ToxStrategies

Systematic Review Protocol:
Characterization of the dose-response relationship between exposure to dioxin-like compounds during sensitive windows of development and reduced sperm count

November 21, 2018

1. Review title

Systematic review and meta-regression to characterize the dose-response relationship between exposure to dioxin-like compounds during sensitive windows of development and reduced sperm count

2. Original language title

English

3. Anticipated or actual start date

January 1, 2018

4. Anticipated completion date

August 1, 2019

5. Stage of review at time of this submission

Preliminary searches – started/completed
Piloting of the study selection process – started/completed
Formal screening of search results against eligibility criteria – started
Data extraction - started
Pilot of select data analysis – started/completed
Risk of bias – not started

Data analysis – not started

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10. Organizational affiliation of the review

ToxStrategies, Inc.

11. Review team members and their organizational affiliations

- Dr. Jonathan Urban, ToxStrategies
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12. Funding sources/sponsors

This work was funded by Dow Chemical.

13. Conflicts of interest

DW, JU, LH, SF, CR, CT, and MS are employed by ToxStrategies, Inc., which is a consulting firm providing services to private and public organizations on toxicology and risk assessment issues. ToxStrategies received consulting fees for tasks related to topic formulation and protocol development, as well as conduct and reporting of the review. None of the authors from ToxStrategies will directly benefit, financially or non-financially, from the conclusions of the SR.

LH has consulted, given presentations to regulatory agencies and at scientific conferences, and published scientific manuscripts regarding dioxin risk assessment issues.

JU has consulted, given presentations at scientific conferences, and published scientific manuscripts regarding dioxin risk assessment issues.

DW has consulted, given presentations at scientific conferences, provided expert testimony, and published scientific manuscripts regarding dioxin risk assessment issues.

CR has consulted, given presentations at scientific conferences, and published scientific manuscripts regarding chemical exposure and dose-response methods and analysis.

CT has consulted, given presentations at scientific conferences, and published scientific manuscripts regarding dioxin risk assessment issues and, more broadly, on dose-response methods and analysis.

MS has consulted and contributed to presentations at a scientific conference regarding dioxin risk assessment issues and, more broadly, on dose-response methods and analysis.

No authors received personal fees.

15. Review question(s)

What is the dose-response relationship between exposure to dioxin-like compounds (DLCs) during sensitive windows of development and reduced sperm count in rats and humans when the collective evidence is evaluated using meta-regression?

16. Searches

Searches will be conducted using PubMed and Embase (exclusive of Medline); hand-searching will also be conducted. Syntax was developed and validated by an information specialist.

PubMed:

(Dioxin OR TCDD OR tetrachlorodibenzodioxin OR tetrachlorodibenzodioxin[MeSH]) AND (sperm OR spermato* OR spermati* OR semen OR spermatozoa[MeSH] OR spermatogenesis[MeSH] OR (in utero AND male))

[no filters applied]

Embase:

('dioxin'/exp OR dioxin OR 'tcdd'/exp OR tcdd OR 'tetrachlorodibenzodioxin'/exp OR tetrachlorodibenzodioxin) AND (sperm OR spermato* OR spermati* OR semen OR spermatozoa OR spermatogenesis OR ('in utero' AND male))

filters: AND [embase]/lim NOT [medline]/lim

17. Condition or domain being studied

Male reproduction and development: sperm count

18. Participants/population

Populations: Male humans and rats.

Include: Experimental animal studies in any strain of rats which involve gestational exposures to TCDD or DLCs.

Include: Epidemiological studies of males that have been exposed to TCDD or DLCs while *in utero* and/or postnatally through the onset of puberty.

Exclude: Studies that report findings from *in vitro* models, and experiments in laboratory species other than rats (e.g., mice, guinea pigs, primates, etc.). Such studies – including those designed to address underlying key events or other mechanistic endpoints – will be retained and consulted for context.

19. Intervention(s), exposure(s)

Exposure to TCDD and/or DLCs during developmentally sensitive windows.

Include: Studies with quantitative measures of exposure to TCDD or DLC-based total or congener-specific toxic equivalency (TEQ). DLCs are those defined by the World Health Organization (PMID 16829543).

Include: Experimental studies in rats in which the study design involves exposure to pregnant rats during gestation. Exposure paradigm must include gestation day 15 (GD15).

Include: Any duration of exposure during gestation; acute, chronic, or intermittent. Experimental animal studies will include those that extend exposures up until weanling.

Include: Human studies in which exposure occurs *in utero* and/or postnatally during pre- and/or peri-pubertal windows.

Exclude: Experimental animal studies that do not provide adequate quantitative information on dose, or studies that utilized exposure paradigms that involve exposure outside of the gestational period (e.g., only premating or mating period for parents, or only post-natal period for offspring). Select studies of potential relevance will be retained and consulted for context.

Exclude: For humans, studies where exposure paradigms to TCDD or DLC mixtures have not been defined prior to puberty and/or adulthood, or studies that do not provide quantitatively defined/measured TCDD or DLC mixture exposure levels (internal measures or external estimates). Select studies of potential relevance will be retained and consulted for context.

20. Comparator(s)/control

Comparator: For rat studies, comparators must include a control group of pregnant rats exposed to appropriate vehicle at the same time period as DLC treatment groups, and their respective male offspring. For human studies, the comparator must involve clear differentiation of exposure (e.g., a group that has lower exposure than others).

21. Types of study to be included initially

Peer-reviewed experimental laboratory studies in rats and human epidemiology studies.

Include: Studies published in peer-review journals; any language.

Exclude: Reviews (unless original data, such as a meta-analysis, were conducted); selected reviews will be retained and consulted for context.

Exclude: Studies reported only in Organohalogen Compounds and not in a peer-reviewed journal.

Exclude: Epidemiological studies in which both exposure and outcome were not evaluated on an individual basis (e.g., ecological studies) and case studies/case series.

22. Context

A qualitative evaluation of dioxin-induced changes in epididymal sperm count were characterized as the most sensitive endpoint for the developing male reproductive system in rats (Foster et al., 2010; PMID 20368131). In rats, GD15 has been identified as the most sensitive time point for the adverse effects of TCDD exposure on spermatogenesis in rats. As such, this review focuses on exposure during this sensitive window in experimental studies in rats. For humans, the period of sensitivity for a chemical affecting spermatogenesis is less well-defined and therefore this review will include and evaluate all human developmental studies for this exposure endpoint, rather than restricting the evidence base to a specific window during development.

Characterization of the dose-response relationship from the entire body of evidence is important as reduced sperm count serves as the critical effect used to develop health-based benchmarks by a number of authoritative bodies. Thus, the overall objective is to conduct a systematic review and meta-regression analysis between exposure to DLCs and reduced sperm count in rats, and to compare such with a similar assessment of the evidence base in humans. This effort will also include an evaluation of the quality of the studies that comprise the evidence base describing the dose-response relationship in rats and humans, thereby characterizing the inherent risk of bias and reliability of each study. The output provides results that could be used to develop health-based benchmarks which accommodate the available body of evidence, including considerations of study quality and relevance.

Secondary topics may include (1) characterization of effects of exposure to DLCs on sperm count in other laboratory animal species, and (2) characterization of other endpoints associated with spermatogenesis and reproductive health in rats (e.g., sperm motility and morphology, pathology [gross and histological] of testicular and epididymal tissue), (3) dose-response relationships between DLCs and key events (e.g., GD16-PND1 pituitary and testicular responses) that provide biological plausibility to the adverse outcome of reduced cauda epididymal sperm number, and (4) dose-response relationships and evidence of an adverse effect, consistent with AHR activation, for human populations with emphasis on the mini-puberty period in males occurring between 6 and 18 months of age. These topics will be qualitatively surveyed and evaluated with the purpose of collectively providing context to the primary review question.

23. Primary outcome(s)

Sperm count.

Include: Studies that provide quantitative assessments of sperm count. Sperm counts conducted either manually or via computer assisted sperm analysis (CASA) methods are included.

Include: Experimental studies in rats in which sperm count was measured at PND 60 or later.

Exclude: Experimental studies in rats in which sperm count was only measured at time points earlier than PND 60. Studies that evaluate sperm at earlier time points, e.g. PND 49, are not reliable because of the large natural variation in sperm production between rats this early in spermatogenic development.

Exclude: Studies assessing endpoints related to sperm viability but not including sperm count. Examples include sperm motility, or sperm morphology, or gross pathology or histopathology of male sex organs. Selected studies on such endpoints will be retained and consulted for context.

24. Secondary outcomes

Data on other sperm endpoints (motility, morphology), or related male reproduction developmental endpoints (e.g., histopathology of testes and the epididymides, testicular and serum testosterone, anogenital distance, preputial separation, information on testes and epididymal weight, and data on pituitary and testicular biology relevant to testicular programming of sperm production), could be used to interpret the primary outcome, and will be collected from studies which evaluate sperm count for contextual interpretation.

25. Study selection and data extraction

Data will be extracted from studies meeting inclusion criteria. Fields will include information reported by authors (e.g., dose level, frequency and duration of exposure, age at outcome observation, outcome measure, n, sperm count results, etc.), as well fields that may require analyst interpretation (e.g., identification of no or low observed effect levels).

26. Risk of bias (quality) assessment

Risk of bias will be evaluated using the U.S. National Toxicology Program: Office of Health Assessment and Translation Risk of Bias Rating Tool for Human and Animal studies (2015). Criteria will be refined based on topic area and piloted prior to appraisal.

27. Strategy for data synthesis

Following critical appraisal of individual studies for both internal and external validity, the body of evidence will be evaluated and integrated using the U.S. National Toxicology Program: Office of Health Assessment and Translation Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review an

Evidence Integration (2015). The process will involve the generation of evidence tables and a qualitative synthesis of the available data. Evidence analysts will use a weight of evidence approach incorporating concepts such as consistency, dose response, imprecision, indirectness, magnitude of effect, confounding, publication bias, and risk of bias to characterize confidence in the body of literature. Analytical tools, such as funnel plots or weight function models, may be used to evaluate publication bias.

Based on the evaluation of study quality and relevance for systematic review, qualified studies will be utilized in meta-regression per the methods described by the National Academy of Sciences (2017), *Application of Systematic Review Methods in an Overall Strategy for Evaluating Low-Dose Toxicity*. Prior to analyses, data from rat and human studies will be adjusted to a common dose metric, respectively. This is anticipated to be a measure of internal dosimetry, though decisions will be based on examination of available data following selection and extraction of study information. Meta-regression will be performed to characterize the dose-response relationship across the group of studies while taking into account heterogeneity between and within studies. In addition, a method that quantitatively accounts for differences in quality between the studies will be developed and evaluated for integration into the meta-regression. In order to perform meta-regression, a common measure of effect size will be calculated across studies and dose groups within studies. For example, for rat studies that measure mean sperm count of male offspring of dams exposed during pregnancy (including a control group and one or more dose groups), effect size may be measured as the difference in mean sperm count between dose group and control group. This common measure of effect size will be used as the response variable in meta-regression. Meta-regression will take into consideration the fact that mean differences for multiple dose groups within a given study cannot be considered independent, since they are all defined with reference to the same control group (see e.g. NAS 2017, and Crippa and Orsini 2016a; DOI 10.18637/jss.v072.c01). Meta-regression is anticipated to be performed using two open-source software packages developed for use with R statistical software: 'metafor' (Viechtbauer, 2010; DOI 10.18637/jss.v036.i03) and 'dosresmeta' (Crippa and Orsini, 2016b; PMID 27485429).

It is anticipated that various dose-response models will be assessed (e.g. linear, log-linear, quadratic, spline) and their goodness of fit will be compared, for example by comparing the model Akaike Information Criterion (AIC), to determine which model is preferred. Meta-regression modeling may also be used to assess the effect of covariates such as strain, age at evaluation of sperm count, or publication year, if relevant in the selected studies.

The results of meta-regression will be used to estimate a benchmark dose (BMD) value across all amendable studies.

28. Analysis of subgroups or subsets

Sensitivity analyses will be performed to examine the influence of specific studies and/or various subsets (e.g., low validity) of studies on the characterization of dose-response via

the meta-analyses. Analytical tools, such as forest plots and descriptive statistical parameters, may be used to aid in the weight of evidence assessments regarding hazard and strength of the data, as well strengths and weaknesses of subgroups of data. Similar considerations will be used in evaluating the epidemiological evidence base if data are amenable.

29. Dissemination plans

Approach and findings will be submitted for publication in the peer-reviewed literature and presented at Scientific Conferences (e.g., Dioxin Conference, Society of Toxicology, and Society or Risk Analysis).