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Review article

Human and animal evidence of potential transgenerational inheritance of health effects: An evidence map and state-of-the-science evaluation

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ABSTRACT

Background: An increasing number of reports suggest early life exposures result in adverse effects in offspring who were never directly exposed; this phenomenon is termed "transgenerational inheritance." Given concern for public health implications for potential effects of exposures transmitted to subsequent generations, it is critical to determine how widespread and robust this phenomenon is and to identify the range of exposures and possible outcomes.

Objectives: This scoping report examines the evidence for transgenerational inheritance associated with exposure to a wide range of stressors in humans and animals to identify areas of consistency, uncertainty, data gaps, and to evaluate general risk of bias issues for the transgenerational study design.

Methods: A protocol was developed to collect and categorize the literature into a systematic evidence map for transgenerational inheritance by health effects, exposures, and evidence streams following the Office of Health Assessment and Translation (OHAT) approach for conducting literature-based health assessments.

Results: A PubMed search yielded 63,758 unique records from which 257 relevant studies were identified and categorized into a systematic evidence map by evidence streams (46 human and 211 animal), broad health effect categories, and exposures. Data extracted from the individual studies are available in the Health Assessment Workspace Collaborative (HAWC) program. There are relatively few bodies of evidence where multiple studies evaluated the same exposure and the same or similar outcomes. Studies evaluated for risk of bias generally had multiple issues in design or conduct.

Conclusions: The evidence mapping illustrated that risk of bias, few studies, and heterogeneity in exposures and endpoints examined present serious limitations to available bodies of evidence for assessing transgenerational effects. Targeted research is suggested to addressed inconsistencies and risk of bias issues identified, and thereby establish more robust bodies of evidence to critically assess transgenerational effects - particularly by adding data on exposure-outcome pairs where there is some evidence (i.e., reproductive, metabolic, and neurological effects).

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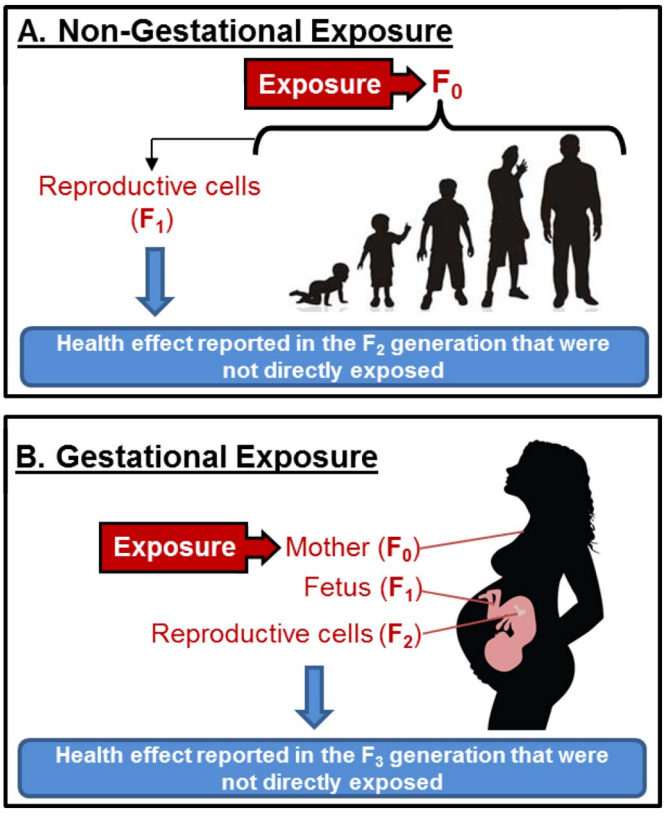

Fig. 1. Transgenerational study exposure paradigm. To fulfill a transgenerational study design, there must be exposure in the F_0 generation that stops and is not continuous across generations. This definition was selected to be consistent with the language in recent research grants at NIEHS as an indication of a one standard in the field. The 2012 developmental research grant encouraged applications "to investigate whether exposure to environmental toxicants can induce adverse phenotypic outcomes that are transmitted to subsequent, unexposed generations, a phenomenon known as transgenerational inheritance." (https://grants.nih.gov/grants/guide/rfa-files/RFA-ES-12-007.html).

1. Introduction

There is evidence that early life exposures can lead to disease outcomes much later in life (Barker et al., 2002). The traditional dogma suggests that negative effects of these early life exposures are not carried forward, such that subsequent generations are unaffected by the exposure history of their parents and grandparents. However, there are reports of exposures that, in certain cases, can have far-reaching consequences affecting multiple generations removed from the original exposure(reviewed in Aiken and Ozanne, 2014; Grossniklaus et al., 2013; Jirtle and Skinner, 2007). This phenomenon is known as "transgenerational inheritance" where there is evidence of a health effect in offspring (e.g., F_3 and beyond following exposure during pregnancy; see Fig. 1) who were not themselves exposed to a chemical or non-chemical stressor.

Fig. 1 outlines the transgenerational inheritance exposure paradigm (Heard and Martienssen, 2014; NIEHS, 2012; Rissman and Adli, 2014; Xin et al., 2015), where there is exposure in the F_0 generation but then exposure stops and is not continuous across generations. Two exposure scenarios are commonly investigated based on exposure timing as gestational (during pregnancy) or non-gestational. In the pregnancy exposure model, a health effect must be evaluated in subsequent generations that have not been exposed, even as germ cells, to be considered transgenerational. This entails direct exposure to the pregnant female (F_0), the fetus (F_1), and the germ cells developing within the fetus (F_2). Therefore, a transgenerational study with gestational exposure requires evaluation of an effect in the F_3 generation (or later) as the first generation not exposed to the initial chemical or stressor. In the other model, exposure occurs at any life stage outside of pregnancy where there is direct exposure to the individual (F_0) with potential impacts of the exposure on the developing germ cells, and a transgenerational study requires evaluation of an effect in the F_2 generation (or later) as the first unexposed generation.

The transgenerational inheritance exposure paradigm described above is more specific than how the term transgenerational has been used generally in the literature to describe an effect transmitted from one generation to the next (Heard and Martienssen, 2014). Using this definition, many studies reported in the literature as "transgenerational" were not considered to be "transgenerational inheritance" studies because effects were examined in animals that may have been exposed during development or as germ cells. This includes continuous exposure across generations (i.e., multi-generational or inter-generational studies) as well as exposure that was discontinued without sufficient generations [e.g., observations in offspring (F_2) following gestational exposure would be from individuals that were not directly exposed but the germ cells contributing to those individuals were exposed].

Transgenerational inheritance is an emerging field of research for which attempts to synthesize findings across studies are complicated by the lack of consistent terminology described above. If transgenerational inheritance is taking place and the effects of exposure can indeed be transmitted to subsequent unexposed generations, this would have major public health implications. Therefore, we conducted a state-of-the-science or scoping review to systematically map the extent of the evidence for transgenerational inheritance of health effects potentially associated with exposure to a wide range of stressors (e.g., environmental chemicals, drugs of abuse, nutrition and diet, pharmaceuticals, infectious agents, or stress) in humans and animals. The first 4 steps of a systematic review were used: 1) problem formulation and protocol development, 2) search and select studies, 3) data extraction, and 4) risk of bias assessment to identify potential study design challenges. This evaluation provides a summary and critical assessment of the literature related to transgenerational inheritance of health effects to map the major health effect categories and principal exposures with a goal of identifying areas of consistency within available "pockets" of research, and areas of uncertainty as well as data gaps and research needs to support decisions on subsequent laboratory research or further evaluation of transgenerational inheritance in a systematic review.

2. Methods

2.1. Problem formulation

The objective, specific aims, and detailed protocol for conducting this scoping review were developed and refined through a series of problem formulation steps including: (1) consultation with an evaluation team comprised of The National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) staff with expertise in reproductive and developmental toxicology, epidemiology, epigenetics, systematic review, and information science; (2) development of an NTP project webpage (https://ntp.niehs.nih.gov/go/38159) and a public request for information in the Federal Register [88 FR 26646 (May 7, 2013)], and (3) consideration of the extent of information available for specific exposures and health outcomes based on initial literature search results. During problem formulation, a preliminary search of PubMed identified over 50,000 references to be screened for relevance and eligibility for this review. Rather than a comprehensive literature search performed during a systematic review developed to reach hazard conclusions, this scoping report considered a single database, PubMed. The goal was not to be comprehensive, as would be necessary to support hazard conclusions, but to have sufficient coverage of the literature to identify areas of consistency, uncertainty, and data gaps. This search method was designed to develop a map of the major health effect categories and exposures for each

Table 1
Transgenerational inheritance PECO (population, exposure, comparators and outcomes) statement.

Element	Evidence
Population	Human or animal (whole organism) without restriction based on age, sex, or life stage at exposure or outcome assessment
Exposure	Any exposure/stressors at any life stage as long as the study design (i.e., outcome measurement stage) was transgenerational in nature (Fig. 1)
Comparators	*Humans*: a comparison population exposed to lower levels (or no exposure/exposure below detection levels) of the stressor than the more highly exposed subjects *Animals*: comparable animal populations exposed to vehicle-only treatment in experimental studies or a comparison animal population exposed to lower levels (or no exposure/exposure below detection levels) of the stressor than more highly exposed animals in wildlife/farm animals *Note: the comparison groups are defined at the time of exposure and therefore apply to the F_0 generation (which in a gestational exposure would include exposure of the offspring (F_1) and its gametes (F_2); see Fig. 1)*
Outcomes	Inherited diseases were excluded. No other restrictions on health outcome or effect measures

evidence stream and thereby identifies major pockets of literature for exposure-outcome pairs as well as major gaps in the literature to support decisions on health effects and exposures for further evaluation in subsequent research or systematic reviews.

We considered similar searches in SCOPUS and Web of Science to broaden the database coverage. However, due to the low specificity of the search inclusion of those databases would have more than quadrupled the number of references (to an estimated 200,000 or more). In an effort to determine the extent of relevant evidence that may have been missed through a PubMed-only search, the search terms were formatted for both SCOPUS and Web of Science and focused on the top five exposures identified in our evaluation. We reviewed over 4000 articles at the title and abstract level and 162 at the full text level and identified four studies (approximately 1.5% addition relative to PubMed) that were not retrieved in the PubMed search that met the PECO criteria (1 Holocaust study, 2 vinclozolin studies, 1 radiation study and no studies for dioxin or high fat diet exposures) (Amoako-Atta et al., 1978; Kreß et al., 2017; McCarrey et al., 2016; Palgi et al., 2015). However, the addition of these studies would not have changed the evidence map or the objectives of this review, as they did not identify new health outcomes. The four studies would be identified and considered in a systematic review of any specific topic for reaching hazard conclusions. Evidence maps are an emerging methodology in the environmental health sciences with different approaches depending on the purpose of the review, including use of a single database to identify major patterns in the evidence and focus follow-up activities (e.g., Berger et al., 2014; Wang et al., 2016). Thus, we limited the search to PubMed to support the objectives outlined below.

An evaluation protocol was developed with detailed methods for conducting this scoping review based on the problem formulation activities, feedback received, and the OHAT Handbook for Conducting a Literature-Based Health Assessment (NTP (National Toxicology Program), 2015). The OHAT systematic review framework consists of a 7-step process, and the first 4 are relevant to produce a scoping review and assess study quality; whereas the last 3 are relevant for synthesizing evidence and reaching hazard conclusions. Therefore, this evaluation is restricted to the first 4 steps: 1) problem formulation and protocol development, 2) search and select studies for inclusion, 3) data extraction, and 4) risk of bias assessment of individual studies. The protocol was posted on the NTP website in June 2015 (http://ntp.niehs.nih.gov/go/38159). A brief summary of the methods is presented below.

2.2. Objective and specific aims

The overall objective of this scoping review was to examine the extent of the evidence for transgenerational inheritance of health effects potentially associated with exposure to a wide range of stressors in humans and animal models. To accomplish this objective, the specific aims of this evaluation were to:

1. Identify human and animal literature within PubMed that utilized a transgenerational study design with no restriction on the type of exposure or type of health outcome.
2. Extract data on potential health effects from relevant studies.
3. Provide an inventory of included studies, focusing on type of exposure and health outcome for both human and animal studies [i.e., develop an evidence map by stream (human or animal studies), type of exposure, and health outcome with a focus on bodies of evidence with the same exposure - outcome pair].
4. Assess risk of bias, as described in section Risk of Bias Assessment of Individual Studies, of individual human and animal studies from subsets of the relevant references prioritized to focus on exposures with the largest number of human and animal studies.
5. Prepare a brief summary of the study results, grouped by exposures for which the same health outcome is reported, in order to identify areas of consistency and areas of uncertainty.
6. Outline key issues or data gaps that could be addressed in future research of transgenerational inheritance of health effects.

2.3. PECO statement

To address our overall objective we developed a PECO (Population, Exposure, Comparators and Outcomes) statement (Table 1), which was used as an aid to identify specific search terms and inclusion/exclusion criteria for identifying transgenerational research and addressing the review question (Higgins et al., 2011).

2.4. Literature search

Search terms were developed by an informationist (SDH) familiar with systematic review methodology to identify all relevant published evidence indexed in the PubMed database (MEDLINE) that addressed the PECO statement for transgenerational inheritance of health effects potentially associated with a wide range of exposures. Since there are no MeSH terms for transgenerational effects or transgenerational inheritance, our search strategy was developed using text words that describe a transgenerational study and terms used to report the topic of transgenerational inheritance in the literature. The resulting strategy was used to search PubMed (specific search terms presented in Supplemental Table S1). No language restrictions or publication year limits were imposed and PubMed was searched up to November 2016.

2.5. Study selection

2.5.1. Study selection criteria

In order to be eligible for inclusion, studies had to satisfy eligibility criteria specified by the PECO statement (Table 1). The following additional criteria were also used for exclusion:

- articles without original data (i.e., reviews, editorials, commentaries)
- studies of non-animal organisms (e.g., plants, fungi)
- in vitro exposure studies
- non-English publications

2.5.2. Screening process

References retrieved from the literature search were screened for relevance and eligibility using DistillerSR® (Evidence Partners; http://www.systematic-review.net), a web-based, systematic-review software program with structured forms and procedures to ensure standardization of the process. Screeners from the evaluation team (AB, KP, RB, PH, AR, VW) were trained with an initial pilot phase undertaken to improve clarity of the evidence selection criteria and to improve accuracy and consistency among screeners. All references were independently screened by two trained screeners at the title and abstract level to determine whether a reference met the evidence selection criteria. Studies that were not excluded by reviewing the title and abstract were screened with a full-text review for relevance (VW and KP). Screening conflicts were resolved through discussion and consensus (or 3rd reviewer, AB or AR).

2.6. Data extraction process and data warehousing

Data extraction was managed with structured forms and stored in a database format using the Health Assessment Workspace Collaborative (HAWC, https://hawcproject.org) tool, an open source and freely available web-based interface application. Data extraction elements are listed separately for human and animal studies and described in the study protocol (http://ntp.niehs.nih.gov/go/38159). Data extraction was performed by contract support and checked by the project lead for completeness and accuracy. Any discrepancies in data extraction were resolved by discussion or consultation with a third member of the evaluation team if the discrepancy was not immediately resolved. Information that was inferred, converted, or estimated during data extraction was annotated, e.g., using brackets [n = 10]. Studies reporting gene expression data, mechanistic data supporting mechanism of action or qualitative summary statements with no quantitative data to extract are noted in HAWC as "data not extracted." OHAT attempted to contact the corresponding author by email to obtain missing data considered important for evaluating key study findings. The extraction details in HAWC note when study researchers did not respond within one month of the attempt.

When multiple studies reported data on the exposure and outcome, select figures were developed in HAWC to graphically display the results. All figures are presented as percent change relative to the control response for which 95% confidence intervals were calculated from the summary data presented in the paper using a Fisher Information Matrix and assumed independent normal distribution. Confidence interval calculations required that the study reported variance measures (i.e., standard deviation or standard error), the n for each dose-group and the control mean was not equal to zero. Statistical significance of a finding as reported by study authors is indicated by a red circle in the HAWC figures.

2.7. Risk of bias assessment of individual studies

2.7.1. Full risk of bias assessment

Risk of bias assessment was performed for individual studies from the two exposures with the largest bodies of evidence from both evidence streams: human studies (Holocaust and radiation) and experimental animal studies (vinclozolin and radiation). These studies were used to illustrate general issues with risk of bias relevant to reaching conclusions on potential transgenerational health effects for environmental exposures.

Risk of bias was assessed using a tool developed by OHAT that takes a parallel approach to evaluating risk of bias from human and animal studies (see details in protocol http://ntp.niehs.nih.gov/go/38159) to facilitate consideration of risk of bias across evidence streams with common terms and categories (Rooney et al., 2014). The tool is comprised of a common set of questions that are answered based on the specific details of individual studies to develop risk of bias ratings for each question (on a four point scale "definitely high", "probably high", "probably low", or "definitely low" risk of bias). When studies did not report the information necessary to answer the risk of bias question, or important details were not reported, a rating of "NR" was recorded. The corresponding author was contacted by email to obtain the necessary information. If study researchers did not respond to an email request within one month of the attempt to contact, that information is noted in the specific risk of bias response in HAWC. Without the ability to determine what was actually done in the study, an NR rating is equivalent to "probably high" risk of bias under this method, and it is not possible to distinguish problems with the quality of the reporting from problems with the study conduct. A conservative standard is used wherein a lack of reporting is considered a lack of conduct.

Study design determines the subset of questions used to assess risk of bias for an individual study. Transgenerational studies involve tracking health effects over multiple generations following an exposure, and controlling for litter effects is critical to address the potential of fetuses from a given litter to exhibit similar responses to chemical exposure (Kupper, 2014). As such, a project-specific question was added to the risk of bias approach for experimental studies to query whether or not the litter was considered the experimental unit for study design and statistical analysis.

Risk of bias assessors were trained with an initial pilot phase undertaken to improve clarity of rating criteria and to improve consistency among assessors. Studies were independently evaluated by two trained assessors who answered all applicable risk of bias questions with one of four options following specific criteria detailed in the protocol (http://ntp.niehs.nih.gov/go/38159). The criteria describe aspects of study design, conduct, and reporting required to reach ratings for each question and specify factors to distinguish among ratings (e.g., what separates "definitely low" from "probably low" risk of bias). After two assessors review a study across all questions, discrepancies are resolved through discussion to reach final risk of bias ratings for each question as well as documented rationale/justification for the rating.

In the OHAT approach, some risk of bias questions are considered potentially more important when assessing studies because there is more empirical evidence that these areas of bias have a greater impact on estimates of the effect size or because these issues are generally considered to affect the credibility of study results in environmental health studies (Rooney et al., 2016). The key questions for observational human studies are: confounding, exposure characterization, and outcome assessment (including blinding of outcome assessors). For experimental animal studies these key questions are: randomization of exposure, exposure characterization, and outcome assessment.

2.7.2. Consideration of key risk of bias questions

In this state of the science review, risk of bias was only assessed for individual studies from the two largest bodies of evidence from the human and animal evidence streams as an example to illustrate potential general bias issues for the transgenerational study design. A complete risk of bias assessment was not conducted for individual studies from all exposures. However, in the results section, when there were two or more studies with the same exposure-outcome pair, the key risk of bias questions (randomization, blinding of outcome assessment, exposure characterization and statistical aspects) were considered when assessing the consistency and uncertainty in the bodies of evidence.

3. Results

3.1. Literature search results

The Pubmed database search retrieved 63,789 references and three additional references were identified by experts or reviewing published reviews and reference lists from the included studies. Ninety-eight percent of the total references retrieved (62,671) were excluded during

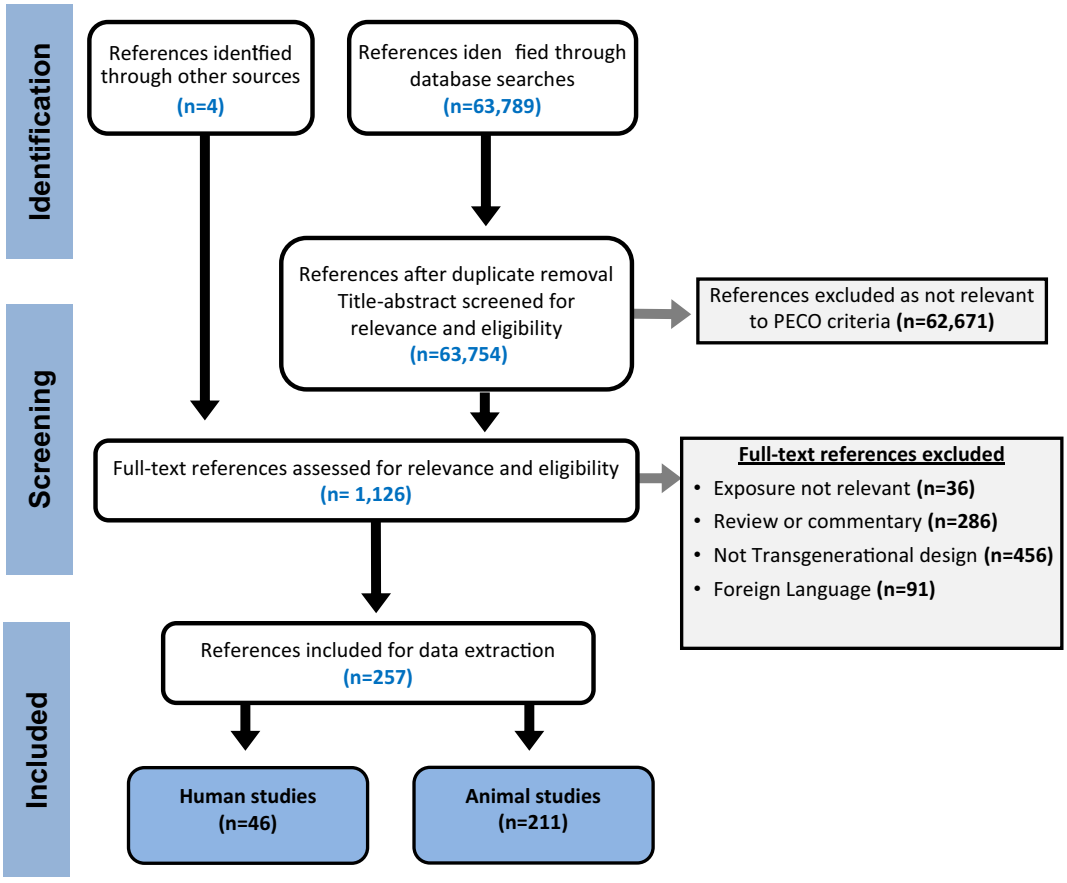

Fig. 2. Study selection diagram.

the title and abstract screening and 869 were excluded during the full text review for not satisfying the PECO criteria. After full text review, 257 studies were considered relevant; 46 human and 211 animal (Fig. 2). List of included references are in Supplemental materials.

This review systematically collected and categorized the literature to develop a systematic evidence map for transgenerational inheritance by broad health effect categories, exposures, types of evidence, and identified areas of consistency, uncertainty, data gaps and research needs. The studies were grouped by broad health outcome categories (e.g., cardiovascular system) to look for potential bodies of evidence with multiple studies of the same exposure agent on the same or related health outcomes (Table 2 and Table 3). Within these health outcome categories, there are few specific exposure agents for which there is more than one study (indicated in bold in the tables). The evidence mapping includes an interactive file that allows the reader to review the mapped data and provides a direct link to the data extraction page in HAWC (Interactive Evidence Map).

3.2. Study quality and risk of bias

Risk of bias assessment was performed for individual studies from the two exposures with the largest bodies of evidence for each evidence stream: Holocaust and radiation for the human studies and vinclozolin and radiation for the experimental animal studies. The subsets of studies were used to illustrate general issues with risk of bias relevant to reaching conclusions on potential transgenerational health effects associated with environmental exposures.

The majority of the human studies were rated probably high or definitely high risk of bias for each of the three key questions: confounding considerations (87%), confidence in the exposure

Table 2
Transgenerational human studies sorted by major health effect categories and exposure agents.

Health outcome category	# of studies	Exposure agents (n)
Cancer	1	1 Exposure: smoking (1)
Reproductive	5	1 Exposure: **radiation** (**5**)
Growth and development	4	8 Different exposures: born out of wedlock (1), diethylstilbestrol (1), education (1), income (1), positive parenting (1), radiation (1), socioeconomic status (1) and food availability (1)
Mortality	6	3 Exposures: **food availability** (**3**), born out of wedlock (1), socioeconomic stress (2)
Mutagenicity	1	1 Exposure: radiation (1)
Neurological or sensory	26	14 Different exposures: abuse (1), anxiety (1), born out of wedlock (1), **behavioral depression** (**4**), diethylstilbestrol (1), education (1), famine (1), **Holocaust** (**8**), income (1), parental communication (1), positive parenting (2), radiation (1), socioeconomic status (1), **substance use/abuse** (**2**)

Number of transgenerational studies (n) in humans sorted by broad health outcome categories as well as individual exposure agents. As defined in the PECO statement the exposures included a wide range of stressors from environmental chemicals to the Holocaust. Few health outcome categories or exposure agents were evaluated in more than one study. Exposure agents in bold text indicate agents with more than one study within a health outcome category.

Table 3
Transgenerational animal studies sorted by major health effect categories and exposure agents.

Health outcome category	# of studies	Exposure agents (n)
Cardiovascular	5	5 Different exposures: bisphenol A (BPA) (1), caffeine (1), low-protein (1), nutrient restriction (1), voluntary exercise (1)
Growth and developmental	60	42 Different exposures: atrazine(1), benzo(a)pyrene (1), chlorpromazine (1), cobalt (1), crowding (1), **cyclophosphamide or cyclophosphamide and vinblastine** (**2**), dichlorodiphenyltrichloroethane (DDT), decitabine (1), di(2-ethylhexyl) phthalate (DEHP)(1), dexamethasone (1), ethinyl estradiol (EE2) (1), **dioxin** (**4**), ethylnitrosourea (ENU) (1), flutamide (1), food restriction (1), G418 antibiotic (1), gold nanoparticles 10 nm (1), heat stress (1), **high fat diet** (**8**), **JP-8** (**2**), low protein (1), l-thyroxine (l-T4) (1), **methoxychlor** (**2**), methylating micronutrients (1), **n-nitrosomethylurea** (**NMU**) (**2**), non-lethal heat stress (1), nonylphenol (1), novel environment: laboratory (1), nutrient restriction (1), **overnutrition** (**2**), polychlorinated biphenyl (PCB) congeners 101 and 118 (1), **pesticide mixture**: **permethrin** + **diethyltoluamide** (**DEET**) (**2**), plastics: BPA + diethylhexyl-phthalate (DEHP) + di-n-butyl-phthalate (DBP) (1), PBBs (1), protein-restricted diet (1), **radiation** (**5**), radiofrequency electromagnetic fields (RF-EMF) (1), sulfamethoxazole (1), temperature (1), **vinclozolin** (**5**), zinc (deficiency) (1)
Disease	7	7 Different exposures: DDT (1), dioxin (1), JP-8 (1), methoxychlor (1), NMU (1), pesticide mixture: permethrin and DEET(1), vinclozolin (1)
Non-reproductive endocrine	16	15 Different exposures: benzo(a)pyrene (1), chronic stress (1), ethanol (1), high-sugar diet (1), I-thyroxine (I-T4) (1), malnourishment (1), morphine sulfate (1), ENU (1), n-nitrosomethylurea (NMU) (1), nonylphenol (1), obesity (1), polybrominated biphenyls (PBBs) (1), prereproductive stress (PRS) (1), protein-restricted diet (1), **vinclozolin** (**2**), voluntary exercise (1)
Female reproductive	55	35 Different exposures: azadirachtin (1), acaricide (1), **benzo(a)pyrene** (**2**), **BPA** (**2**), carboxymethyl cellulose stabilized nanoscale zerovalent iron (1), chlorpromazine (1), cyclophosphamide or cyclophosphamide and vinblastine (1), DDT (1), dexamethasone (1), diet (1), **DEHP** (**2**), DBP (1), **dioxin** (**7**), doxorubicin (DXR) (1), ethanol (1), Ethinyl-oestradiol (EE2) (1), ENU (1), fenpyroximate (1), carbon nanomaterial (1), gonadotropin (1), **high-fat diet** (**2**), **JP-8** (**3**), levonorgestrel + EE2 (1), **low protein** (**2**), l-T4 (1), methoxychlor (1), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) (1), NMU (1), nonylphenol (1), novel environment: laboratory (1), p,p′-DDE (1), PCB congeners 101 and 118 (1), **pesticide mixture**: **permethrin** + **DEET** (**3**), plastics (BPA + DBP + DEHP) (1), plastics: BPA + DEHP + DBP (1), PBBs (1), pregnant mare's serum gonadotropin + hCG (1), **radiation** (**3**), temperature (1), **vinclozolin** (**5**)
Gastrointestinal	3	3 Different exposures: MNNG (1), stress (1), zinc (1)
Hepatic	18	**17 Different exposures**: 5-aza-2′-deoxycytidine (1), dexamethasone (1), dioxin (1), ENU (1), high fat diet (1), methoxychlor (1), methylating micronutrients (1), methylmercury (1), nonylphenol (1), orthoaminoasotouol (1), PBBs (1), protein-restricted diet (1), **radiation** (**2**), rosiglitazone (1), tributyltin (1), vinclozolin (1), voluntary exercise (1)
Immune	14	12 Different exposures: beta-lactoglobulin (1), dengue-3 virus (1), dioxin (1), ENU (1), high-fat diet (1), Neospora caninum Nc-Spain 7 or Nc-Spain 3H (1), nonlethal heat stress (1), poly(Glu52Lys33Tyr15) (1), radiation (1), stress (1), **vinclozolin** (**2**), **zinc** (**deficiency**) (**2**)
Male reproductive	50	32 Different exposures: atrazine (1), benzo(a)pyrene (1), BPA (1), DDT (1), decitabine (1), **DEHP** (**3**), **dioxin** (**5**), DXR (1), ethanol (1), ethinyl estradiol (1), flutamide (1), gonadotropin (1), **high-fat diet** (**3**), hypoxia (1), **JP-8** (**2**), levonorgestrel + EE2 (1), l-T4 (1), methoxychlor (1), ENU (1), MNNG (1), overnutrition (1), p,p′-DDE (1), PCB congeners 101 and 118 (1), pentylenetetrazole (1), **pesticide mixture**: **permethrin** + **DEET** (**2**), plastics: BPA + DEHP + DBP (1), PBBs (1), protein-restricted diet (1), **radiation** (**4**), tetracycline (1), **vinclozolin** (**13**), vorinostat (1)
Metabolic or glucose-related	24	16 Different exposures: alloxan (1), *Areca catechu* (betel nut) (1), **dexamethasone** (**2**), **high-fat diet** (**8**), high-sugar diet (1),malnourishment (1), ENU (1), obesity (1), overnutrition (1), **protein-restricted diet** (**4**), rosiglitazone (1), tributyltin (1), voluntary exercise (1)
Musculoskeletal	11	10 Different exposures: BPA (1), benzo(a)pyrene (1),dioxin (1), **high fat diet** (**2**), isolation (1), methoxychlor (1), methylating micronutrients (1), ENU (1), vinclozolin (1), voluntary exercise (1)
Mutagenicity	7	9 Different exposures: 4-chloromethyl biphenyl (1), 4-hydroxymethyl biphenyl (1), benzyl chloride (1), diethyl sulfate (1), ethyl methanesulfonate (1), ethyl methanesulfonate (EMS) (1), G418 antibiotic (1), ENU (1), phenytoin (1), radiation (1)
Neurological and sensory	32	27 Different exposures: acetophenone (1), **benz(a)pyrene** (**2**), **BPA** (**2**), chronic stress (1), **cyclophosphamide or cyclophosphamide** + **vinblastine** (**3**), corticosterone (1), DEHP (1), ethanol (1), ENU (1), gonadotropin (1), heat stress (1), heroin (1), immune activation (1), isolation (1), methylazoxymethanol acetate (MAM) (1), methylphenidate (1), **morphine sulfate** (**2**), **NMU** (**3**), poly(I:C) (1), pre-reproductive stress (1), pyrolytic (PY) mixture (1), radiation (1), **stress** (**5**), **vinclozolin** (**4**), testosterone (1), voluntary exercise (1)
Renal	10	10 Different exposures: DDT (1), dioxin (1), flutamide (1), JP-8 (1), methoxychlor (1), permethrin and DEET (1), PBBs (1), protein-restricted diet (1), **vinclozolin** (**2**)
Respiratory	4	4 Different exposures: benz(a)pyrene (1), nicotine bitartrate (1), PBBs (1), zinc (1)

Number of transgenerational studies (n) in experimental animals sorted by broad health outcome categories as well as individual exposure agents. For most health outcome categories, the available studies include a diverse set of exposures, but relatively few exposure agents were used in more than one study. Exposure agents in bold text indicate agents with more than one study within a health outcome category. Although not the focus of this review, some mechanism-only studies (e.g., DNA methylation) were included under the PECO statement under a broad definition of outcome. Studies that only presented mechanistic data that did not fit within a health outcome category are included in HAWC (https://hawcproject.org/assessment/73/).

characterization (53%), and confidence in the outcome assessment/ including blinding of outcome assessors to subjects exposure level (87%) (Fig. 3). Fig. 3A presents data across both exposures, and Fig. 3B and C show the risk of bias for individual studies for Holocaust and radiation exposures, respectively. The high risk of bias ratings for multiple risk of bias questions (including all three key questions), across the majority of studies decreases confidence in the bodies of evidence for reaching conclusions on potential transgenerational health effects. The high risk of bias is not unexpected as challenges with confounding and exposure misclassification are common in environmental health studies. However, complications in controlling for confounding variables and other exposures when tracking health effects over multiple generations may make exposure mischaracterization an even greater problem for investigating potential transgenerational health effects.

A clear majority (58–92%) of animal studies were rated probably high or definitely high risk of bias for all three key questions (randomization of treatment/exposure (92%), confidence in the exposure characterization (58%), and confidence in the outcome assessment related to lack of blinding to study groups (73%)) (Fig. 4). In addition, 85% of studies were rated probably high risk of bias for either failing to control for litter effects or not reporting that the litter was the statistical unit of analysis, a critical issue for developmental studies and potentially more so for tracking health effects over multiple generations. Studies were also rated probably high risk of bias for other issues (e.g.,

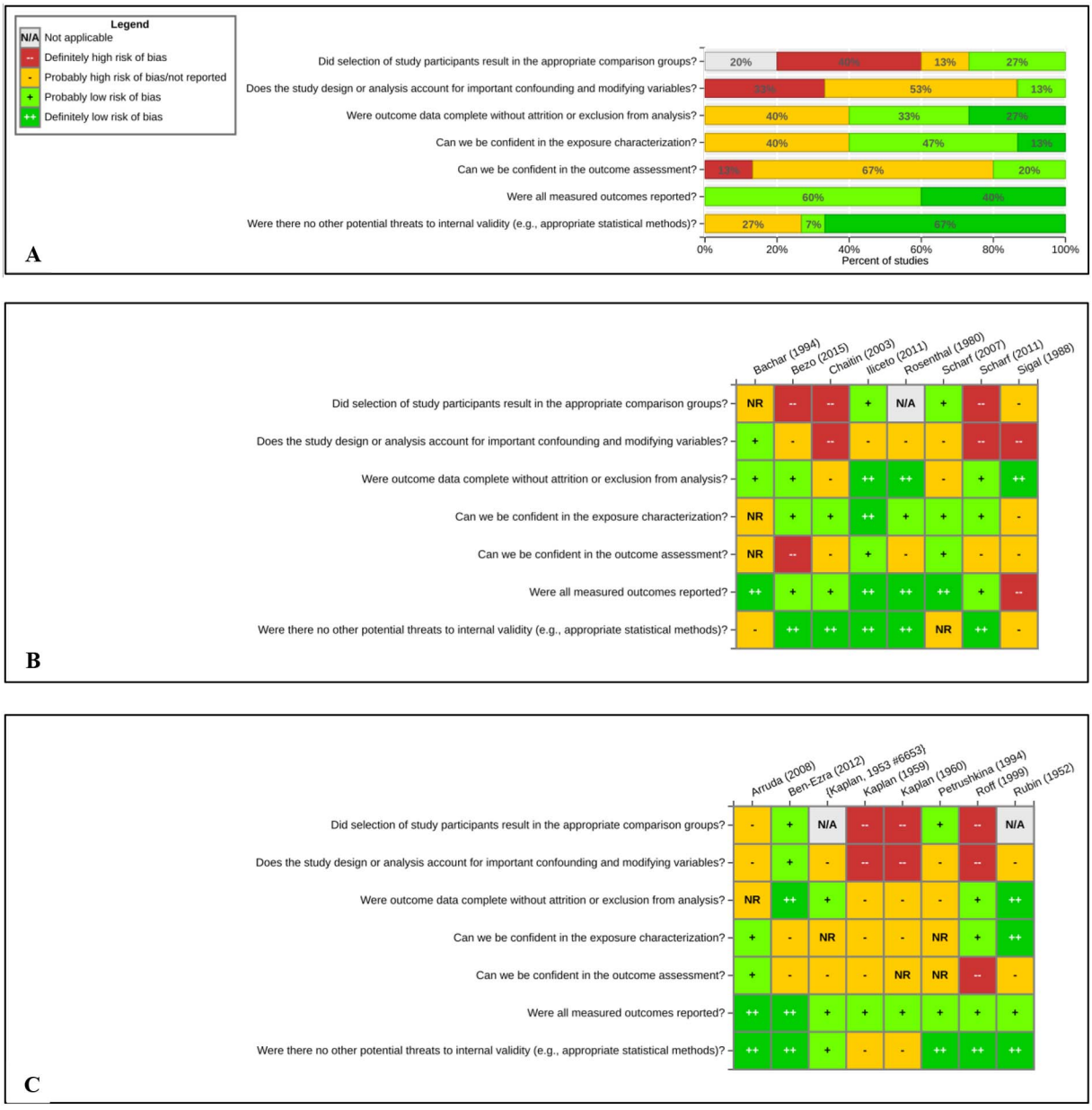

Fig. 3. Risk of bias summary and heatmaps for holocaust and radiation human transgenerational studies.
A) Risk of bias barchart presenting the summary percent ratings for each risk of bias question for the example human transgenerational studies. The Holocaust and radiation exposure studies were used as examples to illustrate internal validity or risk of bias issues for studies of transgenerational design because these exposures were the largest bodies of evidence. B) The risk of bias heatmap of the individual human Holocaust studies. C) The risk of bias heatmap of the individual human radiation studies. *Figure 3A Risk of Bias bar chart*, *Figure 3B Risk of Bias Heat Map of Holocaust Studies* and *Figure 3C Risk of Bias Heat Map of Radiation Studies*.

either not performing or not reporting allocation concealment and blinding of researchers to study groups during the study). High risk of bias ratings, particularly for the key questions, decreases confidence in the body of evidence for reaching conclusions on any health effect based on these transgenerational studies.

It is also worth noting that high risk of bias ratings are common under the current reporting and study design practices in experimental animal studies, despite the increasing uptake of reporting guidelines (e.g., ARRIVE guidelines for animal studies; (Kilkenny et al., 2010)). Although the rating or not reported "NR" is equivalent to probably high risk of bias, it may reflect the quality of the reporting rather than study conduct. For the example subsets, an effort was made to contact study authors for missing information to address specific risk of bias questions and the rating adjusted if information was received. Some of these same risk of bias challenges (e.g., high risk of bias ratings for lack of blinding of outcome assessors to treatment groups or lack of information on whether or not outcome assessors were blind to treatment groups) have been observed when NTP has evaluated other datasets (e.g., Immunotoxicity Associated with Exposure to PFOA or PFOS http://ntp.niehs.nih.gov/go/749926).

3.3. Human studies

There were 46 human studies that fit our PECO criteria for transgenerational inheritance design. These studies were grouped by broad health outcome categories such as within the neurological system to identify potential bodies of evidence on the same or related health effects (Table 2). Many datasets identified in the literature as "transgenerational" (e.g., the Dutch famine studies on diabetes or cardiovascular disease), are not included here because the studies do not include

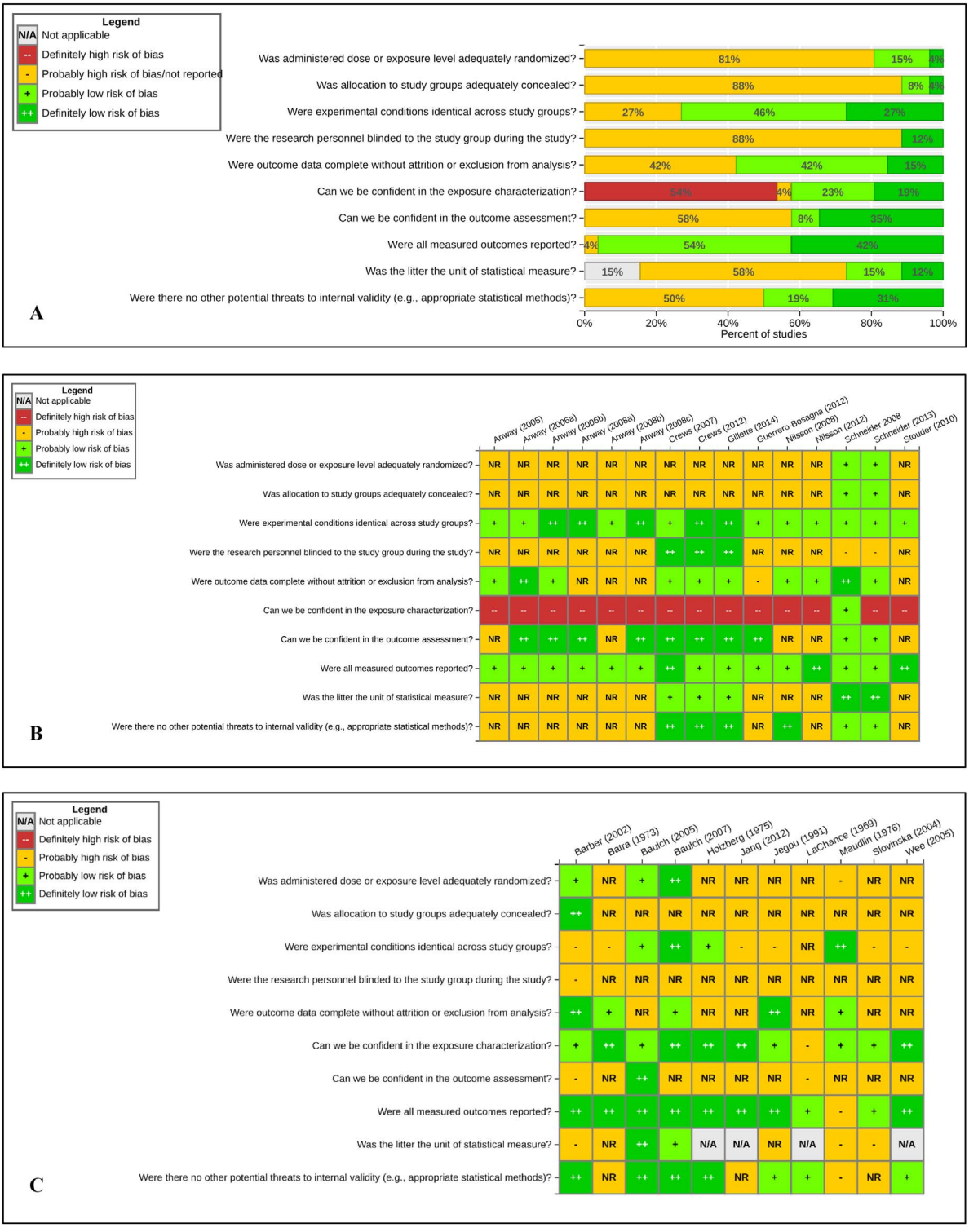

Fig. 4. Risk of bias summary and heatmaps of vinclozolin and radiation animal transgenerational studies.
A) Risk of bias barchart presenting the summary percent ratings for each risk of bias question for the example animal transgenerational studies. The vinclozolin and radiation exposure studies were used as examples to illustrate internal validity or risk of bias issues for studies of transgenerational design because these exposures were the largest bodies of evidence. B) The risk of bias heatmap of the individual studies of animal vinclozolin exposure. C) The risk of bias heatmap of the individual studies of animal radiation exposure. *Figure 2A Risk of Bias Bar Chart of Animal Studies*, *Figure 2B Risk of Bias Heat map of Vinclozolin exposure*, *Figure 2C Risk of Bias heatmap of Radiation studies*.

sufficient number of generations to be considered under our PECO criteria. Although 13 exposures have been investigated in transgenerational studies, only four exposure agents (radiation, food availability, depression, and Holocaust) with more than one publication were identified. Similarly, few health outcomes (reproductive, growth/development, mortality, and neurological/sensory) were identified in more than one study. Data extraction entries for each epidemiological study is available for viewing and downloading in HAWC (https://hawcproject.org/assessment/73/) including bibliographic information, study design details, and results. In addition, an interactive evidence map is available for easy filtering and sorting of the extracted data (access Interactive Evidence Map). Although multiple studies have

examined growth or developmental endpoints, consistency of potential transgenerational effects was not evaluated for this endpoint because there are only single studies of each exposure. The bodies of evidence for potential transgenerational effects from human studies on reproductive, mortality, and neurological/sensory outcomes are summarized briefly below because these exposure agents were reported in multiple studies. Assessment of these datasets included a description of limitations and areas of consistency; and, based on these factors, whether or not the dataset is likely to be sufficient to support a critical evaluation for health effects conclusion in a full systematic review. The human observational studies face a challenging task of reaching conclusions on potential transgenerational effects of exposure across multiple generations and to separate the effects of that exposure from potential confounding from measured variables as well as variables that may be unaccounted for.

3.3.1. Reproductive outcomes

3.3.1.1. Radiation. Five prospective cohort publications investigated women treated with low-dose X-ray therapy for menstrual dysfunction or potential sterility and then followed to determine if treatment resulted in effects in subsequent generations (Kaplan, 1953, 1954, 1959, 1960; Rubin, 1952). There was no evidence of transgenerational reproductive effects of radiation from this body of evidence; however, there are a number of serious limitations in the dataset that make it likely that a critical evaluation of the transgenerational effects of radiation would reach a conclusion of insufficient data to assess potential reproductive effects. The dataset only includes a narrow range of reproductive endpoints, comparison groups were not used in many of the reported analyses, and four of the five studies are from a series of papers on the same population. The authors concluded that low-dose radiation therapy for women does not cause sterility in future generations. Few health outcomes were tracked in these studies and the data are limited to observations of the birth of children or grandchildren and general statements as to "healthy" or "physically well." For example, Kaplan (1959) reported general health observations of 43 grandchildren (physically and mentally well) for a cohort of 644 women who received irradiation for sterility and were followed for periods ranging from 1 to 33 years.

3.3.2. Mortality

3.3.2.1. Food availability. Three studies evaluated mortality risk in grandchildren following food supply effects on grandparents born in 1890, 1905 and 1920 in Overkalix, Sweden, an isolated population with periods of low food availability (Bygren et al., 2014; Kaati et al., 2002; Pembrey et al., 2006). Assessment of the availability of food was based on historical records including harvests and price statistics. The major limitation of this body of evidence to support an evaluation of transgenerational effects of food availability is that the studies are restricted to a single population and there are a large number of parameters (multiple life stages, and multiple outcomes) given the small sample size in the third generation (100–300 individuals). Consequently, even if there were no effects of food availability, analyses would expect to find an effect in 5% of the individuals analyzed (van den Berg and Pinger, 2016). Studies on this population (164 men and 139 women with 1818 children and grandchildren) that reported low food supply (classified as poor, moderate, or good) reported some sex-specific effects on mortality in grandchildren. Pembrey et al. (2006) reported sex-specific effects on mortality, wherein the paternal grandfather's food availability was only linked to the increased relative risk for mortality of grandsons, and the paternal grandmother's food availability was only associated with the granddaughters' relative risk for mortality. Kaati et al. (2002) evaluated the effects of food supply on diabetes-related mortality in grandchildren. The study reported that if the paternal grandfather was exposed to a limited food supply during his slow growth period (i.e., prior to puberty) the grandchild had a tendency to be protected from diabetes as the cause of death; however, if the maternal grandmother had the same exposure the grandchildren were not protected. Using data from the same population, Bygren et al. (2014) reported that drastic changes in the food availability during childhood for paternal grandmothers resulted in increased hazard ratio for cardiovascular mortality in their granddaughters. Effects on cardiovascular mortality were not observed in any other combination of grandchildren and grandparents (Bygren et al., 2014). As with other bodies of evidence from the epidemiological literature, addressing confounding is a principal challenge, and given that the studies are from a single population, there are serious limitations in the dataset to support general conclusions on transgenerational effects of food availability.

3.3.3. Neurological or sensory outcomes

3.3.3.1. Depression. Four studies evaluated neurological and sensory outcomes following a transgenerational exposure of depression experienced by parents and grandparents (Bruder et al., 2007; Cents et al., 2011; Olino et al., 2008; Pettit et al., 2008). Although several of these studies have reported that children are impacted by the mental health condition of grandparents, the body of evidence has serious limitations for supporting a critical evaluation of transgenerational inheritance and it may not be possible to separate depression or behavioral experiences as an exposure to an external condition without distinct start and end dates. Depression and other behavioral disorders have a hereditary component, but children, parents, and grandparents often share environment and experiences (Hancock et al., 2013). Studies reported various behavioral outcomes in grandchildren relating to various disorders (anxiety, mood), abnormalities in alpha symmetry in the brain, and internalizing and externalizing problems. Two studies examined the influence of grandparental depression on internalizing and externalizing problems in grandchildren and report that grandparents with major depressive disorder or anxiety disorders are associated with increased risk of internalizing and externalizing problems in grandchildren (Cents et al., 2011; Olino et al., 2008). Conclusions on potential transgenerational effects of depression would likely have serious challenges addressing confounding and exposure misclassification.

3.3.3.2. Holocaust. Eight studies evaluated neurological and sensory outcomes in grandchildren following "exposure" or experience of the Holocaust by their grandparents (Bachar et al., 1994; Bezo and Maggi, 2015; Chaitin, 2003; Iliceto et al., 2011; Rosenthal and Rosenthal, 1980; Scharf, 2007; Scharf and Mayseless, 2011; Sigal et al., 1988). There is inconsistent evidence across studies for transgenerational effects of the Holocaust on sensory or behavioral outcomes; however, there are serious limitations in the body of evidence due to confounding and heterogeneity of the dataset to support a critical evaluation of behavioral effects in general. Few specific behavioral outcomes were reported in more than one study. In a meta-analysis of the above studies plus additional unpublished data, Sagi-Schwartz et al. (2008) reported there was no evidence for behavioral indicators of trauma in an analysis that combined outcomes to assess the general pattern of behavioral effects (e.g., combining measures of aggression, conduct problems, anxiety, eating problems, and attachment disorders) in third generation Holocaust offspring. Similar to the body of literature for depression discussed previously, exposure to the Holocaust is challenging to evaluate because of genetic or hereditary considerations not associated with the Holocaust, as well as potential confounding effects of socio-economic status, family structure, and potential unaccounted for variables that reflect shared aspects of the environment and experiences between children and the grandparents.

3.4. Experimental animal studies

There were 211 experimental animal studies that fit our PECO

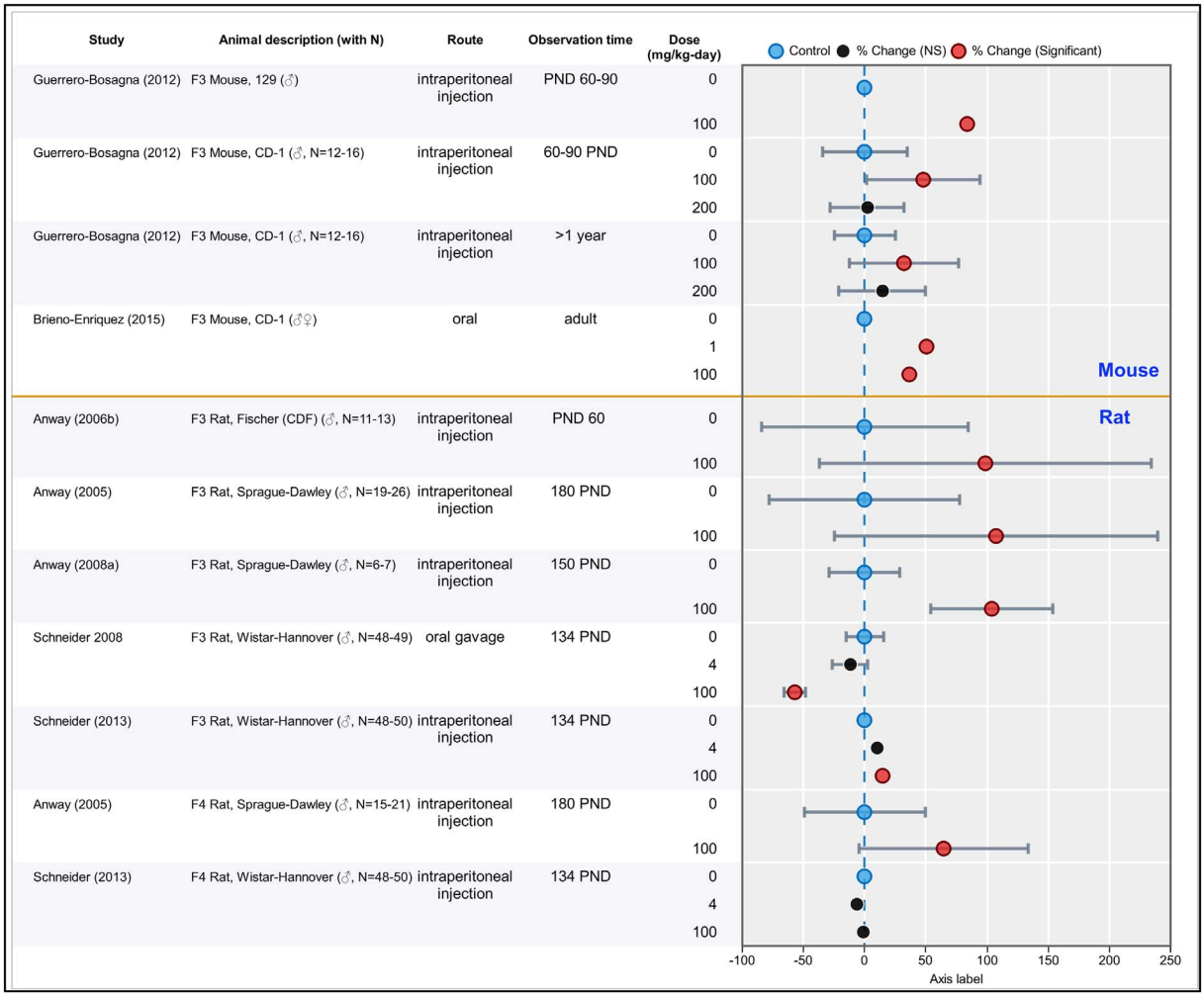

Fig. 5. Apoptosis in the testis following vinclozolin exposure.
Studies are sorted by route of administration, species and generation. The orange line distinguishes species. The results are plotted as % change relative to control (blue circles). Black circles indicate non-significant difference from control and red circles indicate statistically significant differences from control indicated by study authors. The orange line distinguishes species and the dotted blue line indicates zero % change relative to control. Error bars are ± 95% CI and are present in the figure only when standard deviation or standard error and sample size were reported for both treatments and control animals. Link to HAWC figure: *Figure 5. Apoptosis in the testis following Vinclozolin Exposure*.

criteria for transgenerational inheritance design that were grouped by broad health outcome categories (e.g., male reproductive system) in order to identify potential bodies of evidence on the same or related health effects (Table 3). For most health outcomes, the collection of studies was comprised of a diverse set of exposures and relatively few exposure agents were evaluated in more than one publication. Male and female reproductive, growth and developmental, neurological, and metabolic and glucose-related outcomes were the most frequently investigated health outcome categories. We focused our evaluation on the bodies of evidence in these health outcome categories with three or more studies on a given exposure: vinclozolin, radiation, dioxin, cyclophosphamide, jet propellant 8 (JP-8), high-fat diet, protein-restricted diet, diethylhexyl phthalate (DEHP), and a mixture of permethrin + diethyltoluamide (DEET). We examined the consistency of the reported findings and noted limitations in the body of evidence including if publications came from the same research group. Based on these factors, the discussion includes a statement of whether or not the dataset is likely to be sufficient to support a critical evaluation for health effects conclusions in a full systematic review. For bodies of evidence with two publications testing the same exposure [bisphenol A (BPA), dexamethasone, methoxychlor, n-nitrosomethylurea (NMU), zinc, a mixture of BPA+ dibutyl phthalate (DBP) + DEHP, and over nutrition] the results are only discussed if they included common endpoints across studies. Therefore, immune, hepatic, musculoskeletal, and renal health outcome categories are not discussed in detail because the two publications with common exposures did not test the same endpoints. Data for each animal study are available for viewing and downloading from HAWC (https://hawcproject.org/assessment/73/) including bibliographic information, study design details, and results. In addition, an interactive evidence map is available for easy filtering and sorting of the extracted data (access Interactive Evidence Map).

3.4.1. Male reproductive outcomes

Male reproductive outcomes were reported in 50 transgenerational studies evaluating 32 different exposures (Table 3 for list of exposure agents). In this body of evidence, six exposures were reported in multiple studies: vinclozolin (13), radiation (4), dioxin (5), high-fat diet (3), DEHP (3), JP-8 (2), BPA (2) and a mixture of permethrin + DEET (2). The 13 vinclozolin studies were not only the largest body of evidence, but these studies also presented the most commonly reported evidence for transgenerational effects on a male reproductive endpoint: vinclozolin-associated changes in apoptosis of the germ cells within the testis.

3.4.1.1. Vinclozolin. Thirteen transgenerational studies of the fungicide vinclozolin in rodents were identified (11 studies in rats and two in mice) making this the largest body of evidence reporting data on male

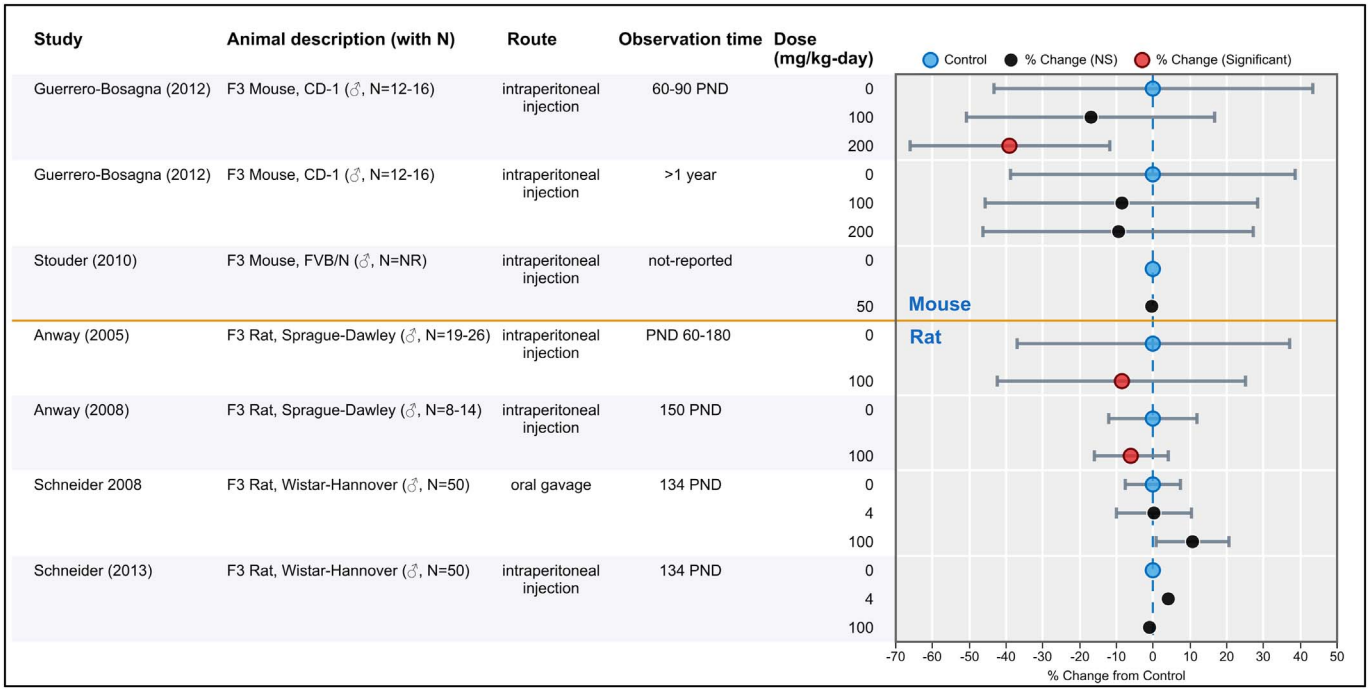

Fig. 6. Sperm concentration following vinclozolin exposure.
Studies are sorted by species and generation. The results are plotted as % change relative to control (blue circles). Black circles indicate non-significant difference from control and red circles indicate statistically significant differences from control indicated by study authors. The orange line distinguishes species and the dotted blue line indicates zero % change relative to control. Error bars are ± 95% CI and are present in the figure only when standard deviation or standard error and sample size were reported for both treatments and control animals. Link to HAWC figure: *Figure 6. Sperm Concentration Following Vinclozolin Exposure*.

reproductive outcomes. These studies were conducted in two independent labs that evaluated a wide range of endpoints, including sperm parameters, reproductive organ weights, prostate disease, fertility, and histology of the reproductive tissues. Few endpoints were examined across multiple studies that would allow an assessment of consistency or robustness of the findings. The 11 rat studies included a common dose of vinclozolin and prenatal exposure (100 mg/kg/day to pregnant dams of the F_0 generation), with 10 studies using an intraperitoneal (IP) injection and a single study using oral gavage. Both mouse studies used IP injection. Internal dose metrics such as blood or tissue levels of vinclozolin were not reported for either route of exposure thus it is not possible to evaluate whether the different results reported are due to variability in internal dose or study design parameters. The most consistently reported finding for reproductive effects of vinclozolin is for changes in apoptosis of the germ cells within the testis. There is also inconsistent evidence of altered sperm parameters. No vinclozolin-related effects were reported for prostate disease, fertility, or histological endpoints. Germ cell apoptosis and sperm parameters are discussed further below; however, there are serious limitations in the dataset for drawing conclusions from this body of evidence because of wide confidence intervals for most endpoints, inconsistency in the results, and risk of bias issues such as failure to consider the litter as the statistical unit of analysis (see Study Quality and Risk of Bias section above for further discussion of risk of bias for transgenerational studies).

3.4.1.1.1. Apoptosis of the germ cells in the testis. Eight studies evaluated apoptosis of the germ cells in the testis following transgenerational inheritance evaluation of vinclozolin exposure: six rat studies (Anway et al., 2005; Anway et al., 2006; Anway et al., 2008; Anway and Skinner, 2008; Schneider et al., 2008; Schneider et al., 2013) and two mouse studies (Brieno-Enriquez et al., 2015; Guerrero-Bosagna et al., 2012). Vinclozolin exposure of mice and rats to pregnant dams via IP injection, resulted in a significant increase in apoptotic germ cells in the testis of animals from the F_3 or F_4 generation. Relative increases in apoptosis across studies ranged from 5 to 105% compared to the control response with much of the data having wide confidence intervals (Fig. 5). In contrast to the IP studies, Schneider et al. (2008) reported a 5–65% decrease of apoptotic germ cells in F_3 Wistar-Hannover rats after administration of vinclozolin by oral gavage to pregnant dams (F_0) (Fig. 5). Although the body of evidence suggests that developmental exposure to vinclozolin may have transgenerational inheritance effects on testicular germ cell apoptosis, there are serious limitations in the dataset to support a critical evaluation of effects of vinclozolin on apoptosis. Even where the study authors indicated significant changes in apoptosis, confidence intervals for almost all endpoints overlap with the null hypothesis. Four of the six studies result in probably high risk of bias for failure to use the litter as the statistical unit of analysis and for lack of randomization of animals into treatment groups (reflecting that this was either not performed or not reported). Therefore, the probably high risk of bias ratings for these key questions would present serious limitations in the dataset to support a critical evaluation of effects (see Study Quality and Risk of Bias section). The two Schneider et al. (2008, 2013) present some of the best designed and reported studies in terms of bias from the risk of bias subset (e.g., Schneider et al. (2008) was rated probably low or definitely low on the key risk of bias questions as well as almost all others). It is interesting to note that Schneider et al. (2013) study reported a significant increase in apoptosis in F_3 rats following IP exposure to the F_0 pregnant dams, but the effect size was much smaller than the studies with more risk of bias issues. The potential effect of risk of bias on effect size as well the effect of risk of bias, imprecision and other factors that may impact confidence in the body of evidence could be examined in detail in a full systematic review of the evidence for transgenerational effects of vinclozolin on male reproductive outcomes.

3.4.1.1.2. Sperm count and motility. Six studies evaluated sperm count or motility following transgenerational vinclozolin exposure: four rat studies (Anway et al., 2005; Anway et al., 2008; Schneider et al., 2008; Schneider et al., 2013) and two mouse studies (Guerrero-Bosagna et al., 2012; Stouder and Paoloni-Giacobino, 2010). The evidence for transgenerational effects of vinclozolin on sperm production and

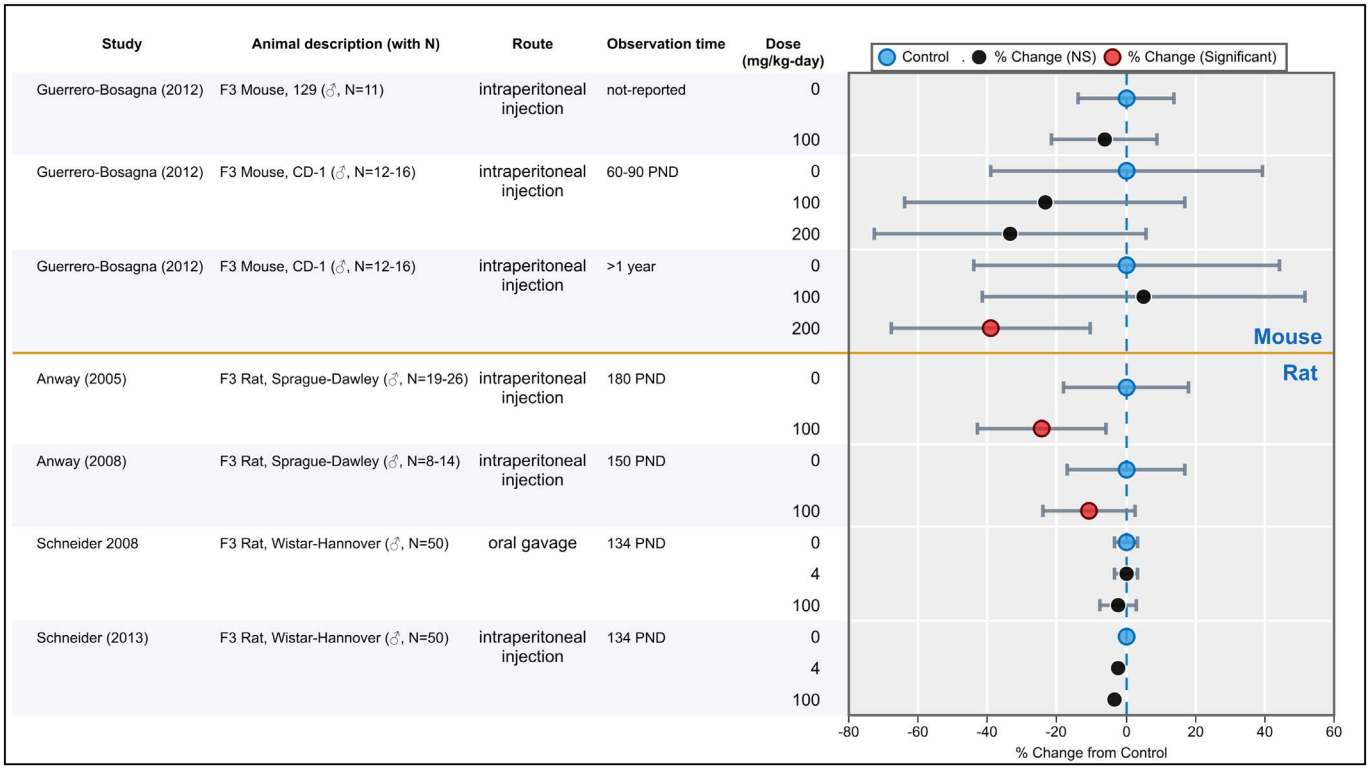

Fig. 7. Sperm motility following vinclozolin exposure.
Studies are sorted by species and generation. The results are plotted as % change relative to control (blue circles). Black circles indicate non-significant difference from control and red circles indicate statistically significant differences from control indicated by study authors. The orange line distinguishes species and the dotted blue line indicates zero % change relative to control. Error bars are ± 95% CI and are present in the figure only when standard deviation or standard error and sample size were reported for both treatments and control animals. Link to HAWC figure: *Figure 7. Sperm Motility Following Vinclozolin Exposure*.

motility is inconsistent, with the majority of the results indicating no effect and the confidence intervals for almost all of the results overlapping with the null. The two rat studies by Anway et al. (2005, 2008) reported reduced sperm concentrations (8–22%) and motility (11–25%) compared to concurrent controls in F_3 and F_4 Sprague Dawley rats after 100 mg/kg/day of vinclozolin was administered by IP injection to pregnant dams (F_0) (Figs. 6 and 7). In contrast, two studies by Schneider et al. (2008, 2013) reported no effect of vinclozolin at the same dose in Wistar-Hannover rats following either IP injection or oral gavage. The results in mice were also inconsistent with most results indicating no effect of vinclozolin at doses of 50, 100, and 200 mg/kg measured at PND 60–90 or over 1 year of age (the specific observation time was not reported in Stouder and Paoloni-Giacobino, 2010). However, a third mouse study reported that vinclozolin decreased sperm concentration at a single dose (200 mg/kg) and wide observation age ranging from PND 60–90 (Guerrero-Bosagna et al., 2012; Stouder and Paoloni-Giacobino, 2010). This body of evidence is from an overlapping set of studies to those discussed for apoptosis above and consideration of all the issues that impact confidence in the body of evidence could be examined in detail in a full systematic review of the evidence. The serious limitations in the dataset are similar to the apoptosis dataset to support a critical evaluation of effects of vinclozolin on sperm parameters. Four of the six studies result in probably high risk of bias for failure to use the litter as the statistical unit of analysis and lack of randomization of animals into treatment groups (reflecting that this was either not performed or not reported); and the two studies that did (Schneider et al. (2008, 2013)), reported no effect of vinclozolin.

3.4.1.1.3. Relative organ weight of testes, prostate and seminal vesicle. Six studies evaluated relative reproductive organ weights following transgenerational vinclozolin exposure: including five rat studies (Anway et al., 2006; Anway et al., 2008; Guerrero-Bosagna et al., 2012; Schneider et al., 2008; Schneider et al., 2013) and one mouse study (Guerrero-Bosagna et al., 2012). These studies evaluated absolute and relative weights of the prostate, epididymis, testes, and seminal vesicles in animals from the F_3 and F_4 generations following exposure of pregnant dams to vinclozolin (Supplemental Fig. S1). Although there are serious limitations in the dataset to support a critical evaluation of effects of vinclozolin as discussed above for other endpoints, exposure was not associated with changes in relative weight of reproductive organs across multiple studies.

3.4.1.2. Radiation. Four studies reported data on male reproductive outcomes in the F_2 generation across multiple animal models (e.g., rodents and insects) following non-gestational radiation exposure in the F_0 generation (Batra et al., 1973; Baulch et al., 2007; Jegou et al., 1991; LaChance and Degrugillier, 1969). This is a small heterogeneous dataset and the studies reported no transgenerational effects of radiation on most endpoints. The endpoints included relative organ weight, chromosomal abnormalities, fertility and sperm parameters; however, only testis and epididymis weight were evaluated across multiple studies (Supplemental Fig. S2). Radiation exposure was not associated with changes in reproductive organ weight across the two studies that evaluated these endpoints. Jegou et al. (1991) reported greater postimplantion loss in female rats mated to F_2 male offspring of radiation exposed males and Baulch et al. (2007) reported altered sperm parameters in F_3 generation 129SvEv mice. The heterogeneity in the endpoints examined and limited number of studies (maximum of two with sample sizes of 5–8 animals) are very serious limitations in the dataset that make it likely that a critical evaluation of the body of evidence would reach a conclusion of insufficient data to evaluate potential transgenerational effects of radiation on any male reproductive outcomes. As discussed for other exposures, lack of randomization and blinding of the outcome assessors also present

limitations for reaching conclusions on the body of evidence(see Study Quality and Risk of Bias section).

3.4.1.3. Dioxin. Five transgenerational studies reported data on male reproductive outcomes in rodents from the F_3 generation following gestational dioxin exposure of the F_0 dam (Baker et al., 2014; Bruner-Tran et al., 2014; Manikkam et al., 2012; Manikkam et al., 2012b; Sanabria et al., 2016). Transgenerational exposure to dioxin had no effect on most reproductive endpoints measured in F_3 males including sperm concentration and motility, apoptosis of germ cells in the testes, prostate and testis disease, luteinizing hormone concentration, reproductive organ weights, and puberty-related outcomes. A single study reported a dioxin-associated reduction in morphologically normal sperm compared to controls following transgenerational exposure to dioxin (10 μg/kg), tail defects were the most common abnormality reported in the F_3 generation (Bruner-Tran et al., 2014). Two studies from the same research group reported dioxin-associated changes in testosterone concentrations with opposite effects in animals of different ages (i.e., decreased testosterone at 3–4 months and increased at 12 months of age following transgenerational exposure to 100 ng/kg-day dioxin (Manikkam et al., 2012; Manikkam et al., 2012b)) (Supplemental Fig. S3). The heterogeneity in endpoints evaluated, the heterogeneity in the results (increase vs. decrease), as well as the limited number of studies are serious limitations in the dataset that make it likely that a critical evaluation of transgenerational effects of dioxin would reach a conclusion of insufficient data to assess male reproductive effects. As discussed for other exposures, risk of bias issues would likely also present limitations for dioxin; consideration of the key risk of bias questions for the dioxin studies found that most of the studies failed to report or did not use the litter as the statistical unit of analysis.

3.4.1.4. Diethylhexyl phthalate (DEHP). Three transgenerational studies were identified that reported data on male reproductive outcomes following exposure to DEHP (Doyle et al., 2013; Chen et al., 2015; Quinnies et al., 2015). Transgenerational exposure to DEHP had no effect on most reproductive endpoints measured in F_3 rodents including most sperm parameters and reproductive organ weights. Reported effects on anogenital distance and sperm count were inconsistent between the rat and mouse studies. Chen et al. (2015) reported that exposure of pregnant Sprague-Dawley dams to 750 mg/kg-day DEHP on GD 7–19 decreased anogenital distance in F_3 male offspring at PND 30 while Quinnies et al. (2015) reported no effects of doses up to 200 mg/kg DEHP on GD 7–14 on anogenital distance in F_3 C57Bl6 male mice at PND 38. Doyle et al. (2013) reported that exposure of pregnant CD-1 mice to 500 mg/kg DEHP from GD 7 to 14 resulted in decreased sperm count in F_3 and F_4 offspring on PND 120, whereas there was no effect of 750 mg/kg-day DEHP on sperm counts in F_3 or F_4 Sprague-Dawley rats on PND 80 (Chen et al., 2015). The heterogeneity in the endpoints examined, differences in animal models evaluated, and limited number of studies are serious limitations in the dataset that make it likely that a critical evaluation of transgenerational effects of DEHP would reach a conclusion of insufficient data to assess male reproductive effects. Similar to other exposures, consideration of the key risk of bias questions documented limitations for DEHP as two of the three studies failed to use the litter as the unit of analysis or randomize animals to treatment groups(reflecting that these factors were either not performed or not reported).

3.4.1.5. High-fat diet. Three transgenerational studies were identified that reported data on male reproductive outcomes following exposure to a high-fat diet (Chambers et al., 2016; de Castro Barbosa et al., 2016; Fullston et al., 2012). Generally, there were no effects of exposure by maternal or paternal grandparents (F_0) to high-fat diet on reproductive endpoints in F_2 animals including relative organ weight, sperm parameters, anogenital distance, and luteinizing hormone concentrations; however, only reproductive organ weights and testosterone were evaluated across multiple studies. Fullston et al. (2012) reported decreased testosterone concentrations in F_2 male C57Bl6 mice at 17 weeks of age with the maternal grandparent exposed to high-fat diet, and no effect from the paternal grandparent. Chambers et al. (2016) reported no effects of high-fat diet exposure to either maternal or paternal grandparents on testosterone in F_2 male Sprague-Dawley rats at 19 weeks of age. Although there was no effect on most endpoints examined, the limited number of studies and the heterogeneity in the endpoints examined are serious limitations in the dataset that make it likely that a critical evaluation of effects of high-fat diet would reach a conclusion of insufficient evidence to assess male reproductive effects in general. In addition, risk of bias issues would likely also present limitations for high-fat diet; consideration of the key risk of bias questions found that two of the three studies failed to use the litter as the unit of analysis (reflecting that this factor was either not performed or not reported).

3.4.1.6. Jet propellant 8 (JP-8), bisphenol A (BPA), and permethrin + DEET mixture. Two transgenerational studies examined male reproductive endpoints after exposure to either JP-8 (Manikkam et al., 2012; Tracey et al., 2013), BPA (Salian et al., 2009a, 2009b), or a mixture of permethrin + DEET (Manikkam et al., 2012; Manikkam et al., 2012c). There is a limited ability to draw conclusions for these chemicals given that the datasets were restricted to two studies that had few or no overlapping endpoints. The BPA and permethrin + DEET studies did not share any endpoints in common and reported no evidence of effects on most male reproductive endpoints. The two JP-8 studies were performed by the same research group with both studies suggesting increased apoptosis of germ cells in the testes of F_3 offspring (only one set of results was statistically significant) (Supplemental Fig. S4). No JP-8-related effects were observed on reproductive organ weights, prostate disease or puberty-related outcomes. Although most of the results are negative, the heterogeneity in endpoints evaluated along with the limited number of studies are serious limitations in the dataset to support a critical evaluation of effects of JP-8, BPA, or the mixture of permethrin + DEET on any male reproductive effects. Consideration of key risk of bias questions found that all of the studies failed to use the litter as the statistical unit of analysis (reflecting that this factors was either not performed or not reported).

3.5. Female reproductive outcomes

Female reproductive endpoints were reported in 55 transgenerational studies evaluating 35 different exposures (Table 3 for list of exposure agents). Only five exposures were evaluated in multiple studies: vinclozolin (8), dioxin (6), JP-8 (3), a mixture of permethrin + DEET (3), and radiation (3). In a study of multiple chemicals, Nilsson et al. (2012) reported treatment-related increases (200–400%) in the number of ovarian cysts and decreases (20–40%) in follicle count in F_3 generation rats for the four chemical exposures listed above (dioxin, vinclozolin, JP-8, permethrin + DEET) as well as a "plastics" mixture of BPA, DBP, and DEHP. This group of researchers has also reported the same effects for DDT and methoxychlor (Manikkam et al., 2014; Skinner et al., 2013). Although, there are only 2 or 3 studies of these endpoints for each chemical, the data suggest that follicle number and ovarian cysts may be useful endpoints to test for potential transgenerational effects. The body of evidence for individual chemicals are discussed in separate sections below; however, looking across exposures there are serious limitations in the data to support a critical evaluation of potential transgenerational effects on follicle count and ovarian cysts. The principal limitations for most studies are in study design, conduct, and reporting that would result in serious risk of bias issues including failure to report the litter as the unit of statistical measure, and lack of reporting randomization of animals into treatment groups which results in probably high risk of bias (see Study Quality and Risk of Bias section). Further robust study (including standardization of methods such as age or timing of observations) of follicle number and

ovarian cysts after transgenerational exposure in rodents to these and other chemicals would increase the utility of these endpoints for drawing conclusions from these bodies of evidence as well as the confidence that these two effects may be useful endpoints to test for potential transgenerational effects of chemical exposure.

3.5.1. Vinclozolin

Seven transgenerational studies reporting data on female reproductive outcomes of the fungicide vinclozolin in rodents were identified from two groups of researchers (Anway et al., 2006; Gillette et al., 2014; Guerrero-Bosagna et al., 2012; Nilsson et al., 2012; Nilsson et al., 2008; Schneider et al., 2008; Schneider et al., 2013). Vinclozolin (100 to 500 mg/kg/day) was associated with decreased follicle counts and increased ovarian cysts in F_3 female rodents in two studies from the same research group (Supplemental Fig. S5). Other than follicle counts and ovarian cysts, the data are largely negative for female reproductive toxicity across the body of evidence. No vinclozolin-related effects were reported in the F_3 generation on fertility, litter size, sex ratio or reproductive organ weights (Schneider et al., 2008; Schneider et al., 2013). Although seven studies were located, the heterogeneity of endpoints evaluated across the different studies limits the utility of a critical evaluation of this body of evidence on female reproductive outcomes following vinclozolin exposure and would likely reach a conclusion of insufficient data to assess outcomes. Specifically, few endpoints were assessed in more than one study (e.g., maximum of two studies assessed fertility or ovarian cysts), there were small sample sizes (n = 7–9), and studies had clear risk of bias issues (see Study Quality and Risk of Bias section). For example, five of the seven studies result in probably high risk of bias for failure to use the litter as the statistical unit of analysis and for lack of randomization of animals into treatment groups (reflecting that this was either not performed or not reported) [including both studies reporting on follicle counts and ovarian cysts (Guerrero-Bosagna et al., 2012; Nilsson et al., 2012)].

3.5.2. Dioxin

Six transgenerational studies were identified that reported data on female reproductive outcomes following dioxin exposure (Baker et al., 2014; Bruner-Tran and Osteen, 2011; Bruner-Tran et al., 2016; Manikkam et al., 2012; Manikkam et al., 2012b; Nilsson et al., 2012). Dioxin (0.001 mg/kg/day) was associated with decreased primordial follicles number (25–37%) and increased number of small cysts in F_3 female rodents in several studies from the same research group (Manikkam et al., 2012; Manikkam et al., 2012b; Nilsson et al., 2012) (Supplemental Fig. S6). Parental exposure (F_0) to dioxin was associated with early onset of puberty in female F_3 rats in two of these studies (Manikkam et al., 2012; Manikkam et al., 2012b). In a single study evaluating adenomyosis, an endometriosis-like uterine phenotype, the microvessel density within the uterus was significantly higher in F_3 offspring of dioxin-exposed (10 µg/kg by oral gavage) F_0 mice compared with F_3control animals (Bruner-Tran et al., 2016). No effects were observed in the rodent studies on other endpoints such as fertility, sex ratio, tumor development, or reproductive organ weights. In zebrafish, dioxin exposure (50 pg/ml) was associated with a significant increase in the % of female offspring, while no effects were reported on the number of ovarian follicles or eggs released per female (Baker et al., 2014). There are serious limitations in the body of evidence that make it likely that a critical evaluation of the effects of dioxin would reach a conclusion of insufficient data to assess female reproductive effects including heterogeneity in endpoints across studies, heterogeneity in the measurement and analysis of endpoints (that make it difficult to compare results across studies), lack of information on the age of animals when observations were taken (to facilitate comparisons across studies), and most of these reports are from the same group of researchers. In addition, consideration of key risk of bias questions showed that five of the six studies failed to consider the litter as the statistical unit of analysis (reflecting that this factors was either not performed or not reported).

3.5.3. Permethrin + DEET mixture

Female reproductive outcomes were reported in three studies from the same laboratory following transgenerational exposure to a pesticide mixture of permethrin and DEET (Manikkam et al., 2012; Manikkam et al., 2012c; Nilsson et al., 2012). Permethrin + DEET (190 mg/kg/day) was associated with decreased follicle counts and increased ovarian cysts in F_3 female rodents in all three studies. These studies also reported inconsistent effects on pubertal abnormalities (or early onset of puberty) with no effects observed in some studies, an increase in the incidence of pubertal abnormalities reported in others and no effects on fertility or ovarian weight. One of the Manikkam et al. (2012) studies reported decreased uterine weight in F_3 animals at 12 months of age. For general reproductive effects, heterogeneity of endpoints across studies, differences in the measurement and analysis of the endpoints, and failure (reflecting that this factor was either not performed or not reported) to consider the litter as the statistical unit of analysis limit the utility of a critical evaluation of the effects of this mixture, which would likely reach a conclusion that there are insufficient data to assess effects.

3.5.4. Jet propellant 8 (JP-8)

Three JP-8 transgenerational studies were identified from the same research group with data on female reproductive outcomes (Manikkam et al., 2012; Nilsson et al., 2012; Tracey et al., 2013). JP-8 (500 mg/kg/day) was associated with decreased follicle counts and increased ovarian cysts in F_3 female rodents in all three studies (Supplemental Fig. S7). Other than follicle counts and ovarian cysts, these studies also reported inconsistent effects on puberty as one study reported no effect while another indicates an increase in the incidence of pubertal abnormalities. These inconsistencies may be a result of the outcome assessment methods or the analysis and presentation of the data (e.g., the same research group presents the data as “pubertal abnormalities” in one study and “onset of puberty” in another). There were no effects on fertility or reproductive organ weights reported. The heterogeneity in the endpoints examined across studies, the lack of standardization of the analysis and reporting (i.e., age at observation), and failure (reflecting that this factor was either not performed or not reported) to use the litter as the statistical unit of analysis are serious limitations in the dataset that make it likely that a critical evaluation of effects of JP-8 would reach a conclusion of insufficient data to assess female reproductive effects.

3.5.5. Radiation

Three studies were identified evaluating transgenerational radiation exposure and female reproductive outcomes, two studies in moths (Jang et al., 2012; Wee et al., 2005) and a single study in Daphnia (Sarapultseva and Dubrova, 2016). The two moth studies exposed F_0 males to 100–300 Gy radiation and tested female fecundity and fertility in the F_2 and F_3 generations (Jang et al., 2012; Wee et al., 2005). These studies investigated inherited sterility as a strategy for management and reported that radiation exposure resulted in a dose-dependent reduction in female fertility (egg hatching) in both generations and decreased fecundity (eggs per female) in the F_2 females (42% reduction at highest dose), but not in the F_3 females. A single study evaluating increasing amounts of ionizing radiation to small planktonic crustacean, *Daphnia magna*, reported effects on fertility at the highest doses. Transgenerational exposure to 10,000 mGy of ionizing radiation resulted in a 14% reduction in the mean brood size and 17% reduction in the mean number of progeny per daphnia in the F_2 females (Sarapultseva and Dubrova, 2016). Radiation exposure was associated with transgenerational inheritance effects (decreased female fertility) in two arthropod species in multiple studies supported by a dose-dependent response in

moths. Other than having a limited number of studies, the principal limitation in the data for drawing conclusions from this body of evidence are from serious risk of bias issues that are evident by examining key risk of bias questions including failure to randomize treatment or blind the outcome assessors.

3.5.6. BPA, benzo(a)pyrene, and high-fat diet

Two transgenerational studies evaluated female reproductive effects following exposure to BPA (Ziv-Gal et al., 2015; Berger et al., 2016), benzo(a)pyrene (Ziv-Gal et al., 2015; Berger et al., 2016), or a high-fat diet (de Assis et al., 2012; Fullston et al., 2012). There is a limited ability to draw conclusions for BPA given that the dataset was restricted to two studies with no overlapping endpoints. Authors reported BPA related effects on delayed age at vaginal opening and age at first estrus (50 μg/kg/day BPA) in F_3 females with no effects on fertility or gestational indices, follicle counts (PND 21), estradiol levels (PND 4 or 21), or germ cells remaining in nests (PND 4) (Berger et al., 2016). The heterogeneity in endpoints across studies, the limited number of studies, and failure to consider the litter as the statistical unit of analysis (reflecting that this factor was either not performed or not reported) limits the utility of a critical evaluation of this body of evidence on effects of BPA on female reproductive outcomes.

3.6. Non-reproductive endocrine outcomes

Non-reproductive endocrine outcomes were investigated in 16 transgenerational studies evaluating 15 different exposures (Table 3 for list of exposure agents). Only vinclozolin (2) was evaluated in multiple studies; and as discussed below, the results are inconsistent across several stress-related endpoints.

3.6.1. Vinclozolin

Two studies from the same research group reported inconsistent results on non-reproductive endocrine outcomes (e.g., stress-related measures including adrenal organ weights and corticosterone concentrations) in rats after transgenerational exposure to vinclozolin (Crews et al., 2012; Gillette et al., 2014). Gillette et al. (2014) reported that exposure of pregnant rats to 100 mg/kg (GD 8–14) resulted in no change to circulating concentrations of corticosterone levels in F_3 male offspring under restraint stress at PND 124 and a reduction in adrenal weights in F_3 males (−8%) and females (−10%); however, Crews et al. (2012) reported an increase in corticosterone levels in F_3 males under restraint stress. The small sample size (n = 5–8 animals) for both studies from the same research group, heterogeneity in endpoints, inconsistency in results, and failure to randomize animals to treatment group make it likely that a critical evaluation of the effects of vinclozolin would reach a conclusion of insufficient data to assess stress-related effects or on non-reproductive endocrine outcomes.

3.7. Metabolic or glucose-related outcomes

Metabolic outcomes associated with diabetes and obesity were investigated in 25 transgenerational studies evaluating 16 different exposures (Table 3 for list of exposure agents). Three exposures were evaluated in multiple studies: high-fat diet (8), protein-restricted protein diet (4), and dexamethasone (2). As discussed further below, these are small datasets and most of the results are inconsistent. Replication and further study of metabolic outcomes (particularly the consistent use of preferred outcome measures such as fasting insulin or glucose levels and the glucose tolerance test) for high-fat diet and other exposures would increase the utility of the data for drawing conclusions from these bodies of evidence as well as the confidence for potential transgenerational effects on metabolic outcomes in general. Some studies have reported sex-specific effects on metabolic outcomes depending both on the sex of the offspring and the sex of the exposed generation. Therefore, studies should be designed to allow for the evaluation of potential effects based on the grand-parental lineage of exposure (maternal or paternal or both exposed).

3.7.1. High-fat diet

Eight transgenerational studies evaluating a high-fat diet in rodents were identified (5 studies in mice, two studies in rats and single study in drosophila). These studies were conducted in multiple labs and evaluated a wide range of endpoints that reflect metabolic status and function of the offspring including body weight, adiposity or fat mass, pancreatic measures (e.g., islet volume and diameter), basal glucose and insulin concentrations, insulin and glucose during a glucose tolerance test, insulin during an insulin tolerance test, leptin, and lipid profiles (cholesterol and triglycerides levels). Across this dataset, there are multiple reports of transgenerational effects; however, there is a limited utility of a critical evaluation of the body of evidence, which would likely reach a conclusion of insufficient data to assess potential effects for this exposure given the diversity of study designs, species, and endpoints tested. The range of high-fat diets consisted of 21%–62% fat compared to control diets ranging from 6%–13.5% fat. Five studies evaluated effects in mice; four of these studies used C57Bl6 or ICR mice (Dunn and Bale, 2011; Fullston et al., 2013; Masuyama et al., 2016; Saben et al., 2016; Steffensen, 2016). In rats, two studies evaluated effects using the Sprague Dawley rat model (Chambers et al., 2016; de Castro Barbosa et al., 2016). A single study evaluated effects of a high-fat diet in drosophila (Dew-Budd et al., 2016).

Glucose was the most widely reported metabolic endpoint, and five studies evaluated either basal glucose or glucose tolerance in offspring of F_0 rodents fed a high-fat diet; three studies in mice and two studies in rats (Chambers et al., 2016; de Castro Barbosa et al., 2016; Dunn and Bale, 2011; Fullston et al., 2013; Steffensen, 2016). Three of the four studies which evaluated glucose tolerance reported a statistically significant effect; although the effects were not consistent in direction or lineage. In mice, Dunn and Bale (2011) reported that F_3 male mice from both grandparental lineages exposed to a high-fat diet exhibited improved glucose tolerance; demonstrated by a reduced rise in plasma glucose and more rapid return to base line levels relative to controls. In contrast, Fullston et al. (2013) reported in C57Bl6 mice that F_2 males (but not females) whose maternal grandfather consumed a high-fat diet had impaired glucose tolerance at 8 and 14 weeks (increased 57% and 29% respectively with glucose challenge); however, this effect was not reported via the paternal grandfather (Fullston et al., 2013). Similar results were reported in Sprague-Dawley rats, where F_2 males (but not females) whose paternal grandfather consumed a high-fat diet had impaired glucose tolerance (de Castro Barbosa et al., 2016) whereas, Chambers et al. (2016) reported no effect on glucose tolerance in Sprague-Dawley rats under a very similar study design. Both mouse studies that evaluated basal glucose levels, reported no effect of developmental exposure of F_0 dams to high-fat diet on glucose in F_3 mice (Saben et al., 2016; Steffensen, 2016).

Two of the three studies that reported adiposity data reported effects. Fullston et al. (2013) found increased adiposity in F_2 male C57Bl6 offspring by the maternal exposure line. Chambers et al. (2016) reported increased adiposity and leptin concentrations in F_2 male Sprague-Dawley rats of maternal grandfathers exposed to a high-fat diet only (Chambers et al., 2016). In contrast, de Castro Barbosa et al. (2016) reported no effects on brown or white adipose tissue in F_2 male Sprague Dawley rats under a similar study design. The heterogeneity in endpoints evaluated along with the limited number of studies and failure to randomize animals to exposure groups are the principal limitations in the dataset to support a critical evaluation of effects of high-fat diet on metabolic outcomes. There is apparent heterogeneity in response (e.g., increase vs. decrease in glucose tolerance) that may be explained by sex differences or lineage of exposure with the most consistent effects associated with exposure from the maternal grandfather; however, there are not enough studies for a detailed evaluation.

3.7.2. Protein-restricted diet

Four transgenerational studies examined metabolic or glucose-related outcomes in rodents after F_0 females consumed a protein-restricted diet (Benyshek et al., 2006; Frantz et al., 2011; Harrison and Langley-Evans, 2009; Hoile et al., 2011). The endpoints included pancreatic measures (e.g., islet volume and diameter), basal cholesterol, triglycerides, glucose, insulin, as well as insulin and glucose concentrations during a glucose tolerance test. Although glucose and insulin were evaluated in multiple studies, only one study (Benyshek et al., 2006) used the glucose tolerance test. Protein-restricted diet did not affect basal glucose in F_3 male Swiss mice at birth or PND 21 (Frantz et al., 2011) or F_3 Wistar rats of either sex at 10 weeks of age (Harrison and Langley-Evans, 2009); however, basal glucose was decreased in female F_3 Wistar rats at 10 weeks of age (Hoile et al., 2011). In addition, Benyshek et al. (2006) reported increased glucose in fasted male F_3 Sprague-Dawley rats and decreased glucose after 30 min of a glucose challenge (no effect in female offspring). Insulin was evaluated in F_3 offspring in two studies after F_0 females consumed a protein restricted diet. Frantz et al. (2011) reported decreased basal insulin in male F_3 Swiss mice on PND 21 and Benyshek et al. (2006) reported increased insulin in fasted F_3 male Sprague-Dawley rats and no change in insulin following glucose challenge. There is a limited utility of a critical evaluation of the body of evidence, which would likely reach a conclusion of insufficient data to assess potential effects of a protein-restricted diet given that only glucose and insulin were evaluated in more than one study and for both endpoints the inconsistency in results may reflect differences in assay protocols (i.e., fasting vs non-fasting vs glucose challenge). Consideration of the key risk of bias questions found that all four studies failed (reflecting that this factor was either not performed or not reported) to consider the litter as the statistical unit of analysis.

3.7.3. Dexamethasone

Two transgenerational studies reported metabolic or glucose-related outcomes after exposure to dexamethasone (Buchwald et al., 2012; Drake et al., 2005). There is a limited utility of a critical evaluation of the body of evidence, which would likely reach a conclusion of insufficient data to assess potential effects of dexamethasone given that the dataset is restricted to two studies with no overlapping endpoints and different species (rat and marmoset). Buchwald et al. (2012) reported that 5 mg/kg dexamethasone given to pregnant marmosets resulted in increased LDL cholesterol and decreased HDL triglycerides and lipoproteins in F_3 female offspring with no effects on fatty acids and other metabolic outcomes. Drake et al. (2005) tested fewer endpoints following exposure of pregnant dams to 100 μg/kg dexamethasone and reported no effects on insulin in a glucose tolerance test given to male F_3 rats at six months of age. Serious risk of bias issues would also contribute to the limitations in this dataset for evaluating metabolic outcomes given that neither study randomized treatment or used the litter as the statistical unit of analysis (reflecting that these factors were either not performed or not reported).

3.8. Growth and developmental outcomes

Growth and developmental outcomes were investigated in 60 transgenerational studies evaluating 42 different exposures (Table 3 for list of exposure agents); however, only 10 exposures were reported in multiple studies: vinclozolin (4), radiation (4), dioxin (3), high-fat diet (3), JP-8 (2), cyclophosphamide (2), methoxychlor (2), n-nitrosomethylurea (NMU) (2), over-nutrition (2), and Permethrin + DEET Mixture (2). Most of the data are limited to measurements of growth or body weight, rather than potential measures of teratogenicity or other developmental endpoints. As discussed further below, most of the evidence is inconsistent for these small datasets; however, all three high-fat diet transgenerational studies reported treatment-related increases in body weight or length. Note that body weight could be used as an indicator of metabolic effects or growth and metabolism. A discussion of potential transgenerational effects of high-fat diet on metabolic and glucose-related outcomes is provided above, and the body weight data are included here in relation to growth and development.

3.8.1. Vinclozolin

Three transgenerational studies reported inconsistent effects on body weight following vinclozolin exposure from two different research groups (Crews et al., 2012; Guerrero-Bosagna et al., 2012; Schneider et al., 2013). In rats, two studies reported no difference in F_3 offspring body weight (Crews et al., 2012; Schneider et al., 2013). Crews et al. (2012) monitored body weight from weaning (PND 21) through PND 90 and reported that non-stressed descendants from the vinclozolin lineage had a significantly higher body weight than the non-stressed control lineage at PND 46–67. The authors also reported that the vinclozolin lineage males gained weight more rapidly and became heavier than control non-stressed males. In mice, a single study reported no effect on body weight in F_3 offspring in two strains of mice; however, the age of observation was not reported (Guerrero-Bosagna et al., 2012). There are serious limitations in the body of evidence including the inconsistency in the results, small sample size and the heterogeneity in the timing of outcome assessment across studies and these limitations make it likely that a critical evaluation of the effects of vinclozolin would reach a conclusion of insufficient data to assess effects on growth and development.

3.8.2. Radiation

Five studies were identified that evaluated developmental effects following non-gestational radiation exposure across a diverse range of animal models (e.g. fish, rodents and moths) (Baulch et al., 2007; Ishikawa and Hyodo-Taguchi, 1997; Jegou et al., 1991; Sarapultseva and Dubrova, 2016; Wee et al., 2005). Although few developmental endpoints were examined, a single study reported that exposure of ciliates (F_0) to 0.1 W/m^2 of either 1 or 10 GHz RF-EMF resulted in a significant decrease in motility in 10–15 subsequent generations (Sarapultseva and Dubrova, 2016), whereas other studies reported no significant effects on malformations, longevity, or body weight in animals. Body weight was the only endpoint evaluated in more than one study, with no effects observed in F_2 rats and F_3 mice in two separate studies following radiation exposure of three Gy and 0.1 Gy respectively to F_0 males (Baulch et al., 2007; Jegou et al., 1991). There are very serious limitations in the dataset that make it likely that a critical evaluation of transgenerational effects of radiation would reach a conclusion of insufficient data to assess growth and development due to inconsistency of results, heterogeneity in the endpoints examined across studies, and failure to randomize treatment or blind the outcome assessors.

3.8.3. Dioxin

Three studies were identified that evaluated growth and developmental outcomes following transgenerational exposure to dioxin in either rats or zebrafish (Baker et al., 2014; Manikkam et al., 2012; Manikkam et al., 2012b). Few developmental endpoints were evaluated and only body weight was reported in more than one study from the same laboratory. Manikkam et al. (2012, 2012b) reported that dioxin (0.1 μg/kg/day) was associated with increased body weight in F_3 male rats at weaning (PND 21) and 12 months of age, with no effect on females. It is not clear from study reporting if these are two independent studies or if animals from a single experiment were followed over time and reported separately impacting the ability to discuss consistency of the findings. Dioxin (50 pg/ml) exposure to zebrafish (F_0) resulted in axial skeletal malformations in the F_2 generation, while other developmental endpoints (i.e., jaw and cranial malformations) were not affected (Baker et al., 2014). There are serious limitations in the dataset that make it likely that a critical evaluation of transgenerational effects of dioxin would reach a conclusion of insufficient data to assess growth

or developmental outcomes including the limited number of studies, heterogeneity in endpoints across studies, and failure to consider the litter as the statistical unit of analysis (reflecting that this factor was either not performed or not reported).

3.8.4. High-fat diet

Seven studies in rodents and a single study in fruit flies evaluated growth and developmental outcomes following transgenerational exposure to a high-fat diet (21–45% fat). Although few growth and developmental endpoints were examined, body weight was evaluated in five studies in mice; three studies reported an increase in offspring body weight in a grandparent-of-origin and sex-specific manner, while two studies reported no effect. In mice, studies reported sex-dependent increases in body weight in offspring following (F_0) consumption of a high-fat (21–45% fat) diet (Dunn and Bale, 2011; Fullston et al., 2012; Fullston et al., 2013). Maternal grandfathers consuming a high-fat diet resulted in an increase in total body weight at 17 weeks of age (11%) and postmortem (10%) in F_2 males, but not females. No effects were reported for offspring whose paternal grandfathers consumed a high-fat diet (Fullston et al., 2012; Fullston et al., 2013). A significant increase in body weight (4%) and length (2%) were reported in F_3 females whose paternal grandfather was exposed in utero to a high-fat diet; however, no effect was reported for females or offspring whose maternal grandfather was exposed to a high-fat diet (Dunn and Bale, 2011). Masuyama et al. (2016) reported an increase in body weight at 24 weeks of age in F_3 females (19%) and males (18%) when great-grandparents both consumed a high-fat diet. A single study reported no effect on body weight in the F_3 offspring following great-grandmother consumption of a high-fat diet (Steffensen, 2016). Both rat studies reported no transgenerational effects on body weight or length, and the body of evidence in mice is inconsistent, although most studies reported a sex-dependent increase in body weight following transgenerational exposure to a high-fat diet in F_0 animals. A single study in fruit flies reported significant increases in pupal weight of F_2 females, but not males following maternal or paternal grandparents consumption of a high-fat diet (Dew-Budd et al., 2016). The differences in fat content of the diets across studies and failure (reflecting that this factor was either not performed or not reported) to consider the litter as the statistical unit of analysis are serious limitations in the dataset that make it likely that a critical evaluation of transgenerational effects of high-fat diet would reach a conclusion of insufficient data to assess growth and development.

3.8.5. Jet propellant 8 (JP-8), cyclophosphamide, methoxychlor, n-nitrosomethylurea (NMU), over-nutrition and permethrin + DEET mixture

Two transgenerational studies were identified for each of the following exposures that examined growth and developmental endpoints: JP-8 (Manikkam et al., 2012c; Tracey et al., 2013), cyclophosphamide (Auroux et al., 1988; Dulioust et al., 1989), methoxychlor (Manikkam et al., 2014; Stouder and Paoloni-Giacobino, 2011), n-nitrosomethylurea (NMU) (Hemsworth, 1991; Tomatis et al., 1975), over-nutrition (Diaz and Taylor, 1998; Pentinat et al., 2010) or a mixture of permethrin + DEET (Manikkam et al., 2012; Manikkam et al., 2012c). There is a limited ability to draw conclusions for these exposures given that the datasets were restricted to two studies that had few or no overlapping endpoints and there were concerns with study design and potential risk of bias. Two JP-8 studies in rats, conducted by the same research group, evaluated transgenerational effects on body weight; one study reported an increase in body weight (age not specified) (Tracey et al., 2013) and the other study reported no effect on body weight at 21 days of age (Manikkam et al., 2012c). In rats, two studies from the same research group reported no effect on F_3 female body weight following cyclophosphamide exposure to F_0 females and a significant increase in F_3 male body weight at 17 and 39 weeks of age (Auroux et al., 1988; Dulioust et al., 1989). Over-nutrition was also evaluated in two studies, one in mice and one in rats. There were no transgenerational effects of over-nutrition reported on body weight in mice at four weeks or six months of age (Pentinat et al., 2010); however, in rats Diaz and Taylor (1998) reported a significant increase in body weight at 21 days of age (Diaz and Taylor, 1998). Body weight was evaluated in two studies from the same group of researchers and reported increased body weight at 12 months of age in F_3 offspring but no effect of exposure on weaning weight (PND 21) in the F_3 offspring exposed transgenerationally to a mixture of permethrin + DEET (Manikkam et al., 2012; Manikkam et al., 2012c). The heterogeneity in endpoints evaluated across studies along with the limited number of studies and failure to consider the litter as the statistical unit of analysis (reflecting that this factors was either not performed or not reported) limit the utility of a critical evaluation of this body of evidence on growth or developmental effects of JP-8, which would likely reach a conclusion that there are insufficient data to assess potential effects.

3.9. Neurological and sensory outcomes

Neurological outcomes were reported in 32 transgenerational studies evaluating 27 different exposures (Table 3 for list of exposure agents). The four exposures that were reported in multiple studies [BPA (2), cyclophosphamide (3), stress (5), and vinclozolin (4)] are discussed further below. These data sets for BPA, cyclophosphamide and stress reported transgenerational effects on several neurological measures. Replication and further study of neurological outcomes (particularly if researchers evaluated similar aspects of neurological function or used a consistent set of assays) for these chemicals and other exposures would increase the utility of the data for drawing conclusions from these bodies of evidence as well as the confidence for potential transgenerational effects on neurological outcomes in general.

3.9.1. Bisphenol A (BPA)

Two studies from the same research group were identified that evaluated neurological effects in the F_4 generation following gestational exposure to BPA, and effects were reported for most endpoints assessed (Wolstenholme et al., 2012; Wolstenholme et al., 2013). Transgenerational BPA exposure (5 mg BPA per kg diet) increased social investigation (interacting with stimulus mice) and locomotor activity (general activity in open field) and decreased non-social behaviors (time spent exploring) in mice. In contrast, no effects were reported for anxiety-like behaviors (elevated plus maze). Although both studies reported effects in subsequent generations of offspring transgenerationally exposed to BPA, heterogeneity in the methods or types of assays used to evaluate neurological outcomes across multiple studies, and limited number of studies are very serious limitations in the body of evidence that make it likely that a critical evaluation of effects of BPA would reach a conclusion of insufficient data to assess neurological effects.

3.9.2. Cyclophosphamide

Three studies from a single research group were identified that evaluated neurological effects following non-gestational exposure to cyclophosphamide in rats (Auroux et al., 1988; Auroux et al., 1990; Dulioust et al., 1989). Adult male (F_0) Wistar rats received cyclophosphamide (10 mg/kg-day) by IP injection for 15 days and following a rest period of 60 days which exceeds a spermatogenic cycle, males were then mated with non-treated females to produce the offspring of subsequent generations. In the F_2 generation, transgenerational exposure to cyclophosphamide was associated with decreased success rates in a learning task independent of the learning performance of the parents (F_1); males with unsuccessful learning parents, exhibited decreased spontaneous activity (Auroux et al., 1988). Biochemical analysis of the brains of the F_2 offspring showed a reduction in the activity of hippocampal choline acetyltransferase and fronto-parietal cortex norepinephrine which authors report may correspond to the observed behavioral deficits (Auroux et al., 1990). Cyclophosphamide exposure to

the F_0 male did not result in transgenerational effects on learning ability or spontaneous activity in the F_3 generation (Dulioust et al., 1989). This series of three studies report transgenerational exposure of cyclophosphamide effects learning and spontaneous activity in the F_2 generation but not the F_3 generation with mechanistic support for biochemical changes in the brain; however, this body of evidence is from a single laboratory which limits the ability to compare the consistency and robustness of the findings for a transgenerational effect.

3.9.3. Stress

Five studies were identified that reported data on neurological outcomes in rodents following non-gestational exposure to stress in animals of the F_0 generation (Franklin et al., 2011; Saavedra-Rodriguez and Feig, 2013; van den Wijngaard et al., 2013; Wu et al., 2017; Zaidan and Gaisler-Salomon, 2015). Most studies reported some type of transgenerational neurological effect of stress; however, it is difficult to evaluate the consistency of findings and therefore likely that a critical evaluation of transgenerational effects of stress would reach a conclusion of insufficient data to assess neurological outcomes because of the heterogeneity in stressors used (e.g., social instability, maternal separation, acute water avoidance, restraint, heat, colon distension (an internal physical stress)) and outcomes assessed (e.g., fear conditioning, hypersensitivity, locomotor activity, olfactory recognition and social behavior).

3.9.4. Vinclozolin

Four transgenerational studies in rats from two laboratories were identified that evaluated data on neurological and sensory outcomes following vinclozolin exposure (100 mg/kg/day to F0 dam) (Crews et al., 2007; Crews et al., 2012; Gillette et al., 2014; Skinner et al., 2008). Behavioral characterization included standard measures of anxiety (open-field test, light-dark box and elevated plus maze) and sociability evaluated in multiple studies (Crews et al., 2007; Crews et al., 2012; Gillette et al., 2014; Skinner et al., 2008). Two studies reported that transgenerational exposure to vinclozolin resulted in a decrease in anxiety-like behavior in F_3 generation males, while the females had an increase in anxiety-like behavior (Skinner et al., 2008; Crews et al., 2012). In addition, Crews et al. (2012) evaluated the response of chronic restraint stress during adolescence in descendants of the vinclozolin exposed lineage and concluded that vinclozolin males responded differently to the stress challenge compared to controls. In contrast to their previous work, Gillette et al. (2014) reported that social behavior was not strongly affected by vinclozolin exposure or chronic restraint stress in the F_3 generation citing differences in laboratory environments, age at testing and limited number of animals derived from few litters may have contributed to the divergent experimental outcomes (Gillette et al., 2014). These studies also provided data on gene expression and metabolic brain activity that provide some mechanistic support of the functional behavioral outcomes. A single study reported a sex specific mate preference in females from the vinclozolin lineage as females preferred mates who did not have a history of vinclozolin exposure; however, males did not exhibit a mate preference (Crews et al., 2007). Furthermore, authors report no effect of vinclozolin exposure in males or females in the odor-salience test. There is apparent heterogeneity in response (increase vs. decrease) that may be explained by sex differences; however, there are not enough studies for a detailed evaluation. The heterogeneity in endpoints evaluated across studies along with the limited number of studies and failure (reflecting that this factor was either not performed or not reported) to use the litter as the statistical unit of analysis limit the utility of a critical evaluation of this body of evidence on neurological outcomes following vinclozolin exposure and would likely reach a conclusion of insufficient data to assess potential effects.

4. Discussion

We identified 257 transgenerational inheritance studies that evaluated health effects in humans and experimental animals following exposures to over 80 different agents. This review collected and categorized the literature into a systematic evidence map for transgenerational inheritance by evidence streams (46 human studies and 211 animal studies), broad health effect categories, and exposures. The majority of exposures were to environmental chemicals; however, exposures ranged from non-chemical stressors such as food availability and the experience of the Holocaust to drugs of abuse, pharmaceuticals and radiation. The principal limitation in the human transgenerational epidemiological literature, even before addressing consistency or confounding, is the availability of studies that have data on enough generations to qualify as transgenerational inheritance studies. There were only four exposure agents (radiation, food availability, depression, and Holocaust) with more than one publication identified in the human studies. Similarly, for the animal studies, there are relative few bodies of evidence for exposure-outcome pairs where multiple studies evaluated the same exposure and the same or similar outcomes to allow for an assessment of the consistency and robustness of the evidence. The evidence mapping of the animal studies indicated that the only health effects categories with more than two studies on a given exposure-outcome pair were for reproductive, metabolic, neurological, and growth or developmental outcomes. Only nine exposures had a body of evidence of more than two transgenerational studies with data on a given health effect category: cyclophosphamide, DEHP, dioxin, high-fat diet, permethrin + DEET, protein-restricted diet, radiation, stress, and vinclozolin. The critical assessment of consistency and uncertainty for these bodies of evidence illustrated that given the limitations outlined for even the most established of these bodies of evidence on exposure-outcome pairs, critical evaluation with a systematic review to reach hazard conclusions of transgenerational effects for most exposure are likely to reach a conclusion of insufficient data to assess transgenerational effects. In many cases, there are potential explanations for the inconsistency of results across a body of evidence that would be examined in a full systematic review. For example, different results between studies could be explained by differences in the animal model used (mice vs. rats), gender, route of exposure between studies, or timing of outcome assessment. These would not then be unexplained inconsistency and would not decrease confidence in the body of evidence. However, multiple studies with each feature would be required in the systematic review to examine these and other factors as sources of heterogeneity or confidence in the body of evidence would be decreased.

The confidence in interpreting an association of exposure with an outcome from a body of evidence depends not only on the consistency of the results, but also on the rigor of the experimental design and the quality of the reporting. The risk of bias assessment of a subset of transgenerational inheritance studies identified multiple sources of bias that would decrease confidence in reaching definitive conclusions from this evidence base. Some of the findings are not unique to this area of research, and multiple calls to define and improve study design and reporting to reduce risk of bias in experimental research have been made (e.g., Thayer et al., 2014; Macleod et al., 2015). Improved reporting and study designs to minimize the potential for bias would increase the ability and confidence in making conclusions on transgenerational health effects, particularly considering the key issues of randomization, blinding of outcome assessors, and exposure characterization. In some cases, the current body of evidence made it difficult to confirm that multiple publications reporting similar effects are true replications, and not multiple reports of endpoints from the same group of experimental animals. At a minimum, transgenerational studies should include procedures for randomization of treatment, blinding of outcome assessors, and controlling for litter effects, which is

an especially important issue for developmental studies and tracking health effects over multiple generations. In addition to controlling for litter effects in transgenerational studies, outcome assessment for developmental exposures and multi-generational studies need to assure that the endpoints being evaluated are done consistently across treatment groups. At a minimum, the age or timing of outcome assessment should be standardized within a study and, across generations, for meaningful comparisons across studies. A recent review by Bohacek and Mansuy addresses the issues in the design and conduct of transgenerational studies and provides considerations and guidelines to improve study design to generate reproducible and high-quality data (Bohacek and Mansuy, 2017).

The transgenerational inheritance field of research is an emerging field and negative findings are as important to the field as positive findings. Evidence of no association from well designed and transparently reported studies can focus future research away from chemicals, health effects, or endpoints that do not appear to be effected under a transgenerational exposure regime. Although not the focus of this evaluation, some authors reported data suggesting mechanisms of transmission for transgenerational effects which are discussed in detail in a recent review by Bohacek and Mansuy (Bohacek and Mansuy, 2015). For instance, studies evaluating transgenerational effects of vinclozolin exposure in animals also evaluated epigenetic reprogramming - methylation patterns and more recently, non-coding RNAs, (Guerrero-Bosagna et al., 2010; Schuster et al., 2016; Skinner et al., 2012; Skinner et al., 2013). In addition, studies of transgenerational effects of high-fat diets in animals evaluated potential epigenetic mechanisms related to glucose regulation and reproductive effects (de Castro Barbosa et al., 2016; Masuyama et al., 2016). Transgenerational studies with mechanistic endpoints that are closely linked to reported health outcomes or provide data on multiple endpoints along a biological pathway would be helpful in the critical evaluation of transgenerational inheritance of health effects for a given exposure and support a systematic review for reaching hazard conclusions.

Radiation was the only exposure for which there were multiple transgenerational studies in both humans and animal models evaluating effects on reproductive outcomes. There is some consistency, as both datasets reported no evidence of transgenerational effects of radiation at the doses tested. However, these studies also illustrate the heterogeneity that is typical of the current transgenerational body of evidence and the challenges for reaching conclusions given the lack of a consistent evidence base. For example, the rodent studies included body weights, relative organ weights, fertility and sperm parameters, with most endpoints evaluated in two studies (Baulch et al., 2007; Jegou et al., 1991). The endpoints tracked in the human studies were limited to observations of the birth of children or grandchildren and general statements that children were in good health from studies of women treated with low-dose radiation for menstrual dysfunction.

Human evidence of transgenerational effects is limited by the number of studies for which the same exposure and health effect were studies. Many datasets commonly identified in the literature as “transgenerational” (e.g., the Dutch famine studies on diabetes or cardiovascular disease) are not included here because the studies do not contain a sufficient number of generations to be considered transgenerational inheritance under our PECO criteria. There are few human studies that have data on enough generations to qualify as transgenerational inheritance studies and no bodies of evidence on a well-characterized exposure (e.g., blood levels of a given chemical) that have been followed up for a sufficient number of generations. The largest body of evidence evaluating effects in humans examines neurological and sensory outcomes in the grandchildren of survivors of the Holocaust; however, the ability to evaluate consistency of the effects was limited given the range of behavioral assays used across studies. There are a number of exposures (e.g., radiation from atomic bomb survivors from world war II) from which there may be sufficient subsequent generations to potentially further study potential transgenerational effects in humans. For now, the majority of epidemiological studies report effects in children (e.g., Grant et al. (2015) study of F_1 generation effects associated with the Chernobyl nuclear accident), rather than examining the second or third generation offspring necessary to be considered transgenerational inheritance under our PECO criteria. (Grant et al., 2015).

Studies of potential male reproductive health effects have been a major focus of transgenerational inheritance research to date. The most consistently reported health effect identified was increased germ cell apoptosis in the testis of F_3 and F_4 male offspring of pregnant dams exposed intraperitoneally to the fungicide vinclozolin. The body of evidence suggests vinclozolin may have transgenerational inheritance effects on testicular germ cell apoptosis; however, this single endpoint was not associated with a reduction in overall testis weight and differences in exposure routes between oral and IP studies presents challenges for interpreting the results. As discussed previously, a full systematic review would examine sources of heterogeneity in the results including route of exposure, and confidence in the body of evidence would not be downgraded if there were sufficient data to explain differences across studies. While evidence for changes in a single endpoint may provide some confidence for a transgenerational effect, the overall confidence could be strengthened with evidence of multiple effects along a biological pathway. Mechanistic studies could add support to the biological plausibility of a transgenerational effect of vinclozolin on germ cell apoptosis if other endpoints associated with apoptosis regulation during male germ cell development (e.g., testosterone levels, expression levels of Bcl-x and Bcl-xL) were also affected. Building on the evidence of potential transgenerational effects, mechanistic studies can also suggest candidate substances for further study. For example, chemicals known to induce germ cell apoptosis (e.g., DEHP, BPA and 4-ter-octylphenol) and thus, disrupt testicular development would be interesting exposures to examine for transgenerational effects. Germ cell apoptosis was also evaluated in transgenerational studies of dioxin, JP-8, and a mixture of permethrin + DEET; however, results were either negative or inconsistent for these exposures.

Female reproductive endpoints were the next largest body of evidence from transgenerational studies; however, the overwhelming majority of exposures were tested in a single study. One finding stands out for potential transgenerational effects, and that is the reports that multiple exposures (e.g., dioxin, vinclozolin, JP-8, and a mixture of permethrin + DEET) decrease the number of ovarian follicles and increase the number of ovarian cysts. These data suggest that follicle number and ovarian cysts may be useful endpoints to test for potential transgenerational effects. However, it also emphasizes the need for further study of potential mechanisms. Multiple classes of environmental chemicals (e.g., organochlorine pesticides - DDT; dicarboximide fungicides - vinclozolin; phthalates - DBP; hydrocarbons - JP-8) with diverse chemical structures and mechanisms of action (e.g., androgen receptor agonists - vinclozolin; testosterone synthesis inhibitor - DBP; and aryl hydrocarbon receptor-pathway activator - dioxin) resulted in the same effect reported in single studies. It would be useful if further transgenerational studies of follicle number or ovarian cysts included measures of mechanistically relevant endpoints such as hormones that control follicle number and cyclicity (e.g., FSH and LH).

The transgenerational body of evidence evaluating neurological effects was small in total number of studies and stressors evaluated; however, most experiments with rodents reported some type of neurological effect. Transgenerational studies of BPA, cyclophosphamide, vinclozolin and various non-chemical stressors all reported behavioral effects including non-social behaviors, anxiety, or learning behaviors. The data suggest that neurological and particularly behavioral assays may be useful endpoints for future assessments of potential transgenerational effects of chemical and non-chemical exposures. Given the wide array of behavioral assays used by different researchers, it would be useful if a common set of assays were used as part of the behavioral test battery considered in future studies to develop bodies of evidence

that would support the evaluation of consistency across studies.

In conclusion, a broad range of exposures and outcomes have been reported to support transgenerational inheritance of health effects. Over 80 different agents have been tested in a transgenerational experimental design; and this state of the science review collected and categorized the literature into a systematic evidence map for transgenerational inheritance by broad health effect categories, exposures, and evidence streams. This scoping review and evidence map identifies serious limitations in the available bodies of evidence to support a systematic review for reaching hazard conclusions or even rating certainty in the bodies of evidence under evidence to decision frameworks such as the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. For most exposures the principal limitations are that there are few studies and considerable heterogeneity in endpoints evaluated. In addition, serious risk of bias issues were apparent from the detailed risk of bias assessment of example datasets as well as assessment of the key risk of bias or study design and conduct features across studies. The overwhelming majority of exposures have been evaluated in only one or two laboratories and few studies have examined the same or related endpoints. To support a robust critical evaluation of transgenerational inheritance of health effects for a given exposure with a systematic review for reaching hazard conclusions, it is necessary to have data on the same or closely related health effects from multiple studies (or a particularly strong, well conducted study, e.g. larger sample size ($n > 50$ per treatment or conducted on multiple species) and ideally across multiple labs. This state of the science review provides a summary and categorization of the current literature base and indicates potential areas that would benefit from further study. In addition, the risk of bias assessment of individual studies for several bodies of transgenerational evidence strongly supports the importance for future studies to minimize bias and produce robust data on potential transgenerational effects through best practices in study design and reporting including randomization of treatment, blinding of outcome assessors to study groups, controlling for litter effects, and standardizing the age of outcome assessment.

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Competing financial interests

The authors declare that they have no actual or potential competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2017.12.032.

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