Project 1.11: AOP for Heritable germ cell derived disease

Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations

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Introduction: Background to the specific AOP

Germ cell/heritable mutations are important regulatory endpoints for international agencies interested in protecting the health of future generations. However, germ cell mutation analysis has been hampered by a lack of efficient tools. With the publication of the OECD test guideline TG488 (rodent transgene mutation assay) and new technologies (including next generation sequencing) this field is experiencing renewed focus. Indeed, regulatory approaches to assess germ cell mutagenicity were the focus of an IWGT workshop in 2013. Of particular concern is the inability to address this endpoint through high-throughput screening assays, because spermatogenesis cannot be carried out in culture, and mutagenesis is an important gap in existing tests. The motivation for developing this AOP was to provide context for new assays in this field, identify research gaps and facilitate the development of new methods. In this AOP, a compound capable of alkylating DNA is delivered to the testes causing germ cell mutations and subsequent mutations in the offspring of the exposed parents. The AOP requires uptake of the parent compound or metabolite in spermatogonia and interaction with DNA in those cells. DNA alkylation in male pre-meiotic germ cells is the molecular initiating event. A variety of different DNA adducts are formed that are subject to DNA repair; however, at high doses the repair machinery becomes saturated or overwhelmed. The fate of remaining adducts includes: (1) attempted DNA repair by alternative DNA repair machinery, or (2) no repair. Key event (KE) 1 is insufficient or incorrect DNA repair. Lack of repair can lead to replication of adducted DNA and ensuing mutations in male pre-meiotic germ cells (KE2). Mutations that do not impair spermatogenic processes will persist in these cells and eventually be present in the mature sperm. Thus, the mutations can be transmitted to the offspring (adverse outcome – inherited mutations). It is well documented that mice and other animals exposed to alkylating agents develop mutations in male pre-meiotic germ cells that are then found in sperm, resulting in the transmission of mutations to their offspring. There is extensive empirical evidence supporting the AOP and the overall weight of evidence is strong. Although there are some gaps surrounding some mechanistic aspects of this AOP, the overarching AOP is widely accepted and applies broadly to any species that produces sperm.

Review Process

Following the internal review by the EAGMST, this AOP was recommended for external review and 8 reviewers (see Annex 1) from Europe, the US and Japan were selected.

The OECD Secretariat provided the reviewers with instructions, webinar training, the list of the 4 charge questions and a printout of the AOP.

All 8 reviewers provided written comments for the 4 charge questions, with the last comments received/entered into the Wiki on October 10th.

To assist both with responding to the comments and also with the group discussion, a spreadsheet table was developed (see Annex 2). To help focus the response to the comments and the group discussion, the comments to the 4 charge questions were further divided as follows, and the comments were placed into one of these 7 questions in the first column of the spreadsheet table :

- Construction of the AOP (MIE, KEs, KERs)—are they appropriate per the OECD Handbook?
- Does additional information need to be added to the MIE, KE, KER or AO descriptions?
- Weight of evidence for KEs, KERs and AOP (are modifications required to the text?)
- Is the cited literature appropriate? Are there major significant papers that are not (and should be) cited?
- Regulatory applicability. Is this adequately described?
- Comments related to the AOP and test methods?
- Scope of the AOP content—should additional issues be included?

The authors of the AOP provided an initial response to the review comments (see Annex 2, column 2).

A group discussion/webinar led by the review coordinator was held on Tuesday October 13th with 3 of the authors and 7 of the 8 reviewers participating. The discussion was focused around the 7 questions (listed above). Summary comments for each of the questions were developed during the call and entered into the spreadsheet table (see Annex 2, column 3).

The AOP authors made modifications to the AOP based on the reviewer comments and entered their final responses to the comments in the 4th column of the spreadsheet table (see Annex 2).

Synthesis of main issues based on reviewers' comments

A number of issues/comments were received and they are summarized as a bulleted list highlighting the main comments according to the 7 questions (the complete set of comments can be found in Annex 2):

- **Construction of the AOP (MIE, KEs, KERs)—are they appropriate per the OECD Handbook?**

  Question as to whether DNA repair should or should not be a KE
  Question as to whether ADME should be included
  Question as to whether cell replication should be a KE
  Should adaptive response be included?

- Does additional information need to be added to the MIE, KE, KER or AO descriptions?

  Suggestion that Mass Spectrometry should be added as an technique to measure DNA adducts
  Suggestion that the comet assay should be included
  Suggestion for discussion of additional chemicals such as ethylene oxide
  Suggestion for including additional information in the discussion of mutational spectra
  Suggestion that additional information should be added for the biological impact of the AO
  Suggestion to include more discussion of somatic cell mutation

- Weight of evidence for KEs, KERs and AOP (are modifications required to the text?)

  Comments that the weight of evidence was appropriate and comments that the weight of the evidence should be reconsidered
  Comments that authors should survey the human evidence for male cancer patients
  Insufficient number of chemicals to generalize

- Is the cited literature appropriate? Are there major significant papers that are not (and should be) cited?

  Additional references suggested for methods to measure DNA adducts and review of human mutations
  Question about route of exposure used for studies
  Statement that the scientific content reflects the scientific knowledge

- Regulatory applicability. Is this adequately described?

  The reviewers were mixed in their responses concerning regulatory applicability with some indicating no regulatory relevance and others saying that it will be useful for applications such as REACH (It should be noted that this is reflective of different regional differences with regard to the consideration of heritable mutation as a regulatory endpoint).

- Comments related to the AOP and test methods?

  Several OECD TGs were listed as relevant 488, 478, 483, 485

- Scope of the AOP content—should additional issues be included?

  Question of target tissue exposure
  The AOP is limited by the fact that it is primarily based on data from one chemical

Summary record of the teleconference (highlighting the main issues, responses and action)

As indicated above, a group discussion/webinar led by the review coordinator was held on Tuesday October 13th with 3 of the authors and 7 of the 8 reviewers participating. The discussion was focused around the 7 questions. Summary comments for each of the questions were developed during the call and entered into the spreadsheet table (see Annex 2, column 3). Following the call the authors updated the AOP based on the recommendations (see Annex 2, column 4 for the full details of the authors' responses).

The group consensus on the main issues and the authors' responses and actions are summarized by the 7 questions as follows:

- Construction of the AOP (MIE, KEs, KERs)—are they appropriate per the OECD Handbook?

  The group recommended that DNA repair be included and that the text be revised (and this was done)
  Clarification to be made on the exact types of alkylation that is covered by the AOP (and this was done)
  The authors to revisit the issue of cell replication as a key event (authors decided that it should not be a KE)

- Does additional information need to be added to the MIE, KE, KER or AO descriptions?

  Clarify that not all adducts lead to mutation (this was done)
  Determine if the ethylene literature might be useful (determined that it was not)
  Additional methods and references to be added (this was done)

- Weight of evidence for KEs, KERs and AOP (are modifications required to the text?)

  Authors to determine if there are more alkylating agents with relevant data (this was done and some additional information added to the AOP)
  Authors to determine if there is relevant information from human cancer survivors (there were no relevant additions based on a review of the literature, please see Annex 2)
  Authors rewrote the biological plausibility of the KER linking mutations to heritable mutations based on revisiting this issue. The authors revised to indicate that the majority of deletions are neutral and some are deleterious, and strengthened statement surrounding the 'strong' call for this KER.
  Suggest that the level be changed for quantitative understanding from strong to moderate for the KER linking mutations to heritable mutations (this was done)

- Is the cited literature appropriate? Are there major significant papers that are not (and should be) cited?

Additional references suggested (and they were added)

- Regulatory applicability. Is this adequately described?
  Regulatory applicability was extensively discussed but ultimately no revisions to the text were recommended

- Comments related to the AOP and test methods?

  No recommendations made for altering the text

- Scope of the AOP content—should additional issues be included?

  Recommended that the authors consider appropriate additions based on the discussion (the authors added some additional information in the background information concerning relevant human exposures)

Recommendation from the reviewers following the discussion: Extensive discussion concerning whether the AOP was ready to be submitted to the EAGMST and the WNT resulted in a group consensus that the AOP should move forward after the authors further considered and modified the AOP (as appropriate) based on the summary group comments.

Review coordinator summary and recommendation

The full details and summary of all of the reviewer comments, the authors' responses before and after the discussion, and the summary of the discussion between the reviewers and authors can be found in Annex 2, which contains four columns that were populated during the process as follows:

- Reviewer comments (entered and then provided to the authors for their initial comments)
- Initial comments from authors (completed by the authors prior to the group discussion)
- Summary of the group discussion comments (completed during the group discussion)
- Action taken by the authors in response to the comments (completed by the authors after the group discussion)

Based on the reviewer comments and the discussion between the authors and reviewers, the authors made modifications to the AOP, entered the revised AOP into the Wiki and provided, to the review coordinator, the completed spreadsheet table (see Annex 2) in which they completed the 4th column indicating how they had dealt with the group discussion comments. The primary AOP author also summarized their major revisions for the review coordinator as follows:

> "I did alter the section on the human genetics in both the background and in the description of the AO, and included more up to date references (including the one recommended). Although we did reduce one of the quantitative WOE calls, after discussion with the core group of authors we did NOT reduce the KER linking mutations in germ cells to heritable mutations to 'moderate' as recommended. This is dogma and the call is based on decades of work in evolutionary biology and genetics. I have inserted a quote from a recent science paper to address this in the revised description of the biological plausibility."

Review coordinator recommendation: The review coordinator has reviewed the responses of the authors to the reviewers' comments, verified that the Wiki has been updated based on the review comments (per the primary AOP authors summary above) and concludes that this AOP is now ready to be submitted to the EAGMST for further consideration for submission to the WNT.

Annex 1. Reviewers (Grouped by their regulatory and research experience)

Both Regulatory and Research Experience:

Bhaskar Gollapudi (BIAC): Exponent

Maria Donner (BIAC): Dupont

Kenichi Masumura (Japan): National Institute of Health Science

Masa Honma (Japan): National Institute of Health Science

Rosalie Elespuru (US): U.S. Food and Drug Administration

Maria Dusinska (Norway): Norwegian Institute for Air Research

Regulatory Experience:

Abigail Jacobs (US): U.S. Food and Drug Administration

Research Experience:

Les Recio (US): Integrated Laboratory Science

Annex 2: Summary of reviewer comments, author responses, reviewers/author discussion and final author responses

The final completed spreadsheet table contains 4 columns which were populated as follows:

- Reviewer comments (entered and then provided to the authors for their initial comments)
- Initial comments from authors (completed by the authors prior to the group discussion)
- Summary of the group discussion comments (completed during the group discussion)
- Action taken by the authors in response to the comments (completed by the authors after the group discussion)