OECD ADVERSE OUTCOME PATHWAY

Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to our generic account (env.tgcontact@oecd.org), and Nathalie Delrue (Nathalie.delrue@oecd.org)

PROJECT TITLE

Early-life stromal estrogen receptor activation by endocrine disrupting chemicals in the mammary gland leading to enhanced cancer risk

SUBMITTED BY (Country / European Commission / Secretariat)

United States

DATE OF SUBMISSION TO THE SECRETARIAT

May 9, 2019

DETAILS OF LEAD COUNTRY/CONSORTIUM

<table>
<thead>
<tr>
<th>Country/Organisation:</th>
<th>United States/ Silent Spring Institute, Newton, Massachusetts, 02453</th>
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</thead>
<tbody>
<tr>
<td>Agency/ministry/Other:</td>
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PROJECT CATEGORY

☑ Development of an AOP - applicable to a chemical category

Select the development tool to be used
☑ AOP-Wiki ☐ Effectopedia

☐ Guidance document related to AOP development including its evaluation

☐ Knowledge management tool for supporting AOP development including its evaluation

☐ Other, please specify below

If other category, please specify:
**PROJECT DESCRIPTION**

Please provide sufficient information to facilitate the review of the project submission by the OECD secretariat and the Extended Advisory Group with respect to its suitability to be included in the workplan of the AOP programme.


Note: For AOP Development projects please indicate the extent of the pathway to be described (i.e. the anchor points), the intermediate events that are likely to be addressed, the state of current development, the degree to which this pathway is already understood and described in the literature, and the expectation on the availability of evidence to support the AOP. Proposers should also indicate if and how the AOP is associated to any regulatory toxicological endpoints (e.g. acute or chronic toxicity, toxicity to reproduction etc.) Please provide references, links or attachments for supplementary information.

**PROJECT PLANNING**

In this section, please provide an indication of when the project is likely to commence and the expected duration. Please also make reference to any particular milestones or external factors that will influence project planning, and if the project is linked to programmes of particular organisations or consortia.

See attached pdf.
FLOW DIAGRAM

In this section, please provide a flow diagram of the proposed AOP, including the MIE, KEs at the various stages (molecular interaction, cellular response, organ response) and the AO.

Title: Adverse outcome pathway on early-life endocrine disrupting chemical activation of estrogen receptor in the mammary gland leading to enhanced cancer risk

AOP: 295 [https://aopwiki.org/aops/295]

Authors: Andrea R. Hindman ††, Alissa P. Link † and Ruthann A. Rudel †

Author affiliations: † Silent Spring Institute, Newton, MA; †† Social Science Environmental Health Research Institute, Northeastern University, Boston, MA; †‡ Research and Instruction, Northeastern University, Boston, MA

Contact persons: Andrea R. Hindman (hindman@silentspring.org) and Ruthann A. Rudel (rudel@silentspring.org), 320 Nevada Street, Suite #302, Newton, MA 02460

PROJECT DESCRIPTION:

Breast cancer risk background: Breast cancer is a significant health concern as the second leading cause of death in women [1]. Only 5-10% of breast cancers are attributable to genetic predisposition and substantial evidence indicates many lifestyle and environmental factors contribute to lifetime risk [2-5]. Early-life developmental disruption by hormone-like, or endocrine disrupting chemicals (EDCs), heightens age-related breast cancer risk. A human model of this disruption emerges from the treatment of pregnant women with synthetic estrogen, diethylstilbestrol (DES), beginning in the 1940’s with the intent to prevent miscarriages. This practice ceased when women exposed in gestation – “DES Daughters” – had a 40x increased incidence of cervical and vaginal cancers [6, 7], highlighting in utero development as a critical window of exposure. The later finding that “DES Daughters” also had a 2-fold increased incidence of breast cancer only detected in women ≥30 years post-exposure, underscores the latency of this disruption in causing disease [7-9]. Studies of the reproductive tract and mammary gland of rodent models have recapitulated these increased risks [10-12]. While synthetic estrogens are no longer prescribed to pregnant women, human biomonitoring data show widespread exposure to EDCs that include weak estrogens [13, 14] and their ability to cross the placental barrier [14-18]. Many EDCs are present at human-relevant exposures in the environment, but these chemicals can act together on the same adverse health outcomes [19], including estrogen action as a relevant target for breast cancer [20-23]. Taken together, this evidence raises concerns that early-life EDC exposure enhances later-life breast cancer risk.

AOP description: This adverse outcome pathway (AOP) links gestational EDC exposure to enhanced breast cancer risk. The molecular initiating event (MIE) is gestational estrogen receptor (ER) activation; particularly, stromal activation at in utero time of exposure. The ER is a master transcriptional regulator, with proliferation as its primary effect, and is the main mediator of breast development [24, 25]. Human-relevant EDC exposure triggers transcriptional activity that promotes altered signaling between the epithelial and stromal tissue compartments leading to disrupted tensitional homeostasis [26] and
tissue architecture. Inflammation and altered cellular differentiation are major cell- and tissue-level key events (KEs) mediating these disruptions. The pathway converges on the following mammary gland adverse outcomes (AOs) at the tissue- and organ-levels: altered density, structure and hormone sensitivity along with hyperplasia. Epigenetic alterations are a cellular-level AO that propagate gestational EDC exposure to later-life risk through cellular memory that directs ER-mediated gene expression and altered mammary development. Risk of tumorigenesis follows from these AOs.

The industrial estrogen, bisphenol A (BPA) is one of the most data-rich chemicals related to breast cancer and altered mammary gland development [11]. As such, studies in model rodent strains following gestational EDC exposure to bisphenol A (BPA) or DES provide experimental support for this AOP and human-relevance. A thorough search of the literature yielded experimental evidence for this AOP as directed by a mix of natural and MeSH term search logic specifying rodents and non-human primates (population); human-relevant, in utero exposure to BPA or DES (exposure); and mammary gland AOs (outcome) [27-32] (see PECO statement, Table 1 below). Most studies investigating EDC-effects on mammary development heavily described altered growth and structure, resulting in limited mechanistic understanding. This AOP integrates knowledge and tools from investigations of established breast cancer risk factors such as density and obesity to enhance understanding of the molecular- and cellular-driven etiologies of altered mammary structure and growth. Integrating this knowledge promotes the development of in vitro assays capable of predicting high-risk phenotypes and offers efficient alternatives to in vivo mammary gland evaluation. Ultimately, making these links in the knowledge base will improve screening to identify chemicals that act on gestational development and will more specifically target chemical contributions to later-life breast cancer risk in toxicity testing. Productive intermediate testing endpoints would follow ER-binding, -activation and steroidogenesis (OECD TG-455; EDSP TG-890(33, 34)), precede carcinogenicity (OECD TG-451, and -453) and connect these with EDC-effects on breast cancer due to prenatal exposure (OECD TG-414, -415, -416, -422, -443). This AOP will also describe ‘missed opportunities’ in the existing evidence; not reporting or measuring traditional toxicity testing endpoints, like uterine weight and body weight alongside more sensitive mammary gland growth and structural changes. Failure to do this in parallel within the same study undermines the sensitivity of these endpoints to predict later-life breast cancer risk.

PROJECT PLANNING:

AOP development for gestational EDC exposure leading to enhanced breast cancer risk has started. It is in the earlier stages of development, though the concept and direction are fully realized and it has been approved for wiki inclusion (AOP:295, https://aopiwiki.org/aops/295). As such, the enclosed diagram does not have arrows as the evidence is being assembled. From the time of this submission, the anticipated duration of development is 4 months (June 2019). Our approach was more systematic in evidence identification. Building off the expertise of the authors in mammary gland development and chemical exposure influence on breast health and cancer risk, an appraisal of the review literature focused the scope of the AOP and formed the basis of the AOs of risk and the PECO statement that dictated evidence inclusion [19, 27, 28, 30, 31, 35]. A risk of bias protocol was written, using the National
Toxicology Programs Office of Health Assessment and Translation (OHAT) tool. Risk of bias criteria was added to the standard tool for the purposes of this AOP to address sensitivities related to EDCs. Then search logic, developed in collaboration with a librarian (author, Link), was built using both gold standard publications and authoritative reviews related to the AOIs of interest, using a mixture of both MeSH and natural terms. The screening phase of the identified literature is complete (see literature tree, Figure 1) and now risk of bias assessment of individual studies is being performed using the Health Assessment Workspace Collaborative (HAWC)[36]. The literature screen also was performed using HAWC. Further endpoint assessment are planned to be facilitated on the HAWC platform and the rest of the AOP development will follow the OECD AOP guidance [37].

Table 1. PECO statement [27, 28]. A statement of the Population, Exposure, Comparators and Outcomes was prepared to direct objective experimental study collection for this AOP synthesis on breast cancer risk from early-life EDC exposure. The Organization for Economic Co-operation and Development does not cite systematic review methods or objective identification of included evidence in its guidance for AOP development. A narrowed survey of review articles in PubMed, published after 2006 and until November 2018, was performed to assess the state of mechanistic evidence connecting EDC exposure to breast cancer risk and altered mammary gland growth and structure. This step assisted problem formulation by situating human-relevant EDC exposures in the hallmarks of cancer via ‘important reviews.’ There were no systematic reviews. This initial survey of the review literature assisted search logic development and supported an initial sketch of the AOP.
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<th><strong>INCLUSION CRITERIA</strong></th>
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<tbody>
<tr>
<td><strong>Population (Experimental animal, in vivo studies)</strong></td>
<td><strong>Human and non-rodent animals and organisms, including wildlife, aquatic species and plants</strong></td>
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<tr>
<td>- Female laboratory rodents</td>
<td>- Males</td>
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<tr>
<td>- Female laboratory non-human primates</td>
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<tr>
<td><strong>Exposure</strong></td>
<td><strong>High-dose or pharmacological-dose exposures to BPA or DES</strong></td>
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<tr>
<td>- Human-relevant exposure to BPA, related BPA analogues or DES</td>
<td>- Any other EDC</td>
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<tr>
<td>- <em>In utero</em> exposure. <em>In utero</em> exposure is a requirement but studies that extend exposure to the perinatal period are also included</td>
<td>- Exposure to chemical mixtures in animals</td>
</tr>
<tr>
<td>- Exposure to controlled doses of BPA via an exposure method (e.g. – diet, drinking water, gavage, injection)</td>
<td>- Exposures during other developmental windows of risk</td>
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<tr>
<td><strong>Comparators</strong></td>
<td><strong>No controls</strong></td>
</tr>
<tr>
<td>- Vehicle-only, concurrently run treatment controls</td>
<td>- Historical controls</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td><strong>Any other organs</strong></td>
</tr>
<tr>
<td>- Determination of mammary gland disruption via any methodology intended to address mechanisms mapped in the AOP (see Figure) including to alterations of tissue density, epigenetics, gland morphology, hormone sensitivity and hyperplasia as precursors to tumorigenesis</td>
<td>- Any other stage-of-life</td>
</tr>
<tr>
<td>- Assessed in virgin, female laboratory rodents or non-human primates at any stage-of-life (e.g. - postnatal, pubertal or adult development)</td>
<td></td>
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<tr>
<td>- Uterine weight</td>
<td></td>
</tr>
<tr>
<td>- Body weight</td>
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<td><strong>Publication parameters</strong></td>
<td><strong>Non-peer reviewed; gray literature (e.g. - conference presentations or other studies published in abstract form only, grant awards/ proposals and theses/ dissertations</strong></td>
</tr>
<tr>
<td>- Peer-reviewed</td>
<td>- Retracted articles</td>
</tr>
<tr>
<td>- Original data</td>
<td>- Review articles (only considered for initial survey of available mechanistic data)</td>
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<td>- Studies must be published in English</td>
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Figure 1. Literature tree and study flow. A comprehensive literature search was performed in direct collaboration with a STEM librarian (APL). The PECO statement guided search logic and decisions were tracked using the project management tool Trello (https://trello.com). Search logic was a mix of MeSH and natural language terms to ensure the largest sweep of literature. The core search logic yielded a body of literature ($n = 1923$) describing organ, chemical and animal models. DES and BPA were specified due to the wealth of mechanistic information available for these chemicals. The core search logic yield was further refined to 6 different adverse outcomes (AOs). These outcomes were based on expert knowledge, informed through the initial review literature survey and in part developed using search terms from a set of ‘gold-standard’ publications and ‘important reviews’ describing normal biology.
Evidence stream (in vitro vs. in vivo) was not specified in the search logic and was used for discovery of supporting in vitro studies. Health Assessment Workspace Collaborative (https://hawcproject.org) was used to facilitate screening, data extraction and analysis.

FLOW DIAGRAM (attached). Again, there are no arrows yet. This AOP is in under development and assembly of evidence is in progress.

References

33. OECD, Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists. 2016.
