**Internal review charge questions - November 2017**

## AOP Information

* AOP title: Chronic Cyp2E1 Activation Leading to Liver Cancer
* Author: Carole Yauk, Francina Webster
* Associated wiki page: <https://aopwiki.org/aops/220>

## Reviewers

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

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#### Date review completed:

## 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:**  **SR1:**  **SR2:** Not quite and I think this AOP highlights two issues for the EAGMST to consider in its guidance.  Firstly, the guidance doesn’t mention how to handle a requirement for time-dependence in an AOP title – and in my view AOPs don’t handle time very well in general. So although the use of the word “chronic” in the title isnt excluded by the Guidance, I think it is implicit that temporal requirements are handled in the temporal concordance details of the AOP (ie in the KERs) rather than in its title. This is debateable. But if that was the intent of the guidance perhaps “chronic” should be removed; however, if the sustained nature of the MIE is essential to this AOP argueably it should remain.  Secondly, when MIEs have used activation or inhibition of a protein in the past I think they meant that the intrinsic functional activity of the protein is changed due to the effects of some stressor. In this case, I think the authors intent is not quite this so perhaps the use of activation isnt appropriate? Are you saying that an increase in the catalytic activity of Cyp2E1 is the MIE (perhaps due to substrate-dependent stabilisation of the enzyme), or that the sustained production of reactive metabolites due to a chemical reaction catalysed by Cyp2E1 is the MIE (that is chronic substrate metabolic acitvation ie the MIE), or is it both of these?  Perhaps it would be better described as (chronic) Cyp2E1-catalysed production of reactive oxygen and electrophilic metabolites leading to liver cancer. |

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

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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:**  **SR1:**  **SR2:** 2017-11-17 10:02 |

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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:**  **SR1:**  **SR2:** there are some differences. In the abstract the authors mention Cyp2e1-dependent generation of reactive metabolites and decoupling produced reactive oxygen species, however in the detailed MIE discription they also mention substrate-dependent stabilisation of cyp2e1. Is this the “activation” of cyp2e1 catalytic activity they mention. |

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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:**  **SR1:**  **SR2:**  *Is a MIE described?* ***YES*** *If yes, then:*  *Is the MIE description clear and is it biologically plausible?* **yes**  *Is the MIE described in a way that allows its use in other AOPs?* **yes**  *Are measurement/prediction methods specified and adequately described/referenced?* **Somewhat superficial in detail but adequately referenced.**  *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?* **Not really, they only include information on presence in primates and rodents. It isnt clear what wider taxa orthologous genes exist.**  *Have chemical initiators (prototypical chemicals or chemical features) been identified?* ***A few examples are named and many more referenced in papers.*** |

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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:**  **SR1:**  **SR2:**  ***Key event 1: oxidative stress***  *Are the KE descriptions clear on how the events work and are they biologically plausible?* **Yes. (reuse of an existing KE)**  *Are the KEs described in a way that allows their reuse in other AOPs?*  ***Yes***  *Are measurement methods specified and adequately described/referenced?*  **A bit superficial and only based on NRF2. In this case they might have considered adding to the KE “how it is measured” section the regulation of thioredoxin-ASK1/ Jnk/p38 pathway as an additional option for the CYP2e1 mediated mechanism (see Wu D, Cederbaum A. Activation of ASK-1 and downstream MAP kinases in cytochrome P4502E1 potentiated tumor necrosis factor alpha liver injury. Free Radic Biol Med. 2010 Aug 1;49(3):348-60)?**  *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?*  ***Yes***  ***Key event 2: hepatocytotoxicity***  *Are the KE descriptions clear on how the events work and are they biologically plausible?* **yes**  *Are the KEs described in a way that allows their reuse in other AOPs?*  ***No*, this Key event is actually a group that describes 3 separate processes and each is qualitatively different in terms of how it is detected/diagnosed by pathologists and what causes it. Used as described here, it prevents people specifically proposing only one of these as a key event in another AOP. I would recommend these be split out.**  *Are measurement methods specified and adequately described/referenced?*  **Partly. A bit superficial in the detail and lacks any details on how the ASK1 pathway would be measured. The authors make the comment that H&E sections examined for the presence of cytotoxicity, which is true by heptocyte toxicities are typically described as apoptosis or degeneration – the term cytotoxicity is not used. Therefore this could be confusing.**  *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?*  *yes*  ***Key event 3: regenerative proliferation***  *Are the KE descriptions clear on how the events work and are they biologically plausible?* AND *Are the KEs described in a way that allows their reuse in other AOPs?*  **I don’t think they are clear or allow reuse. Regenerative proliferation isnt a description of a pathology observation made in hepatocytes: ie it isnt an acutal thing that you can measure and therefore isnt a key event. Regenerative proliferation is typically used as a summary description of the process by which necrosis/apoptosis induced loss of hepatocytes is counterbalanced by proliferation of hepatocyte- i.e. a description of the key event relationship. I havent seen any evidence that the key event you measure: the proliferation induced by a cytotoxic stimulus is qualitatively different from the proliferation induced in hepatocytes by other stimuli such as hepatocyte mitogens. If the proliferation is qualitatively different included the evidence for it, if it isnt just call this key event hepatocyte proliferation and move the regeneration process description to the key event relationship. This will allow proliferation to be a reusable key event.**  **The description is focused on liver regeneration after partial hepatectomy and is highly superficial for a complex process (see for instance recent reviews Alvarez-Sola et al: Bile acids, FGF15/19 and liver regeneration: From mechanisms to clinical applications. Biochim Biophys Acta. 2017 Jul 12. pii:S0925-4439(17)30222-3 or Michalopoulos: Hepatostat: Liver regeneration and normal liver tissue maintenance. Hepatology. 2017 Apr;65(4):1384-1392).**  *Are measurement methods specified and adequately described/referenced?*  **For proliferation yes. For the processes stimulating that proliferation, no. Nor is there any proposed measure for the proliferation and transdifferentiation of stem cells that could occur.**  *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?* |

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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:**  **SR1:**  **SR2:**  *Is an AO described?* ***YES***  *Is the AO description clear and is it biologically plausible?* **YES**  *Is the AO described in a way that allows its use in other AOPs?* **NO, other AOPs have used liver adenoma or carcinomas to describe their liver cancers. Perhaps reuse of these terms would be a better way to enable reuse?**  *Are measurement methods specified and adequately described/referenced?* **Lacking in specific detail but referenced yes**  *Is the biological context (inc. taxonomic applicability/relevance, level of biological organisation) specified and explained sufficiently?* **yes**  *Has the regulatory relevance of the AO been described?****no*** |

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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:**  **SR1:**  **SR2: I think the descriptions of the KERs were generally well described. I would say that there is lot of detail hidden in the KERs that could be used to describe additional key events in this AOP but for some reason the authors have chosen to chunk and not split KE and KERs. As the mechanisms of the hepatostat continue to be uncovered it is likely that there will be more MIEs identified and they will need KE and KERs at a highly grnaular detail to enable reuse in AOP networks- so I would encourage you to split KE and KERs as much as you can given the available data.**  **When describing the quantative nature of the regenerative proliferation KER (cytotoxicity to proliferation) they use the term “significant amounts of hepatotoxcity” but do not define what this means.** |

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

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| **Author response:** |

**General Observations and Conclusions of the Reviewer**

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| **Reviewers' responses and comments**  **PR:**  **SR1:**  **SR2: I think this is a well written AOP that reviews and integrates a complex and well studied area of science. I think our goal to enable reuse of key events would benefit from the authors splitting their propsed KE into their component parts to capture some of this complexity. Methods are often described in a superficial way. Their use of the term CYP2e1 activation is ambiguous in meaning and should be clarified.** |

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| **Author response:** |