OECD ADVERSE OUTCOME PATHWAY``

Project Submission Form
(Revised 11 February 2013)`
If you require further information please contact the OECD Secretariat
Return completed forms to Lisa Savary (Lisa.SAVARY@oecd.org) and Camilla Francis (Camilla.FRANCIS@oecd.org)

PROJECT TITLE

AOPs for RONS and DNA damage leading to increased risk of Breast Cancer

SUBMITTED BY (Country / European Commission / Secretariat)

DATE OF SUBMISSION TO THE SECRETARIAT

5/10/19

DETAILS OF LEAD COUNTRY/CONSORTIUM

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PROJECT CATEGORY

X☐ Development of an AOP - applicable to a chemical category

☐ Development of an AOP Case Study - applicable to a single chemical or a very limited number of chemicals

☐ Guidance document related to AOP development including its evaluation

☐ Knowledge management tool for supporting AOP development including its evaluation

☐ Other, please specify below

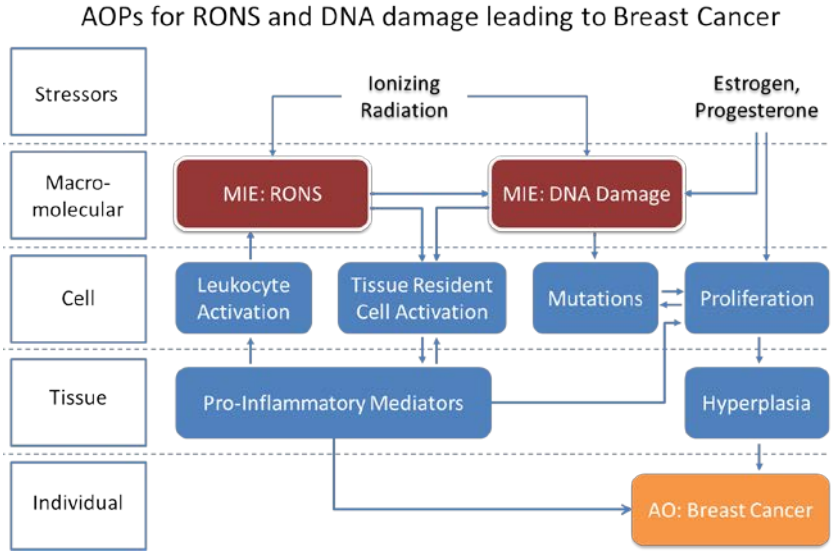
If other category, please specify:

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 and AOP Case Study 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.

Knowledge about established breast carcinogens can support improved 21st century toxicological testing methods by identifying key mechanistic events. Ionizing radiation (IR) increases the risk of breast cancer, especially for women and for exposure at younger ages. We propose and describe two AOPs for increased risk of breast cancer following ionizing radiation. Based on the identification of two essential MIEs, we have developed two interrelated AOPs shown below: AOP 293, "Increased DNA damage leading to increased risk of Breast Cancer" at https://aopwiki.org/aops/293 and AOP 294, "Increased reactive oxygen and nitrogen species (RONS) leading to increased risk of Breast Cancer" at https://aopwiki.org/aops/294. These AOPs describe the major measurable key events leading to the adverse outcome of Breast Cancer via the activation of RONS or DNA damage.



We used the Adverse Outcome Pathway (AOP) framework to outline and evaluate the evidence linking ionizing radiation with breast cancer from molecular initiating events (MIE) to the adverse outcome (AO) through intermediate key events (KE). We identified prospective key events using recent literature on ionizing radiation and carcinogenesis, focusing on review articles. We searched PubMed for each key event and ionizing radiation, and used references cited in the resulting papers and targeted searches with related key words to identify additional papers. We manually curated publications and evaluated data quality. Based on the essentiality, biological plausibility, and empirical evidence linking the two events following IR or RONS stressors, we evaluated the strength of evidence for each Key Event, KER, and the overall AOPs and found the evidence to be strong overall. The AOP identifies areas for additional research, including better description of the time and dose-dependence of MIEs and KEs in mammary tissues directly and indirectly exposed to IR.

Many aspects of this pathway have been studied or discussed in the literature, but this AOP provides a major review for the many of the key event and key event relationships. The two AOPs are populated and await EAGMST and public input for further refinement. Some additional refinement may take place before June, but the content is ready for evaluation now.

In the two AOPs, the stressor (ionizing radiation or IR) increases production of reactive oxygen and nitrogen species (RONS, MIE for AOP 294), and directly and indirectly causes DNA damage (MIE for AOP 293). DNA damage leads to mutations, proliferation, and hyperplasia (KEs), leading to increased risk of breast cancer (the Adverse Outcome). RONS also increases inflammation-related key events: tissue resident cell activation, increased production of pro-inflammatory mediators, and recruitment and adhesion of leukocytes (KEs). These inflammation-related key events contribute to direct and indirect effects (effects in cells not directly reached by IR) via positive feedback to RONS. They also separately increase the Adverse Outcome through increased proliferation of cells (KE) and increased tumor growth and invasion in tissue (AO). Recognizing that some of these key events reflect a measure of adversity, we include DNA damage, mutation, and hyperplasia in the AOPs as adverse outcomes.

The risk of breast cancer from ionizing radiation also depends on the developmental and lifetime presence of estrogen and progesterone. These reproductive hormones affect DNA damage and proliferation, and we link these stressors directly to these key events in the AOP. These and other KEs overlap at multiple points with events characteristic of "background" induction of breast carcinogenesis, including hormone-responsive proliferation, oxidative activity, and DNA damage. These overlaps make the breast particularly susceptible to ionizing radiation and reinforce the importance of including these MIEs and KEs in toxicological panels for carcinogenicity.

In the interest of making this AOP relate to and interconnect with other AOPs, we selected existing key events when possible. Multiple key events (KEs) in aopwiki.org address specific types of DNA damage, however only two are general enough to encompass the multiple types of DNA damage discussed in this AOP (nucleotide damage, double strand breaks, and complex damage). We therefore modified the following existing key events: *1194, Increase in DNA Damage, 1182 Increase in Proliferation (Epithelial Cells), 1192 Increase in Hyperplasia, and 1193, Breast Cancer.* These key events are included in several AOPs including *AOP 200: Estrogen receptor activation leading to breast cancer* but had not been populated with data. We also integrate key event *185, Increase in Mutation* as well as three key inflammatory events proposed and developed in (Villeneuve, Landesmann et al. 2018): *1492 Tissue Resident Cell Activation, 1493, Increase in Pro-Inflammatory Mediators, and 1494, Leukocyte Recruitment/ Activation*. Although the key event increase in reactive oxygen and nitrogen species has common elements with existing key events, none of these include reactive nitrogen species and a new key event was created to encompass the contribution of these species to the AOP.

While these AOPs were developed based on evidence from ionizing radiation stressors, we also briefly evaluated the relevance of the AOP to other DNA damaging agents. This material is included in the AOP stressor "Other DNA damaging agents".

Relevance to guideline tests

Because of the long latency of mammary tumors, the two-year rodent carcinogenicity bioassay is the primary assay for the adverse outcome of breast cancer. The assay is included in the OECD Test No. 451 and 453 for carcinogenicity and combined toxicity and carcinogenicity. Mammary tumors are also reported in short term, sub-chronic, and chronic toxicity tests, but these tests are less sensitive due to their shorter duration.

This AOP is relevant to guideline tests addressing DNA damage and mutation. MIE2: Increase in DNA damage is relevant to OECD Test Nos. 473, 475, 483, 487, and 489, which detect DNA damage in the form of single and double strand breaks, chromosomal damage and micronuclei, as well as some forms of nucleotide damage. KE1: Increase in mutation is relevant to OECD Test Nos. 471, 476, 488, and 490 for in vitro and in vivo mutations. To our knowledge no guideline tests address increases in RONS, proliferation, or inflammation, although some in vitro tests in ToxCast or in development elsewhere may reflect changes in these key events.

References:

Villeneuve, D. L., B. Landesmann, et al. (2018). "Representing the Process of Inflammation as Key Events in Adverse Outcome Pathways." Toxicological sciences : an official journal of the Society of Toxicology **163**(2): 346-352.

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.

Project Timeline:

Inclusion of project into the AOPs workplan	Q2 2019
Drafting of the AOP	largely completed
Review of AOP by EAG-MST, WNT, etc	Q2 2019
Additional review, commenting, drafting as needed	Q3 2019