Transgenerational Inheritance of Health Effects: A State-of-the-Science Evaluation

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Definition of transgenerational effects
Objectives and methods for systematic review
Results
Summary
What is a Transgenerational Effect?

- Exposure of the $F_0$ generation
  - Exposure stops – not continuous, not across generations
- Health effect is evaluated in generation(s) not directly exposed

A. Non-Gestational Exposure

- Exposure
- Reproductive cells ($F_1$)
- Health effect reported in the $F_2$ generation that were not directly exposed

B. Gestational Exposure

- Exposure
- Mother ($F_0$)
- Fetus ($F_1$)
- Reproductive cells ($F_2$)
- Health effect reported in the $F_3$ generation that were not directly exposed
• “Transgenerational” has not been defined consistently in literature

• Transgenerational effects are reported
  – Are they transgenerational under this definition?
  – Strength and consistency of the findings?
  – Controversial topic (no evidence or clear evidence?)
  – NIEHS is actively funding research in this area

• What is the nature and extent of the evidence for transgenerational inheritance of health effects?
Objectives and Systematic Review Methods
• Objective

- Systematically collect and map transgenerational studies by evidence stream, health effects, and exposures
- Assess the risk of bias (study quality and reporting) for subset of studies to identify potential issues to consider when evaluating this literature and in designing future transgenerational studies
Goals of the Evaluation

- Identify literature utilizing a transgenerational study design
- Identify and map exposures and health outcomes evaluated
- Extract and share data for reported exposures and outcomes
- Synthesize, summarize and critically assess the evidence for exposures evaluating similar outcomes
  - Areas of consistency and uncertainty
  - Key factors of risk of bias for transgenerational study design
Search Strategy

• Transgenerational studies are not indexed

• We used a text word - concept based approach
  – Transgenerational
  – Multigenerational or intergeneration
  – Grandparent, grandmother, grandfather, grandchild
  – Successive generations and offspring

• Limited the search to PubMed database only
Study Selection

Based on PECO statement developed inclusion/exclusion criteria

• Inclusion criteria
  – Transgenerational design
  – Human or whole animal model system
  – An exposure or stressor
  – A health outcome
  – Must contain original data

• Exclusion criteria
  – Plants
  – Cell and organ cultures
  – Studies with continuous exposure
  – Selective breeding studies
  – Foreign language
Results
Literature Search and Study Selection

**Identification**
- References identified through other sources (n=3)
- References identified through database searches (n=63,789)

**Screening**
- References after duplicate removal
- Title-abstract screened for relevance and eligibility (n=63,753)

**Included**
- Full-text references assessed for relevance and eligibility (n=1,12)
- References included for data extraction (n=2)

- Human studies (n=49)
- Animal studies (n=232)

98% excluded

References excluded as not relevant to PECO criteria (n=62,671)

Full-text references excluded n=844
- No Exposure or Outcome (n=36)
- Not Transgenerational design (n=437)
- Review or commentary (n=286)
- Foreign Language (n=85)
Data Extraction Files are Publicly Available

HAWC Link: hawcproject.org/study/assessment/73/

Experimental protocol and dose regimen

### F3 males

<table>
<thead>
<tr>
<th>Name</th>
<th>F3 males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Strain</td>
<td>C57BL/6</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Source</td>
<td>Harlan Sprague-Dawley Laboratories (Indianapolis, IN)</td>
</tr>
<tr>
<td>Lifestage exposed</td>
<td>no exposure</td>
</tr>
<tr>
<td>Lifestage assessed</td>
<td>adult</td>
</tr>
<tr>
<td>Generation</td>
<td>F3</td>
</tr>
<tr>
<td>Parents</td>
<td>• F2 generation</td>
</tr>
</tbody>
</table>

### Dosing regimen

<table>
<thead>
<tr>
<th>Dosed animals</th>
<th>P0 females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of exposure</td>
<td>Oral gavage</td>
</tr>
<tr>
<td>Number of dose-groups</td>
<td>2</td>
</tr>
<tr>
<td>Positive control</td>
<td>Unknown</td>
</tr>
<tr>
<td>Negative control</td>
<td>Not-reported</td>
</tr>
</tbody>
</table>

### Endpoint Summary

#### epididymal sperm counts

**Endpoint Details**
- **Endpoint name**: epididymal sperm counts
- **System**: male reproductive system
- **Organ**: testis
- **Effect**: sperm count
- **Effect subtype**: non-mutagenic chemical
- **Diagnostic description**: phase contrast microscopy
- **Observation time**: 190 PND
- **Data reported?**
- **Data extracted?**
- **Values estimated?**
- **Location in literature**: Figure 1B
- **LOEL**: 100 mg/kg-day
- **Monotonicity**: N/A, single dose level study
- **Statistical test description**: two-way ANOVA
- **Trend result**: not reported
- **Results notes**: Sperm numbers were reduced minimally, 20%, and sperm forward motility was reduced about 25 to 35% for vinclozolin generation animals.
- **General notes/methodology**: Animals were sacrificed and cauda epididymal sperm motility was determined using cauda epididymal sperm. Briefly, the...
References identified through other sources (n=3)

References identified through database searches (n=63,789)

References after duplicate removal Title-abstract screened for relevance and eligibility (n=63,753)

Full-text references assessed for relevance and eligibility (n=1,125)

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References excluded as not relevant to PECO criteria (n=62,671)

References included for data extraction

Human studies (n=49)

Animal studies (n=232)

Risk of Bias

Litter the statistical unit
Randomization
Outcome Assessment
Exposure Characterization

Other Exposures
2 Largest Exposures
Other Exposures
2 Largest Exposures
Emerging Research Focus

Publication Trends

Number of Studies vs Publication Year

- Publication Year
  - 1946
  - 1956
  - 1966
  - 1976
  - 1986
  - 1996
  - 2006
  - 2016

- Number of Studies
  - 0
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

The graph shows an increasing trend in the number of publications from 1946 to 2016, with a significant rise after 2006.
Human Studies: Outcomes

- Neurological and Sensory: 27 studies
- Mortality: 8 studies
- Growth and Development: 7 studies
- Reproductive: 5 studies
- Cancer: 2 studies
- Cardiovascular: 2 studies
- Immune: 1 study
- Mutagenicity: 1 study
Human Studies: Outcomes

Few Studies of Same Exposure – Outcome Pair

<table>
<thead>
<tr>
<th>Category</th>
<th># of Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological and Sensory</td>
<td>27</td>
</tr>
<tr>
<td>Mortality</td>
<td>8</td>
</tr>
<tr>
<td>Growth and Development</td>
<td>7</td>
</tr>
<tr>
<td>Reproductive</td>
<td>5</td>
</tr>
<tr>
<td>Cancer</td>
<td>2</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>2</td>
</tr>
<tr>
<td>Immune</td>
<td>1</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td>1</td>
</tr>
</tbody>
</table>

8 different “exposures”
- Holocaust (n=8)
- Behavioral depression (n=4)
- Stress (n=6)
- 5 other exposures (n=1)
Reproductive outcomes following radiation exposure (5 studies)

- Single cohort of women treated with low-dose radiation therapy for menstrual dysfunction
- Few outcomes tracked, evidence limited to observational findings and reported in a series of publications
Mortality risk in grandchildren following food availability (3 studies)

- 3 studies – from the same population in Sweden
- Reported sex-specific effects on mortality in grandchildren following low food supply of grandparent(s)
Neurological and sensory outcomes in grandchildren whose grandparents experienced behavioral depression (4 studies)

- Impact of grandparent’s mental health on grandchild's behavior
- Could be considered hereditary
Neurological and sensory outcomes in grandchildren whose grandparents experienced the Holocaust (8 studies)

- Evaluated behavioral effects in 2\textsuperscript{nd} and 3\textsuperscript{rd} generation Holocaust survivors
- Meta analysis reports no evidence for behavioral indicators of trauma in an analysis that combined behavioral outcomes

Bottom line: Very few epidemiological studies
Animal Studies: Wide Range of Outcomes

- Growth and Development: 60 studies
- Female Reproductive: 55 studies
- Male Reproductive: 50 studies
- Neurological and Sensory: 32 studies
- Metabolic or glucose related: 25 studies
- Non-Reproductive Endocrine: 16 studies
- Hepatic: 18 studies
- Immune: 14 studies
- Renal: 11 studies
- Musculoskeletal: 11 studies
- Disease: 8 studies
- Mutagenicity: 7 studies
- Cardiovascular: 5 studies
- Respiratory: 4 studies
- Gastrointestinal: 3 studies
Animal Studies: Wide Range of Outcomes

Few Studies With the Same Exposure and Similar Health Outcome

42 different “exposures”

- High fat diet (n=7)
- Radiation (n=5)
- Vinclozolin (n=3)
- Dioxin (n=3)
- Methoxychlor (n=2)
- JP-8 (n=2)
- NMU (n=2)
- DEET + permethrin (n=2)
- Cyclophosphamide (n=2)
- 33 other exposures (n =1)
Animal Studies: Wide Range of Outcomes

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No exposure with more than 2 studies on these outcomes, (even broadly defined outcome categories)
Example Endpoint Reported Across Multiple Exposures

Reduction in Primordial Follicles and Increase in Ovarian Cysts

Jet Propellant 8

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal description (with N)</th>
<th>Route</th>
<th>Observation time</th>
<th>Dose (mg/kg-day)</th>
<th># of Cysts</th>
<th>% of Follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilsson (2012)</td>
<td>F3 Rat, Sprague-Dawley (♀, N=9)</td>
<td>intraperitoneal injection</td>
<td>not-reported</td>
<td>0</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Manikkam (2012a)</td>
<td>F3 Rat, Sprague-Dawley (♀, N=88-131)</td>
<td>intraperitoneal injection</td>
<td>120 PND</td>
<td>0</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Nilsson (2012)</td>
<td>F3 Rat, Sprague-Dawley (♀, N=9)</td>
<td>intraperitoneal injection</td>
<td>not-reported</td>
<td>0</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

- Few studies—same group of researchers
- Same effects reported across different classes of environmental chemicals

Dioxin

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal description (with N)</th>
<th>Route</th>
<th>Observation time</th>
<th>Dose (ng/kg-day)</th>
<th># of Cysts</th>
<th>% of Follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilsson (2012)</td>
<td>F3 Rat, Sprague-Dawley (♀, N=9)</td>
<td>intraperitoneal injection</td>
<td>not-reported</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manikkam (2012b)</td>
<td>F3 Rat, Sprague-Dawley (♀, N=84-131)</td>
<td>intraperitoneal injection</td>
<td>120 PND</td>
<td>0</td>
<td>100</td>
<td></td>
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<td>100</td>
<td></td>
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</tbody>
</table>
Animal Studies: Exposure x Outcome Pairs

Drilling Down Identifies Specific Chemicals as potential “pockets”

- Vinclozolin
- Radiation
- High-Fat Diet
- Dioxin (TCDD)

Number of Studies

Growth and Development (N=60):
- Vinclozolin: 3
- Radiation: 5
- High-Fat Diet: 7
- Dioxin (TCDD): 3

♀ Reproductive (N=55):
- Vinclozolin: 3
- Radiation: 6
- High-Fat Diet: 2
- Dioxin (TCDD): 4

♂ Reproductive (N=50):
- Vinclozolin: 13
- Radiation: 4
- High-Fat Diet: 3
- Dioxin (TCDD): 5
Example: Individual Outcomes within Broad Category

Few Outcomes Evaluated in More than One Study

- **sperm parameters**
- **motility**
  - sperm parameters mRNA expression
  - tetraploid cells (meiotic cells)
- **morphology**
- **sperm parameters**
- **DNA damage**
  - DNA methylation
  - sperm parameters abnormality
  - sperm vitality
- **serum luteinizing hormone (LH)**
- **follicle stimulating hormone (FSH)**
- **DNA fragmentation**
- **testes and epididymides histopathology**
- **cryptorchidism**
- **puberty**
- **preputial separation**
- **spermatogonia cell count**
- **diameter of seminiferous lumen**
- **degeneration of seminiferous tubules**
- **composition of testes**
- **1C (spermatids)**

- **apoptosis**
  - disease
  - mutagenicity
  - spermatocytes weight
  - haploid cells
  - ratio of sertoli spermatogonia
  - sperm parameters spermatids
  - reactive oxygen species (ROS)
  - pubertal abnormality
  - percentage of spermatids
  - sperm average path velocity
  - pubertal onset
  - progesterone

- **weight**
  - testosterone
  - testes weight
  - puberty
  - sertoli cell count
  - sperm parameters binding
  - sperm straight line velocity

- **sperm parameters concentration**
  - accessory sex glands weight
  - diameter of seminiferous tubules
  - seminal vesicle weight
  - tumor development
  - height of seminiferous epithelium
  - sperm parameters acrosome reacted
  - reproductive function
  - female conceptions
  - spermatogonial cells mutations
  - composition of testes
  - HC (elongated spermatozoa)
Apoptosis of Germ Cells in the Testis

Example: Vinclozolin - Male Reproductive Outcomes

- 6 studies – from 2 groups of researchers
- Effects reported in both the mouse and rat
- 95% CI overlap with null
Animal Studies: Risk of Bias Evaluation

Subset of Transgenerational Studies Identify Concerns with Study Conduct and Reporting

Legend
- N/A: Not applicable
- : Definitely high risk of bias
- : Probably high risk of bias/not reported
- : Probably low risk of bias
- : Definitely low risk of bias

- Was administered dose or exposure level adequately randomized?
  - Yes: 81% (N/A: 15%)
  - No: 8% (Definitely low risk of bias: 27%)

- Was allocation to study groups adequately concealed?
  - Yes: 88% (Definitely low risk of bias: 8%)
  - No: 8% (Definitely low risk of bias: 8%)

- Were experimental conditions identical across study groups?
  - Yes: 46% (Definitely low risk of bias: 27%)
  - No: 42% (N/A: 18%)

- Were the research personnel blinded to the study group during the study?
  - Yes: 88% (N/A: 12%)
  - No: 8% (Definitely low risk of bias: 15%)

- Were outcome data complete without attrition or exclusion from analysis?
  - Yes: 42% (Definitely low risk of bias: 18%)
  - No: 58% (N/A: 8%)

- Can we be confident in the exposure characterization?
  - Yes: 58% (Definitely low risk of bias: 23%)
  - No: 4% (N/A: 19%)

- Can we be confident in the outcome assessment?
  - Yes: 8% (N/A: 90%)
  - No: 58% (Definitely low risk of bias: 4%)

- Were all measured outcomes reported?
  - Yes: 54% (Definitely low risk of bias: 4%)
  - No: 8% (N/A: 48%)

- Was the litter the unit of statistical measure?
  - Yes: 58% (Definitely low risk of bias: 15%)
  - No: 19% (N/A: 15%)

- Were there no other potential threats to internal validity (e.g., appropriate statistical methods)?
  - Yes: 50% (N/A: 19%)
  - No: 19% (Definitely low risk of bias: 15%)

Percent of studies vs. Risk of Bias
Majority Of Studies Result in *Probably High Risk* of Bias for Key Factors in Study Design And Reporting

### Randomization
- Was allocation to study groups adequately concealed? 81% (15%), 88% (8%), 27% (27%)
- Were experimental conditions identical across study groups? 46% (46%), 27% (27%), 27% (27%)

### Outcome Assessment
- Were data complete without attrition or exclusion from analysis? 42% (42%), 42% (42%), 15% (15%)

### Exposure Characterization
- Can we be confident in the outcome assessment? 58% (58%), 8% (8%), 35% (35%)
- Were all measured outcomes reported? 54% (54%), 42% (42%)

### Litter as statistical unit
- eats to internal validity (e.g., appropriate statistical methods)? 50% (50%), 19% (19%), 31% (31%)
Summary
Male reproductive
• Main exposures: vinclozolin (13), dioxin (5) radiation (4)
• Main outcomes: sperm parameters, organ weights, germ cell apoptosis

Female reproductive
• Main exposures: vinclozolin (8), dioxin (6)
• Main outcomes: ↑ ovarian cysts, ↓ follicle counts

Neurological
• Main exposures: stress (5), vinclozolin (4)
• Main outcomes: Social investigation, locomotor activity, anxiety-like behavior, olfactory recognition

Metabolic or Glucose-related
• Main exposures: high-fat diet (8), protein-restricted diet (4)
• Main outcomes: glucose tolerance, adiposity
How Deep are the “Pockets” of Evidence?

- A broad range of exposures and outcomes report transgenerational inheritance of health effects

- Evidence mapping illustrates that there are serious limitations in the available bodies of evidence to support a systematic review for reaching hazard conclusions
  - Very few human studies of sufficient generations
  - Few studies of same exposure and outcome pair
  - Problems in study design, conduct, and reporting (ROB)
Considerations for Reaching a Conclusion

What Data Would Strengthen a Critical Evaluation?

• For a given exposure, consistent assessment of the same or closely related health effects in multiple studies (and ideally across multiple labs)

• Minimize bias to produce robust data on potential transgenerational effects
  
  – Best practices in study design, conduct and reporting
    • Randomization of treatment
    • Blinding of outcome assessors to study group
    • Control for litter effects - litter as statistical unit of analysis
    • Consistent age/timing of outcome assessment within a study
What is the Nature and Extent of Transgenerational Literature?

- SR methods to map literature by exposures and health effects
  - Evaluation website (protocol, etc.; [https://ntp.niehs.nih.gov/go/38159](https://ntp.niehs.nih.gov/go/38159))
  - Identify studies (included study list)
  - Extract data (publicly available; [https://hawcproject.org/assessment/73/](https://hawcproject.org/assessment/73/))

- Evidence map
  - Extent of evidence by evidence stream
  - Exposures
  - Health effects

- Critical analysis
  - Strengths or challenges of bodies of evidence to support reaching a hazard conclusion on transgenerational inheritance of health effects
Evaluation Design Team

- Division of National Toxicology Program
  - Andrew Rooney, Acting Director OHAT
  - Katherine Pelch
  - Andrew Shapiro
  - Chad Blystone
  - Michael Devito
  - Retha Newbold
  - Vicki Sutherland
  - Abee Boyles

- Office of Data Science
  - Stephanie Holmgren

- Division of Extramural Research and Training
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  - Fred Tyson
  - Jerry Heindel
  - Lisa Chadwick

- Division of Intramural Research
  - Paul Wade

- EPA/NCEA/IRIS
  - Kris Thayer

- ICF International
  - Pamela Hartman
  - Susan Goldhaber
  - Cara Henning
  - Robyn Blain
• Please comment on NTP’s overall approach for this state-of-the-science or scoping review. Did it yield a trackable product for addressing this public health question?

• What value do you envision by NTP providing the output from this (and other) reviews in HAWC for public access? What strategies might NTP use to facilitate use and awareness about this resource?