DEVELOPMENTAL LEAD EXPOSURE ALTERS RODENT MATERNAL PUP RETRIEVAL DISRUPTING ADOLESCENT SOCIAL-PLAY

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INTRODUCTION: HUMAN LEAD (PB) TOXICITY IN REVIEW

Adverse Health Effects of Lead Exposure

- Death
- Encephalopathy
- Nephropathy
- Frank Anemia
- Colic
- Decreased hemoglobin synthesis
- Increased vitamin D metabolism
- Increased risk of hypertension in adulthood
- Increased nerve conduction velocity
- Increased level of erythrocyte protoporphyrin
- Decreased vitamin D metabolism
- Decreased calcium homeostasis
- Developmental toxicity
  - Delayed puberty
  - Decreased growth & hearing
- Developmental toxicity
  - Decreased IQ levels & academic abilities
  - Attention-related behaviors
  - Anti-social behaviors

Lead poisoning

Lead buildup in the body causes serious health problems

Symptoms
- Headaches
- Irritability
- Reduced sensations
- Aggressive behavior
- Difficulty sleeping
- Abdominal pain
- Poor appetite
- Constipation
- Anemia

Additional complications for children:
- Lead is more harmful to children as it can affect developing nerves and brains
  - Loss of developmental skills
  - Behavior, attention problems
  - Hearing loss
  - Kidney damage
  - Reduced IQ
  - Slowed body growth

Source: MedlinePlus/Mayo Clinic

100 mL’s = 1 dL
## Chronological Review of the United States CDC’s Threshold for Pb Poisoning Interventions

<table>
<thead>
<tr>
<th>Year of CDC Revision</th>
<th>Number of Years Between CDC Revision</th>
<th>CDC BLL Listed as Threshold</th>
<th>CDC BLL Considered to be Safe /Actionable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>10</td>
<td>60 µg/dL</td>
<td>≤ 59 µg/dL</td>
</tr>
<tr>
<td>1970</td>
<td>10</td>
<td>40 µg/dL</td>
<td>≤ 39 µg/dL</td>
</tr>
<tr>
<td>1975</td>
<td>5</td>
<td>30 µg/dL</td>
<td>≤ 29 µg/dL</td>
</tr>
<tr>
<td>1985</td>
<td>10</td>
<td>25 µg/dL</td>
<td>≤ 24 µg/dL</td>
</tr>
<tr>
<td>1991</td>
<td>6</td>
<td>10 µg/dL</td>
<td>≤ 9 µg/dL</td>
</tr>
<tr>
<td>2018</td>
<td>27</td>
<td>5 µg/dL</td>
<td>≤ 0 µg/dL</td>
</tr>
</tbody>
</table>

Today BLL’s of ≥39 µg/dL or greater require immediate chelation therapy.

*BLL = Blood Lead Levels*

*CDC = Center for Disease Control*
Pb produced consistent aberrant behaviors related to attention, visual-motor reasoning skills, social skills, mathematics and reading abilities at exposures of ~ 10µg/dL (Canfield et al., 2003; Lanphear et al., 2000; 2005; Wasserman et al., 1997).

Predicts for lifetime educational and emotional problems, delinquent and anti-social behaviors (Nevin, 2007). Rodent models simulate these human IQ deficits (Neuwirth et al., 2017; Neuwirth, 2014).
REAL WORLD PROBLEMS: LEAD RESURGENCE IN FLINT MICHIGAN 2014-2018...AN ONGOING ISSUE

Lead Sediments

Flint River

All Red Balloons =
A public home with leaded water exceeding 15ppb, the federal action level
Neurobehavioral
Social & Emotional
Cognition

Lead Poisoning Research Has Focused Entirely On These two Domains That Contribute to IQ Deficits.

This Domain's relationship With IQ in Response To Lead Poisoning Remains To be elucidated

IQ IS COMPRISED OF THE FOLLOWING DOMAINS

DISRUPTION OF ONE OR A COMBINATION OF DOMAINS CONTRIBUTES TO DEVELOPMENTAL DISABILITIES
In early childhood, social-play skills are important developmental skills that increase the rate of learning from the environment.

Social-play also increases communication skills along with the ability to work with others cooperatively and during times of conflict.

Being able to work with others through appropriate social interactions are important for acquiring functional communication skills.
Social play is an important developmental behavior in juvenile rats.

The engagement of social play helps to shape reward-based brain circuitry.

The stimulation of these limbic centers of the brain influence the emotional value assigned to stimuli rats encounter across development that last into adulthood.


If early developmental lead exposure produces cognitive deficits in learning and memory related to IQ, then would it also produce social emotional developmental deficits that could also influence different domains of intellect?

Would the developmental timing of lead exposure cause differences in the pups weight and maternal retrieval latency?

Would developmental lead exposure causes sex-based differences dependent upon dose (i.e., ppm) and time-period of exposure (i.e., Perinatal vs. Early Postnatal) in pup weight and maternal retrieval latency?

Would a correlation between the pup weight and maternal retrieval latency predict differences in adolescent social play behaviors?
Lead Acetate was administered in the Dam’s drinking water at 25ppm, 150ppm, and 1,000ppm during these developmental time-periods.
## SAMPLE SIZES PER EXPERIMENT

Table 1. Pup Weight Test

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N-Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>59</td>
</tr>
<tr>
<td>25 ppm</td>
<td>35</td>
</tr>
<tr>
<td>150 ppm</td>
<td>65</td>
</tr>
<tr>
<td>1,000 ppm</td>
<td>53</td>
</tr>
<tr>
<td>Males</td>
<td>104</td>
</tr>
<tr>
<td>Females</td>
<td>108</td>
</tr>
</tbody>
</table>

Table 2. Maternal Pup Retrieval Test

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N-Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>63</td>
</tr>
<tr>
<td>25 ppm</td>
<td>35</td>
</tr>
<tr>
<td>150 ppm</td>
<td>55</td>
</tr>
<tr>
<td>1,000 ppm</td>
<td>44</td>
</tr>
<tr>
<td>Males</td>
<td>102</td>
</tr>
<tr>
<td>Females</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 3. Social Play Test

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N-Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>30</td>
</tr>
<tr>
<td>25 ppm</td>
<td>0</td>
</tr>
<tr>
<td>150 ppm</td>
<td>14</td>
</tr>
<tr>
<td>1,000 ppm</td>
<td>25</td>
</tr>
<tr>
<td>Males</td>
<td>37</td>
</tr>
<tr>
<td>Females</td>
<td>42</td>
</tr>
</tbody>
</table>

25 ppm Lead Acetate

**Produces The Following:**
- Early Post Natal (EPN)
  - Rat BLLS ~ 0-3 \( \mu g/dL \)
- Perinatal (Peri)
  - Rat BLLS ~ 5-7 \( \mu g/dL \)

150 ppm Lead Acetate

**Produces The Following:**
- Early Post Natal (EPN)
  - Rat BLLS ~ 5-7 \( \mu g/dL \)
- Perinatal (Peri)
  - Rat BLLS ~ 10-13 \( \mu g/dL \)

1,000 ppm Lead Acetate

**Produces The Following:**
- Early Post Natal (EPN)
  - Rat BLLS ~ 15-21 \( \mu g/dL \)
- Perinatal (Peri)
  - Rat BLLS ~ 28-46 \( \mu g/dL \)
EXPERIMENTAL PROCEDURES

- **Pup Weight Test**: Pups were weighed on a digital scale (measured in grams) every day from postnatal Day 1 to 7 (PND 1-7).

- **Maternal Pup Retrieval Latency Test**: During PND 1-7, pups were briefly separated from the Dam’s cage and reintroduced one at a time for a 2-minute interval. Latency (measured in seconds) was recorded for the time it took the Dam to pick up her pup and bring it back to its nest. If a Dam did not retrieve her pup, trials were repeated up to 3 times before a failure was determined.

- **Social Play Test**: At postnatal Day 22 (PND 22) rats were weaned 3 to 4 in a cage across litters of the same treatment group to control for any maternal carry-over effects. Rats were naturally observed 3-4 weeks following the removal of lead exposure between PND 36-50, within their home cages under red light (30 Lux) and the frequency of attacks, defends, pins, counters, and climbs were recorded during 1 hour natural observations.
QUALITATIVE DIFFERENCES IN MATERNAL PUP RETRIEVAL BEHAVIOR AS A FUNCTION OF DEVELOPMENTAL Pb EXPOSURE

Control Dam (0 ppm Lead Acetate Exposure)                      Perinatal Dam (150 ppm & 1,000 ppm Lead Acetate Exposures)
The first few graphs will compare both pup weights and maternal retrieval latency as a function of developmental time-period of exposure and dose response of lead acetate exposures using an ANOVA. The X-axis will show the postnatal day of the pups, whereas the Y-axis will show in set one the pup weight (measured in grams) and in set two the maternal retrieval latency (measured in seconds).

The next set of graphs will illustrate the correlations using a Pearson’s r between pup weight and maternal retrieval latency for each treatment group. The X-axis will show the pup weight and the Y-axis will show the maternal retrieval latency.

The last set of graphs illustrate the adolescent social-play behavioral data following 3-4 weeks following the removal of lead acetate exposures using an ANOVA. The X-axis will show the type of social-play behavior, whereas the Y-axis will show the number of behaviors observed during a 1-hour natural observation.
**Lead 25ppm Data Summary**

*Lead Time Period Effect* $F_{(3,146)} = 2.659, p = 0.073$ N/S

*Gender Effect* $F_{(1,146)} = 0.189, p = 0.665$ N/S

*Lead Time Period X Gender Interaction* $F_{(3,1,146)} = 5.424, p < 0.01**$

(*) = Age Effect & (#) = Lead Time Period X Gender Interaction

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**Lead Dose Response ppm Data Summary**

*Lead ppm Effect* $F_{(3,204)} = 0.556, p = 0.645$ N/S

*Gender Effect* $F_{(1,204)} = 7.455, p < 0.01**$

*Lead ppm X Gender Interaction* $F_{(3,1,204)} = 0.364, p = 0.779$ N/S

(*) = Age Effect & (#) = Lead ppm X Gender Interaction
**Lead 25ppm Data Summary**

**Lead Time Period Effect** $F_{(3,177)} = 4.196, p < 0.007**$

**Gender Effect** $F_{(1,177)} = 0.550, p = 0.459 \text{ N/S}$

**Lead Time Period X Gender Interaction** $F_{(3,177)} = 0.113, p = 0.952 \text{ N/S}$

(* = Age Effect & (#) = Lead Time Period X Gender Interaction)

**Lead Dose Response ppm Data Summary**

**Lead ppm Effect** $F_{(3,189)} = 5.277, p < 0.002 **$

**Gender Effect** $F_{(1,189)} = 0.098, p = 0.754 \text{ N/S}$

**Lead ppm X Gender Interaction** $F_{(3,189)} = 0.299, p = 0.826 \text{ N/S}$

(† = Age X Lead Time Period X Gender Interaction)
Control Rat Pups

Peri-0 (25ppm) Rat Pups

Peri-22 (25ppm) Rat Pups

EPN (25ppm) Rat Pups

$r = 0.701, p = 0.079$ N/S

$r = 0.612, p = 0.144$ N/S

$r = -0.792, p < 0.05^*$

$r = 0.700, p = 0.080$ N/S
Control Rat Pups

Peri-22 (25ppm) Rat Pups

Peri-22 (150ppm) Rat Pups

Peri-22 (1,000ppm) Rat Pups

$r = 0.701, p = 0.079$ N/S

$r = 0.792, p < 0.05^*$

$r = 0.586, p = 0.167$ N/S

$r = 0.011, p = 0.098$ N/S
Male Lead Dose Response ppm Data Summary

Lead ppm Effect $F_{(2,75)} = 25.211, p < 0.001$ ***

Social-Play Effect $F_{(4,75)} = 6.847, p < 0.001$ ***

Lead ppm X Social-Play Interaction $F_{(2,4,75)} = 1.075, p = 0.391$ N/S

Female Lead Dose Response ppm Data Summary

Lead ppm Effect $F_{(2,55)} = 0.826, p = 0.445$ N/S

Social-Play Effect $F_{(4,55)} = 2.200, p = 0.086$ N/S

Lead ppm X Social=Play Interaction $F_{(2,4,55)} = 0.167, p = 0.994$ N/S

(*) = Lead ppm Effect
Pup weights were influenced by gender and developmental time-period of lead exposure.

The maternal pup retrieval latency and social play tests were shown to be a very sensitive behavioral assays to investigate developmental lead toxicity in rats.

Depending upon the developmental time-period of lead exposure, maternal social pup retrieval behaviors are disrupted with implications for causing abnormal maternal care that may, in turn, influence the social-emotional development of the pups behaviors across their lifespan.

Developmental lead exposure caused sex-based differences in social play with males being more sensitive to higher doses of lead exposure.

The impacts of 25ppm lead exposure on social play are underway in our lab and we would need to know what the outcomes of this dataset to complete the study.
LEAD POISONING DISRUPTS IQ BY ALTERING SOCIAL & EMOTIONAL DEVELOPMENT: WORKING THEORETICAL MODEL

Lead Induced Social & Emotional Deficits May Contribute Directly & Indirectly to Neurobehavioral & Cognition Problems Across Development

DISRUPTION OF ONE OR A COMBINATION OF DOMAINS CONTRIBUTES TO DEVELOPMENTAL DISABILITIES
CONCLUSION & LIMITATIONS

- Lead differentially effects both males and females rats in a gender specific manner.

- Moreover, lead effects rats differentially based upon the developmental time-period of exposure (i.e., EPN vs. Peri).

- Taken together, the pup weight, maternal pup retrieval, and social play tests are a valid model for testing social-emotional developmental problems in the rat to better understand the effects of environmental toxins on the development and life-long consequences of lead exposure in humans.

- Our study was limited as we need to increase the number of 25ppm rats in all conditions to publish the results of our study.

- Future studies can look into the molecular targets in the amygdale, hippocampi, and prefrontal cortices which are collectively responsible for regulating social-emotional behaviors associated with the social-play test.
ACKNOWLEDGEMENTS

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- CSTEP Statewide Student Conference Organizers and Faculty
References


THANK YOU!

Think about FLINT!!!!