medical Centers	Meconium, urine, cord and maternal blood

Time Activity Exposure Estimates

CHAD: Consolidated Human Activities Database

http://everest.sdc-moses.com/chaddev/

- Coding of all data followed identical NHAPS format
- Ages 0 - 98, 2 genders, 5 Race Categories, etc.
- Over 875,000 pre-existing records were input
- 114 location codes
- 144 activity codes



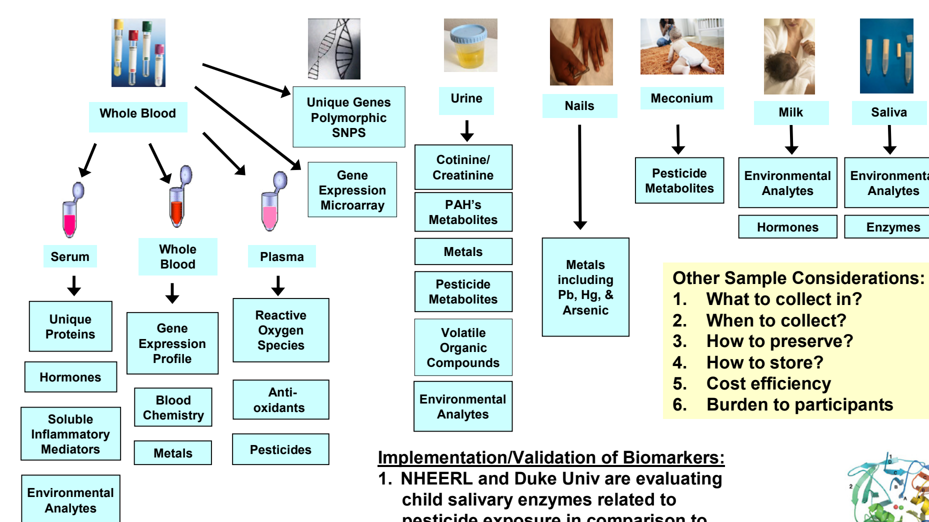
Recovery of Pyrethroid Pesticides from Standard Composite Diets

Analyte	% Recovery (RSD) HFCD[1]		MFCD[1]		LFCD[1]	
	10 µg kg⁻¹	50 µg kg⁻¹	10 µg kg⁻¹	50 µg kg⁻¹	10 µg kg⁻¹	50 µg kg⁻¹
Allethrin 1a	NR [2]	87.8 (7.9)	NR [2]	92.6 (6.0)	NR [2]	84.2 (1.1)
Allethrin 1b	NR [2]	88.1 (10)	NR [2]	85.0 (10)	NR [2]	92.3 (4.2)
Allethrin 2a	72.9 (3.5)	88.6 (5.8)	88.9 (3.4)	85.3 (5.7)	NR [2]	88.8 (4.3)
Allethrin 2b	85.2 (8.1)	88.7 (17)	101 (13)	90.0 (14)	NR [2]	90.3 (6.8)
Resmethrin	65.1 (14)	80.3 (8.4)	50.2 (19)	26.2 (29)	59.7 (11)	70.3 (4.1)
Bifenthrin	90.5 (4.8)	96.0 (6.2)	90.3 (11)	91.8 (1.7)	85.0 (9.5)	97.9 (2.3)
Tetramethrin	105 (6.5)	93.3 (3.5)	91.3 (8.7)	91.1 (1.3)	91.6 (3.3)	94.6 (1.9)
Phenothrin	86.7 (9.0)	88.5 (6.2)	88.9 (10)	89.8 (1.4)	97.1 (27)	90.9 (1.4)
L-Cyhalothrin 1	112 (4.2)	95.3 (3.9)	79.6 (8.9)	92.0 (1.8)	79.2 (16)	95.4 (1.6)
Mirex (Surrogate)	63.5 (18)	79.1 (5.3)	67.1 (18)	79.4 (6.8)	81.9 (2.2)	83.0 (3.0)
L-Cyhalothrin 2	89.7 (14)	101 (7.2)	94.0 (10)	92.9 (2.3)	77.0 (8.1)	93.8 (1.8)
cis- Permethrin	92.9 (4.2)	90.3 (2.4)	94.5 (11)	92.7 (3.1)	93.5 (2.5)	93.2 (2.3)
trans- Permethrin	88.0 (8.5)	91.9 (2.1)	63.9 (9.5)	93.5 (6.2)	93.4 (4.6)	93.8 (2.6)
Cyfluthrin 1	80.4 (26)	81.7 (14)	92.0 (16)	96.3 (4.9)	86.7 (5.4)	95.2 (2.1)
Cyfluthrin 2	NR [2]	NR [2]	NR [2]	NR [2]	NR [2]	124 (25)
Cyfluthrin 3	NR [2]	NR [2]	NR [2]	NR [2]	NR [2]	NR [2]
Cyfluthrin 4	NR [2]	NR [2]	NR [2]	97.0 (4.4)	NR [2]	100 (8.5)
Cypermethrin 1	104 (6.8)	91.8 (4.5)	103 (11)	93.7 (3.6)	102 (4.9)	92.7 (2.0)
Cypermethrin 2	126 (4.8)	92.7 (6.3)	106 (8.6)	91.0 (17)	109 (10)	89.4 (9.8)
Cypermethrin 3	104 (9.0)	90.1 (4.8)	103 (5.2)	94.8 (3.8)	105 (8.0)	94.4 (1.7)
Cypermethrin 4	96.6 (7.0)	91.6 (3.9)	92.5 (4.9)	93.8 (6.8)	89.0 (8.9)	91.2 (5.5)
Fenvalerate	95.1 (16)	91.0 (11)	98.2 (8.7)	92.8 (13)	147 (11)	89.9 (11)
Esfenvalerate	85.9 (16)	81.9 (4.8)	68.6 (11)	80.5 (9.7)	90.8 (7.2)	88.2 (8.1)
Tralomethrin	84.4 (9.6)	79.0 (5.7)	66.1 (16)	84.5 (14)	88.7 (12)	95.0 (12)
Deltamethrin	128 (24)	91.5 (13)	119 (24)	92.1 (17)	99.7 (7.3)	86.3 (12)

[1] HFCD = high fat composite diet, MFCD = medium fat composite diet, LFCD = low fat composite diet, [2] NR = No Result

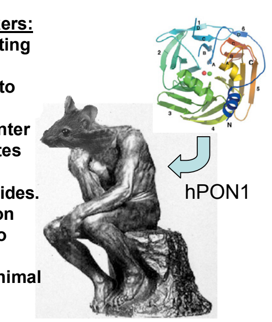
Putting Biomarkers to the Test

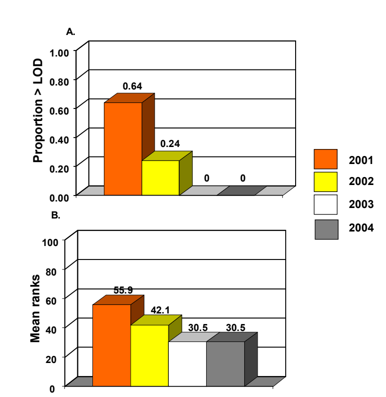
A Variety of Biomaterials are used in Biomarker & Method Development



Other Sample Considerations:
1. What to collect in?
2. When to collect?
3. How to preserve?
4. How to store?
5. Cost efficiency
6. Burden to participants

Implementation/Validation of Biomarkers:
1. NHEERL and Duke Univ are evaluating child salivary enzymes related to pesticide exposure in comparison to measured urinary pesticide levels.
2. Columbia University Children's Center is evaluating the utility of metabolites in meconium as indicators of the infant's *in utero* exposure to pesticides.
3. UC Berkeley and Univ of Washington are evaluating the utility of PON1 to predict susceptibility of individual children to effects of pesticides. Animal models have been developed to further understand the mechanism.





Chlorpyrifos metabolite in meconium after EPA deregistration. (A) Percent of meconium samples with TCPy concentrations > LOD and (B) Mean TCPy ranks by year of sample collection.

Spearman's rank correlation of TCPy levels in meconium and chlorpyrifos in maternal and umbilical cord blood and TCPy in spot urine samples collected from the mother during pregnancy and after delivery.

Chlorpyrifos in blood samples (pg/g)	TCPy levels in meconium (ng/gram)
Maternal blood	r=0.25, p=0.03, n=79
Umbilical cord blood	r=0.33, p=0.01, n=56
TCPy in maternal urine (ug/gram creatinine)	
1st prenatal	r=0.22, p=0.06, n=74
2nd prenatal	r=0.35, p=0.003, n=71
3rd prenatal	r=0.28, p=0.06, n=45
4th prenatal Average prenatal After delivery	r=0.40, p=0.10, n=17 r=0.31, p=0.006, n=78 r=0.32, p=0.01, n=60

Sample size 2001 (n=28); 2002 (n=21); 2003 (n=21); 2004 (n=13).
[a] Chi-square (2001-2002 vs 2003-2004): 22.1, p<0.001.
[b] Mean ranks (2001-2002 vs 2003-2004): 50.0 versus 30.5, p<0.001 (Kruskal Wallis);
Mean rank 2001 vs 2003, p<0.001, 2001 vs 2004, p=0.001, 2002 vs 2003, p=0.02, Mann-Whitney U.

Impact and Outcomes

- Assist in development of protocols, choice of assays, and recruitment and advertisement tactics for longitudinal studies like the NCS
- Creation and evaluation of unique tools and methods allows the NCS to choose the bio-specimens, environmental samples, and methods that provide the most useful exposure or effect information while minimizing costs and participant burden.
- Products integrated into the NCS protocols, to measure exposure for pregnant and lactating women and their children, will inform other observational and longitudinal studies
- Efforts have led to:
  1. improved exposure models used in OAQPS risk assessments
  2. inclusion of high use pesticides in the NHANES nationwide biomonitoring survey
- Validation of predictive biomarkers of exposure or effect will reduce uncertainty in risk assessments and lead to effective intervention and disease treatments.

Future Directions

- Produce high quality, low cost and low burden tools and methods for longitudinal cohort studies
- Improve sensitivity, accuracy, and utility of current collection methods and assays
- Create a decision tree for surrogate sample analyses that can be used in longitudinal children's studies
- Translate information from Children Health Center grantees to formulate retention tactics and other "lessons learned" for use in NCS
- Develop a plan to integrate ORD research into the NCS–define participant exposure routes and pathways, exposure-related hormone levels, oxidative stress, inflammatory reactions, and specific gene perturbations
- Provide data for exposure models and risk assessments

Researchers Involved

NHEERL- David Demarini, Suzanne Fenton, Jane Gallagher, Gary Hatch, Erin Hines, Danelle Lobdell, Suzanne McMaster, Stephanie Padilla; NCER Grantees- Brenda Eskanazi (UC Berkeley), Elaine Faustman (Univ of Washington), Frederica Perera (Columbia Univ), Robin Whyatt (Mt. Sinai); NERL-M. Scott Clifton, Maribel Colon, Tom McCurdy, Lisa Melnyk, Jeff Morgan, Dan Stout, Elin Ulrich, Stephen Vesper, Walter Weathers, Don Whitaker. Rich Callan (NCER), Pauline Mendola (NCHS) and Sherry Selevan (US EPA-retired).

Susceptible Populations