Meeting Report

Report of the 3rd and 4th Mystery of Reactive Oxygen Species Conference

doi: 10.14573/altex.2307041

1 Introduction

The interconnected function of reactive oxygen species (ROS) in the adverse outcome pathway (AOP) framework is being discussed in an international consortium, the Mystery of ROS (MoR). At the MoR I and II conferences in 2021, the consortium created harmonized key events (KEs) on ROS (Tanabe et al., 2022a). Continuing discussions are focused on refining the description of ROS-related KEs in the AOP framework, terminologies, the mechanistic understanding of ROS production by endogenous and exogenous stimuli, and the stress responses associated with ROS. MoR (III and IV) were held on May 10, 2022, and May 11, 2023, respectively.

An AOP begins with a molecular initiating event (MIE), leading through sequenced key events (KEs), which are linked with key event relationships (KERs), to an adverse outcome (AO). The AOPs containing ROS-context have been developed in the AOP Knowledge-Base as part of the Organisation for Economic Co-operation and Development (OECD) projects[1] and the AOP-Wiki[2], where harmonization of the KE nodes to create grouped and representative KEs is in great demand. We have created KE1940[3], mainly focused on the upregulation of ROS, and KE1869[4], mainly focused on the depletion of the protective oxidative stress response.

2 Summary of MoR III

The meeting consisted of four presentations (Tab. 1), followed by free discussion and surveys.

Based on surveys, a discussion and a vote, the title of KE1940 was changed to "Increases in cellular ROS" from "Up-regulation of ROS", which better captures the positive production of ROS versus increased ROS in the disease progression state. Also based on surveys, a discussion and a vote, the title of KE1869 was changed to "Diminished protective response to ROS" from "Depletion of protective oxidative stress response". For KE1869, the enzymes are the main object, including those involved in the Nrf2-mediated oxidative stress response. The measurements in KE1869 distinguishing between oxidative stress response depletion and ongoing oxidative stress response were described.

The main MoR discussion led to further suggestions on KE terminology, including ensuring coherence to directionality in terms of the KE descriptions (e.g., specifying increase, decrease, altered, no direction, etc.) and clarifying differences in ROS and reactive oxygen and nitrogen species (RONS), and enzymatic and non-enzymatic events. The consortium highlighted the importance of the role of ROS as a KE and an associative event in the AOP framework. Additionally, participants highlighted modification to macromolecules from the resultant RONS generation (e.g., lipid peroxidation) as a relevant endpoint to include in the KE. The possibility of grouping ROS-related KEs in the AOP framework needs to be discussed further.

Tab. 1: Outline of MoR III

	Title	Presenter
1	ROS-related KEs (KE1940 and 1869) in development of AOPs	Dr Shihori Tanabe
2	Ang II induces ROS production mediated by NADPH oxidase	Ian Choi and Dr Young-Jun Kim
3	"Multicellular 3D neurospheres" responses to oxidative stress: Generation of an AOP for developmental neurotoxicity	Prof. Dr Ellen Fritsche
4	ROS from the radiation perspective	Prof. Dr Knut Erik Tollefsen

3 Summary of MoR IV

The meeting consisted of four presentations (Tab. 2).

The concept of ROS-associated events was introduced (Tanabe et al., 2022b), and ROS-related KEs in the AOP-Wiki were analyzed in terms of the KE components used to describe them. KE components include controlled vocabulary terms for the process, object and action covered in the KE description. The controlled vocabulary terms are sourced from a collection of 22 biological ontologies (Ive et al., 2017; Developers' Handbook 2.6[5]). Each KE can be described by at least 2 KE components, process and/or object terms and an action term (Tab. 3).

[1] https://aopkb.oecd.org/ (accessed 23.06.2023)
[2] https://aopwiki.org/ (accessed 23.06.2023)
[3] https://aopwiki.org/events/1940 (accessed 23.06.2023)
[4] https://aopwiki.org/events/1869 (accessed 23.06.2023)
[5] https://aopwiki.org/handbooks/4 (accessed 04.07.2023)

Tab. 2: Outline of MoR IV

	Title	Presenter
1	Current status of increased ROS and diminished protective oxidative stress response	Dr Shihori Tanabe
2	Key event components in AOP development	Dr Julija Filipovska
3	Emphasizing the role of test methods in the AOP-Wiki	Dr Clemens Wittwehr
4	Lessons learned from the AOP collaboration on COVID-19	Dr Laure-Alix Clerbaux

A single KE can be described by more than one combination of KE descriptors (e.g., KE1869). Table 4 represents the ROS-related KEs that have been incorporated in AOPs adopted within the OECD or undergoing development.

Inclusion of the KE components in the AOP-Wiki aims to facilitate the generation of AOP networks by using shared KE elements in synonymous KEs, making them more machine-readable. However, KE component descriptions have not been widely used yet by developers (also for ROS-related KEs, see Tab. 4) for various reasons, including the diversity and complexity of the different ontologies available and the difficulty in assessing the hierarchical aspects of ontologies in the current AOP-Wiki lists. Many other reasons, like the narrative preference of human developers and chronic lack of resources for AOP development, may also contribute.

At MoR IV, the alignment between the KE components and the KE description and how it is measured or detected was explored for several KEs. Despite the slightly different KE titles, the KE descriptions in several KEs, particularly those within the oxidative stress and ROS class (Tab. 4), are almost identical in their biologically relevant content.

Further analysis and alignment of the content of the KE with its KE components represents an opportunity for harmonization of the ROS-related KEs. The use of multiple combinations of KE components that would cover all the described objects and relevant processes in the KE may be particularly useful in delineating subtle differences to address the needs of some of the developers of the different KEs (e.g., KE1753 has been created because it is important to have chronic ROS, not just instantly increased ROS) while also harmonizing the knowledge and references on ROS and oxidative stress perturbations under common terms if not under common KEs. There is a possibility to develop KERs between combinations of KE components instead of entire KEs that may be helpful and encourage the use of these controlled vocabularies. Further work is needed to evaluate all ROS-related KEs from the perspective of KE component descriptions.

The initiative Methods2AOP (Wittwehr et al., 2023), which will emphasize the role of test methods in the AOP framework (and consequently in the AOP-Wiki), will result in an increased application of ontologies across KE components, test method descriptions, and actual substance data (ideally described in the OECD Harmonised Template format). Highlighting the role of test methods as the missing link between AOPs and substance data has the potential to underpin the association of ROS-relevant compounds with their corresponding AOPs.

The final presentation shared the lessons learned from the CIAO project[6], an interdisciplinary AOP-based collaboration that aimed at organizing the dispersed and fast-evolving knowledge on COVID-19 pathogenesis (Nymark et al., 2021; Wittwehr et al., 2021; Clerbaux et al., 2022c,d). Over three years (2020-2023), CIAO involved over 70 scientists from different countries and backgrounds, including toxicology, virology, computer science, and pharmacology. Numerous COVID-19 related AOPs were developed, resulting in various AOs such as respiratory symptoms (e.g., acute respiratory distress and lung fibrosis), short-term anosmia and other neurological syndromes (Hogberg et al., 2022; Shahbaz et al., 2022), intestinal disorders (Clerbaux et al., 2022c), gut dysbiosis (Clerbaux et al., 2022b), or liver disorders (Vinken, 2021). Oxidative stress has been identified

Tab. 3: Key event (KE) components in the AOP-Wiki

Process	Object	Action
Dynamics of the underlying biological system (e.g., receptor signaling, biomolecule synthesis process)	Subject of the perturbation (e.g., a specific biological receptor/enzyme that is activated or inhibited)	Direction of perturbation of this system (process of object) Phenotypic quality ontology (PATO) based: increase, decrease, morphological change, functional change, occurrence, disrupted, delayed, premature, abnormal, and pathological
Context of perturbation (movement, relevant interactions)	What is perturbed	How it is perturbed
Includes indirect measurements of the object	What is measured	Result of the measurement compared to steady state or no stress

6 https://www.ciao-covid.net (accessed 23.06.2023)

Tab. 4: ROS-related key events (KEs)

KE ID	Title	Level of organization	Process	Object	Action	# of AOPs[a]	Level of development
1392	Oxidative stress	Molecular	oxidative stress		increased	17	Y[b]
1088	Increased, oxidative stress	Molecular	oxidative stress		increased	12	empty
211	Propagation, oxidative stress	Molecular	oxidative stress		increased	2	empty
1869	Diminished protective oxidative stress response	Cellular	1. cellular response to oxidative stress 2. response to reactive oxygen species	1. reactive oxygen species 2. reactive oxygen species	1. increased 2. increased	5	Y
1279	Increase, oxidative stress / Activation, PMK-1 P38 MAPK	Cellular	1. oxidative stress 2. signalling	1.– 2. MAP kinase p38	1. increased 2. increased	1	empty
1487	Binding, thiol/seleno-proteins involved in protection against oxidative stress	Molecular				2	Y[b]
1969	Increase, oxidative stress	Molecular				3	Y
1510	Oxidative stress in brain	Molecular				2	Y
1538	Decreased protection against oxidative stress	Cellular				2	Y[b]
1753	Chronic reactive oxygen species	Molecular	response to reactive oxygen species	reactive oxygen species	increased	1	Y
257	Increase, reactive oxygen species production	Molecular	reactive oxygen species biosynthetic process	reactive oxygen species	increased	9	empty
1940	Increases in cellular reactive oxygen species	Molecular	reactive oxygen species biosynthetic process	reactive oxygen species	increased	1	Y
249	Production, reactive oxygen species	Tissue	reactive oxygen species biosynthetic process	reactive oxygen species	increased	1	empty
1632	Increase in RONS	Molecular				5	Y
1364	Increase, reactive oxygen species	Molecular				4	empty
1546	Increased reactive oxygen species (in the mitochondria)	Cellular				1	empty
1634	Increase, oxidative damage to DNA	Molecular	regulation of response to reactive oxygen species	reactive oxygen species	occurrence	4	Y[b]
356	Increased, oxidative damage	Individual	oxidative stress		increased	1	empty
1608	Increase, oxidative DNA damage	Molecular				2	empty

[a] as of June 5, 2023; [b] part of OECD-endorsed AOP (as of June 5, 2023); Y: Yes

as an event involved in the pathophysiology of COVID-19. In AOP379, the viral replication of SARS-CoV-2 (AOP430) was proposed to lead to a diminished protective oxidative stress response (KE1869) inducing coagulation and thrombosis. In AOP319, following binding to the angiotensin-converting enzyme 2 (ACE2) receptor and consequent increase of angiotensin II, the activation of angiotensin II receptor type 1 receptor (AT1R) leads to increased ROS (KE1115) activating nuclear factor kappa B (NF-kB) with ultimately collagen accumulation and lung fibrosis as outcome. Applying this AOP-aligned approach in the pandemic context enabled the identification of current knowledge gaps, orientating further research, and allowed to better understand how different known risk factors mechanistically influence COVID-19 progression and outcomes (Clerbaux et al., 2022a). As in the MoR consortium, the CIAO project showcased that the AOP framework facilitates expertise synergy from different disciplines to address public health issues and works as an efficient conceptual mediator for a crowdsourcing model of collaboration (Carusi et al., 2023).

4 Conclusion

More than 70 people from over 20 countries have been involved in the MoR consortium. The MoR consortium aims to harmonize ROS-related KEs, and outcomes of these efforts will be incorporated into the AOP framework to elucidate ROS-related diseases and associated outcomes.

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Conflict of interest

Anna Goralczyk and Jorge Pereira are employed by Philip Morris International (PMI).

Acknowledgments

The authors would like to acknowledge Dr Claudia Torero Gutierrez, Prof. Timothy W. Gant, Dr Sabina Halappanavar, Dr Dan Villeneuve, Prof. Pietro Ghezzi, Dr Ed Perkins, Dr Penny Nymark, Dr Maria João Amorim, Dr Jennifer E. Kay, Nathalie Delrue, Sofia Batista Leite, Anna Lanzoni, all participants in MoR, CIAO members, OECD AOP Coach Team members, and OECD EAGMST members. The author would like to thank members of the National Institute of Health Sciences (NIHS), Japan. This work was supported by Japan Agency for Medical Research and Development (AMED) Grant Number JP21mk0101216 (S.T.) and JP22mk0101216 (S.T.), JP23mk0101216 (S.T.), Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 21K12133 (S.T.), Research Council of Norway (RCN) through its Centre of Excellence (CoE) funding scheme (grant

no. 223268), and NIVAs Computational Toxicology Program, NCTP (www.niva.no/nctp grant no. 160016) (K.E.T.), RCN through the research project RiskAOP (grant no. 301397) (Y.S.), the European Union's Horizon 2020 research and innovation programme under grant agreement No 857560 (I.S.), and the National Research Council of Science & Technology (NST) grant by the Strategies for Establishing Global Research Networks (No. Global-22-000) (Y.K., I.C.). This work was supported by the Ministry of Health, Labour, and Welfare (MHLW), Japan.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the authors' organization, the European Commission in European Union (EU), JSPS, Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), or MHLW in Japan.

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