**Internal review charge questions - February 2018**

## AOP Information

* AOP title: Histone deacetylase inhibition leading to testicular toxicity
* Author: Shihori Tanabe, Akihiko Hirose, Takashi Yamada (t-yamada@nihs.go.jp)
* Associated wiki page: <https://aopwiki.org/aops/212>

## Reviewers

**Primary Reviewer (PR):** Name: Dan Villeneuve; OECD Country/Org.: United States;

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#### Date review completed: 04/25/2018

## Review

**Section 1:**

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| **AOP identifier/Title***Does the name of the AOP follow the right convention (MIE or first KE leading to AO)?* *Does the name of the AOP reflect its content/domain?* |

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| **Reviewers' responses and comments** **PR:** Yes,broadly speaking the AOP title follows the guidance and reflects its content.**SR1:****SR2:** |

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| **Author response:** |

**Section 2:**

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| **Authors***Is it clear who the authors/developers of the AOP are?* *Contact information for one or more corresponding author(s) should be included.* |

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| **Reviewers' responses and comments** **PR:** Yes, the authors identity and affiliation information is provided. The point of contact and contributors are clearly noted.**SR1:****SR2:** |

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| **Author response:** |

**Section 3:**

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| **Date of updating***Reviewer should indicate the date stamp on the PDF snapshot under review.* |

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| **Reviewers' responses and comments** **PR:** Reviewing the content in the AOP-Wiki, last modified January 30, 2018, 20:54**SR1:****SR2:** |

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| **Author response:** |

**Section 4:**

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| **Abstract***Does the abstract concisely describe the main content of the AOP?* |

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| **Reviewers' responses and comments** **PR:** Yes. The abstract describes the main content and relevance of the AOP. It could probably benefit from some light editing to improve clarity.**SR1:****SR2:** |

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| **Author response:** |

**Section 5:**

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| **Molecular Initiating Event***Is a MIE described? If yes, then:* *Is the MIE description clear and is it biologically plausible?* *Is the MIE described in a way that allows its use in other AOPs?* *Are measurement/prediction methods specified and adequately described/referenced?* *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?* *Have chemical initiators (prototypical chemicals or chemical features) been identified?* |

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| **Reviewers' responses and comments** **PR:** The MIE is Event 1502. The MIE description is relatively clear and biologically plausible. See comment below regarding measurement method. The taxonomic applicability is defined quite narrowly based on where clear evidence exists. The authors might consider also defining the expected domain of applicability based on the conservation of HDAC as a target. Life stage and sex applicability of the MIE have not been defined. Those should be added if possible. A number of chemical initiators were identified using both structured chemical name fields and free text. * Event component terms should be added.
* Structured annotation terms for life stage and sex applicability should be selected if possible.
* The authors suggest it is measured/detected by measuring a decrease in histone acetylation. This seems both counter-intuitive and directly contradicts the next KE in the pathway which is an increase in histone acetylation. It also begs the question of whether this MIE can be measured independent of event 1503. Even if it can’t be measured directly, I guess I would favor still including this MIE, as one could conceivably used structure based approaches to identify chemicals likely to directly inhibit HDAC. This make a cleaner connection to a chemical category than the general measurement of histone acetylation, but if there are additional methods for more directly measuring the activity of HDAC, it would be nice to cite those.
* Please provide both first author and year information when citing references.

**SR1:****SR2:** |

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| **Author response:** |

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| Key Events*Are the KE descriptions clear on how the events work and are they biologically plausible?* *Are the KEs described in a way that allows their reuse in other AOPs?* *Are measurement methods specified and adequately described/referenced?* *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?*  |

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| **Reviewers' responses and comments** **PR:** Specific comments are provided for each KE. Comments that apply to all KEs are listed as “All”* All – Structured ontology terms defining the Key Event Components should be selected.
* All – structure terms for life stage, sex, and taxonomic applicability should be selected if possible. The authors cite taxa for which specific evidence of KE applicability is known. At least in the narrative, it would also be useful to identify the probable domain of applicability based on conservation.
* Event 1503 – description is adequate and plausible. Measurement approaches are specified. More details regarding the domain of applicability would be helpful.
* Event 1504 – description is adequate and plausible. Measurement approaches are specified. More details regarding biological context could be added.
* Event 1505. Rather than focusing on a description of cell cycle, the current description makes reference to upstream and downstream KEs and their connection to cell cycle. This may hinder the ability to use this KE description for other AOPs. Consider focusing the description more narrowly on cell cycle. The measurement methods (flow cytometry, FACS, live cell imaging) should be described without specific to HDAC inhibition. See general comments on domain of applicability.
* Event 1262. Description makes reference to both specific chemicals and other stressors that may cause apoptosis rather than simply describing what apoptosis is. Consequently, this event is not written in a particularly modular fashion and confounds content that should go in the KER descriptions with more general description of the biology that should be provided in the KE description. Relative to the methods, it is not clear how the last two sentences regarding proliferative/viability of NHDFs or proliferation of HDAC-/- cells represent a specific measure of apopotosis. More explanation is needed. See general comments regarding domain of applicability.

**SR1:****SR2:** |

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| **Author response:** |

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| **Adverse Outcome***Is an AO described? If yes, then:* *Is the AO description clear and is it biologically plausible?* *Is the AO described in a way that allows its use in other AOPs?* *Are measurement methods specified and adequately described/referenced?* *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?* *Has the regulatory relevance of the AO been described?*  |

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| **Reviewers' responses and comments** * **PR:** Event 1506 is the adverse outcome.
	+ The event title could probably just be “testicular toxicity” with spermatocyte depletion and testis atrophy/weight loss included as ways that testicular toxicity is measured.
	+ This KE description should not include reference to the effects of specific chemicals on sperm production.
	+ This KE is currently not described in a way that allows for/facilitates use in other AOPs.
	+ The text under how it is measured or detected appears to perhaps be cut and pasted directly from other papers in which these measurements were made. This section should be simplified. For example, state that testicular toxicity can be measured through documentation of spermatocyte depletion and/or testis atrophy/weight loss. Then describe generally how each of those are measured. Reference to specific chemicals, fixatives, reagents, etc. is probably too detailed. Readers can seek out the cited papers to find all those details. This is a case where too much information makes it difficult to use.
	+ Similarly, the domain of applicability statement should be much more clear and concise – this KE is applicable to these species, because……. – cite supporting literature.
	+ Regulatory significance of the AO is not addressed.

**SR1:****SR2:** |

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| **Author response:** |

**Section 6:**

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| **Key Event Relationships***Are the KERs well described and in a way that allows their use in other AOPs?* *Are the KERs biologically plausible and is there sufficient evidence presented?* *Is the level of empirical support adequately described in accordance with the OECD AOP Handbook?* *Are inconsistencies, uncertainties and level of confidence adequately described?* *Is the quantitative understanding of the KER described?"* *[refer to Tables 2 & 3 in the handbook]* |

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| **Reviewers' responses and comments** **PR:**General comments: Please select taxa, sex, and life-stage applicability terms for each KER. Generally speaking, much of the right types of information has been captured, but the information could be organized in a way that would make it much easier to digest and understand. The domain of applicability section in particular is a place where the authors should aim to provide a general statement clearly stating what they think the domain of appicability is, and then support that with specific data, citations, or lines of evidence.Relationship 1709: * The KER description contains redundant text. This could be reduced for example by stating that HDAC inhibition leads to increased histone acetylation and gene expression and citing papers that support that. There is no need to repeat the same statement for multiple references that essentially make the same point.
* Biological plausibility section lacks any supporting references. There is also reference to other post-translational modifications which do not appear to have immediate relevance to this KER or the AOP.
* The empirical evidence section would be easier to read if it were presented as a set of bulleted statements rather than a long paragraph essentially listing the same. A number of the statements do not specifically address the relationship between HDAC activity and histone acetylation. For example, “MAA-induced spermatocyte death is associated with histone acetylation increase” would be better suited to support a KER (non-adjacent) linking “histone acetylation increase” to testicular toxicity.
* Nice use of the uncertainties and inconsistencies section.
* The information in the quantitative understanding of the linkage section is good, but clarity to the reader would likely be increased if you were to start the section with the statement “A database containing high-accuracy, species-specific phophorylation and acteylation site predictors that allows for in silico prediction of sites…..”. An important part of the relationship also involves defining how much change in HDAC activity is needed to yield some unit/percentage change in histone acetylation. To the extent possible, it would be nice if the various subsections of the quantitative understanding of the linkage could be addressed. If there is no information available (for example for known modulating factors), please just indicate “no information currently available”
* The domain of applicability section contains some of the relevant information, but also some information that’s not relevant to this section. Some of the information provided here refers to the applicability of a single KE (e.g., hyperactylation or inhibtioin of HDAC), but not necessarily the connection between the two. Consider moving some of the support that pertains to just single KEs into the KE descriptions. Also, an organization of the text that starts with a clear statement of the applicable taxa, “the relationship between HDAC inhibition and hyperacetylation is likely well conserved between species from lower organisms to mammals”, and then placing bullets providing specific supporting information below.

Relationship 1710: * The KER description should provide just a brief summation and any specialized context that might be needed. The current version includes much of what would be viewed as empirical support for the linkage, rather that a brief descriptive summation.
* Empirical support section makes reference to a lot of evidence that links HDAC inhibition (exposure to HDIs) with p21 expression. Strictly speaking that information should align with a non-adjacent KER linking the MIE to p21 expression increase (relationship 1714). Empirical support for this relationship should focus on the connection between measurement of increased acetylation and increased p21 expression only.
* Nice use of the uncertainties and inconsistencies section.
* Relative to quantitative understanding, the authors are capturing the right kind of information, but it needs to be synthesized and presented in a more straight-forward manner for the reader. How long after histone aceytlation occurs can increased p21 expression be observed? How much increase in acetylation is needed for an increase in p21 to be detected? Clear statements addressing these kinds of questions are needed, with supporting information provided to back it up. Right now, the reader gets lost in the details. The text doesn’t extract the relationship for the reader.
* The domain of applicability information provided here is more relevant to relationship 1714. No reference to increased aceytlation is made.

Relationship 1711.* The description text here is pretty good. Sticks closely to the relationship between p21 and cell cycle.
* The biological plausibilty is not real clear though. Some of the KER description text could be moved into biological plausibility, with just a clear statement of what the relationship is and the context in which it operates in the KER description section.
* The first sentence clearly supports the link between p21 and cell cycle disorder. The others link p21 with apoptosis and cell death without a clear explanation of how either of those relate to cell cycle.
* Uncertainties and inconsistencies – it was very easy to get lost in the detail here. I wasn’t sure what the take home message was in terms of the potential uncertainties I should be aware of were.
* Quantitative understanding – the informaiton contained here needs to be synthesized and extracted into a form that’s clearer and easier for a reader to use.
* Domain of applicability – again, details without a sythesizing thesis statement. Provide a clear statement regarding domain, then provide detailed support as bullets, where possible.

Relationship 1712.* In terms of biological plausibility, the right kind of information was present, but it is not organized in a way that makes it real accessible.
* It was unclear how the empirical evidence cited speaks to this particular KER, same applies to the uncertainties and inconsistencies section.
* The authors provide methods for measuring apoptosis and caspase activity – if anything, these should go on the event pages. They do not provide quantitative understanding of what magnitude of cell cycle disorder leads to apoptosis and under what conditions.
* Domain of applicability. Support for applicability to humans and mice is provided, but a clear statement regarding the applicability of this KER is lacking.

Relationship 1713.* The KER description should summarize the basic structural or funcational association between the two KEs being linked together. The information there right now is better suited for empirical evidence.
* Biological plausibility – at present only describes what apoptosis is. It does not describe how increased apoptosis can result in testicular toxicity.
* The first sentence under uncertainties and inconsistencies refers to the realtionship between HDAC inhibition and testicular toxicity, not between apoptosis and testicular toxicity. Not appropriate content for this KER page.
* The information provided in the quantitative understanding does not relate a magnitude of measured apoptosis with decreased spermatocytes or reduced testis size.
* Clear statement regarding the domain of applicability is lacking.

Relationship 1714.* Consider making the KER description simpler and moving the supporting data to the empirical evidence section.
* The information currently presented under biological plausibility would be more appropriate under empirical evidence (in my opinion).
* The current information under empirical evidence requires more explanation. At present it is unclear how either of these sentences/statements support the KER.
* The uncertainties and inconsistencies section refers to KEs that are not part of the present KER. Focus should be on the link between HDAC inhibition and p21 expresion, with possible mention of biology lying in between, but not biology that is further downstream.

Relationship 1715.* Information provided in the KER description is better suited for empirical support. The description section should briefly define what the connection is between HDAC inhibition and cell cycle regulation.
* Information in the biological plausibility section would fit better under empirical evidence.

Relationship 1716.* The first two sentences of the KER description provide a useful summation. The rest of it should be organized under empirical evidence.
* Information provided under biological plausibility is empirical evidence.

Relationship 1718.* Information in the KER description and biological plausibility sections are probably more suited to empirical evidence.
* Although the sentence under quantitative understanding provides some quantitative information, it does not address how much inhibition of HDAC is needed to yield testicular toxicity, how quickly that occurs, and whether other factors are known to modulate that relationship.

**SR1:****SR2:** |

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| **Author response:** |

**Section 7:**

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| **Overall Assessment of the AOP** *Is the domain of applicability of the AOP defined appropriately?* *Is the level of support for essentiality of the KEs adequately described and assessed?* *Has the degree of quantitative understanding of KERs been assessed properly?* *Has consideration been given to the overall weight of evidence for the AOP?* *Are the calls on Overall WoE and Quantitative Understanding supported?* |

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| **Reviewers' responses and comments** **PR:*** Life stage and sex applicability are defined in the structured fields on the AOP page, even though that information is missing on the KE and KER pages.
* The level of support for essetiality of the KEs and the overall rationale for the evidence calls provided in the KER table is not very well described here. I would urge the authors to take a closer look at Section 4 of the handbook, and the guiding questions provided in Annex 1 and Annex 2. It is not necessary to repeat supporting data here. What is most important is to provide the rationale for assigning calls of high, moderate, or low.
* There is no need to copy the structured domain of applicability tables to the free-text domain of applicability section. This section should be used to provide any additional rationale or characterization that is not readily expressed in the machine-readable structured fields.
* The overall assessment of the AOP needs some work.

**SR1:****SR2:** |

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| **Author response:** |

**Section 8:**

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| **Potential application of the AOP (optional):** *Is any context provided as regards the reason for development or the intended use?* |

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| **Reviewers' responses and comments** **PR:** Yes, some context and rationale is provided.**SR1:****SR2:** |

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| **Author response:** |

**General Observations and Conclusions of the Reviewer**

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| **Reviewers' responses and comments** **PR:**Generally speaking the AOP seems plausible and lots of good supporting information has been collected. However, at present much of it is just presented as long lists of facts/supporting information. This is particularly true on the KER pages. The facts provided are often not translated in a way that makes the key points clear to a reader who is unfamiliar with all the details. It is recommended that the authors should focus on re-organizing many of the sections into a format in which a clear statement about the domain of applicability, different types of support, etc. are made for a KER, then the supporting data/details are provided as bullets below. As suggested by SR2, organizing the empirical evidence into subsections for data that address dose-response concordance, temporal concordance, etc. specifically may help. Overall, the goal is not to simply throw up as much data as possible and then let the reader sort it out, but rather to communicat the story as simply as possible, while also providing the data and references that back up the clear concise statements. In its present form, much of this AOP will be inaccessible to many readers, even those with a reasonable background in biology. That will make the AOP difficult to use. Consequently, time spent making the key points easier to pick out and understand will have significant benefits in the longer term.With regard to key events, the probable taxonomic domains of applicability should probably be broadened. Even though data supporting this AOP may have been primarily derived from human, mouse, and rat models, these are pathways that are likely conserved across a broad range of eukaryotic organisms. Early key events are probably even relevant to a number of species that lack testes. While a narrow range of taxonomic applicability may be defined using structured fields, it would be useful to try to define the probable taxonomic domain and a rationale for it in the free text section of the pages.SR2 raises a good point that there are numerous existing KEs for apoptosis in the AOP-Wiki. It might be worth taking a look at some of those other pages and see if there is an existing one, already connected to other AOPs in the wiki that would be suitable for the current AOP. If so, such consolidation can aid in network building etc.The authors have done a nice job of filling in the KER tables with “calls” of High/Moderate/or Low for Evidence and Quantitative Understanding. However, it is important that the rationale/justification for these calls be presented on the AOP page (overall assessment of the AOP section). It is not necessary to reiterate all the data assembled on the KER pages, etc. However, the authors should consider the guiding questions laid out in Annex 1 and Annex 2 of the “User’s Handbook” ( [https://one.oecd.org/document/ENV/JM/MONO(2016)12/en/pdf)](https://one.oecd.org/document/ENV/JM/MONO%282016%2912/en/pdf%29), and explain how consideration of those questions led to selection of high/moderate/or low weight of evidence.Finally, although this was perhaps not described in the Handbook or available in the AOP-Wiki when this AOP description was first developed, it would be useful for the authors to select “Key Event Component” terms relevant to their KEs. This aids in making the AOPs more machine-readable for the purpose of AOP network construction.**Overall, I would recommend that the AOP (and associated KE and KER) description be revised before moving to the external review stage.** However, based on what the authors have assembled, with some reorganization and a bit more synthesis and explanation of the current content, I would envision that this AOP may be able to move to external review after the major comments outlined here and by SR1 and SR2 have been addressed.Thank you for your patience. My apologies for the short delay in providing my review.**SR1:****SR2:** |

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| **Author response:** |