

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

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NTP Board of Scientific Counselors Meeting
June 29, 2017



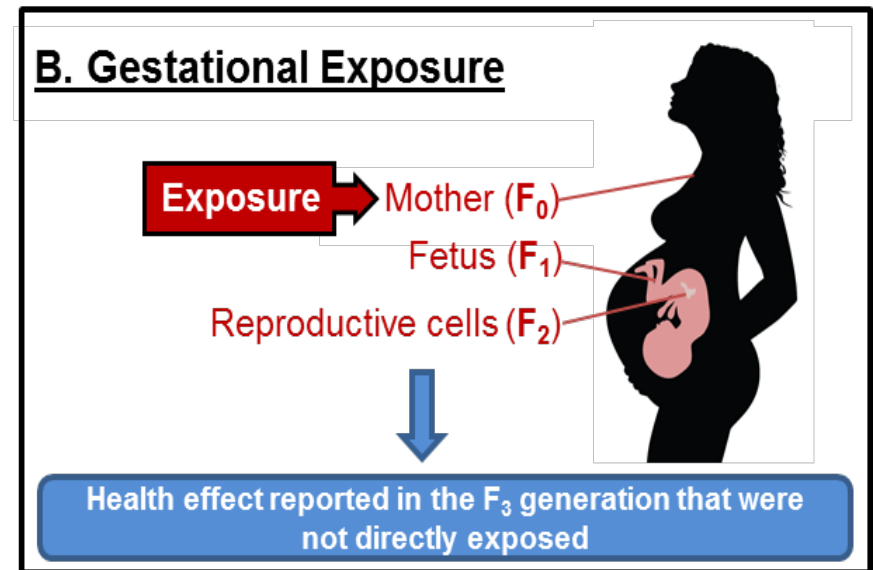
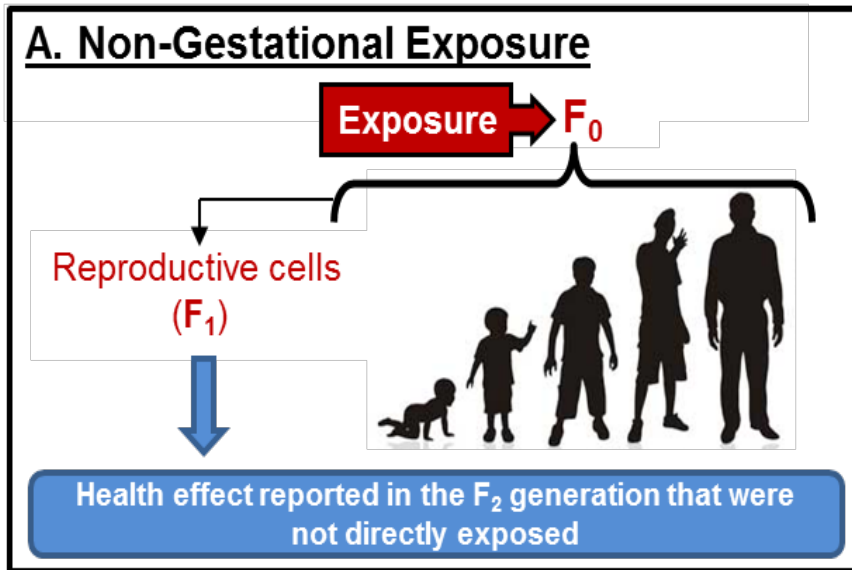


- Definition of transgenerational effects
- Objectives and methods for systematic review
- Results
- Summary



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

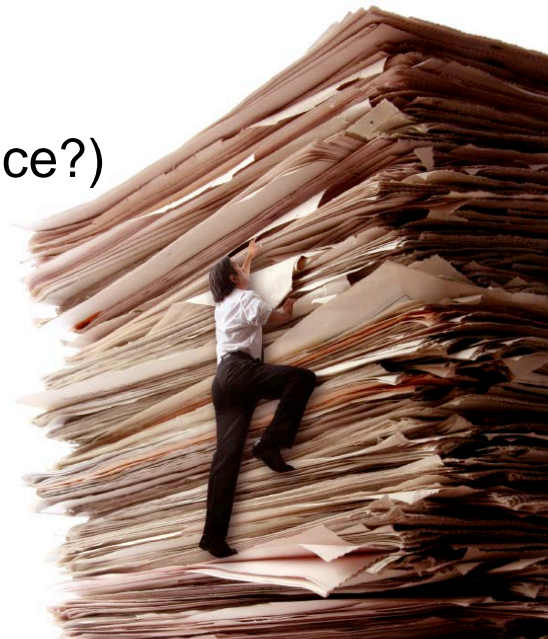




Investigating Transgenerational Inheritance

Complex topic and challenging literature base

- “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?





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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

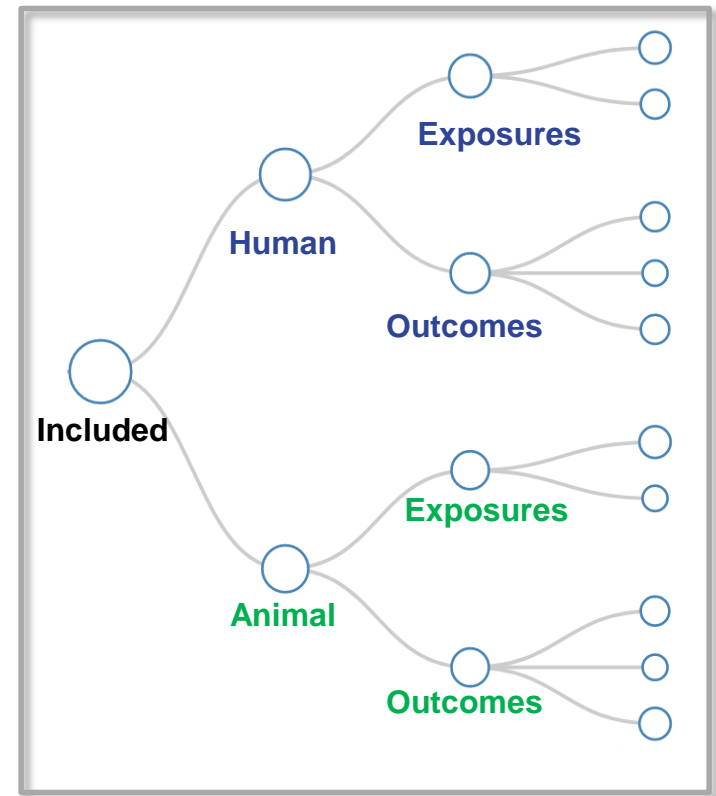


NTP State of the Science Evaluation

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

Systematic Evidence Map





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**





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

Population

Exposure

Comparator

Outcome



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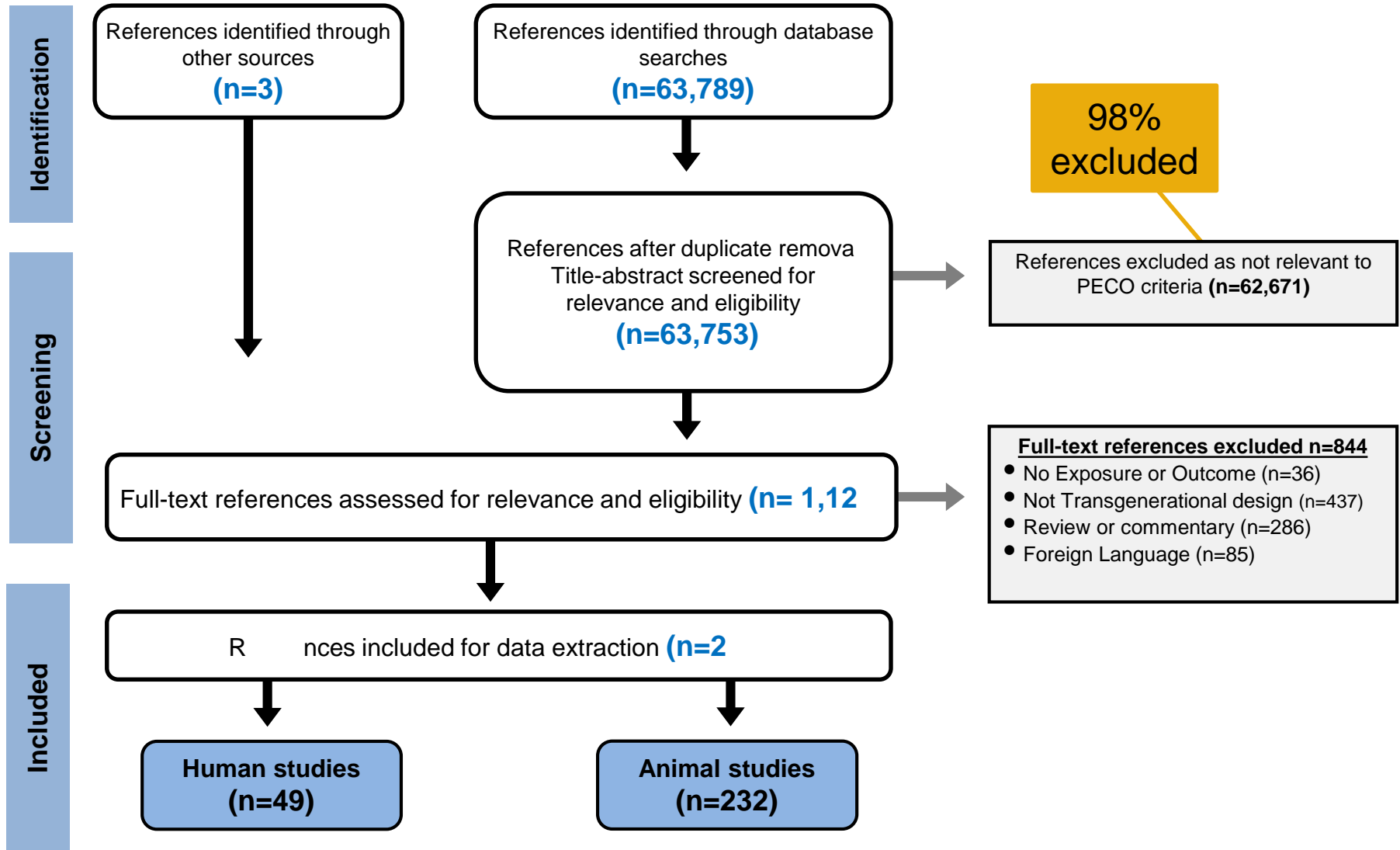
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Results





Literature Search and Study Selection





Data Extraction Files are Publicly Available

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



Experimental protocol and dose regimen

F3 males

| | |
|--------------------|---|
| Name | F3 males |
| Species | Mouse |
| Strain | C57BL/6 |
| Sex | Male |
| Source | Harlan Sprague-Dawley Laboratories (Indianapolis) |
| Lifestage exposed | no exposure |
| Lifestage assessed | adult |
| Generation | F3 |
| Parents | <ul style="list-style-type: none">F2 generation |

Dosing regimen

| | |
|-----------------------|--------------|
| Dosed animals | P0 females |
| Route of exposure | Oral gavage |
| Number of dose-groups | 2 |
| Positive control | Unknown |
| Negative control | Not-reported |
| Doses | ug/kg |
| | 0 |
| | 10 |

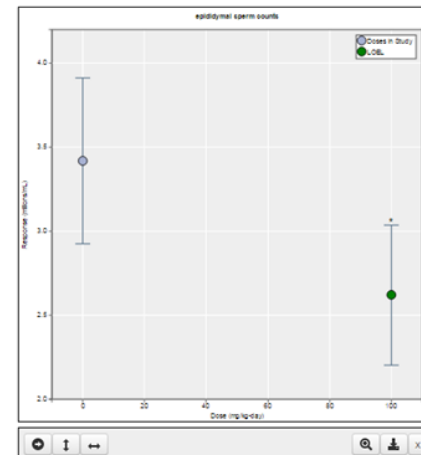
Endpoint Summary

epididymal sperm counts

Endpoint Details

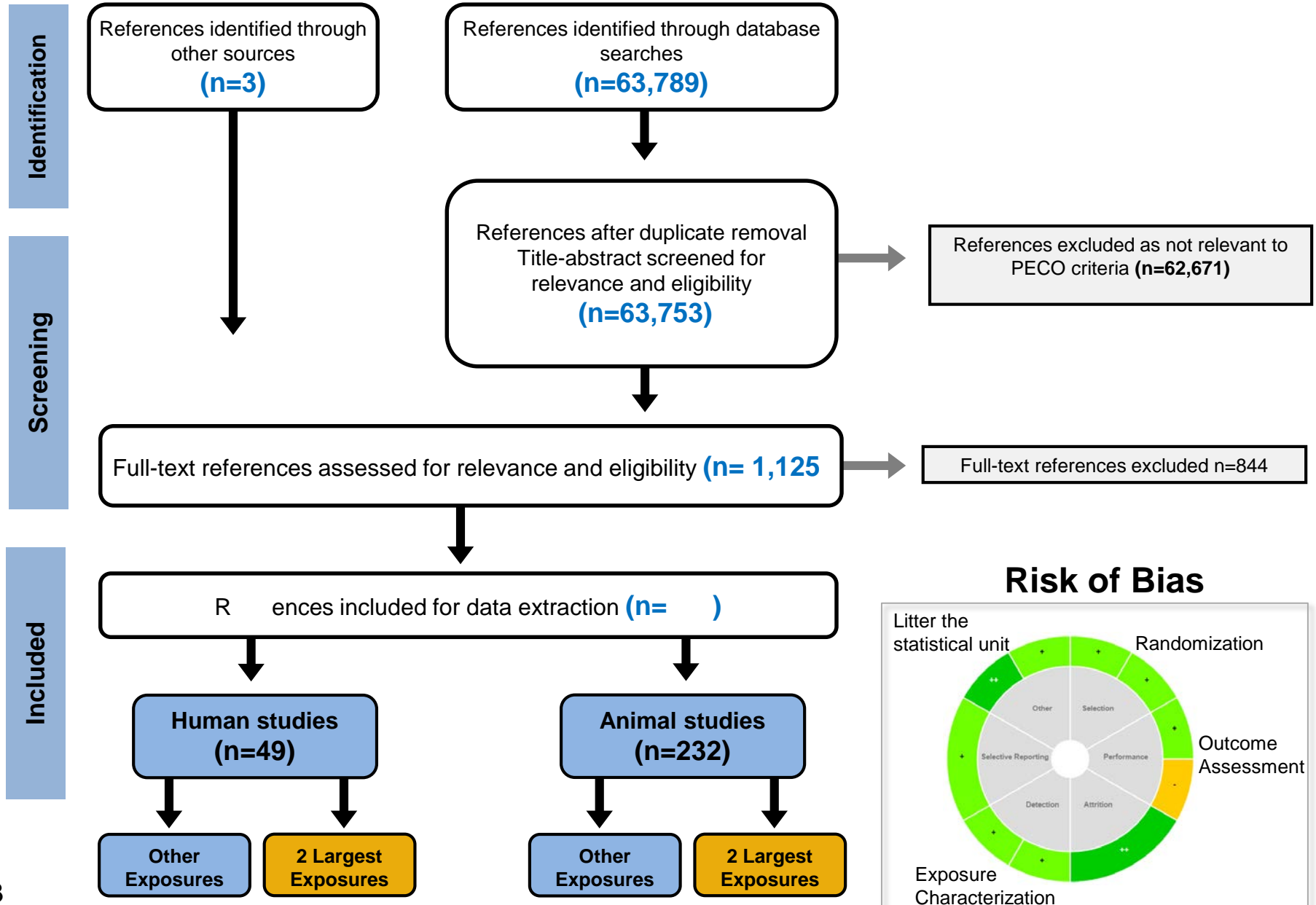
| | |
|------------------------------|---|
| Endpoint name | epididymal sperm counts |
| System | male reproductive system |
| Organ | testis |
| Effect | sperm count |
| Effect subtype | non-mutagenic chemical |
| Diagnostic description | phase contrast microscopy |
| Observation time | 180 PND |
| Data reported? | <input checked="" type="checkbox"/> |
| Data extracted? | <input checked="" type="checkbox"/> |
| Values estimated? | <input checked="" type="checkbox"/> |
| 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 |

Plot



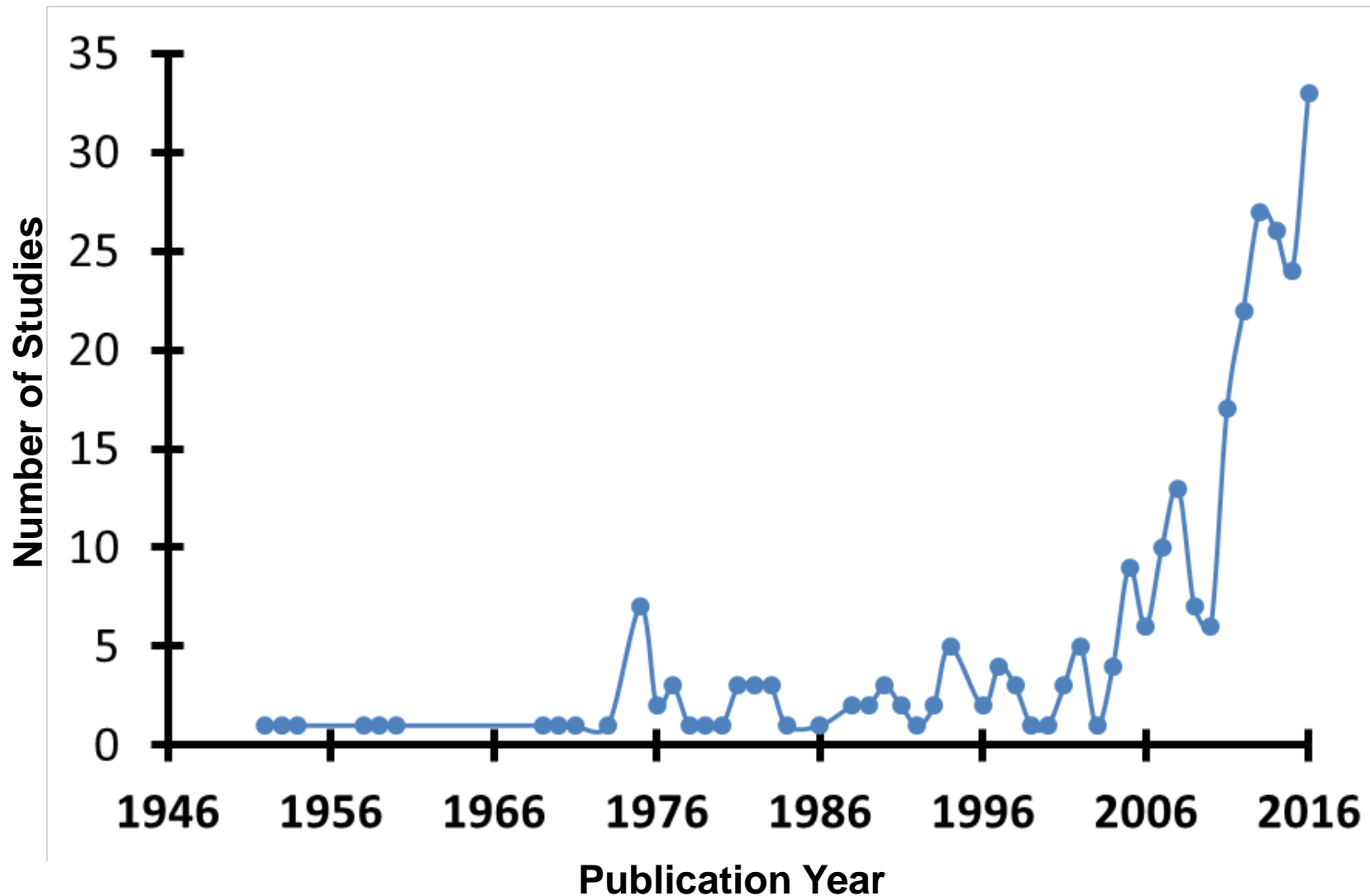


Study Selection and Risk of Bias



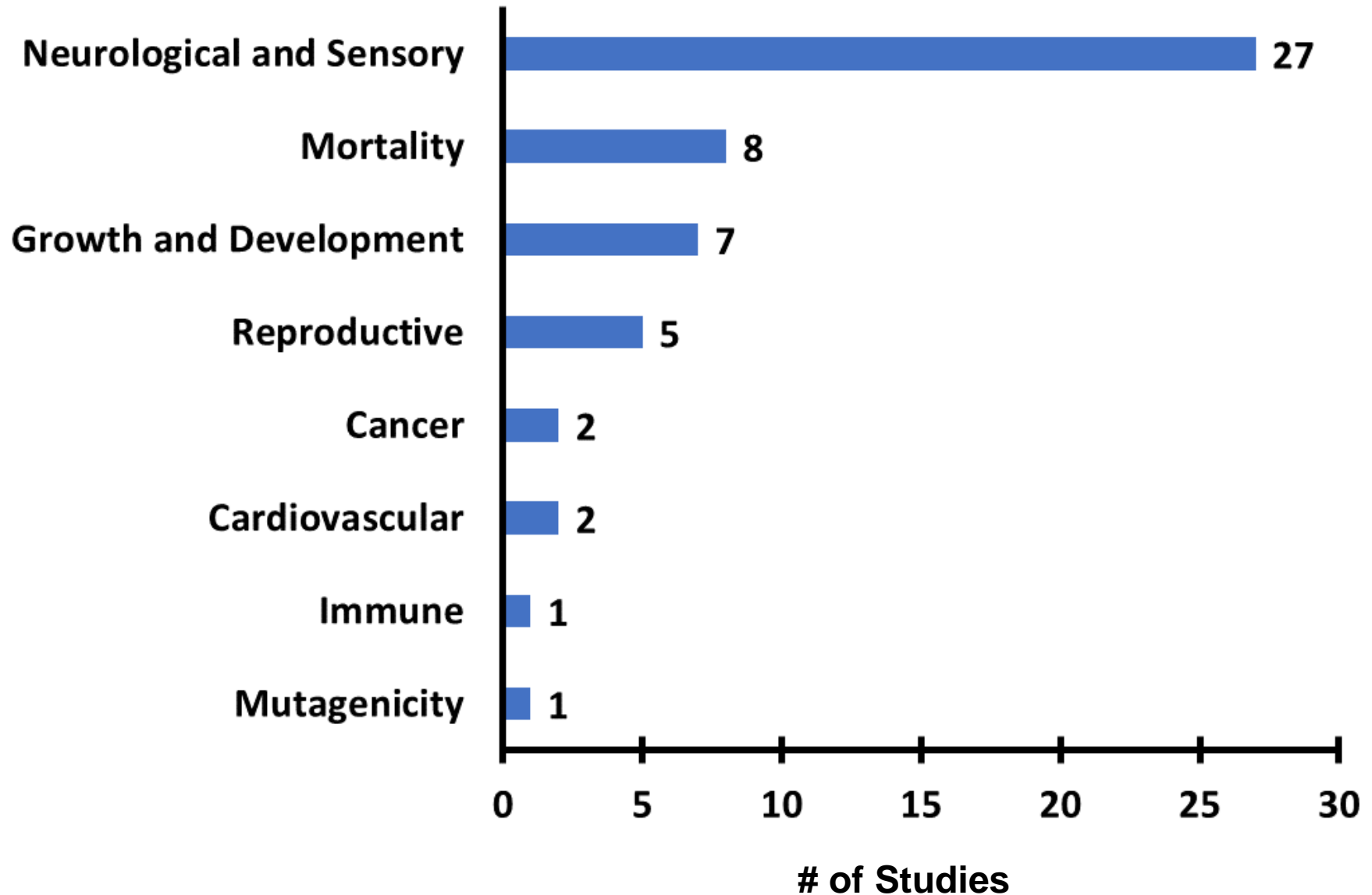


Emerging Research Focus





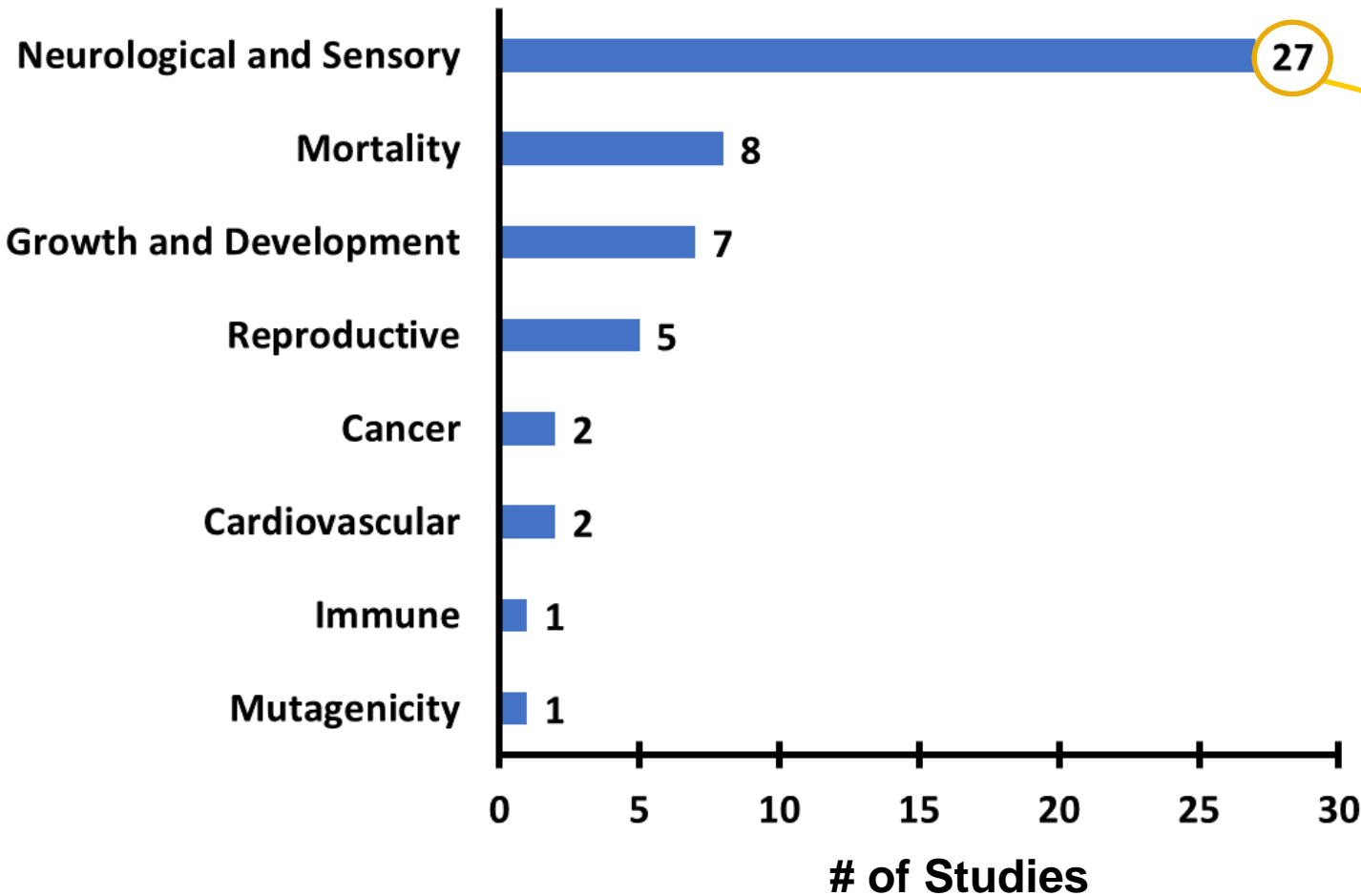
Human Studies: Outcomes





Human Studies: Outcomes

Few Studies of Same Exposure – Outcome Pair



8 different “exposures”

- Holocaust (n=8)
- Behavioral depression (n=4)
- Stress (n=6)
- 5 other exposures (n =1)



Few Studies of Same Exposure – Outcome Pair

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



Few Studies of Same Exposure – Outcome Pair

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)



Few Studies of Same Exposure – Outcome Pair

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





Few Studies of Same Exposure – Outcome Pair

Neurological and sensory outcomes
in grandchildren whose grandparents
experienced the Holocaust
(8 studies)

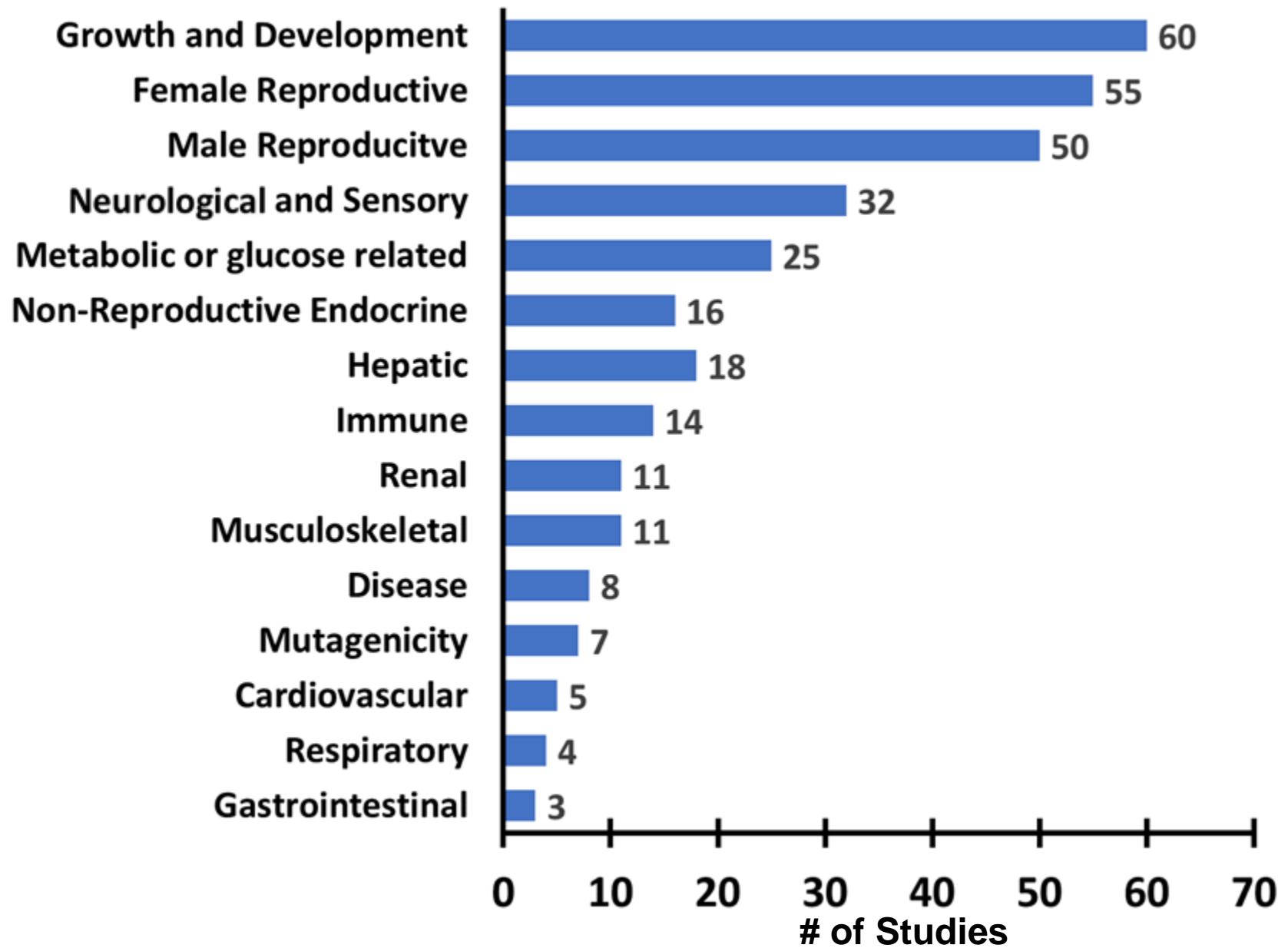
- Evaluated behavioral effects in 2nd and 3rd generation Holocaust survivors
- Meta analysis reports no evidence for behavioral indicators of trauma in an analysis that combined behavioral outcomes



Bottom line- Very few epidemiological studies



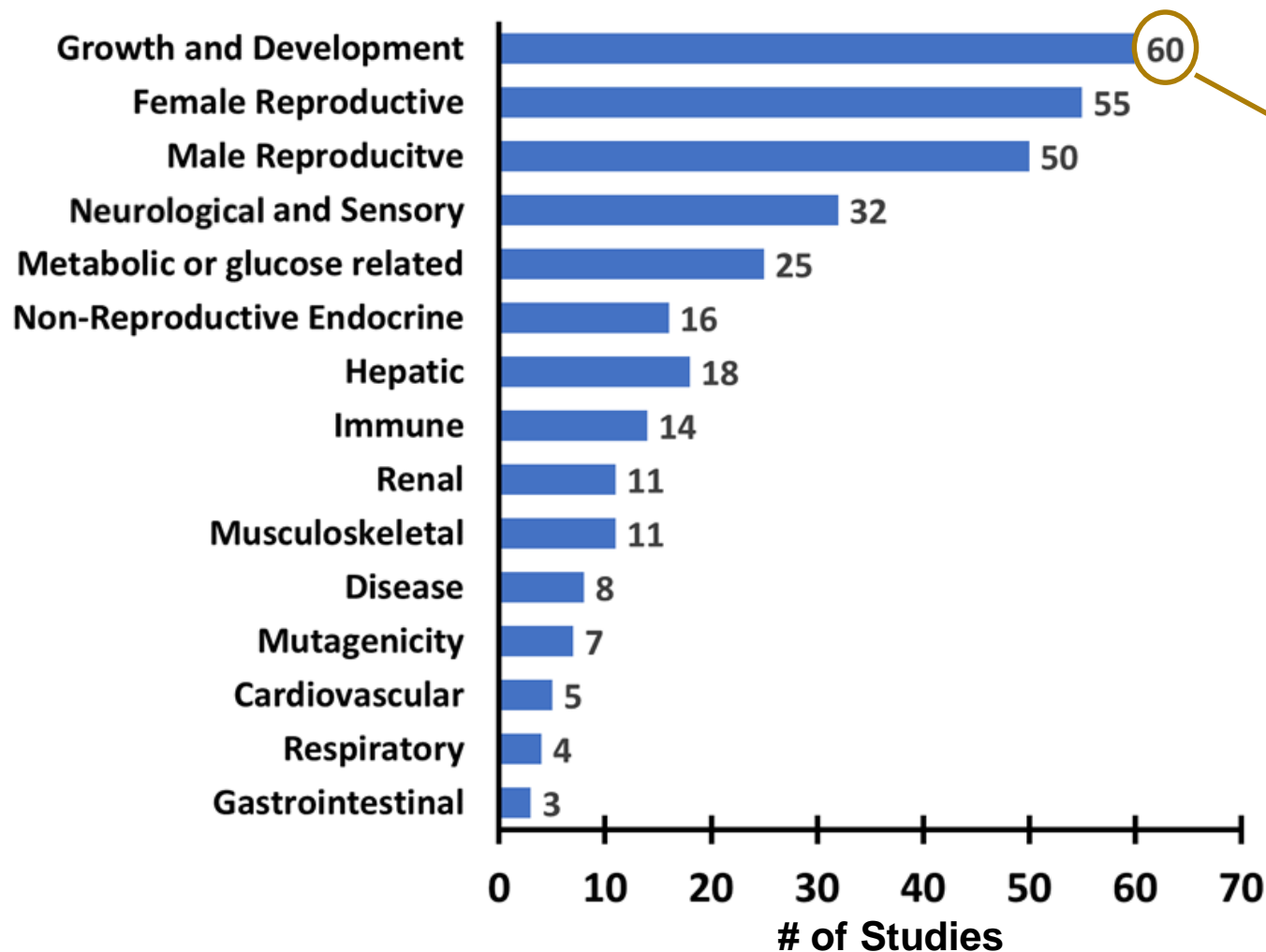
Animal Studies: Wide Range of Outcomes





Animal Studies: Wide Range of Outcomes

Few Studies With the Same Exposure and Similar Health Outcome



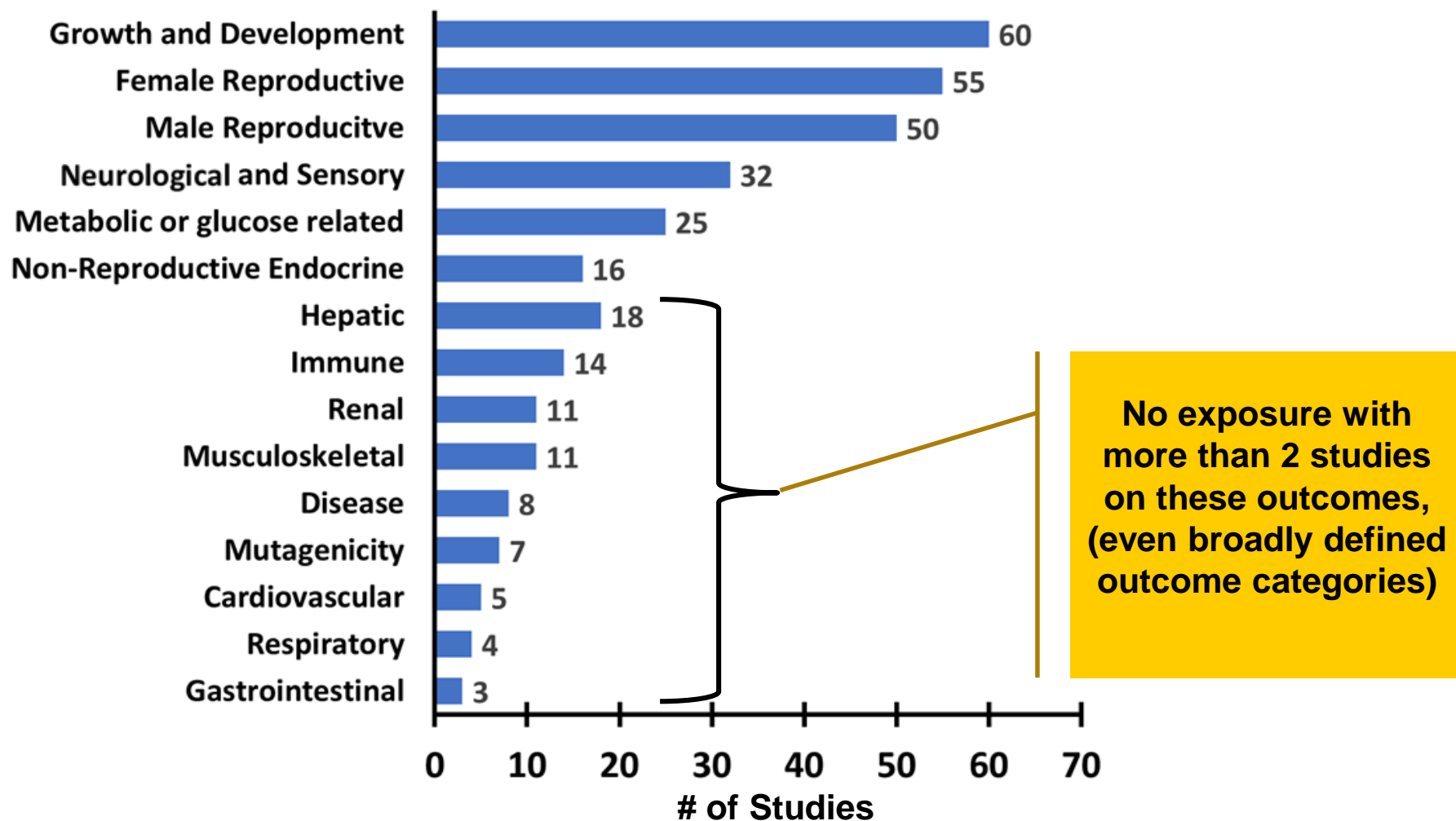
42 different “exposures”

- 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

Few Studies With the Same Exposure and Similar Health Outcome

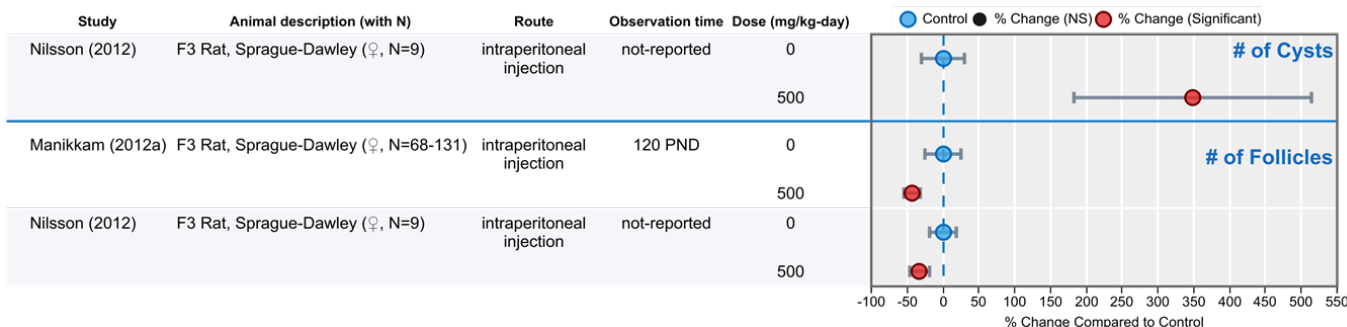




Example Endpoint Reported Across Multiple Exposures

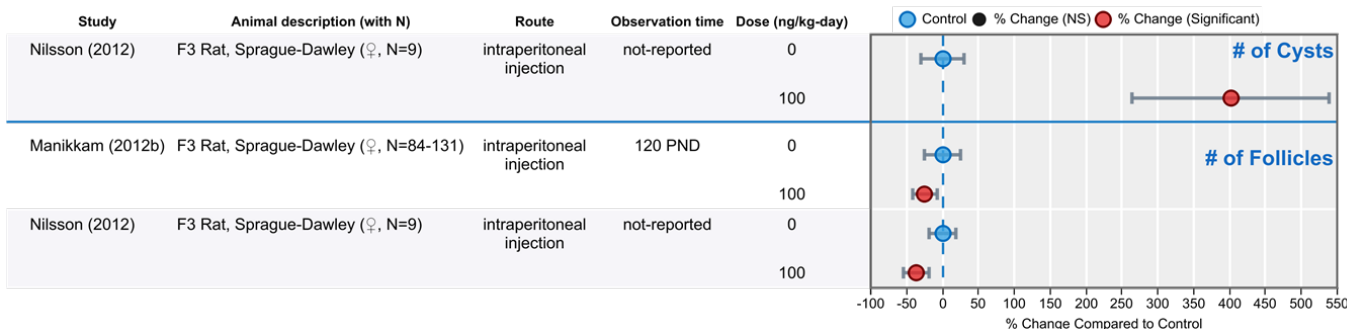
Reduction in Primordial Follicles and Increase in Ovarian Cysts

Jet Propellant 8



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

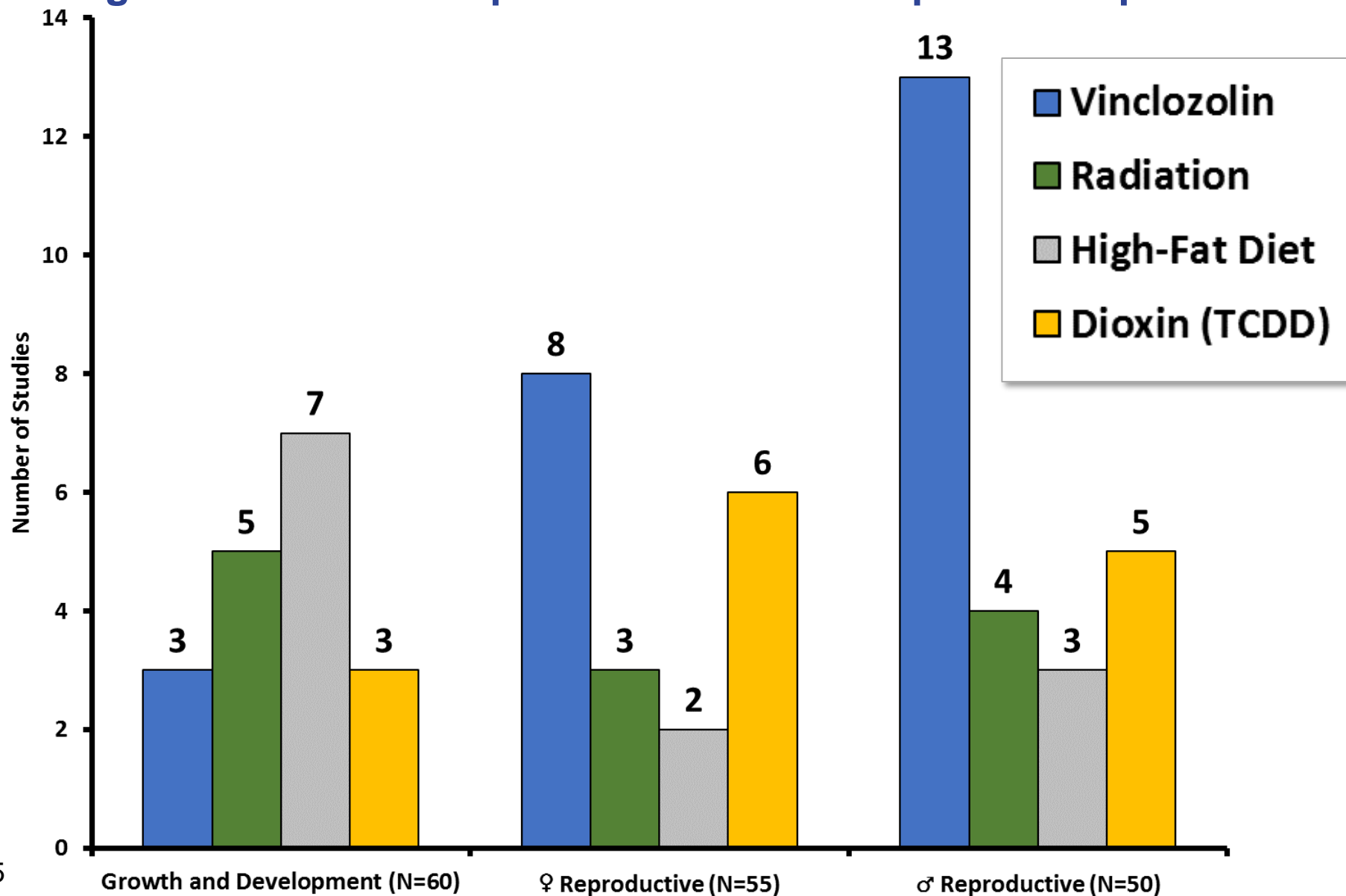
Dioxin





Animal Studies: Exposure x Outcome Pairs

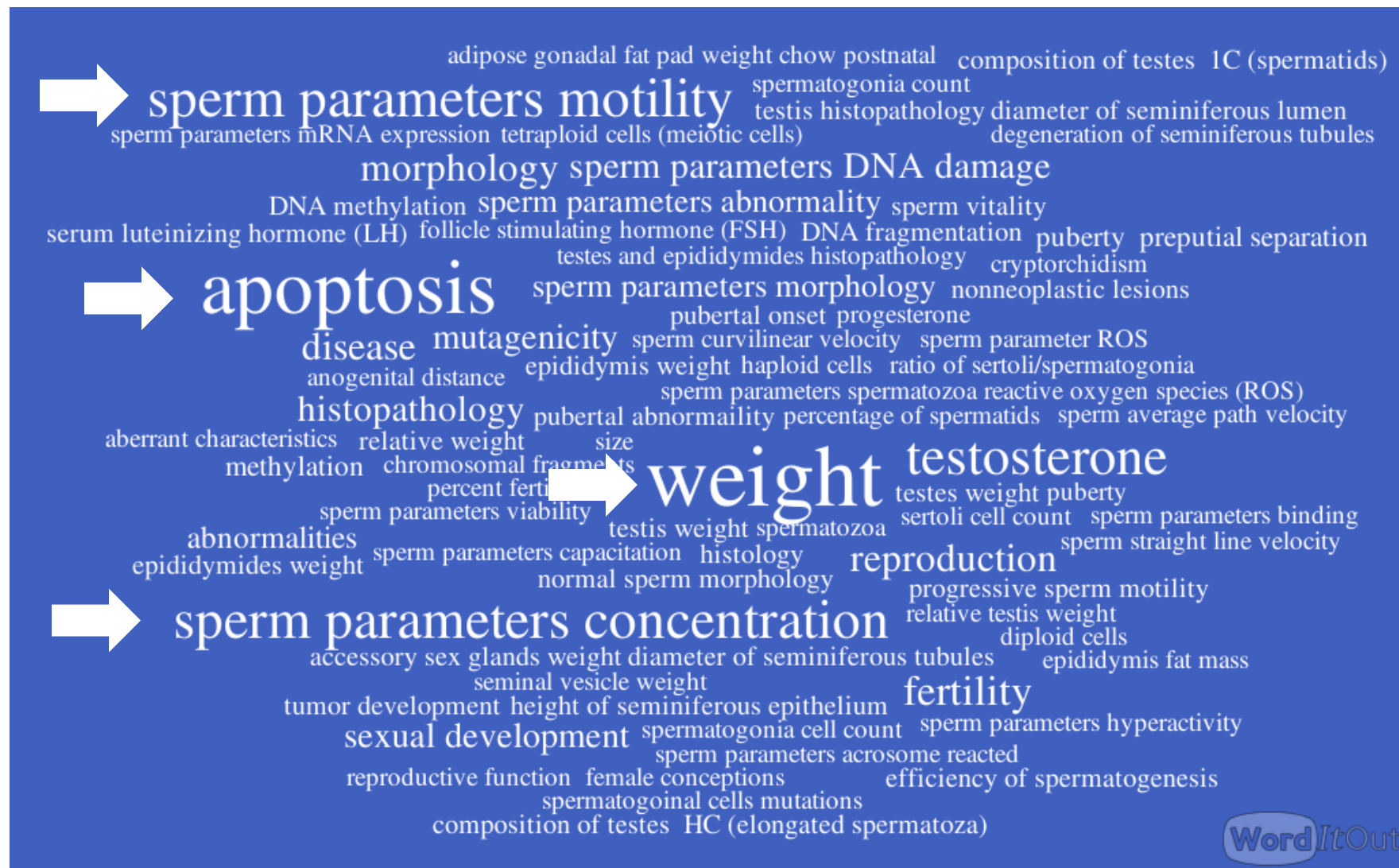
Drilling Down Identifies Specific Chemicals as potential “pockets”





Example: Individual Outcomes within Broad Category

Few Outcomes Evaluated in More than One Study

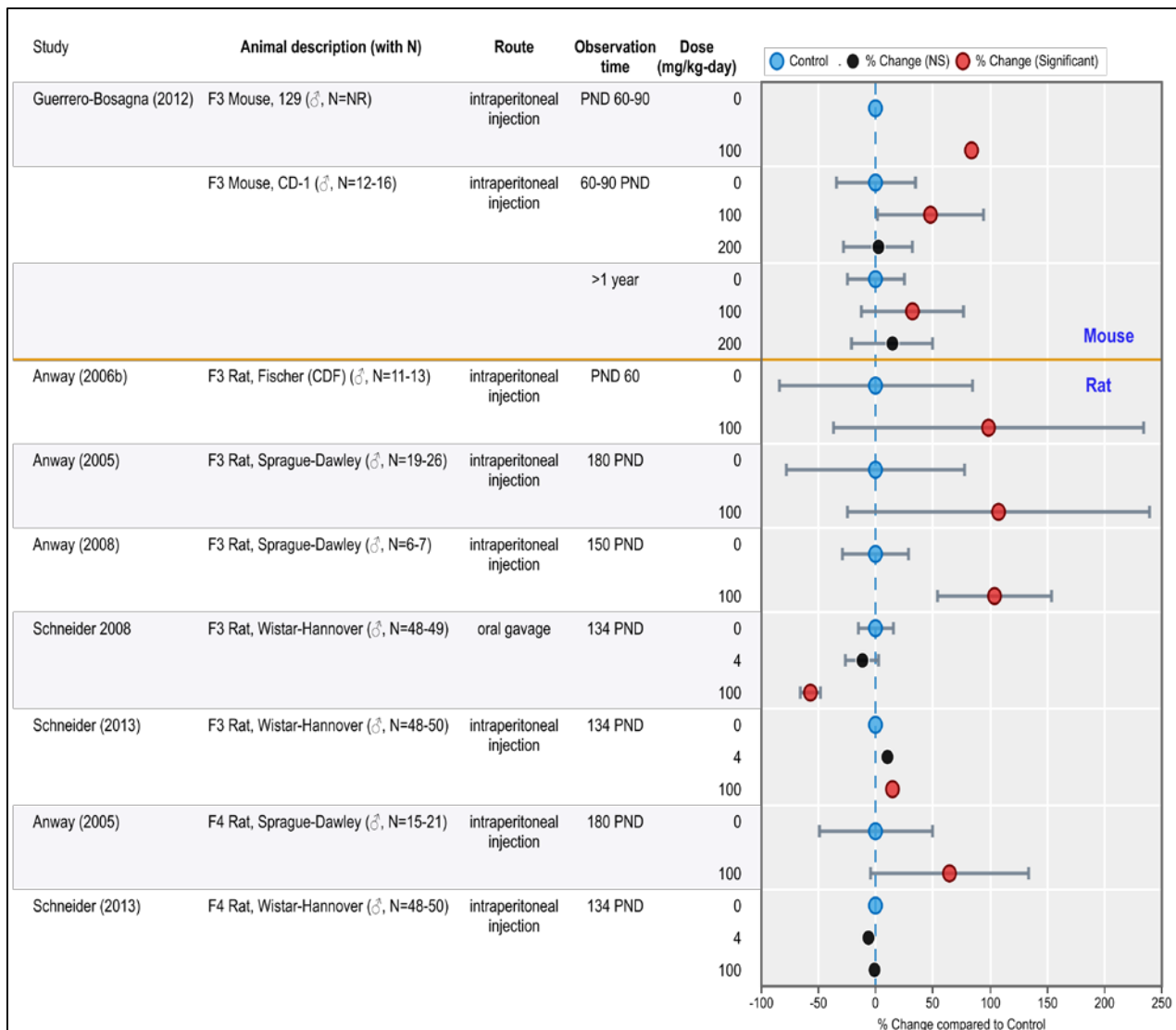


WordItOut



Example: Vinclozolin - Male Reproductive Outcomes

Apoptosis of Germ Cells in the Testis

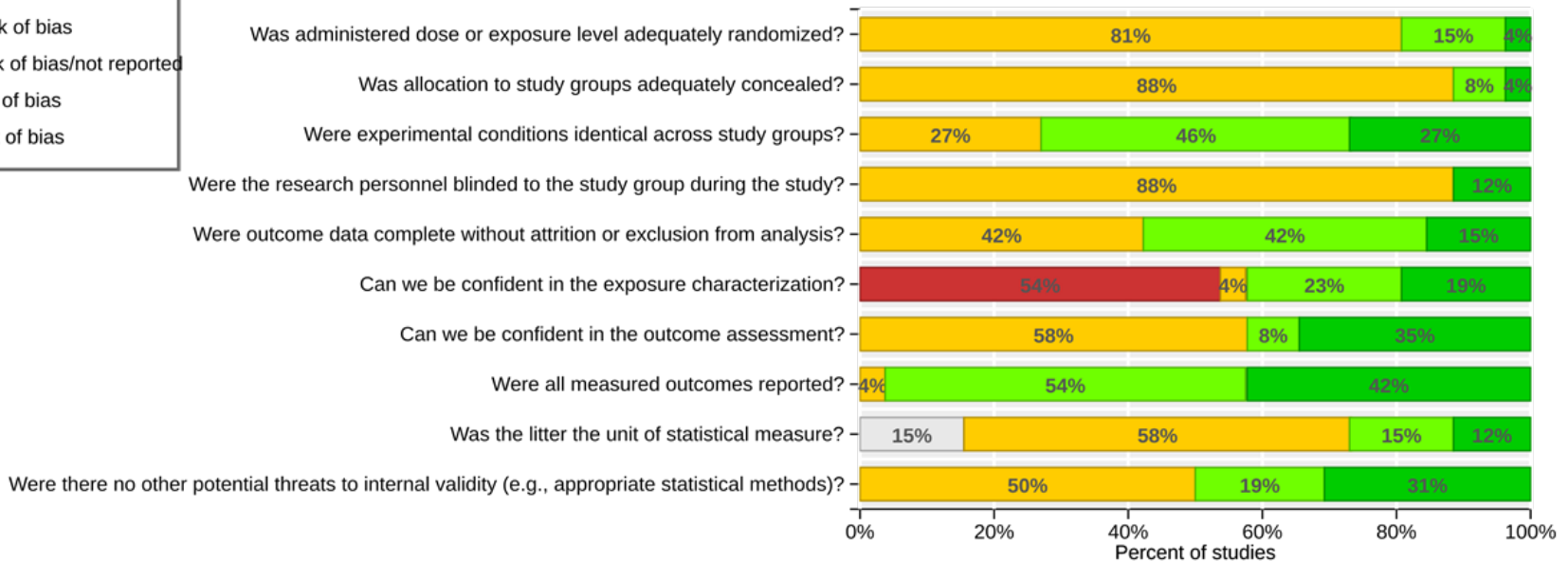
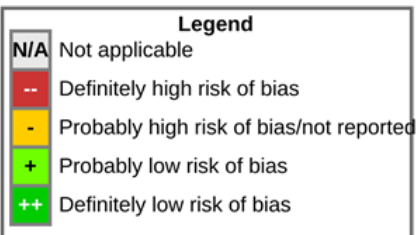


- 6 studies – from 2 groups of researchers
- Effects reported in both the mouse and rat
- 95% CI overlap with null



Animal Studies: Risk of Bias Evaluation

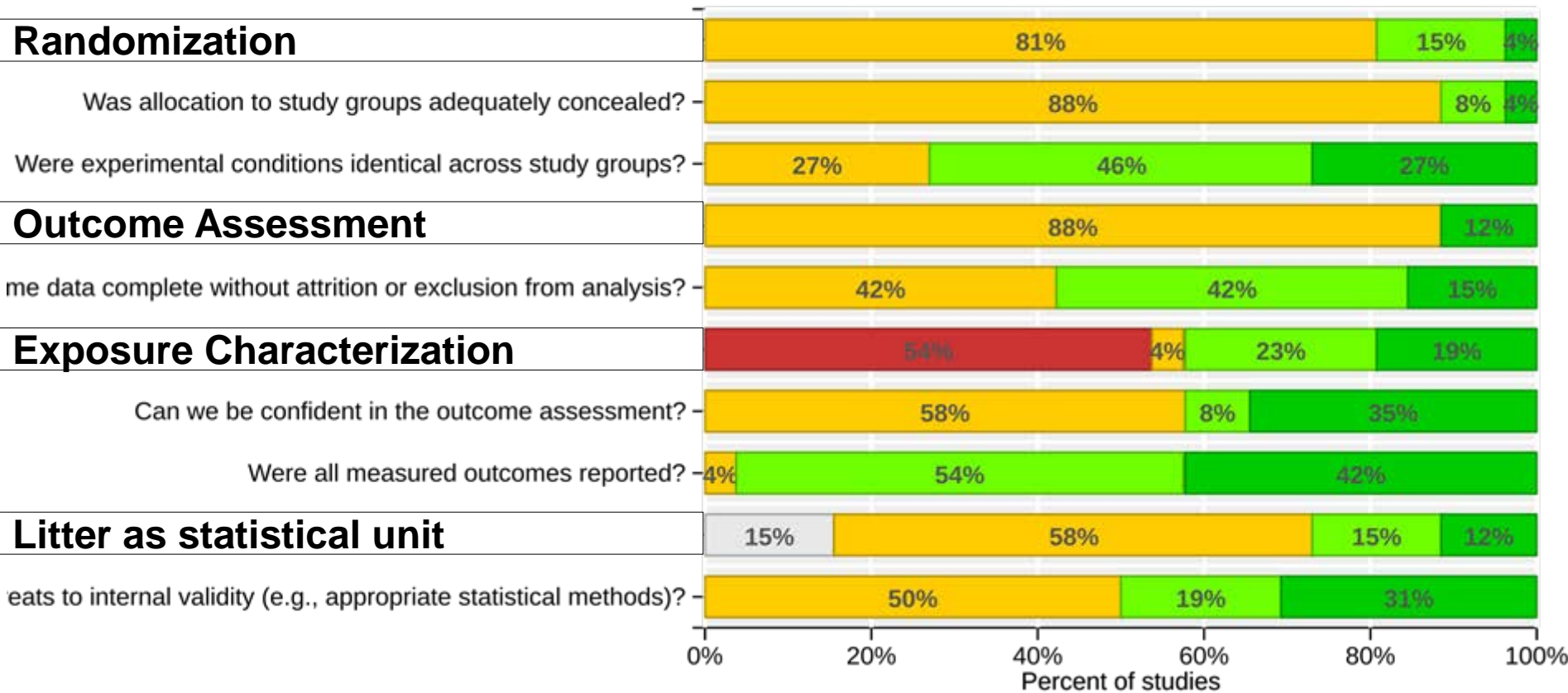
Subset of Transgenerational Studies Identify Concerns with Study Conduct and Reporting





Animal Studies: Risk of Bias Evaluation

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





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Summary





Outcome of Evidence Mapping

“Pockets” of Transgenerational Evidence

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



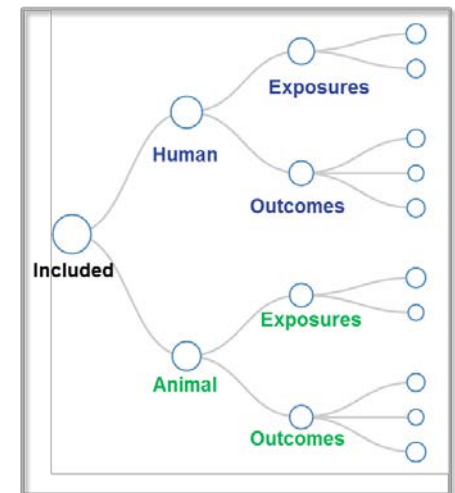
- **Main exposures:** high-fat diet (8), protein-restricted diet (4)
- **Main outcomes:** glucose tolerance, adiposity



Outcomes of Evidence Mapping

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?

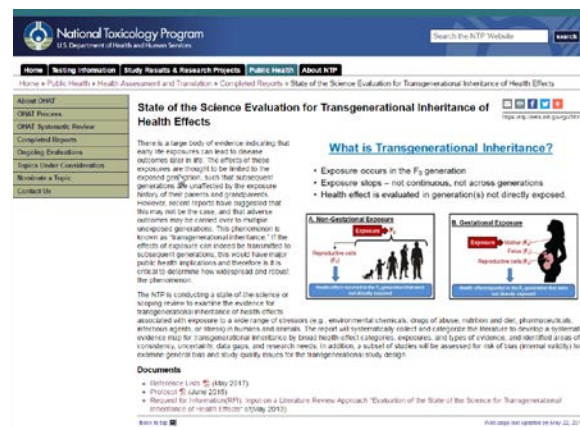
- For a given exposure, consistent assessment of the same or closely related health effects in multiple studies (and ideally across multiple labs)
- Minimize bias to produce robust data on potential transgenerational effects
 - Best practices in study design, conduct and reporting
 - Randomization of 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



NTP State of the Science Evaluation

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>)
 - Identify studies (included study list)
 - Extract data (publicly available; <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
 - **Abee Boyles**
 - **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?