RAMAZZINI INSTITUTE
and
MOUNT SINAI SCHOOL OF MEDICINE:
IN VIVO BIOASSAY MODEL FOR
ENDOCRINE DISRUPTIVE CHEMICALS
(EDCs) STUDIES

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This study is part of the NIH founded project “Breast Cancer Genomics in Windows of Susceptibility to Endocrine Disruptors”.

It combines animal experiments and epidemiological investigations.

Epidemiological data were drawn from the population-based Long Island Breast Cancer Study Project (LIBCSP) a population-based study of breast cancer focusing on female residents in Long Island (NY).

High urinary concentrations of Diethylphthalate (DEP), Methylparaben (MPB) and Triclosan (TRC) were observed in the LIBCSP women and an increased risk of breast cancer associated with higher levels of these commonly-used EDCs was observed.
Aim: explore whether DEP, MPB, TRC and a mixture of them act in specific mammary gland developmental windows (prenatal, postnatal, pre-pubertal, pubertal, parous, nulliparous) and whether they exert their biological effects in breast tissue leading to breast cancer development.
Challenges

- Windows of Susceptibility (WOS) not clearly elucidated in humans
- Experimental doses orders of magnitude higher than human exposure
- Testing one ED at a time
Dose calibration study
Summary of orally administered ED doses

- Six doses per chemical were selected based on its **NOAEL** (No Observed Adverse Effect Level).

<table>
<thead>
<tr>
<th>Doses</th>
<th>Diethyl Phthalate (NOAEL=1735 mg/kg)</th>
<th>Methyl Paraben (NOAEL=1050 mg/kg)</th>
<th>Triclosan (NOAEL=50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOAEL / X</td>
<td>Dose (mg/kg b.w.)</td>
<td>Dose (mg/kg b.w.)</td>
<td>Dose (mg/kg b.w.)</td>
</tr>
<tr>
<td>Vehicle only (olive oil)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NOAEL/100,000</td>
<td>0.01735</td>
<td>0.0105</td>
<td>--</td>
</tr>
<tr>
<td>NOAEL/10,000</td>
<td>0.1735</td>
<td>0.105</td>
<td>0.005</td>
</tr>
<tr>
<td>NOAEL/1,000</td>
<td>--</td>
<td>--</td>
<td>0.05</td>
</tr>
<tr>
<td>NOAEL/200</td>
<td>86.75</td>
<td>5.25</td>
<td>0.25</td>
</tr>
<tr>
<td>NOAEL/100</td>
<td>17.35</td>
<td>10.5</td>
<td>0.05</td>
</tr>
<tr>
<td>NOAEL/10</td>
<td>173.5</td>
<td>105.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Dose calibration study: urinary collection

- Treatment lasted 5 days
- Following the last treatment (day 5), rats were housed individually in metabolic cages
- 24 h urine was collected and placed in phthalate-free vial
- Urine samples were placed in cryoboxes and frozen at −20°C, then shipped on dry ice to the Centers for Disease Control and Prevention (Atlanta)
- Rats were sacrificed by carbon dioxide at day 6, serum was collected
Dose-calibration study: results on diethyl phthalate

Mono-Ethyl-phthalate = metabolite of Diethylphthalate (DEP)

- **LI max**
- **LI 95%**
- **LI median**

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>NOAEL (mg/Kg/bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.01735</td>
</tr>
<tr>
<td>Low</td>
<td>0.01735</td>
</tr>
<tr>
<td>Medium</td>
<td>0.1735</td>
</tr>
<tr>
<td>High</td>
<td>8.675</td>
</tr>
</tbody>
</table>

**Notes:**
- NOAEL: No Observed Adverse Effect Level
Dose-calibration study: results on methyparaben

Methylparaben (MPB)

- Vehicle: olive oil
- Low NOAEL / 100,000: 0.0105 mg/kg/bw
- Medium NOAEL / 10,000: 0.105 mg/Kg/bw
- High NOAEL / 200: 5.25 mg/Kg/bw

LI max
LI 95%
LI median
Dose-calibration study: results on triclosan

- Vehicle: Olive oil
- Low: NOAEL /10,000 mg/Kg/bw (0.005 mg/Kg/bw)
- Medium: NOAEL /1,000 mg/Kg/bw (0.05 mg/Kg/bw)
- High: NOAEL /200 mg/Kg/bw (0.25 mg/Kg/bw)
This study has identified a range of oral doses of three EDCs that result in a range of urinary biomarker concentrations in a rat model that are consistent with the range of biomarker concentrations measured in the female US population.

- Based on growing evidence suggesting low-dose health effects, there is an urgent need for studies utilizing doses in the range of typical human exposures (Birnbaum, 2012).

- The results of our study could provide a foundation for future rodent-based health risk assessment studies for human exposure to low-doses of chemicals.
Main Study
The experiment

Significantly higher litter mortality in the treated groups compared to control group
Clinical observation: lower or even absent development of lactating mammary glands in some treated dams
Histopathology of mammary gland
Effects on mammary glands development: results

- Mammary glands from lactating female rats (LD 28), treated from birth and continuously through pregnancy and lactation until weaning of their pups.

**OIL:** the alveoli are filled with secretions containing lipid vacuoles, epithelial cells are flattened and acini are filled with secretions.

**TRICLOSAN (TRC):** In treated cases (especially for TRC) a low development of the alveoli with higher presence of adipose tissue support is evident even though the secretory activity is still present.
EDCs exposure results in profound changes in gross phenotypes (e.g. reproductive mortality and mammary gland morphology) at levels comparable to those of human scenario.

By using human equivalent exposures to three EDCs and a mixture of them, this animal study highlights the heightened sensitivity to EDCs of the MGs during pregnancy and lactation, suggesting an impact on pup survival possibly due to reduced milk production.
Effects on mammary transcriptome

- Whole-transcriptomes of mammary glands were profiled on microarrays.
- Differentially expressed genes were identified by linear models.
- Whole genome expression profiling of mammary tissue also revealed that in the course of development, the number of differently expressed genes was lower in ED-treated rats compared to controls, suggesting developmental delay or suppression by ED exposure (paper in submission).
The teams

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