History of the project

• 2010 NIH funded 2 year pilot project
• UMass Lowell partnered with Mahidol University Faculty of Public Health in Bangkok Thailand
• Hypothesis: low maternal iodine and pesticide exposure may act synergistically to alter thyroid hormone status resulting in deficiencies in child neurobehavioral development
**Methods**

- Recruit pregnant women from 3 provinces in Thailand representing a range of risks for pesticide exposure and iodine deficiency disorders.
Methods

– At recruitment (7 months)
  • Urine for iodine & pesticide metabolite (organophosphates (OP))
  • Extensive health history, exposure history, dietary questionnaire

– At birth
  • Urine for maternal iodine, pesticide metabolites (OP’s)
  • Blood for maternal thyroid hormone & hemoglobin levels
  • Placental cord blood for thyroid hormone
  • APGAR and other birth data
  • Brazelton Newborn Behavioral Assessment Survey (BNBAS)
Newborn Behavioral Assessment Scale (NBAS)

• NBAS covers 28 behavioral items and 16 primitive reflexes.
• Cluster scored into 7 domains:
  – habituation,
  – orientation,
  – motor,
  – range of state,
  – regulation of state,
  – autonomic stability,
  – number & type of abnormal primitive reflexes
• Used Certified NBAS trainer to train Thai pediatricians
• Test in first 4 days of life
  – (avg 1.8 days; 91% < 3; max 4)
Further Data Collection

- **Age 2 months:** maternal urine & breast milk for pesticides and iodine & questionnaires

- **Age 5 months:** Neurobehavioral testing with observational Bayley Scales and physiological testing with EEG/ERP (Electroencephalogram/Event Related Potential) & questionnaires

- **Questionnaires:** infant health, behavior, food intake, mothers nutrition and exposure to pesticides
- **EEG/ERP**
  - Infants view images of mother vs stranger (500ms each)
  - EEG/ERP data is collected for 1500 ms after each picture appears on the screen
  - ERP components relate to perceptual processing
- **Bayley Scales**
  - Observational assessment of motor skills (fine & gross) and cognitive development (attention, memory, learning)
    - Mental Development Index
    - Psychomotor Development Index
Unforeseen Complications

• In Oct 2010 the MoPH began providing iodine supplement tablets to pregnant women.

• Major flooding Sept 2011-Jan 2012
  – 13.6 million people affected. 65/77 provinces were declared flood disaster zones, > 20,000 km² (7,700 mi²) land damaged, seven major industrial estates flooded to 3 m (10 ft)
Results

• Recruited 112 women at 7 months pregnancy
  – 27 agricultural workers
  – 30 live with agricultural workers or worked in fields less than 1 day per week for only 1 trimester.
  – 55 unexposed to agricultural pesticides
    • 30 (55%) reported applying insecticide in home during pregnancy
• NBAS data collection on 82 newborns
  – 27% loss to follow-up from recruitment due to cesarean section, prematurity (<37 weeks), delivered elsewhere (some due to flooding).
Pesticide Use

Pregnant agricultural workers report:

- 81% used insecticides on their farm
  - 41% used organophosphates
  - 4% report using pyrethroids
- 85% used herbicides on their farm
  - 41% used glyphosate
  - 59% used paraquat
  - 26% use 2,4-D
Pesticide Use

During pregnancy Agworkers reported:

- 19% used pesticides to treat seeds
- 59% mixed pesticides
- 63% applied pesticides to crops
- 37% applied pesticides to animals
- 63% entered fields sprayed with pesticides on the same day
- 74% washed clothes used to mix or apply pesticides (42% wash with regular clothes)
- 57% never use rubber gloves when mixing pesticides
Newborn Behavioral Assessment Scale (NBAS)

- 3/7 Thai test results significantly poorer than CHAMACOS longitudinal birth cohort in California (85% Mexican-American; 64% < poverty level; many agricultural workers)
- 2/7 Thai tests results better than CHAMACOS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Δ CHAMACOS – our data</th>
<th>t-test with CHAMACOS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habituation</td>
<td>7.3</td>
<td>1.2</td>
<td>-0.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Orientation</td>
<td>6.1</td>
<td>1.5</td>
<td>1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Motor</td>
<td>5.7</td>
<td>0.8</td>
<td>0.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Range of State</td>
<td>3.9</td>
<td>0.5</td>
<td>-0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regulation of State</td>
<td>5.2</td>
<td>1.7</td>
<td>0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Autonomic Stability</td>
<td>6.1</td>
<td>1.1</td>
<td>0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal Reflex</td>
<td>1.8</td>
<td>2.3</td>
<td>-0.1</td>
<td>0.08</td>
</tr>
</tbody>
</table>
NBAS

- Each NBAS cluster score examined for association with potential covariates in linear or poisson regression (reflex). Covariates included in final model if $p \leq 0.15$
  - Mother’s age, income, education, parity, marital status, alcohol use, cough medicine use, caffeinated soda use, nonprescription medicine use, babies gender, NBAS tester

- Final models include Agworker & Iodine ($0/1$) variables
  - Agricultural worker (n=21): at least 2-3 days/month in field in any trimester for mother
  - Iodine tablet supplement (n=71): medical staff report of date tablets provided & subject reported receiving tablets during at least 1 trimester.
## Final NBAS Model Estimates

<table>
<thead>
<tr>
<th></th>
<th>Orientation</th>
<th>Motor</th>
<th>Range of State</th>
<th>Regulation of State</th>
<th>Habituation</th>
<th>Autonomic Stability</th>
<th>Abnormal Reflexes Rel Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Agworker</td>
<td>-0.41</td>
<td>-0.22</td>
<td>0.27</td>
<td>-0.03</td>
<td>-0.45</td>
<td>-0.07</td>
<td>0.77 ChiSq=0.31</td>
</tr>
<tr>
<td></td>
<td>p=0.30</td>
<td>p=0.24</td>
<td>p=0.04</td>
<td>p=0.95</td>
<td>p=0.10</td>
<td>p=0.72</td>
<td></td>
</tr>
<tr>
<td>Iodine tablets provided</td>
<td>0.02</td>
<td>0.22</td>
<td>0.17</td>
<td>0.78</td>
<td>-0.24</td>
<td>-0.05</td>
<td>0.45 ChiSq=0.09</td>
</tr>
<tr>
<td></td>
<td>p=0.97</td>
<td>p=0.36</td>
<td>p=0.34</td>
<td>p=0.17</td>
<td>p=0.55</td>
<td>p=0.87</td>
<td></td>
</tr>
</tbody>
</table>

- Agworker parameter direction (N.S.) for 5/7 tests suggests being Agworker results in poorer newborn neurological function.
- Iodine parameter direction (N.S.) for 5/7 suggests tablets improve newborn neurological function.
### Newborn Demographics

- Birth data on 78 of 82 NBAS babies.
- Using linear and logistic regression with covariates: **Agworker or iodine tablet supplements were not significant predictors of birth outcomes.**

<table>
<thead>
<tr>
<th>Newborn</th>
<th>Mean</th>
<th>SD</th>
<th>% &lt; norm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>3.08</td>
<td>0.41</td>
<td>11</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>51.6</td>
<td>2.3</td>
<td>9</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>22.3</td>
<td>1.4</td>
<td>9</td>
</tr>
<tr>
<td>Apgar 1 min</td>
<td>8.9</td>
<td>1.0</td>
<td>7</td>
</tr>
<tr>
<td>Apgar 5 min</td>
<td>9.7</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>Apgar 10 min</td>
<td>9.8</td>
<td>0.4</td>
<td>5</td>
</tr>
</tbody>
</table>
Cord Blood Thyroid Hormone

• 35% of newborns were out of normal TSH range.
  – 6.5% had severely high TSH levels
  – Compared to 4.6% of >5000 neonates at a Bangkok hospital in 2003
• 34% of newborns had low free T4 levels.
• 11.6% newborns clinically hypothyroid based on cord blood
Thyroid Hormone, Iodine supplements & Agworker status

- Using linear and logistic regression with covariates: Agworker or iodine tablet supplements were not significant predictors of thyroid levels or hypothyroid status of either the mother or newborn cord blood.
The latency and amplitude differences for the ERPs suggest slower processing of novel stimuli (stranger’s faces) from the infants of agricultural workers.

Single child of an agricultural worker who did not receive iodine supplementation showed no discernible ERP components, suggesting very delayed development.
Future Directions

• For NBAS, Thyroid hormone, Birth data
  – Add organophosphate (OP) urinary metabolite levels at 7 months and birth to models
  – Add estimates of iodine uptake from food frequency questionnaire and tablet intake
• Complete collection/analysis of Bayley & EEG/ERP :
  – OP urine metabolite & breast milk levels
• Increase power by recruitment in follow-up study
• Follow subjects longitudinally
• Examine herbicide exposures