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SKIG Report 2023-2024

2023-2024 Report of the
Society for the Advancement of AOPs Knowledgebase Interest Group

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Contents

Abstract

The Society for the Advancement of AOPs Knowledgebase Interest Group (SKIG) is a vibrant assembly of more than 40 international experts focused on advancing the Adverse Outcome Pathways (AOP) framework. SKIG operates through regular online meetings, where two or three presentations per session—followed by lively Q&A interactions—delve into both technical and scientific topics critical to the AOP domain, with a particular focus on AOP-Wiki related issues. Presentations cover a broad spectrum of subjects, such as ontology-based harmonization, AI tools for AOP development, the integration of omics data, incorporating temporal aspects into AOPs, automated access to AOP-Wiki contents, the role of physiological maps and other approaches in establishing biological relevance, and many more. This caters to a diverse audience of scientists, regulators, and policymakers, and this dynamic approach allows readers to engage with specific subjects of interest rather than following a chronological sequence when perusing this report. The document also offers a concise summary of practical adaptations implemented in the AOP-Wiki following meeting discussions. Covering the meetings held in 2023 and 2024, the report reflects SKIG's ongoing contributions and indicates that the group will continue to operate in 2025 and beyond. SKIG's work to foster the development and application of AOPs is pivotal in supporting the European Union's policy on the protection of animals used for scientific purposes (Directive 2010/63/EU) and the European Commission's Roadmap towards phasing out animal testing for chemical safety assessments.

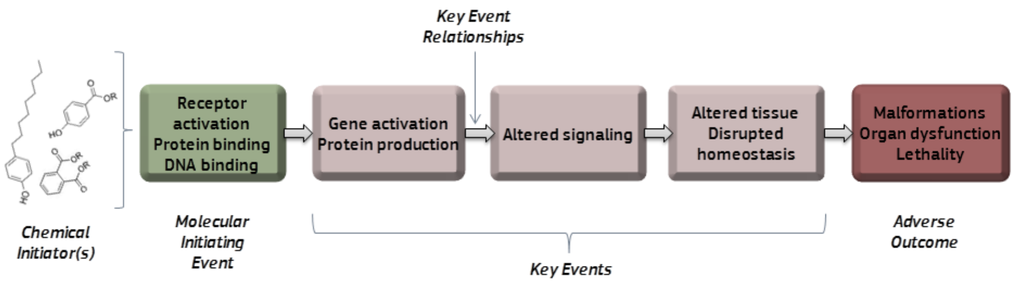
1 Introduction

1.1 Adverse Outcome Pathways

1.1.1 Background

Regulatory Toxicology encompasses the collection, processing, and evaluation of epidemiological as well as experimental toxicology data to permit toxicologically based decisions directed towards the protection of health against harmful effects of chemical substances. In regulatory toxicology, an Adverse Outcome Pathway (AOP) is a conceptual construct that portrays existing knowledge concerning the linkage between a direct Molecular Initiating Event (MIEs) and an Adverse Outcome (AOs), via Key Events (KEs), connected by Key Event Relationships (KERs) at a biological level of organization relevant to hazard and risk assessment.

Figure 1. Adverse Outcome Pathways

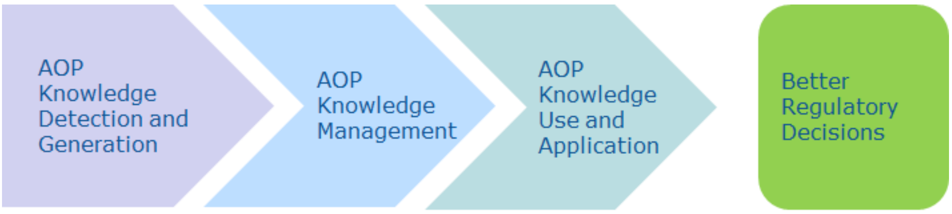


Source: adapted from freely available material for this publication

Efforts are underway to transform regulatory toxicology and chemical safety assessment from a largely empirical science based on direct observation of apical toxicity outcomes in whole organism toxicity tests to a predictive one in which outcomes and risk are inferred from accumulated mechanistic understanding. The AOP Framework provides a systematic approach for organizing knowledge that may support such inference. The framework is championed by the Organisation for Economic Co-operation and Development (OECD), and organisations like the Systems Toxicology Unit of the European Commission's Joint Research Centre (JRC) and the US Environmental Protection Agency (US EPA) play a crucial role in its further development and promotion.

AOP knowledge is detected, generated, processed, managed, and applied in three steps to improve regulatory decisions:

Figure 2. The three steps to improve regulatory decisions



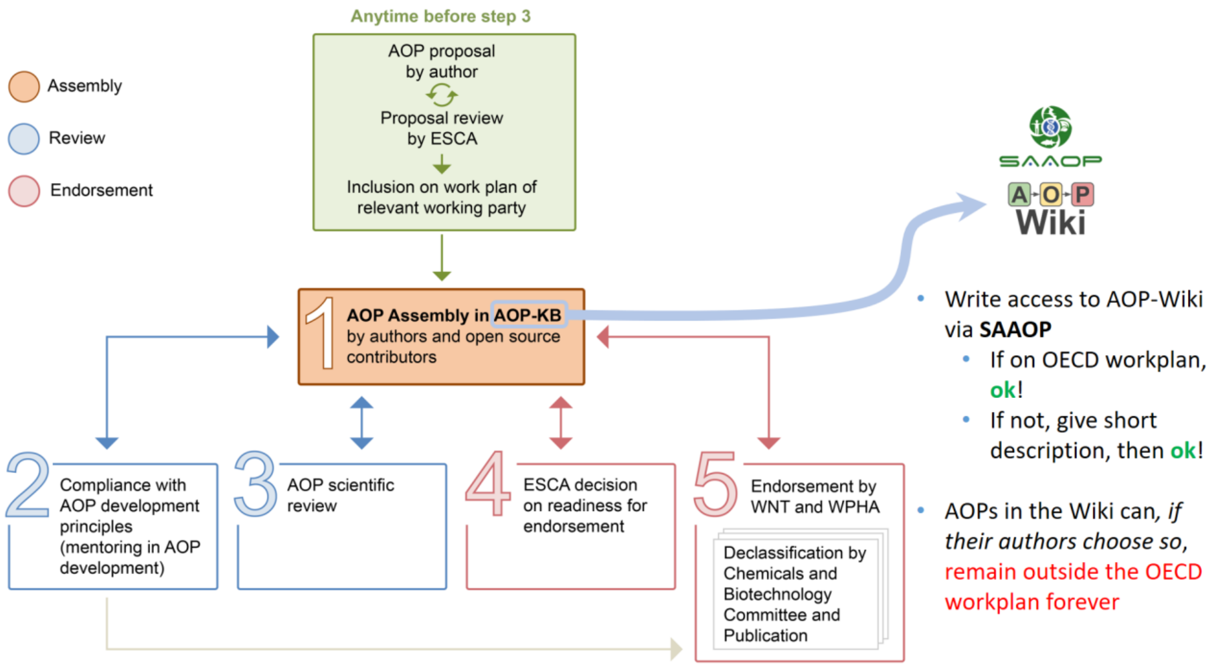
Source: created for this publication

1.1.2 The AOP development and review workflow

Parties who want to support any of these three steps by developing AOPs are requested follow the rules laid out in (OECD, 2018), and subsequent updates published at the AOP-Wiki[1], which are detailed guidelines for the development and review of AOPs.

In their individual lifecycles, AOPs typically follow the workflow shown in **Figure 3**:

Figure 3. AOP Lifecycle



Source: based on OECD website[2], adapted for this publication

1. Parties who intend to develop an AOP need to apply for write access to the AOP-KB/Wiki (see Section 1.2). It is important to note that potential AOP developers can freely choose whether they want their AOPs to become part of the OECD workplan or not. Regardless, the application for AOP-Wiki write-access requires review and approval by the Society for the Advancement of AOPs (SAAOP, see 1.3.1.2).
2. AOP developers start their work in the AOP-Wiki.

At this point, the author can decide to apply for the AOP to become part of the OECD workplan or not.

Only for AOPs on the OECD workplan:

3. The AOP is scientifically reviewed via the OECD.

[1] https://aopwiki.org/handbooks/

[2] https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/adverse-outcome-pathways.html

4. The Advisory Group on Emerging Science in Chemicals Assessment (ESCA, see 1.3.1.1) assesses the readiness of the AOP for endorsement.

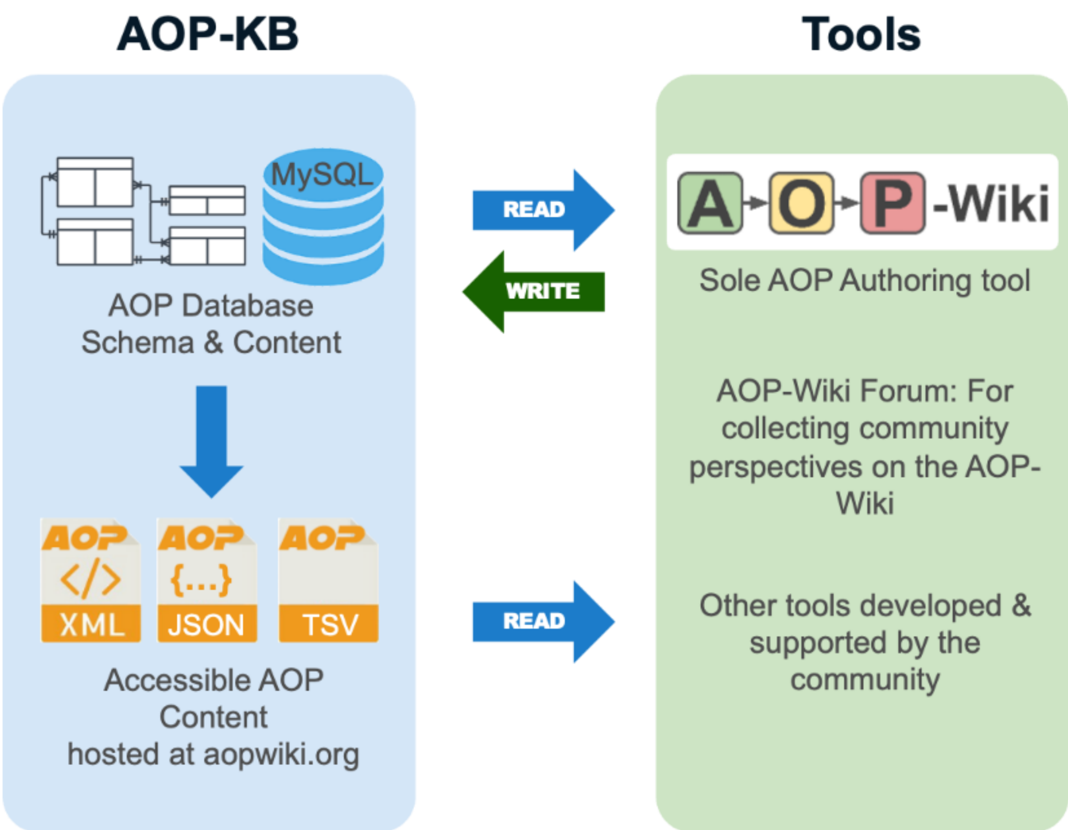
5. As a final step, the AOP can be endorsed by the OECD, representing a significant level of recognition in an AOP's lifecycle.

Scientifically, AOPs within and outside the OECD workplan are equally valuable, and they can freely share knowledge (KEs, KERs, ...) with other AOPs.

1.2 The Adverse Outcome Pathway Knowledgebase

In order to manage the process laid out in section 1.1.2 in one central location, the OECD launched a project in 2013 to develop the "Adverse Outcome Pathway Knowledge Base" (AOP-KB). The AOP-KB allows all stakeholders to build AOPs by entering and then linking information about MIEs, KEs, AOs and Chemical Initiators. Knowing that pathway elements are not necessarily unique to a single AOP, value is added to existing knowledge by facilitating the re-use of MIE, KE and AO information in multiple AOPs, which prevents redundancy and make the collective knowledge about those entities available in all AOPs in which they appear:

Figure 4. The AOP-KB and the AOP-Wiki



Source: https://aopwiki.org/info_pages/10

The AOP-KB (left side of **Figure 4**) is the primary central repository for all AOPs developed either as part of the OECD AOP Development Programme, or by the larger scientific community. The AOP-KB Tools (right side of **Figure 4**) are a collection of web-based resources that are constantly undergoing development and refinement. Together, the AOP-KB and accompanying tools aim to bring together knowledge and evidence pertaining to how chemicals and other stressors (radiation, nanomaterials, viruses, …) induce adverse effects on humans and ecosystems.

The AOP-Wiki serves as the primary authoring tool and user interface for submitting AOPs and their building blocks (KEs and KERs) to the AOP-KB. AOP contributions to the AOP-KB have been crowd sourced from the international AOP community, which represents many research and regulatory organizations, including government agencies and academic institutions. Financial support for ongoing maintenance and evolution of the AOP-KB and AOP-Wiki framework has come from the European Commission - DG Joint Research Centre (JRC), the U.S. Environmental Protection Agency (EPA), Environment and Climate Change Canada (ECCC), and the OECD.

1.3 Towards AOP-Wiki 3.0

The AOP-Wiki evolved continuously over the past twelve years, as shown in **Table 1**:

Table 1. Evolution of AOP-Wiki

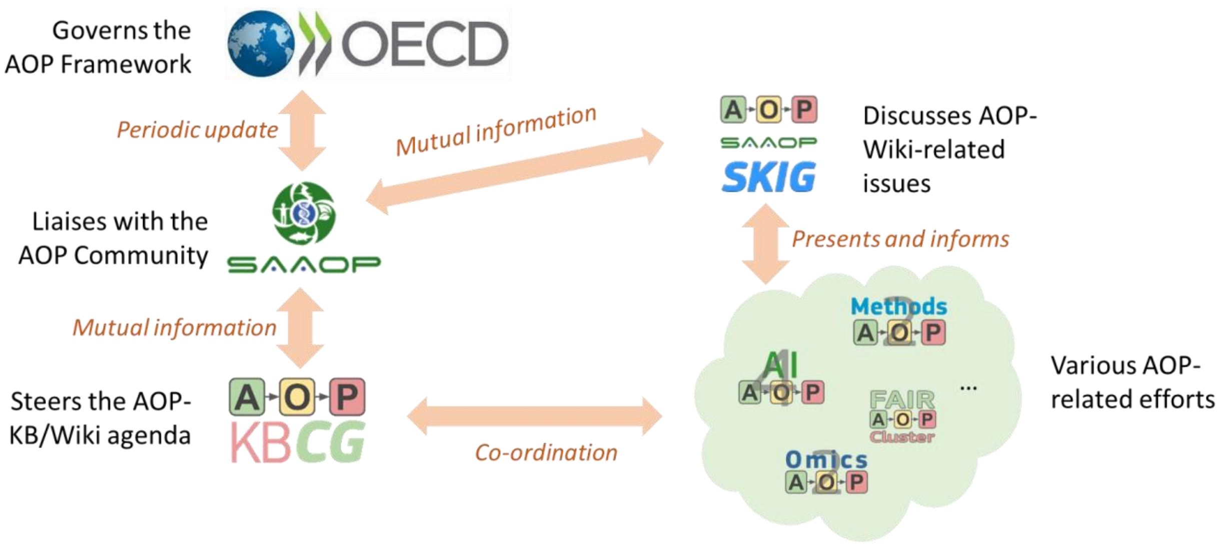
Date	*Version*	*Major improvements*
2013-05		Demo for OECD
2013-06		Beta Release
2014-10	1.0	First publicly accessible version of the AOP-Wiki
2016-01	1.5	Snapshot tools introduced, improved page loading, search tables
2016-12	2.0	Text fields as separate entities
2017-03	2.1	Full support for snapshots
2018-01	2.2	XML downloads
2020-12	2.3	User interface redesign; mobile friendly; CC-0 license
2021-04	2.4	Check for existing KEs, patches and bug fixes
2022-07	2.5	Electronic handbook, AOP Table filter tools, Strategy and evidence fields, Third party tools, Training page, Prototypical stressors
2023-04	2.6	Licensing improvements, Prototypical Stressors, Handbook versioning, Coaches assignment, AOP Table improved, Recaptcha
2024-03	2.7	Consolidation of OECD applications, Getting ready for 3.0

While the current version of the AOP-Wiki is stable and working smoothly, technology advances and a changing requirements environment make a major update to the AOP-Wiki necessary. This further evolution of the AOP-Wiki will culminate in the conception, design and ultimately development of the AOP-Wiki 3.0.

1.3.1 Bodies involved in the AOP-Wiki management

In order to properly manage, maintain and further develop the AOP-Wiki, various bodies and organisations collaborate:

Figure 5. Bodies involved in the AOP-Wiki management



Source: created for this publication

1.3.1.1 OECD

The Organisation for Economic Co-operation and Development (OECD) is an international organisation that works closely with policymakers, stakeholders, and citizens of its member countries to establish evidence-based international standards and find solutions to social, economic, and environmental challenges. The OECD is a unique forum and knowledge hub for data, analysis, and best practices in public policy. Its core aim is to set international standards and support their implementation.

The OECD Environment Directorate steers the AOP Framework, which is the basis for all work done in the AOP-KB/Wiki area. To achieve this, the OECD Advisory Group on Emerging Science in Chemicals Assessment (ESCA) collaborates with the SAAOP.

1.3.1.2 SAAOP

The purpose of the Society for the Advancement of AOPs (SAAOP) is to promote and advance scientific research that fosters the development and use of adverse outcome pathways. The SAAOP maintains the AOP-Wiki under the guidance of the OECD ESCA. Externally, the SAAOP serves as the primary interface between the OECD ESCA and the broader AOP community of practice. Internally, the SAAOP collaborates with the AOP-KB Coordination Group (AOP-KB CG).

1.3.1.3 AOP-KB CG

A dedicated AOP-KB Coordination Group (AOP-KB CG) is in place to ensure the maintenance and further development of the AOP-KB/Wiki and to facilitate interoperability with OECD and third-party tools that draw upon information from the AOP-KB.

The AOP-KB CG consists of organizations that finance, or have financed, the AOP-KB/Wiki maintenance and development. Ultimate decisions on features implemented in the annual AOP-KB (Wiki) work plans and priorities for maintenance lie with the AOP-KB CG. This governance model is adopted to ensure that the financial resources contributed by organizations toward the development and maintenance of the AOP-KB are invested in their top priorities rather than diverted to other interests.

1.3.1.4 Various AOP-related efforts

Various independent efforts are underway to examine specific aspects of the AOP-Wiki:

- "Methods2AOP", dealing with an improved role of test method information in the AOP-Wiki,
- "FAIR AOP Cluster", a group addressing the FAIRness of the AOP-Wiki, i.e. increasing the alignment between the AOP framework and FAIR principles (findable, accessible, interoperable, and reusable) (Wittwehr et al., 2024)
- "Omics2AOPs", a project looking at the role of omics data in the AOP-Wiki (see 2.16), and
- "AI4AOP", an initiative exploring the role of AI in the AOP domain (see 2.19)

are examples.

While completely independent from any central management, these efforts are nevertheless coordinated by the AOP-KB CG to ensure coordination of all groups' efforts.

Initiatives like the ones mentioned above are invited to present their (including intermediate) work results in the SKIG.

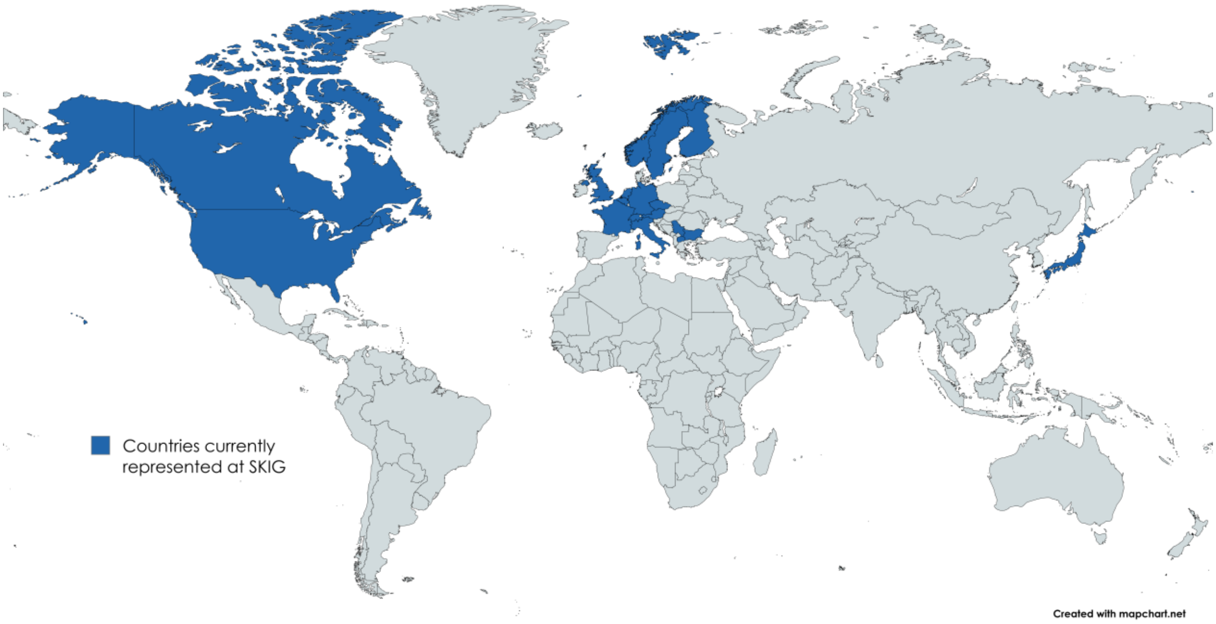
1.3.2 SKIG

The SAAOP Knowledgebase Interest Group (SKIG) is a welcoming assembly open to all AOP-Wiki stakeholders and users, regardless of their position within the framework. Unlike e.g. the AOP-KB CG (section 1.3.1.3) where only funding organizations are members, SKIG invites participation from a diverse range of individuals active in the AOP(-Wiki) domain. Established in June 2023, SKIG serves as a platform for presenting and discussing various aspects related to the AOP-Wiki. While its primary focus is on AOP-Wiki-related issues, discussions frequently extend to topics concerning the AOP Framework, as the two are often inseparable. SKIG informs SAAOP and other bodies about the latest developments and provides a forum for new ideas and the discussion of existing issues in front of a knowledgeable audience.

1.3.2.1 SKIG Membership

SKIG is open to all parties willing to engage in meaningful and productive discussions about a wide range of AOP-Wiki-related issues. As shown in **Figure 6**, at the time of writing, SKIG has more than 40 members from government, research, and academic organizations across 18 countries:

Figure 6. Countries currently represented at SKIG



Source: SKIG membership table and mapchart.net

1.3.2.2 Meetings

SKIG meets every six weeks for 90 minutes. In each meeting, two or three volunteers present a topic related to the AOP-Wiki or the AOP-Framework. Discussions and a Q&A session complement the presentations.

> The purpose of this document is to summarize the presentations and discussions from the founding of SKIG in 2023 until the end of 2024. It aims to familiarize readers with relevant AOP-related topics, encourage them to participate in discussions in 2025 and beyond, and serve as a foundation for their own AOP-related considerations.

2 Topics discussed in SKIG meetings 2023-2024

This section provides a summary of all 21 presentations shown in SKIG meetings from 16 June 2023 until 11 December 2024. Where possible and available, links and/or references are provided for more in-depth information.

For quick access to presentations relevant for specific readers, the presentations were classified along eight categories and scored according to their relevance for the various categories (5 = highly relevant, 0 = not relevant at all), see **Table 2**. This table was created using AI, as laid out in the Annex.

The classification is based on an interpretation of the articles' topics and their potential emphasis on various aspects like *scientific content, technical details, implementation strategies, innovation, community involvement, policy or regulatory relevance, data management techniques, and interdisciplinary collaboration*:

Table 2. SKIG Report presentations and their classifications

	SKIG Report Presentation	*Classification (5 = highly relevant, … 0 = not relevant at all)*							
		Scientific	*Technical*	*Implementation*	*Innovation*	*Community Engagement*	*Policy and Regulation*	*Data Management*	*Interdisciplinary Collaboration*
2.01	Opportunities and challenges of integrating non-chemical stressors in AOPs	5	3	3	4	3	2	2	4
2.02	Ontology-based harmonisation of ROS-related KEs	5	4	3	3	2	2	4	3
2.03	Ontology-based KE components as enablers of Umbrella KEs	4	5	4	4	2	2	3	4
2.04	AOP-Wiki content curation	3	5	5	3	3	2	5	3
2.05	AOP-helpFinder 2.0	4	5	3	5	3	2	5	4
2.06	AOP-KB 3.0 workshop discussion about Umbrella KEs	4	4	3	4	3	2	4	4
2.07	How to enter temporal aspects into the AOP-Wiki	5	4	3	4	2	2	3	3
2.08	An approach for applying ChatGPT in AOP development, and umbrella KEs related to the Mystery of ROS	4	5	3	5	3	2	4	4
2.09	Applying a systematic approach to the development of evidence-based adverse outcome pathways (AOPs) for regulatory use	5	3	4	3	3	5	3	4
2.10	Update on "Guide for Coaches"	3	4	5	3	4	3	3	3
2.11	An SOP developed to translate literature AOPs into AOPs in the AOP-Wiki	4	4	5	3	3	3	4	4
2.12	Adverse Outcome Pathways for Safe and Sustainable by Design approaches	5	3	3	5	3	5	4	5
2.13	Automated access to AOP-Wiki contents through knowledge graph and API	4	5	4	5	3	2	5	4
2.14	The SCAHT AOP_HUB	4	3	3	4	5	3	3	4
2.15	When to report an AOP network	5	3	4	3	3	3	3	3
2.16	Omics2AOPs	5	4	3	5	3	3	4	5
2.17	Tools and strategies used in the development of an AOP network leading to permanent DNA damage	5	4	3	4	3	4	4	5
2.18	Multi-endpoint Data Reporting Format (DRF) and dedicated user-interface (UI) for dose response-data	4	5	4	4	2	3	5	4
2.19	AI4AOP	5	5	3	5	4	3	4	4
2.20	ChatAOP: A chat interface for the AOP-Wiki	4	5	3	5	3	3	4	4
2.21	Physiological maps (and how to use them to establish or assess biological relevance in AOPs)	5	4	3	4	3	3	3	5

Source: created for this publication with the help of ChatGPT 4.o

Note: All AOPs, KEs and KERs mentioned in this report can be accessed in the AOP-Wiki by using the URLs laid out in **Table 3**.

Table 3. URLs to access AOPs, KEs, KERs

AOPs	`https://aopwiki.org/aops/`[AOP number]	e.g. https://aopwiki.org/aops/327 for AOP 327
KEs	`https://aopwiki.org/events/`[KE number]	e.g. https://aopwiki.org/events/1753 for KE 1753
KERs	`https://aopwiki.org/relationships/`[KER number]	e.g. https://aopwiki.org/relationships/377 for KER 377

2.1 Opportunities and challenges of integrating non-chemical stressors in AOPs

Presenter: Laure-Alix Clerbaux, UCLouvain (Belgium), ORCID 0000-0003-2707-482X, email: laure-alix.clerbaux@uclouvain.be

Date: 2023-06-16

2.1.1 Issue addressed

The AOP framework emerged in the context of regulatory toxicology to guide an effective paradigm shift towards mechanism-based chemical risk assessment, addressing the challenges of assessing large numbers of data-poor chemicals with minimal but effective testing. Because of this stressor-agnostic, pragmatic, and mechanistic-based approach, AOPs became attractive to other fields. In the last five years, the concept of AOPs has expanded to include non-chemical stressors such as nanomaterials, radiation, viruses, cells used to treat patients, and microorganisms employed as pesticides. This increase in interest and activities outside the chemical field generates opportunities but also new challenges.

2.1.2 Presentation content

The presentation outlined the opportunities and challenges for the different types of stressors (Clerbaux et al., 2023).

2.1.2.1 Nanosized material stressors

At least eighteen AOPs present in the AOP-Wiki report a nanosized material as a stressor (AOP 144, AOP 173, AOP 207, AOP 208, AOP 209, AOP 210, AOP 237, AOP 241, AOP 296, AOP 303, AOP 302, AOP 392, AOP 319, AOP 241, AOP 409, AOP 451, AOP 463, AOP 481), of which nine relate to lung toxicity ((Halappanavar et al., 2020); (Clerbaux et al., 2023)). Four main challenges have arisen during the development of AOPs related to nanomaterials: non-specific MIEs due to the high reactivity of nanomaterials with the biological milieu, toxicity associated with a wide variety of mechanisms, limited understanding of nanomaterial biodistribution, and the need for adaptations of in silico modeling and testing systems ((Gerloff et al., 2017); (Halappanavar et al., 2020); (Halappanavar, Nymark, et al., 2021); (Halappanavar, Ede, et al., 2021)). Several European projects focused on the safety of nanomaterials and other advanced materials have actively advanced the development of AOPs relevant to nanosized materials, including e.g. HARMLESS ((Pousen et al., 2022); (Ulla Vogel, 2022)).

2.1.2.2 Physical stressors (radiation)

The first ionizing radiation AOP (AOP 272) was endorsed in 2023 by the OECD Working Party on Hazard Assessment - WPHA (Chauhan et al., 2021). The development of AOPs for radiation faces challenges in incorporating ionizing event types, dose rates, energy deposition, and accounting for the fact that ionizing agents target multiple macromolecules.

2.1.2.3 Viral stressors

Around 15 AOPs have been developed to model the pathogenesis triggered by the SARS-CoV-2 virus (Nymark et al., 2024). Developing AOPs for COVID-19 required the inclusion of SARS-CoV-2-specific replicative steps to capture the essential events driving the disease, which might contrast with the stressor-agnostic principle. The virus-specific KEs were embedded within a 'hub AOP', a reusable unit that can trigger further downstream KEs. Hence, those KEs are specific to the stressor, but the following KEs adhere to the stressor-agnostic principle[3].

2.1.2.4 Cellular stressors

Putative AOPs provided a framework for the integration of new testing methodologies by linking the understanding of mechanisms triggered by engineered cell immunotherapies, such as CAR-T cells, with appropriate test systems (Mazein et al., 2023). Developing AOPs to evaluate the efficacy and toxicity of cell therapies necessitates addressing the cellular nature and the therapeutic function of the stressor.

2.1.2.5 Emerging biologicals

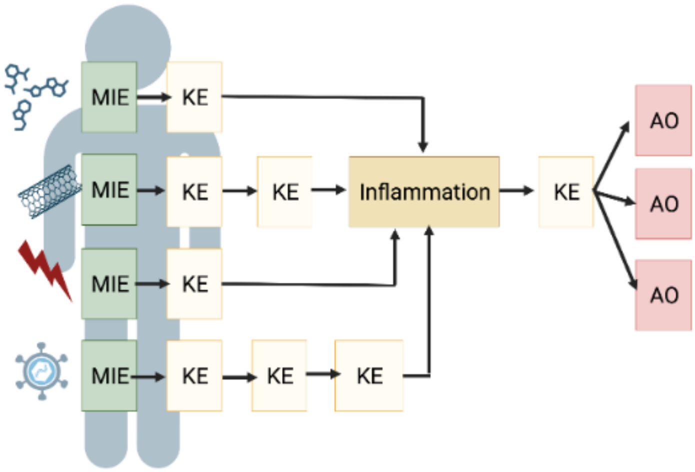
Currently, microbial pesticide hazard testing adheres to the same procedures as chemical pesticide testing, which fails to account for the unique characteristics of microbial pesticides, such as proliferation, infectivity, or secondary metabolite toxicity. The experience gained from developing COVID-19 AOPs can provide valuable insights for assessing the toxicity of emerging biological stressors.

2.1.2.6 Conclusion

Considering different types of AOP stressors could be a way to bridge toxicology and disease biology. In most real-world scenarios, humans and animals are exposed to multiple stressors of different natures (chemical, physical, viral, or biological) rather than just one at a time. The adaptations needed to expand AOP applicability beyond chemicals are mainly at the molecular and cellular levels, while downstream key events at the tissue or organ level, such as inflammation, are shared by many AOPs initiated by various stressors, with late key events tending to converge towards a common adverse outcome (**Figure 7**).

[3] The "stressor agnostic principle" in the Adverse Outcome Pathway (AOP) Framework refers to the idea that AOPs are constructed without being specific to any particular stressor or chemical. Instead, they focus on the biological mechanisms and processes leading to adverse outcomes, allowing for broader applicability across different stressors.

Figure 7. Inflammatory KEs are shared by many AOPs initiated by different types of stressors



Source: Clerbaux et al., 2023

In conclusion, although challenging, integrating non-chemical stressors within AOPs expands opportunities to account for real-world scenarios, identify vulnerable individuals, and bridge knowledge on mechanisms of adversity.

2.1.3 Links and references

(Halappanavar, Ede, et al., 2021; Halappanavar, Nymark, et al., 2021; Ulla Vogel, 2022), (Halappanavar et al., 2020), (Chauhan et al., 2021), (Nymark et al., 2024), (Mazein et al., 2023; Pousen et al., 2022).

2.2 Ontology-based harmonisation of ROS-related KEs

Presenter: Shihori Tanabe, National Institute of Health Sciences (NIHS, Japan), ORCID 0000-0003-3706-0616, email: stanabe@nihs.go.jp

Date: 2023-06-16

2.2.1 Issue addressed

As the AOP-Wiki continues to expand with a wealth of information, there is a growing need to address the issue of KEs that cover similar concepts. An international consortium, known as the "Mystery of ROS" (MoR), is actively discussing the scientific roles and interconnected functions of reactive oxygen species (ROS) within the AOP framework. The MoR workshops have highlighted that many KEs in the AOP-Wiki address ROS. In particular, KEs 1940 and 1869 are promising candidates to serve as "umbrella" KEs (see section 2.6), under which similar KEs could be merged or at least associated. This approach will also increase the focus on ontologies.

2.2.2 Presentation content

The presentation outlined the lessons learnt from the "Mystery of ROS" consortium ((Tanabe, Beaton, et al., 2022; Tanabe et al., 2023; Tanabe, O'Brien, et al., 2022)).

2.2.2.1 ROS-related KEs

The “Mystery of ROS” consortium identified KE 1753 *Chronic ROS*, KE 1364 *Increase, ROS*, KE 257 *Increase, ROS production*, KE 1115 *Increased, ROS*, KE 249 *Production, ROS*, KE 1632 *Increase in reactive oxygen and nitrogen species* as ROS-related KEs. Oxidative stress-related KEs included KE 1869 *Oxidative stress response*, KE 1392 *Oxidative stress*, KE 1538 *Decreased protection against oxidative stress*. DNA damage-related KEs included KE 1634 *Increase, oxidative damage to DNA* and KE 1194 *Increase, DNA damage*.

2.2.2.2 Mystery of ROS collaboration network

The ROS collaboration network consists of AOP 327-330, AOP 299, AOP 386, AOP 387 starting at KE 257, AOP 298 starting at KE 1753 and KE 1980, AOP 293 and AOP 294 starting at KE 1194 and mediated by KE 1632. AOP 382 and AOP 383, AOP 384, starting at KE 1115, AOP 296 starting at KE 1634, and AOP 379 mediated by KE 1869 were also included in the network.

2.2.2.3 ROS- and oxidative stress- related AOPs

AOP 17, 39, 207, 213, 220, 257, 263, 264, 266, 267, 268, 282, 293, 294, 296, 298, 362, 386, 387, 389, 396, 406, 413, 450, 451, 457, 462, 470, 472 and 483 were identified as ROS-related AOPs in the AOP-Wiki (as of May 2023). AOP 17, 457, 296, 210, 220, 149, 413, 284, 406, 462, 492, 470, 482, 31, 478, 425, 260, 213, 209, 447, 411, 450, 207, 483, 424, 37, 38, 159, 313, and 27 were identified as oxidative stress-related AOPs in the AOP-Wiki (as of May 2023).

2.2.2.4 Development of AOP 379

AOP 379 “Binding to angiotensin converting enzyme 2 (ACE2) leading to thrombosis and disseminated intravascular coagulation” was introduced in the context of the relationship in ROS, coagulation and SARS-CoV-2. Literature studies have suggested that ROS, oxidative stress responses, blood coagulation, and thrombosis are involved in COVID-19. AOP 379 has been developed in the effort of CIAO. AOP 379, originally created as “Increased susceptibility to viral entry and coronavirus production leading to thrombosis and disseminated intravascular coagulation” has been developed as “Binding to ACE2 leading to thrombosis and disseminated intravascular coagulation” in an OECD project.

2.2.2.5 Conclusion

According to the demand where ROS-related KEs need to be harmonised, KE 1940 *Increases in cellular ROS* (now called *Increases in ROS*) and KE 1869 *Diminished protective oxidative stress response* were created in the collaboration of the “Mystery of ROS” consortium. To better network the AOPs related to ROS, ontology-based harmonisation of the related KEs is needed in a concept of like the one of “Umbrella KE” which is discussed in section 2.6.

2.2.3 Links and references

Presentation slides: (Tanabe, 2024b)

(Tanabe, Beaton, et al., 2022; Tanabe et al., 2023; Tanabe, O’Brien, et al., 2022)

2.3 Ontology-based KE components as enablers of Umbrella KEs

Presenter: Julija Filipovska, independent (North Macedonia), ORCID 0000-0001-7395-214X, email: julija4CIAO@yahoo.com

Date: 2023-07-28

2.3.1 Issue addressed

The need for harmonization, grouping, or effectively structuring KEs to enable the emergence of functional AOP networks transcends the issue of the “Mystery of ROS” (MoR) discussed in Section 2.2. This presentation (Filipovska, 2024) explored a more general approach based on the use of KE components and the ontology-defined 'tags' of the KEs as an objective and efficient method for addressing the growing number of similar and potentially redundant KEs within the AOP-Wiki.

2.3.2 Presentation content

The Key Event components represent KE identifiers based on structured, pre-defined terms from 22 biological ontologies currently available in the AOP Wiki (Ives et al., 2017). They are intended to capture the detailed free-text KE description within three machine-readable components: ‘Process’, ‘Object’, and the type/direction of the perturbation (‘Action’). Depending on complexity, one or more sets of two (Object/Action; Process/Action) or three (Process/Object/Action) ontology terms can be used to describe a KE.

The potential utility of the pre-defined KE components has been considered for the development and application of AOP networks (Knapen et al., 2018) and for conducting network analytics (Villeneuve et al., 2018). However, these KE components have not yet been systematically or extensively applied to define or group similar KEs. The application of user-defined ‘freestyle’ tags for marking similar KEs has also been proposed within the SKIG. While this approach may be useful for identifying similar KEs, it may be limited in its effectiveness for addressing the need for harmonization or grouping. The proliferation of similar and potentially redundant KEs stems, in part, from users’ attempts to capture biological complexity through free-text descriptions and naming of the KE (Section 2.4.2.5).

The presentation explored the complexity and difficulties of the current use of KE ontology terms in the context of MoR-relevant KEs. It also proposed a new approach to better align the human-generated free-text content with relevant and informative KE components, based on greater use of (i) information related to the methods for measuring the KE perturbations, and (ii) Large Language Models for more objective analysis of the free text. Additionally, it is proposed to define distinct sets of composite tags of KE components (‘tokens’) containing two or three ontology terms. Such machine-readable ‘tokens’ would potentially enable more objective and efficient grouping of similar KEs.

Overall, this approach aims to facilitate the functional harmonization of similar KEs in an objective, high-throughput, machine-assisted manner, without compromising the integrity, complexity, or creativity of the human-generated free-text content. However, further discussion and training for AOP developers are necessary to enhance understanding and utilization of the available ontologies. Additionally, the practical implementation of such a ‘tokenized’ structure of key events and sub-events into the AOP Wiki needs to be explored. Some of these aspects were discussed at the AOP-KB 3.0 workshop (Section 2.6).

2.3.3 Links and references

Presentation slides: (Filipovska, 2024)

(Ives et al., 2017; Knapen et al., 2018; Villeneuve et al., 2018)

2.4 AOP-Wiki content curation

Presenter: Laure-Alix Clerbaux, UCLouvain (Belgium), ORCID 0000-0003-2707-482X, email: laure-alix.clerbaux@uclouvain.be

Date: 2023-07-28

2.4.1 Issue addressed

The AOP-Wiki faces significant curation challenges. The presence of incorrect information, duplicated entries, and empty pages undermines the platform's credibility and usability. The unclear distinction between validated information and unverified or redundant content discourages users. Therefore, ensuring proper curation is essential to foster trust in AOP-Wiki among both scientific and regulatory communities.

2.4.2 Presentation content

The presentation focused on two key aspects of AOP-Wiki content curation: identifying the types of errors present in the AOP-Wiki and determining who or how to address and correct them.

2.4.2.1 Biologically incorrect content

Curation may be necessary to address scientifically inaccurate content. For instance, in the prototypical stressors section, both SARS-CoV and SARS-CoV-2 were listed alongside COVID-19, which, as a disease, cannot be categorized as a stressor. It was suggested that such erroneous entries could be removed by the gardeners, who have full administrative rights on the AOP-Wiki.

Figure 8. Example for biologically inaccurate content

526	SARS-CoV		320, 377, 430
527	COVID-19		

Source: AOP-Wiki

2.4.2.2 Accidentally incorrect content

Some examples were discussed where authors might have accidentally entered incorrect content. Such entries could be corrected by the authors when feasible or by the gardeners. It might be necessary to create a body that can authorize proposed changes in case the AOP author is unresponsive. It was proposed that engaged individuals within the AOP-Wiki community (AOP-Wiki curators) could perform complex refactoring, such as “de-duplicating” or merging content, so that such issues are readily resolved.

Figure 9. Example for accidentally incorrect content

943	Hypersecretion, Mucus	Hypersecretion, Mucus	Organ
1245	Mucus , Hypersecretion	Mucus , Hypersecretion	
1244	Mucus hypersecretion,	Mucus hypersecretion,	

Source: AOP-Wiki

2.4.2.3 Empty content

The issue of missing or incomplete terms was a major point of focus for the CIAO Ontology Group (Nymark et al., 2024). In the AOP-Wiki, authors are required to fill in several fields, and a great majority of those are left empty. The development of a bug tracking system would help users and developers report quality issues, keep track of them, and finally resolve them. Any such initiative, however, should be coupled with a policy for addressing quality issues. For conflict resolution, one could envision a committee that can make final decisions on how a particular issue should be fixed or which solution should be applied.

2.4.2.4 Duplicated content.

The AOP-Wiki contains numerous similar and almost identical, but separate KEs. This major blocking factor for the Wiki to fulfil its full potential will be further discussed in section 2.6. The bug tracking system described above would also allow for warning AOP authors and developers about this issue. Another recommendation could be to develop a systematic way to identify duplicate KEs extracted from the AOP-Wiki and apply it as a filter.

2.4.2.5 Biologically complex content

Challenges might be rooted in biology itself. For instance, many KEs cover cell death, but this process might need to be described in separate KEs. In such cases, a collaborative effort, similar to the 'Mystery of ROS' consortium, may be required to bring together experts to harmonize and establish consensus on these issues (Tanabe et al., 2023). This has already been discussed in sections 2.3 and 2.6.

2.4.3 Links and references

(Nymark et al., 2024), (Tanabe et al., 2023)

2.5 AOP-helpFinder 2.0

Presenter: Karine Audouze, Université Paris Cité (France), ORCID 0000-0001-7525-4089

Date: 2023-09-08

2.5.1 Issue addressed

The growing volume of scientific literature in the PubMed database makes investigating potential connections between stressors and events increasingly challenging. To address this, the AOP-helpFinder method was introduced in (Carvaillo et al., 2019) to expedite the identification of links between environmental stressors and the biological events associated with AOPs. AOP-helpFinder is an artificial intelligence-based method that leverages natural language processing techniques, such as text mining, along with graph theory.

2.5.2 Presentation content

Building on the strong interest from the scientific and regulatory communities in this method and its web interface launched in 2021, an updated version, AOP-helpFinder 2.0, has been released. This new version introduces several features designed to enhance AOP development. First, an "event-event" module has been added, enabling the automatic screening of PubMed abstracts to identify and extract relationships between different types of events (MIE-KE, KE-KE, and KE-AO). These event-event relationships correspond to connections between events based on existing knowledge, referred to as KERs in the AOP-Wiki. Second, a new scoring system called the Confidence Score (Cs) was introduced to aid users in interpreting the results of the literature screening. The Cs classifies evidence into five categories—"Low," "Quite Low," "Moderate," "High," and "Very High"—and supports the Weight of Evidence (WoE) concept by providing insights into the volume of scientific evidence retrieved for stressor-event or event-event relationships. Finally, a visualization module was developed to improve the accessibility and interpretation of the AOP-helpFinder results.

2.5.3 Links and references

The AOP-helpFinder 2.0 tool source codes are fully accessible via GitHub[4], and searches can be performed via the dedicated web interface[5]. AOP-helpFinder is also available as a third party tool under AOP-Wiki.

(Carvaillo et al., 2019; Jaylet et al., 2023; Jornod et al., 2022).

2.6 AOP-KB 3.0 workshop discussion about Umbrella KEs

Presenter: Clemens Wittwehr, European Commission, Joint Research Centre (JRC, Italy), ORCID 0000-0003-2760-7702

Date: 2023-09-08

2.6.1 Issue addressed

The AOP-Wiki contains numerous similar and almost identical, but separate KEs. This leads to problems when building AOP networks, as they rely on shared Key Events. If the knowledge covered in such shared KEs is not consolidated into a single KE (but is instead spread over various similar KEs), AOP networks will not emerge.

2.6.2 Presentation content

The issue was discussed in the 2023-08-16 SAAOP AOP-Wiki 3.0 Workshop, and the presentation reported the results of a breakout group dealing with a notion called "Umbrella Key Event."

[4] https://github.com/systox1124/AOP-helpFinder

[5] https://aop-helpfinder.u-paris-sciences.fr/

Participants in this breakout group were Shihori Tanabe (NIHS, Japan), staff from the University of Antwerp (Dries Knapen and colleagues), the University of Maastricht (Marvin Martens and a colleague), and JRC (Clemens Wittwehr and Selfa Aspiroz Lucia).

Summary of the main points:

- An Umbrella Key Event is a kind of container for several (very) similar, but distinct Key Events.
- "Similar" in that sense can mean, e.g. "describing similar mechanisms", or "covering similar biological phenomena" (like "altered TH level" as umbrella for "decreased TH" and "increased TH", as shown in (Haigis et al., 2023).
- The current word used for KEs that are included in an Umbrella Key Event is "Member KE". A better expression might be necessary.
- An Umbrella Key Event does not "absorb" the Member KEs, i.e. the Member KEs keep existing.
- The gardening activity of combining similar KEs on the one hand and the introduction and the use of Umbrella KEs are complementing each other:
    - Gardening is the "hard" solution of actually merging two or more KEs and let only one survive.
    - Umbrella KEs are the "soft" solution if the peculiarities of the individual KEs need to be preserved.
    - Gardeners can use Umbrella KEs and their members as "first suspects" when looking for double entries.
- Umbrella KEs should not become members of other Umbrella KEs
- There is an n:n relationship between Umbrella KEs and Member KEs:
    - One Umbrella KE can cover several Member KEs
    - Any KE (not Umbrella KEs) can be member of several Umbrella KEs
- Implementation ideas:
    - A new type of page (data model object?) = Umbrella KE to be introduced
    - The fields and the instructions to fill them would lay out the conditions under which a KE can/should/must be considered to be put "under" an umbrella KE
    - A low tech solution would be to use the already foreseen (but not yet implemented) tagging mechanism = when a user wants to put their KE "under" an existing Umbrella KE, they would tag their KE with the name (or the number) of the Umbrella KE
    - Visualisation of Umbrella KEs: Smaller boxes (Member KEs) within a larger shaper (Umbrella KE), ideally not a box, but something more appealing.

Open points:

- Do KERs link to the Umbrella KE or still to the Member KE? Is both possible?
- Can Umbrella KEs be part of an AOP, or only their Member KEs?

- Fine line between Member KEs containing the required specificity for separate AOPs, and the Umbrella KEs allowing the collection of common information.
- In what way would Umbrella KEs be measurable?
- If two AOPs feature two distinct KEs that happen to be member of the same Umbrella KE: Is this then a valid "match" in the sense of building an AOP network or an emerging AOP?
- The exact fields, free texts and instructions coming with the new page type "Umbrella KE" need further discussion
- Workflows (how to create an Umbrella KE, how to link/unlink a Member KE to/from an Umbrella KE, etc.) and corresponding user interface still need to be designed.
- How would the user of an AOP (e.g., a regulator) view/interact with the varying levels of specificity beneath an Umbrella KE?

2.6.3 Comments during the meeting

- Umbrella Key Events might be one of several solutions to the underlying problem: the duplication of information in the AOP-Wiki.
- A more thorough analysis of this underlying issue is necessary before concrete solutions are proposed or even considered for implementation.

2.6.4 Links and references

(Haigis et al., 2023)

2.7 How to enter temporal aspects into the AOP-Wiki

Presenter: Alexandra Schaffert, Finnish Hub for Development and Validation of Integrated Approaches (FHAIVE), Faculty of Medicine and Health Technology, Tampere University (Finland); Medical University of Innsbruck (Austria), ORCID 0000-0001-8159-1292, email: alexandra.schaffert@tuni.fi

Date: 2024-01-12

2.7.1 Issue addressed

The AOP-Wiki does not yet formally incorporate temporal aspects, such as the duration it takes for a KE to proceed to the next KE or how the timing and duration of exposure (e.g., chronic vs. acute) influence downstream events. However, the scientific community widely acknowledges that time is critically important for understanding how MIE ultimately lead to adverse outcomes. Currently, these temporal dynamics are often discussed in the scientific literature supporting the AOP or in specialized modeling efforts, rather than being embedded in the AOP's standardized description.

2.7.2 Presentation content

Incorporating time in AOPs is challenging for several reasons. Biological processes rarely occur in a linear, uniformly timed fashion. Different pathways may proceed at different rates or modulate each other's kinetics. In reality, few studies collect sufficiently detailed time-course data to pin down precise kinetic or dynamic parameters. The AOP framework is often used as a qualitative, conceptual approach to assemble weight-of-evidence as a way to link mechanistic knowledge to regulatory endpoints.

Emerging efforts to incorporate the aspect of time into AOPs include the development of quantitative AOPs, which aim to incorporate dose-response data, time-course data, and biological variability into AOP descriptions (Perkins et al., 2019). Toxicogenomics and computational toxicology are being leveraged to add a temporal dimension ((Gao et al., 2015; Serra et al., 2020)).

How could the AOP-Wiki incorporate "time" into its framework? A practical, less resource-intensive solution would be to expand KER descriptions with broad temporal classifications—labelling them, for instance, as either acute or chronic—rather than developing fully quantitative AOPs, which can be more time-consuming and data-demanding. Likewise, KE pages could also note approximate onset times or durations (e.g., minutes to hours versus weeks to months), offering a clearer sense of when each KE typically unfolds. A good example can be found in AOP 479, where both "Increase, cardiac remodeling" and "Decrease, contractility" are depicted as parallel KEs stemming from a shared upstream event, and both ultimately contribute to the adverse outcome "Decrease in LV function." Although these KEs appear at the same level in the pathway, they unfold on very different timescales—"Decrease, contractility" can manifest within minutes under sufficient insult, while "Increase, cardiac remodeling" typically requires weeks to become observable.

Explicitly noting the timescales within each KE or KER can help users better interpret how these events progress—and prevent misinterpretation of the biological timeline.

2.7.3 Links and references

(Gao et al., 2015; Perkins et al., 2019; Serra et al., 2020)

2.8 An approach for applying ChatGPT in AOP development, and umbrella KEs related to the Mystery of ROS

Presenter: Shihori Tanabe, National Institute of Health Sciences (NIHS, Japan), ORCID 0000-0003-3706-0616, email: stanabe@nihs.go.jp

Date: 2024-01-12

2.8.1 Issue addressed

As the generative AI technique is rapidly growing, an approach to apply ChatGPT in AOP development is of great interest. Since the Mystery of ROS develops umbrella KEs related to ROS, an attempt to use ChatGPT in describing the contents of KEs were conducted.

2.8.2 Presentation content

Regarding the discussion in previous SKIG meetings, ChatGPT 3.5 was utilized by asking about the description of the KE on NRF2 activity to add the detection method for NRF2 activity in the KE 1869 in AOP 379 on October 30, 2023.

2.8.2.1 Description for detection method of NRF2 activity for KE entitled "Diminished protective oxidative stress response" in AOP

The "Diminished Protective Oxidative Stress Response" is a critical KE in the AOP framework that plays a central role in understanding how exposure to various stressors can lead to adverse outcomes. One essential component of this KE is the activity of Nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor that plays a pivotal role in the regulation of the oxidative stress response. Detecting NRF2 activity is crucial for assessing the status of the oxidative stress response, and in this description, we will outline a method for measuring NRF2 activity.

Measuring NRF2 activity involves assessing its transcriptional activity or protein abundance. Several methods can be employed to detect NRF2 activity, and were identified using ChatGPT 3.5. These include:

- Luciferase Reporter Assay:
    - This widely used method involves creating a reporter plasmid containing NRF2-responsive antioxidant response element (ARE) sequences and a luciferase gene.
    - Cells of interest are transfected with the NRF2-ARE luciferase reporter construct.
    - After exposure to the stressor of interest, cells are lysed, and luciferase activity is measured.
    - Increased luciferase activity indicates NRF2 activation, while decreased activity suggests diminished NRF2 activity.
- Quantitative PCR (qPCR):
    - Assessing NRF2 activity at the transcriptional level can be achieved through qPCR.
    - Specific NRF2 target genes (e.g., NQO1, HO-1) are selected and their mRNA levels are quantified.
    - Increased expression of these genes is indicative of NRF2 activation, while reduced expression suggests diminished NRF2 activity.
- Western Blotting:
    - This method allows the detection of NRF2 protein levels in cell or tissue samples.
    - After exposure to a stressor, proteins are extracted, separated by electrophoresis, and transferred to a membrane.
    - Specific antibodies against NRF2 are used to detect its abundance.
    - Increased NRF2 protein levels suggest NRF2 activation, while reduced levels indicate diminished activity.
- Immunofluorescence:
    - Immunofluorescence can be used to assess the cellular localization of NRF2.
    - Cells are fixed and probed with antibodies specific to NRF2, followed by fluorescently labeled secondary antibodies.

- NRF2 localization within the cell (e.g., cytoplasm or nucleus) can indicate its activation status.

— Electrophoretic Mobility Shift Assay (EMSA):

- EMSA is a technique that measures the binding of NRF2 to ARE sequences.
- Radioactively labeled ARE sequences are incubated with nuclear extracts, and the formation of DNA-protein complexes is visualized on a gel.
- The intensity of the complex can indicate NRF2 binding activity.

2.8.2.2 Mystery of ROS collaboration network

AOP 379 *Binding to ACE2 leading to thrombosis and disseminated intravascular coagulation* is included in the "Mystery of ROS" collaboration network shown in the paper, where harmonised KE 1940, an umbrella KE for up-regulation of ROS is created (Tanabe, Beaton, et al., 2022).

2.8.2.3 A concept of the ROS as KE and associative event in AOPs

A paper entitled "Reactive Oxygen Species in the Adverse Outcome Pathway Framework: toward Creation of Harmonized Consensus Key Events" was published in Frontiers in Toxicology in 2022 as a collaborative work of the Mystery of ROS consortium (Tanabe, O'Brien, et al., 2022).

2.8.2.4 An umbrella KE in decreased protective oxidative stress response

KE 1869 *Diminished protective oxidative stress response* is another candidate for an umbrella KE in the point of view of the decreased protective oxidative stress response. AOP 379 *Binding to ACE2 leading to thrombosis and disseminated intravascular coagulation* is in the coaching process.

2.8.3 Links and references

Presentation slides: (Tanabe, 2024a)

(Tanabe, Beaton, et al., 2022; Tanabe, O'Brien, et al., 2022)

2.9 Applying a systematic approach to the development of evidence-based AOPs for regulatory use

Presenter: Barbara Viviani, Università degli Studi di Milano (Italy), ORCID 0000-0002-0935-0459

Date: 2024-02-21

2.9.1 Issue addressed

One requirement for AOPs in regulatory applications is the confidence and accuracy in extrapolating data measured at lower levels of biological organization to predicted outcomes at higher levels of organization, and how measurements of biological effects are linked to their specific causes. The AOP 503, "Activation of uterine estrogen receptor-alpha leading to endometrial adenocarcinoma via epigenetic modulation,"[6] is an example of an evidence-based approach methodology that integrates literature evidence into mechanistic pathways, enhancing transparency, reproducibility, and objectivity (Viviani et al., 2023).

2.9.2 Presentation content

The process is divided into four phases. The first phase involves designing an a priori protocol that formulates the problem, plans the methods, and ensures transparency while allowing flexibility to deal with justified deviations.

The second phase uses topic modeling, an AI-based method, to map evidence from 84,722 papers into 400 topics, which are then grouped into MIEs and KEs, highlighting novel knowledge for further expert evaluation. MIEs, KEs, and poorly established relationships were prioritized for systematic review and evidence appraisal.

The systematic review (phase three) gathered information on the biological plausibility, dose and time concordance (consistency), and human relevance of the mechanistic pathway from in silico, in vitro, in vivo, epidemiological, and clinical studies.

The application of a risk of bias analysis, using a customized NTP OHAT RoB[7] tool, identified limitations and potential inaccuracies in the results, thereby increasing confidence in the selected evidence.

In phase four, all evidence was synthesized according to OECD guidelines[8], and the certainty of the KER was quantified using a probability distribution (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2023).

2.9.3 Links and references

(EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2023; Viviani et al., 2023)

2.10 Update on "Guide for Coaches"

Presenter: Shihori Tanabe, National Institute of Health Sciences (NIHS, Japan), ORCID 0000-0003-3706-0616, email: stanabe@nihs.go.jp

Date: 2024-03-27

2.10.1 Issue addressed

For anyone creating an AOP in the AOP-Wiki, a team of coaches is available to help them get started and to support them throughout the creation process. For the coaching team itself, a *Guide for Coaches* was created and is now constantly revised according to the progress in the AOP coaching program.

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[6] https://aopwiki.org/aops/503

[7] National Toxicology Program, Office of Health Assessment and Translation, Risk Of Bias

[8] Latest version of the handbook: https://aopwiki.org/handbooks/5

2.10.2 Presentation content

2.10.2.1 Structure of the Guide for Coaches

The guide is divided into several parts, including coaching responsibilities, guidance for AOP development, technical matters in the AOP-Wiki, and AOP review and publication. Frequently asked questions related to coaching are compiled in a Questions and Answers format.

2.10.2.2 Updates on the Guide for Coaches

Recent updates include adding guidance documents for the scientific review of AOPs, a section on follow-up activities for coaches, and technical updates to the AOP-Wiki, such as linking to coaching-related documents and updating ontology descriptions. Upcoming updates include clarifying the difference between authors and contributors in the AOP-Wiki. There is also a discussion about improving how coaches are managed within the AOP-Wiki, potentially tracking contributions by coaches and reviewers in a more quantitative manner, which could be useful for professional credentials.

2.10.2.3 Future of the Guide for Coaches

It was suggested that linking the Guide to the AOP developer's handbook[9] could be useful. Clarification is necessary on the specific needs for coaches in the AOP-Wiki, as there may be a gap in understanding what is currently available for coaches and what the desired changes might be. There's also a need for a system to recognize reviewers' contributions, as they may not always be registered users like coaches are.

2.10.3 Links and references

- General Coaching Documents[10]
- More documents for Coaches[11]

2.11 An SOP developed to translate literature AOPs into AOPs in the AOP-Wiki

Presenters: Travis Karschnik, US EPA (USA)

Dan Villeneuve, US EPA (USA), ORCID 0000-0003-2801-0203,
email: villeneuve.dan@epa.gov

Date: 2024-05-15

[9] https://aopwiki.org/handbooks/5

[10] https://nihs357.sharepoint.com/:f:/s/CoachingDocument748/EgVXTom1v0VFvC9Uv5m-kRsB2fEoEo1gFGnXD4mkPZBpzA?e=T0CdZZ

[11] https://drive.google.com/drive/folders/1ld3TX04tXCHm4IfGlVoGprxEt99Uaw1U?usp=share_link

2.11.1 Issue addressed

Given broad scientific interest in AOPs, many academics have engaged in AOP development and published well documented AOPs in peer-reviewed scientific journals. However, for a variety of reasons such as lack of time, funding, familiarity with the AOP-Wiki, recognition for the effort required, etc. they have not entered or documented their AOPs in the AOP-Wiki. Unfortunately, AOPs dispersed in the literature can be difficult to find and may often be overlooked for relevant applications. Likewise, AOPs presented in a peer-reviewed publication alone are static, making them difficult to update or leverage in developing other AOPs within the networks in which they operate. Consequently, the potential impacts of these AOPs is not being maximized.

Starting in 2023, US EPA initiated a project to fund contract scientific support staff (General Dynamics Information Technology; GDIT) to contact the authors of AOPs published in peer-reviewed manuscripts and assist them in transferring relevant information and supporting evidence into the AOP-Wiki. Where contacted authors are interested and willing to contribute their information to the AOP-Wiki, contract staff aid them in creating their own AOP descriptions. However, in cases where the contacted authors do not have the interest or time, the contract staff directly take the lead in entering the relevant evidence and information, being sure to provide appropriate recognition of the scholarly contributions of the original authors through appropriate citation and attribution. To date (ca. December 2024), this project has resulted in addition of 18 novel AOPs to the AOP-Wiki. In roughly one third of cases, that has been a cooperative/collaborative process with the original authors.

2.11.2 Presentation content

In order to guide the process of entering AOP information from peer-reviewed publications into the AOP-Wiki, GDIT staff developed a standard operating procedure (SOP). Key elements of that SOP were presented to the SKIG. In brief, the GDIT scientists first review a paper of interest to confirm that one or more AOPs are presented in the manuscript. Second, they evaluate the extent of evidence that has been identified or assembled as part of the source publication. In the event that the article presents a single AOP, a determination is made as to whether sufficient evidence has been assembled to support inclusion in the AOP-Wiki (hypothesized AOPs consisting of little more than a box and arrow diagram are not included). In the event that a network of AOPs is presented in the source publication, GDIT scientists evaluate which branches in the network (and associated individual AOPs) are best supported by the evidence presented in the paper and focus their efforts on those for which the strongest weight of evidence is available. After this initial determination is made, GDIT scientists contact the author(s) to inform them of the desire to enter their AOP information into the AOP-Wiki and invite them to lead, contribute to, or participate in the process to the extent that they are interested and able to do so. If information transfer is to be led by GDIT, a detailed evaluation of evidence cited in the original source, primary sources, secondary sources, etc. is performed, organized into concordance tables, and contents of appropriate KE, KER, and AOP pages added to the AOP-Wiki and populated. Wherever possible, existing Event and Relationship pages are utilized, with new/refined content identified using a different font. Once completed, these entries to the AOP-Wiki are reviewed by EPA scientists and/or the original authors (depending on whether they wish to be involved), and revised based comments provided. The overall SOP developed by GDIT scientists, continues to be refined and updated periodically as additional experience is gained.

2.11.3 Links and references

Examples of AOPs resulting from this effort, to date:

https://aopwiki.org/aops/442

https://aopwiki.org/aops/499

https://aopwiki.org/aops/500

https://aopwiki.org/aops/504

https://aopwiki.org/aops/505

https://aopwiki.org/aops/513

https://aopwiki.org/aops/517

https://aopwiki.org/aops/518

https://aopwiki.org/aops/521

https://aopwiki.org/aops/526

https://aopwiki.org/aops/527

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https://aopwiki.org/aops/548

https://aopwiki.org/aops/561

https://aopwiki.org/aops/565

2.12 Adverse Outcome Pathways for Safe and Sustainable by Design approaches

Presenters: Thomas Exner, Seven Past Nine (Switzerland), ORCID 0000-0002-1849-5246

Penny Nymark, Karolinska Institute (Sweden), ORCID 0000-0002-3435-7775

Date: 2024-05-15

2.12.1 Issue addressed

Several European strategies have been put in motion to address the wide variety of existential challenges faced by humanity, including the EU Chemical Strategy for Sustainability (CSS)[12], the Zero Pollution Action Plan[13], the Circular Economy Action Plan[14]. To address needs described within these startegies the European Commision recently adopted a recommendation to promote research and innovation for safer and more sustainable chemicals and materials. The European Safe and Sustainable by Design (SSbD) framework[15] is central in the move towards increased protection of human health and the environment against hazardous substances as well as to reduce negative environmental and social impacts and improve the circularity of chemicals and materials.

2.12.2 Presentation content

The SSbD framework encourages industry to consider safety and sustainability aspects of chemicals and materials as early as possible during the innovation process, before regulatory requirements become relevant. However, successful operationalization of the SSbD framework relies on transparent assessment processes supported by readily available and machine-actionable (FAIR) data, along the entire life cycle of the chemical or material (Karakoltzidis et al., 2024). The data used for assessments during SSbD supports decisions on whether or not to proceed with the innovation, or alternatively what is needed to re-design the innovation to be safer and more sustainable.

Data for SSbD can be obtained through in silico modelling based on existing knowledge from similar substances (e.g. QSAR and read-across) and/or testing with high-throughput in vitro assays, i.e. new approach methodologies (NAMs). This is where the AOP framework comes in, and which, if FAIR itself, has the potential to serve as a transparent framework providing a basis for inclusivity of data and methods, and supporting improved integration, visibility and increased trust in NAMs data (Wittwehr et al., 2024) by relating the results to mechanistic knowledge.

The EU project PINK[16] is one of several ongoing projects aiming to develop novel modelling and simulation approaches addressing industrial SSbD needs. PINK is building an open innovation platform which will implement open science and FAIR principles to strengthen knowledge transfer and boost the innovative capacity of industry whilst also making them more agile to respond to external and internal influences like economic pressures and/or political priority changes. The project's key focus lies on supporting data-driven approaches, including AOP-based evidence generation building on integrated knowledge graphs linking and streamlining widely diverse data, relevant to the SSbD approach. AOPs have the potential to provide a basis for safety assessments based on relevant exposure levels and toxicity pathways/endpoints (Carusi et al., 2018; Nymark et al., 2020), and simultaneously provide excellent means for intuitive and trusted visualization through informed simplicity (Knapen, 2021; Wittwehr et al., 2024).

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[12] https://ec.europa.eu/environment/strategy/chemicals-strategy_en

[13] https://ec.europa.eu/environment/strategy/zero-pollution-action-plan_en

[14] https://ec.europa.eu/environment/strategy/circular-economy-action-plan_en

[15] https://research-and-innovation.ec.europa.eu/news/all-research-and-innovation-news/recommendation-safe-and-sustainable-chemicals-published-2022-12-08_en

[16] https://pink-project.eu/

2.12.3 Links and references

(Carusi et al., 2018; Karakoltzidis et al., 2024; Knapen, 2021; Nymark et al., 2020; Wittwehr et al., 2024)

2.13 Automated access to AOP-Wiki contents through knowledge graph and API

Presenter: Marvin Martens, Maastricht University (Netherlands), ORCID 0000-0003-2230-0840, email: marvin.martens@maastrichtuniversity.nl

Date: 2024-06-26

2.13.1 Issue addressed

The presentation addressed the challenges of accessing and utilizing data from the AOP-Wiki in a streamlined and interoperable manner. Given the vast amount of data and knowledge captured within the AOP-Wiki, and its steady growth, the challenge of optimally using this data becomes increasingly important. While access to content is currently possible via the webpage and XML exports, these methods are limited in flexibility and automatability. They often require significant manual effort or custom scripting to effectively utilize the data. This limitation hinders the potential for utilizing AOP knowledge in computational modeling, integration with other databases, systems toxicology, and the automation of risk assessment applications or tools. Overall, the aim of the presented work is to make AOP-Wiki content more accessible and versatile, addressing the needs of users requiring advanced tools and automated workflows and facilitating the development of targeted services and enhancing interoperability across the AOP community.

2.13.2 Presentation content

The presentation described how Resource Description Framework (RDF) and SPARQL technologies facilitate linked open data, with a focus on their application in the AOP-Wiki. It explained the principles of RDF and SPARQL, emphasizing their role in creating interconnected data and knowledge networks, and highlighted examples of biomedical databases already utilizing these technologies, setting the context for the AOP-Wiki RDF development (Martens et al., 2022), continued in the VHP4Safety[17] project (Kienhuis et al., 2024).

Two primary methods for accessing RDF-based AOP-Wiki content were showcased. The SPARQL endpoint enables flexible data queries, supports exporting in various formats, and integrates seamlessly with coding environments like Python and R. Its reliability is further underscored by its high ranking on Yummydata.org, which monitors life science SPARQL endpoints across the web. The SNORQL interface[18] complements the SPARQL endpoint by offering enhanced user functionalities, such as a preloaded query panel linked to a GitHub repository and the ability to share SPARQL queries via permalinks. Practical examples demonstrated its versatility, from basic data retrieval to advanced federated SPARQL queries integrating AOP-Wiki content with external databases like

[17] https://vhp4safety.nl/

[18] https://aopwiki.rdf.bigcat-bioinformatics.org/

WikiPathways (Waagmeester et al., 2016) and AOP-DB (Mortensen et al., 2022). The technical workflow for generating and deploying these services—using Jupyter notebooks for RDF generation and Docker for deployment—was also presented.

Another highlighted service, a grlc REST API loaded with AOP-Wiki endpoints, simplifies data access by wrapping SPARQL queries into easy-to-use API endpoints, making the data accessible without requiring users to understand SPARQL. Examples illustrated how this approach balances simplicity and functionality.

The presentation concluded with the AOP-networkFinder service (Yarar et al., 2025), which uses the SPARQL endpoint to construct AOP networks. This example demonstrated the endpoint's value in supporting downstream applications, showcasing the broader potential of these technologies in advancing scientific workflows.

2.13.3 Links and references

(Kienhuis et al., 2024; Martens et al., 2022; Mortensen et al., 2022; Waagmeester et al., 2016; Yarar et al., 2025)

Presentation slides: (Martens, 2025)

Funding: This research project is funded by the Netherlands Research Council (NWO) 'Netherlands Research Agenda: Research on Routes by Consortia' (NWA-ORC 1292.19.272).

2.14 The SCAHT AOP_HUB

Presenter: Ellen Fritsche, SCAHT (Switzerland), ORCID 0000-0002-7454-679X

Emre Çörek, SCAHT (Switzerland), ORCID 0000-0002-2088-7556

Date: 2024-08-07

2.14.1 Issue addressed

The development of AOPs, including the assembly of putative AOPs and the construction of KERs, may initially appear straightforward when following the guidelines presented in the OECD handbook. However, the true complexity emerges in the finer details, and researchers quickly recognize that AOP development requires a high level of precision and expertise. This challenge is particularly pronounced for early-career toxicologists, who may encounter difficulties in systematically integrating relevant research data into the AOP framework.

At present, there are limited opportunities for open discussion or dedicated support networks focused on hands-on AOP development for early- and mid-career researchers, including those at the Master's, PhD, and postdoctoral levels. In response to this need, we have established the SCAHT AOP_HUB as a platform to bridge this gap (for logo, see **Figure 10**).

The AOP_HUB is a collaborative platform designed to foster an inclusive and dynamic environment for pre- and postgraduate students, as well as postdoctoral researchers. It supports the generation of AOPs, knowledge sharing, networking, capacity building, and community engagement. Through regular online meetings, the platform provides practical assistance in developing AOPs for real-world challenges. Its goal is to help students from diverse backgrounds, disciplines, and cultural contexts translate their research into AOPs, utilizing existing public data or generating new data to fill gaps in AOP development.

Peer interactions, group discussions, and shared experiences create a supportive and motivating atmosphere, where students feel valued and inspired to pursue their academic and professional aspirations.

Figure 10. Logo of the SCAHT_HUB

Source: SCAHT

2.14.2 Presentation content

At first, the SCAHT – Swiss Centre for Applied Human Toxicology was introduced. The SCAHT represents an interface for regulatory toxicology that spans across research, education, and interaction with regulatory bodies. Due to its integration of research and regulation, the SCAHT is the ideal centre for promoting AOPs. Considering the complexity of AOP development, the SCAHT identified early-career researchers as a target group that we would like to support. Therefore, the SCAHT AOP_HUB was founded. The presentation contextualized the AOP_HUB foundation with the information highlighted above (2.14.1). The AOP_HUB was kicked off by a virtual meeting on August 22nd, 2024, and will be collaborating with other AOP initiatives like the Organization for Economic Cooperation and Development (OECD), the Society for the Advancement of AOPs (SAAOP), and the SKIG.

In a second presentation, the principles and examples for AOPs concerning neurotoxicity were addressed. Modes of action and clinical outcomes differ between developmental (DNT) and adult neurotoxicity (ANT; (Tigges et al., 2021)). Therefore, AOP development follows different paths for DNT and ANT. As for DNT, most MIEs are enigmatic, and hence cellular KEs, which integrate a large variety of MIEs, can be intuitive starting points (Bal-Price et al., 2015). In contrast, a vast amount of textbook knowledge is available for ANT, e.g., concerning activation or inhibition of neurotransmitter receptors (Spencer et al., 2000). Therefore, MIEs are much easier to implement into ANT AOPs. New approach methodologies (NAMs) are very well suited for producing mechanistic understanding of chemicals' adverse effects on the cellular level. Therefore, NAMs for DNT and ANT were briefly explained. In contrast to ANT, there is a DNT in vitro battery available that covers a large variety of cellular KE relevant for DNT[19]. Many of these NAMs have been developed and scientifically validated within collaborative projects like the H2020 projects ONTOX (Vinken et al., 2021) and RISKHUNT3R (Pallocca, 2022), the Horizon Europe projects PARC (https://doi.org/10.3030/101057014[20]) and CHIASMA, and, especially for DNT, with regulatory support from the European Food Safety Authority (EFSA; (Blum et al., 2023)) and the US Environmental Protection Agency (US-EPA; (Carstens et al., 2022)). These NAMs have been supporting AOP network building for DNT and ANT (www.aopwiki.org). However, despite the amount of work that has been contributing to the development of AOPs concerning neurotoxicity, there are still large gaps concerning KE like neural progenitor cell proliferation, myelination, or human disease adverse outcomes to be filled in the future.

2.14.3 Links and references

(Bal-Price et al., 2015; Blum et al., 2023; Carstens et al., 2022; Pallocca, 2022; Spencer et al., 2000; Tigges et al., 2021; Vinken et al., 2021)

2.15 When to report an AOP network

Presenter: Tanja Burgdorf, Federal Institute for Risk Assessment (Germany), ORCID 0000-0002-7810-5545

Date: 2024-08-07

2.15.1 Issue addressed

Topic of the presentation was how AOP networks are to be treated in the AOP-Wiki. According to the AOP developers' handbook[21], each linkage from an MIE to an AO should become a separate AOP. However, this could massively increase the number of AOPs in the AOP-Wiki if several different steps can take place at the cellular and organ levels, as is the case for AOP 439 "Activation of AhR leading to metastatic breast cancer" (Benoit et al., 2022). Based on this example, the question arose of when a series of KEs should be classified as a single AOP versus when they should be considered a network of independent AOPs, and if the AOP developers' handbook provides sufficient information on this issue.

2.15.2 Presentation content

AOP 439[22], which recently completed coaching, presents a complex picture with one MIE and one AO but includes numerous KEs at both cellular and organ levels. So far, the entire network is represented in one AOP but could be divided into a series of six linear AOPs, each combining one cellular and one organ-level KE. However, AOP networks are acknowledged in the developers' handbook when multiple upstream KEs lead to a given AO or when a perturbation may cause a range of AOs. It is recognized that in some cases, there may be exceptions where representing a

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[19] OECD 2023, https://one.oecd.org/document/ENV/CBC/MONO(2023)13/en/pdf

[20] https://cordis.europa.eu/project/id/101057014

[21] AOP Developers' Handbook 2.7, released May 30, 2024; https://aopwiki.org/handbooks/5

[22] https://aopwiki.org/aops/439

simple AOP network on an AOP page is a more pragmatic unit of development and evaluation. For example, representation of branching on an AOP is acceptable when there are multiple KEs, causally linked to the MIE and AO, that are occurring concurrently and acting in concert to drive the downstream effects. In such cases, the various KEs cannot be placed neatly into a single temporal sequence because they are effectively occurring simultaneously. Likewise, it cannot be determined which of the concurrent KEs is most essential or critical, because there are multiple KEs contributing jointly such that it cannot be effectively determined whether one could cause the pathway to progress without the other. The AOP developers' handbook provides sufficient information in this regard.

For AOP 495[23], "Androgen receptor activation leading to prostate cancer," which is not in coaching yet, a representation similar to AOP 439 has been chosen, and there are shared KEs at the cellular level. AOP 200[24], "Estrogen receptor activation leading to breast cancer," is another example of a cancer-related AOP network with extensive cellular and tissue-level responses, separating early effects (initial, primary) at the cellular level in one AOP.

On the other hand, a classical AOP network affecting the thyroid hormone system, which clearly illustrated independent AOPs proceeding via different KEs and occurring at distinct developmental stages in fish, was presented. This network featured three different MIEs leading to the AO of increased mortality in fish, justifying its status as a network of five separate AOPs represented as AOPs 155 to 159 in AOP-Wiki, which are also published in the OECD Series on Adverse Outcome Pathways (Vergauwen, 2022a, 2022b, 2022c, 2022d, 2022e).

It was stated as important in the discussion that AOPs clearly depict what is known in a linear manner. One possibility to achieve this could be incorporating 'and' functions into the KER descriptions rather than entangling them into complex networks. On the other hand, the resulting separate linear AOPs might be overly simplistic, depending on the available weight of evidence for separate pathways within the network.

The discussion highlighted the challenge of determining when to depict a series of KEs as a single AOP or a network of AOPs, particularly in complex biological scenarios like cancer initiation or progression. There was a consensus on the need for further discussion among experts to refine the guidance and application of AOPs in these contexts. The level of detail needed in cancer-related AOPs needs further discussion, as does how tumor initiation, progression, and metastasis should be differentiated and addressed in separate AOPs.

2.15.3 Links and references

(Benoit et al., 2022; Vergauwen, 2022a, 2022b, 2022c, 2022d, 2022e)

2.16 Omics2AOPs

Presenter: Julia M. Malinowska, European Commission, Joint Research Centre (JRC, Italy), ORCID 0000-0001-6565-2980

[23] https://aopwiki.org/aops/495

[24] https://aopwiki.org/aops/200

Date: 2024-09-18

2.16.1 Issue addressed

The AOP framework systematizes current biological knowledge and is an important resource in toxicology. However, examples of incorporating omics data (e.g., transcriptomics, proteomics, or metabolomics) within the AOP framework are scarce. The new project "Omics2AOPs," launched by the European Commission's Joint Research Centre (EC-JRC) and the Finnish Hub for Development and Validation of Integrated Approaches (FHAIVE, Tampere University), aims to annotate genes, proteins, and metabolites to KEs and AOPs by building on existing and well-established ontologies from the two domains (i.e., omics and AOPs).

2.16.2 Presentation content

The presentation started by outlining challenges in using omics data in regulatory toxicology. One such challenge is data interpretation, a crucial step that lacks standardization. Omics2AOPs was initiated to contribute towards efforts addressing this problem and aims to link genes, proteins, and metabolites to KEs and AOPs by using well-established ontologies. The project is inspired by previous work (Saarimäki, Fratello, et al., 2023; Saarimäki, Morikka, et al., 2023) which demonstrated linking gene sets to KEs and AOPs by applying Natural Language Processing (NLP), followed by manual curation and incorporation into the Unified Knowledge Space. This resulted in the annotation of genes to KEs. For example, two biological processes of Gene Ontology ("thyroid hormone generation" and "thyroid hormone metabolic process") were "matched" to the KE 277 "Thyroid hormone synthesis, Decreased," enabling the linking of the genes underpinning these biological processes to the KE (Saarimäki, Fratello, et al., 2023). Such methodology builds upon the wealth of information enclosed in freely available resources commonly used to interpret omics data and allows linking this to KEs and AOPs. The presentation also outlined the potential impact of the project, including 1) improving and standardizing the interpretation of omics data, 2) supporting FAIRification efforts (as different data streams become integrated with each other), and 3) accelerating the development of new in vitro assays through knowledge generated about relationships between genes, proteins, or metabolites and KEs. For these reasons, EC-JRC and FHAIVE have been actively working on the project proposal for submission to the Working Party on Hazard Assessment (WPHA) at the OECD. As the project requires multidisciplinary expertise across several distinct areas, the SKIG facilitated connecting with the AOP community to seek their feedback and involvement in the next steps.

2.16.3 Links and references

(Saarimäki, Fratello, et al., 2023; Saarimäki, Morikka, et al., 2023)

2.17 Tools and strategies used in the development of an AOP network leading to permanent DNA damage

Presenter: Birgit Mertens, Sciensano, Belgian Institute of Health (Belgium), ORCID 0000-0003-2230-0840

Emmanuel Demuynck, Sciensano, Belgian Institute of Health (Belgium), ORCID 0009-0003-1531-968X

Date: 2024-09-18

2.17.1 Issue addressed

When two or more AOPs share at least one KE, they can be integrated into a network. An AOP network is generally more representative of the multiple effects that a stressor can induce on a living organism and allows for a more accurate study of the adverse outcomes of such a stressor compared to single AOPs. However, while seemingly straightforward, constructing an AOP network presents several challenges.

In this context, a practical exercise was undertaken to compile AOPs that lead to genotoxic adverse outcomes (AOs) (i.e., "Increase, gene mutations," "Increase, structural chromosome aberrations," and "Increase, numerical chromosome aberrations") into an AOP network to support the development of AOP-based Integrated Approaches for Testing and Assessment (IATAs) for genotoxicity. The challenges encountered and opportunities identified during this process were discussed.

2.17.2 Presentation content

The development of the AOP network started by compiling AOPs present in the AOP-Wiki and related to genotoxicity into an inventory. Nineteen AOPs were found, ten of which are included in the OECD workplan. The AOPs not included in the OECD workplan did not provide additional information. Therefore, a first draft network was constructed based on these ten AOPs.

Preliminary investigations of the inventory highlighted several challenges:

- Many KEs were similar or duplicated (e.g., KE 1636 "Increase, Chromosomal aberrations" and KE 1554 "Increase Chromosomal aberrations"). The KEs used in the AOPs with the most advanced status of development were selected.
- All ten AOPs included KEs or AOs downstream of the genotoxic AOs of interest (i.e., "Increase, gene mutations," "Increase, Structural chromosome aberrations," and "Increase, Numerical chromosome aberrations"). The downstream KEs were filtered out of the inventory, although they could be integrated later when constructing larger AOP networks leading to, for example, cancer or other genotoxicity-related adverse outcomes.
- Some AOPs were chemical-specific (e.g., AOP 46 "AFB1: Mutagenic Mode-of-Action leading to Hepatocellular Carcinoma (HCC)"). This is not in line with the recommendations provided in the AOP Developer's Handbook (OECD, 2018), which highlights that an AOP is an analytical construct that describes a sequential chain of causally linked events that can be triggered by any stressor able to initiate the MIE.
- Important work on genotoxicity-related AOPs reported in the literature (Sasaki et al., 2020) was not yet included in the AOP-Wiki. Therefore, this information was added to the inventory.

Even after removing downstream and duplicate KEs, integrating the remaining KEs into a network remained challenging, partly due to discrepancies in the description of events associated with genotoxic processes in the different AOPs. While some KEs described precise and unique biological events (e.g., KE 1461 "Increase, DNA double-strand breaks"), others encompassed distinct biological events (e.g., KE 1669 "Increased, DNA damage and mutation"). The discrepancy between AOPs, probably caused by their stand-alone nature, resulted in inconsistencies in the global AOP network. Several additional modifications were therefore made to the draft AOP network. Overall, building an AOP network requires several reviewing steps and additional literature investigations.

One major opportunity of compiling AOPs into a network includes the appearance of new relationships. For example, in the network, the KE “Increase, Structural chromosome aberrations” (KE 1636) is now indirectly linked to the MIE 97 “Alkylation, DNA.” Similarly, the AOP “Formation, Bulky DNA adducts leading to Increase, Mutations” is now also connected to the AO “Increase, Structural chromosome aberrations.” Moreover, the AOP 202 starting with “Binding to topoisomerase II enzyme” now also has a link with “Increase, Mutations.”

The current version of our AOP network will be published during the first half of 2025 and will serve as a basis for building IATAs for genotoxicity. However, significant efforts will be needed to document and update the AOP network on the AOP-Wiki. The full development of an AOP on the AOP-Wiki is a complex process, not always as straightforward as explained in the AOP Developer’s Handbook. Although this document is useful to guide developers, it allows a lot of free choices, leading developers to take different directions, complicating the development of AOP networks. Furthermore, the current context of AOP development progress does not outline the strategy to follow for correctly developing AOPs. Indeed, the consistent progress in AOP development tools adds uncertainties and variability to this task. In our work, we used a basic systematic search approach to further document some specific KEs and KERs (i.e., the development of the AOP linking “Formation, Bulky DNA adducts” with “Increase, Mutations” and “Increase, Structural chromosome aberrations”) in our AOP network in the AOP-Wiki. The steps of our workflow are as follows:

1. Definition of the search string (by a group of experts and with the support of a librarian);
2. Assessment of retrieved papers based on the title/abstract with the Rayyan review tool by two independent reviewers following a pre-defined decision flow chart to streamline the process (everyone thinks differently). In case of conflicting results, a third reviewer was consulted to make a final decision;
3. Data extraction in a dedicated template based on the full manuscript;
4. Writing of the AOP document based on the data extraction table.

In our exercise, about fifteen scientists were involved. Even with the pre-defined decision flow chart to guide choices and instructions for completing the data extraction template, the progress was not as smooth as expected. Therefore, if an AOP development project involves several people/actors, a clear management strategy (including a formal problem formulation) should be defined at the start to make the progress efficient.

2.17.3 Links and references

(OECD, 2018; Sasaki et al., 2020)

https://aopwiki.org

https://aopwiki.org/events/1636

https://aopwiki.org/events/1554

https://aopwiki.org/events/1461

https://aopwiki.org/events/1669

https://aopwiki.org/events/97

https://aopwiki.org/aops/202

https://aopwiki.org/aops/46

2.18 Multi-endpoint Data Reporting Format (DRF) and dedicated user-interface (UI) for dose response-data

Presenter: Knut Erik Tollefsen, Norwegian Institute for Water Research (NIVA, Norway), ORCID 0000-0002-7534-0937

Date: 2024-10-30

2.18.1 Issue addressed

Effect-based methods generate dose-response (DR) data spanning multiple levels of biological organization, often characterized by substantial heterogeneity in format, content, and scope of studies/utilization. These data are typically produced using various enabling technologies across the molecular scale (e.g., genomics, transcriptomics, metabolomics, proteomics, etc.), phenotypical scale (e.g., observable characteristics or traits of an organism), and apical or adverse scale (e.g., survival, growth, development, reproduction, etc.). Reporting of such data often follows criteria or standardized Data Reporting Formats (DRFs) tailored to each enabling technology. While individual DRFs offer valuable guidance for reporting, they may exhibit significant variability in requirements, making common integration into AOPs and AOP networks (AOPNs) challenging and hampering efficient use in developing quantitative AOPs and AOPNs for use in Next Generation Risk Assessment (NGRA).

Developing a multi-endpoint DRF to provide simplified and standardized reporting of effects data spanning the AOP continuum could help address these challenges. Such a solution would align with FAIR (Findable, Accessible, Interoperable, and Reusable) principles and incorporate controlled vocabularies, ontologies, and specialized terminologies. To this end, the Norwegian Institute for Water Research (NIVA), the Norwegian University of Life Sciences (NMBU), and partners in the EU project PARC have collaborated to develop standardized approaches that can FAIRify DR data.

2.18.2 Presentation content

The presentation outlined the importance of standardization in managing the diversity of DR data. It introduced the Multi-endpoint Data Reporting Format (DRF) to systematize metadata, stressor characteristics, and biological observations. Additionally, it presented a prototype web-based User Interface (UI) called “qData,” designed to assist users with data entry, metadata management, and data integration.

Key Functionality of the qData UI:

- Data Structure: Provides standardized fields for stressors, exposure routes, study designs, and assay formats.
- Metadata Management: Ensures alignment with controlled vocabularies, ontologies, and unique chemical identifiers, ensuring compatibility with AOP data fields.
- Host Database Functionality: Supports statistical analysis and modeling for quantitative AOP development and NGRA.

The presentation and discussions also emphasized the linkage between qData and AOP data fields, its complementarity with other FAIRification approaches, and its potential alignment with the OECD

Omics Reporting Framework as standardized guidelines for documenting and reporting AOP-relevant data.

2.18.3 Links and references

- qData Prototype: https://stop-q-data.t.niva.no/
- OECD OMICS reporting Format: https://www.oecd.org/chemicalsafety/testing/omics.htm
- EU PARC Project: https://www.eu-parc.eu/
- FAIR principles: https://www.go-fair.org/fair-principles/

2.19 AI4AOP

Presenter: Clemens Wittwehr, European Commission, Joint Research Centre (JRC, Italy), ORCID 0000-0003-2760-7702, email: clemens.wittwehr@ec.europa.eu

Date: 2024-10-30

2.19.1 Issue addressed

The AOP-Wiki is a collaborative platform where researchers organize and capture AOP knowledge following specific principles. As of December 2024, the AOP-Wiki contains 508 AOPs, 1822 KEs, 2901 KERs, and has 337 users with author privileges. Despite the increasing number of AOPs and the established status of the AOP concept, achieving a critical mass of AOPs for a paradigm shift in science may not occur unless new approaches facilitate growth in AOP numbers. Additionally, AOPs must be easily accessible, understood, and applied in real-life scenarios to be effective. Community building within the AOP framework is also crucial, as it involves engaging stakeholders and fostering collaboration.

2.19.2 Presentation content

To address the aforementioned issues, the use of Artificial Intelligence (AI) is proposed as an innovative solution to support key processes in the AOP domain. The initiative, AI4AOP, is envisioned as a crowd effort to explore the potential of AI in various aspects of AOP development and application. AI can provide support across several domains (as laid out in (OECD, 2018)), including the development, assessment, and review of AOPs, enhancing usage and understanding, and community building.

- For AOP development, AI can identify emerging toxicological issues, locate relevant literature, distill literature into KEs, connect KEs to identify KERs, and assist in systematic reviews and evidence mapping. AI can also detect AOP networks and facilitate data integration and harmonization. When it comes to peer review and consensus building, AI can streamline the process by matching AOPs with potential reviewers and synthesizing feedback.
- In terms of AOP usage, AI can improve the AOP-Wiki's usability and interactivity, including enhancing training and educational tools, quality control, and providing translation and multilingual support. AI initiatives such as ChatAOP in the Harmless project and an AI4AOP JRC initiative are already identified as steps towards these goals.

— For AOP community building, AI can enhance stakeholder engagement, identify experts, track contributions, and facilitate community-driven AOP curation and validation. It can also analyze community discussions for conflict resolution and consensus analysis, send personalized communications, and assist in virtual collaboration and workshop facilitation.

The presentation outlined a possible roadmap for the AI4AOP effort, which includes reaching out to potential team members, elaborating on expected outcomes, deciding on outreach strategies, identifying ongoing AI initiatives, and defining the project scope. The AI4AOP group is expected to work together to leverage AI capabilities to overcome the bottleneck of specialized expertise and accelerate the growth of AOPs.

2.19.3 Links and references

Presentation slides: (Wittwehr, 2024a)

AI4AOP thought starter document: (Wittwehr, 2024b)

(OECD, 2018)

2.20 ChatAOP: A chat interface for the AOP-Wiki

Presenter: Nina Jeliazkova, ORCID 0000-0002-4322-6179, Ideaconsult Ltd., Sofia (Bulgaria)

Evgeni Marinov ORCID 0000-0002-9684-9142, Ideaconsult Ltd., Sofia (Bulgaria)

Date: 2024-10-30

2.20.1 Issue addressed

The development of the ChatAOP proof-of-concept was initiated as part of the HARMLESS project[25] data management Work Package 1 (WP1). The goal is to facilitate the interpretation of complex material safety data through the AOP framework and explore future integration of natural language queries into eNanoMapper[26] data solutions. While the Nanosafety Data Interface (Jeliazkova et al., 2021) https://enanomapper.adma.ai provides free text and faceted search through a web browser, as well as programmatic access, the data complexity poses challenges to users. Data generated through research protocols often requires expert interpretation. ChatAOP aims to assist by enabling intuitive querying and generating summaries of toxicological knowledge to support decision-making and risk assessment.

2.20.2 Presentation content

FAIR data management tools for material safety, developed by Ideaconsult Ltd. through extensive collaboration within EU-funded and industry projects, were briefly outlined. The accumulation and integration of diverse characterization and safety data into the NanoSafety Data Interface

[25] HARMLESS https://www.harmless-project.eu/ European Union's Horizon 2020 research and innovation programme grant agreement 953183

[26] https://www.enanomapper.net/

(Jeliazkova et al., 2021) is enabled through data entry tools like Template Wizard (Jeliazkova et al., 2024) and subsequent conversion into a common data model (Kochev et al., 2020).

The presentation continues with an example of a natural language question to the ChatAOP proof-of-concept tool, asking about the role of oxidative stress in liver fibrosis. ChatAOP identifies AOP 38 and AOP 144 and includes the following summary in its answer: "*Oxidative stress plays a significant role in the development of liver fibrosis. In both AOPs, oxidative stress acts as a crucial KE that drives the progression of liver fibrosis by promoting inflammation, cell damage, and activation of fibrogenic cells.*"

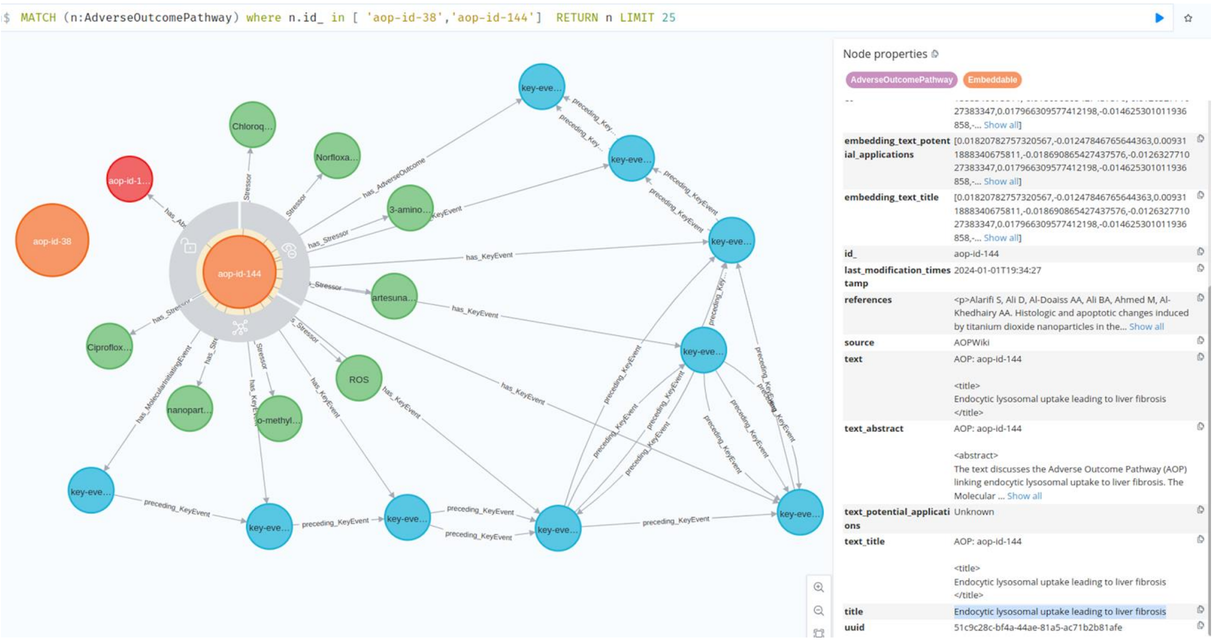
ChatAOP leverages the same technology as the now popular "chat-with-your-pdf" applications, namely Retrieval-Augmented Generation (RAG) (Zhao et al., 2024). The main steps of a RAG process are: (1) the user inputs a query, (2) relevant text and/or data is retrieved from a source (a set of documents, database, etc.) and is added to the query as context, and (3) a Large Language Model synthesizes the results based on both the query and the context, and the generated response is returned to the user. RAG is a very active area, with different implementations of each step. Step 2 typically uses semantic similarity through vector embeddings of the query text and documents. Graph RAG improves retrieval quality by considering connections between items, i.e., taking into account the connections between stressors, KEs, AOs, biological processes, etc.

The ChatAOP implementation[27] includes processing the AOP-Wiki XML file using Python to serialize key elements, including AOPs, MIEs, KEs, AOs, stressors, and their relationships, into JSON format. These objects, along with the corresponding text embeddings, are stored in a Neo4j graph database[28]. The resulting Graph-RAG pipeline combines structured data with advanced natural language querying and graph-based visualization (Figure 11).

[27] https://github.com/ideaconsult/ChatAOP

[28] Neo4j Graph Database: https://neo4j.com

Figure 11. AOP representation in a graph database (Neo4j)



Source: screenshot made for this publication

Additionally, the proof-of-concept has been extended to query multiple sources (e.g., AOP-Wiki through Neo4j, PubMed, and Google search) and synthesize the answer via a multi-agent system[29]. A comparison of results was presented.

2.20.3 Conclusions

ChatAOP demonstrates the potential of applying RAG technology to query a toxicology knowledge base using natural language. This approach reduces hallucinations that may arise when querying a Large Language Model directly. While the results are promising, further testing and validation are necessary. To support this effort, the authors are conducting a survey[30] to gather relevant questions and, where possible, corresponding answers. The next steps are to extend the framework to enable querying and interpretation in the eNanoMapper database (Jeliazkova et al., 2021) based on the AOP knowledgebase.

2.20.4 Links and references

(Jeliazkova et al., 2021, 2024; Kochev et al., 2020)

[29] CrewAI Multi-Agent System: https://www.crewai.com

[30] https://forms.gle/yz2K8UyDMuRw9CLG9

2.21 Physiological maps (and how to use them to establish or assess biological relevance in AOPs)

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2.21.1 Issue addressed

Recent advancements in the field of in silico toxicology emphasize a mechanistic understanding of chemical-induced adverse effects, primarily through (quantitative) AOPs. Despite this progress, there is a growing need for deeper mechanistic insights at the gene and cellular levels, addressing both pathological and physiological processes. Mapping the physiology of specific biological processes through "physiological maps" is envisioned as a valuable complement to the development of AOPs, particularly with regard to the biological plausibility of KER. Besides serving as a literature-supported and annotated graphical representation of molecular processes, these maps also aim to address critical needs for improved data integration, interoperability, and standardization (Staumont et al., 2025).

2.21.2 Presentation content

The presentation covered the development of physiological maps as part of the ONTOX project[31] (Vinken et al., 2021), which aims to develop animal-free strategies for predicting repeated-dose chemical toxicity. These maps are graphical representations of biological processes, functioning as digital navigation tools for exploring molecular and cellular interactions—much like Google Maps, but for the biological landscape (Ladeira et al., 2024).

2.21.2.1 Map Development and Structure

The creation and usage of physiological maps follow a four-phase workflow: planning, curation, update, and application. They are built using the Systems Biology Graphical Notation (SBGN) and the Systems Biology Markup Language (SBML) through tools like CellDesigner (http://www.celldesigner.org/) and the MINERVA (Molecular Interaction NEtwoRk VisuAlization) platform (https://minerva.pages.uni.lu/doc/), which enable interactive features such as zooming and information retrieval, as well as data annotation. The maps integrate various data sources, including research papers, databases, and existing maps.

[31] https://ontox-project.eu/

2.21.2.2 Case Study: Brain Development

A specific case study focusing on oligodendrocyte development in the developing human brain was presented. The maps were created through collaboration with domain experts who manually curate scientific literature, which is then integrated into the map. The oligodendrocyte submap includes detailed sections on inputs, interactions taking place on cellular membranes, and intracellular mechanisms, all leading to the activation or inhibition of key neurodevelopmental processes focusing on the development of a specific cell type (oligodendrocytes).

2.21.2.3 Connection to AOPs

It was demonstrated how physiological maps, represented by the oligodendrocyte development submap, connect to AOPs, particularly through a curated network of 16 AOPs related to cognitive function defects. While physiological maps provide detailed molecular and cellular information in greater granularity than KEs, AOPs typically focus on broader tissue and organ-level effects that are absent in physiological maps. By overlapping distinct processes on the oligodendrocyte development submap with several KEs of the network related to oligodendrocyte development and function, the map helps to establish biological plausibility in KERs and identify knowledge gaps in neurodevelopmental toxicity.

2.21.2.4 Applications and Future Direction

The maps serve multiple purposes, including literature collection, knowledge representation, data integration and analysis, structured ontology exploration, and supporting modeling efforts. Furthermore, various types of (quantitative) data, such as transcriptomic profiles and disease-related information, can be overlaid onto the nodes and processes depicted on the maps. Once available for interactive exploration on the MINERVA platform, they can be leveraged by AOP developers. The same framework is also being used to represent AOPs and AOP networks, allowing easy integration of resources.

2.21.3 Links and references

(Ladeira et al., 2024; Staumont et al., 2025; Vinken et al., 2021)

3 Hands-on lessons learned from the SKIG presentations and discussions for short-term AOP-Wiki implementation

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3.1 Issue covered

The 2023-2024 SKIG presentations featured in this report encompass a broad range of advances and discussions within the AOP community. While they offered insights into how the AOP-KB and AOP-Wiki can be improved to better serve users and enhance the use of AOPs for regulatory decision-making, they also addressed more general scientific and other issues associated with the development and application of AOPs. As mentioned in section 1.3, the AOP-Wiki is working smoothly, but the interoperability, accessibility, and utility of AOPs for a wide range of applications could be further enhanced via a redesign and rebuild of the AOP-Wiki that incorporates advanced technology and concepts that have emerged over the last decade of AOP-Wiki use. This next great leap in AOP-Wiki development is branded as *AOP-Wiki 3.0*.

The ideas and tools presented in the SKIG presentations inform plans for how we will transition from the current AOP-Wiki to a more semantically aligned data model with improved user interface enhancements in AOP-Wiki 3.0. The purpose of this section of the report is to share some of the AOP-Wiki-specific lessons learned from the SKIG meetings and propose ideas for features that can be introduced to the AOP-Wiki in its current form, as an "AOP-Wiki 2.x" release.

In determining which issues to prioritize for implementation in the AOP-Wiki, several key considerations were taken into account. The AOP-KB CG (see 1.3.1.3), which holds the ultimate responsibility for decisions regarding the AOP-Wiki, applied these criteria to ensure that the most relevant updates are addressed first (depending on financial feasibility). Recurring themes identified in SKIG discussions were given particular attention, as they reflect ongoing concerns and opportunities for improvement. Additionally, aspects deemed highly relevant to regulatory applications were prioritized, ensuring that the AOP-Wiki continues to effectively support decision-making processes. Through this prioritization process, the AOP-KB CG aims to enhance the functionality and utility of the AOP-Wiki for all stakeholders:

- To support efforts to scale up annotation of AOPs with terms from ontologies and controlled vocabularies – collectively referred to as knowledge organization systems (KOSs) – *new KOS interfaces* with search and sort options can be introduced to reveal term selections already associated with AOPs.
- A *collections feature* could be used to showcase the many AOP curation efforts presented in this report, which would benefit AOP network developers and help facilitate AOP community involvement in the transition to AOP-Wiki 3.0.

3.2 Lessons learned

The SKIG presentations described in section 2 covered a wide breadth of topics ranging from new tools developed using AOP-Wiki XML-based content exports to structured approaches for AOP development in response to regulatory needs and more. Alongside some of the scalable efforts enabled by specific technologies and tools, other presenters described ways that manual AOP curation approaches have revealed limitations in the existing AOP-KB content (see sections 2.4, 2.6, and 2.18). For example, redundancies exist between KEs, and inconsistent terminology usage can negatively impact network formation, computability, and jeopardize confidence in AOP-Wiki-derived information and tools. To tap into the full potential offered by systematic approaches (section 2.8) and generative AI (sections 2.8, 2.19, and 2.20) to contribute to AOP development, new solutions will be needed within the AOP-Wiki to address challenges associated with terminology inconsistencies. Plans for AOP-Wiki 3.0 are focused on how the data model and workflows can be designed to prevent some of the challenges that have been noted. In the interim period, we have used "low-tech" strategies, like the coordination efforts done by the AOP-Wiki Gardener to mitigate some of the language-based problems that have been identified. To support the AOP-Wiki Gardening efforts and others in the community, the AOP-Wiki development team can implement features that help reduce time spent on content curation processes.

3.3 Suggested new features

3.3.1 Proposed KOS Interfaces to Support AOP Ontology Efforts

As described in section 2.3, and as laid out in (Ives et al., 2017), annotation of KEs with ontology or KOS terms (i.e., KE components) defining biological object, process, and action terms are important for improving machine readability of AOPs and have value for some AOP network development strategies. This is also stressed in (Mortensen et al., 2021; Pittman et al., 2018). However, KE components are not extensively applied to all KEs and a comparative assessment of KOS term usage across multiple AOPs and KEs is not a straightforward process with current AOP-Wiki search interfaces. At present, access to the source ontology and ontology IDs for selected terms requires an approach for processing the available export formats (XML, JSON, and TSV). To help advance more systemic usage of KOS terms across all KEs by AOP authors and the AOP-Wiki Gardeners, KOS details can be revealed on KE pages and a new KOS search page can be added to enable comparison of term usage across KEs. Implementation of these proposed KOS-based updates would involve exposure of existing content and not require any changes to the underlying data model that supports the AOP-KB and AOP-Wiki.

3.3.2 Proposed Collections Feature

Throughout this report, there are numerous examples of groupings of AOPs and their core entities (the KEs and KERs) based on shared biological systems, mechanisms, or stressor impacts, as well strategies for AOP and AOP network development. Unfortunately, the AOP-Wiki does not currently offer a way to host these groupings. A collections feature would provide a way to showcase these groupings within the AOP-Wiki, which could bring more visibility to the efforts underway and improve alignment with FAIR principles. A collections feature would allow AOP-Wiki users to define, create, and showcase their collections of AOPs and/or KEs and KERs within the AOP-Wiki. The collection creators would be able to share the theme for their collection and specific inclusion and exclusion criteria used to define collection elements. A collection export feature could be designed which would make the AOP content ready for import into a network visualization tool such as cytoscape.

A specific use case for how a collections feature could be used can be derived from the details on the DNA damage AOP network development process (section 2.18). Mertens and Demuynck describe how they originally found 19 AOPs on genotoxicity but narrowed the scope of their first draft network to 10 out of the original 19 AOPs that were included in an OECD workplan. The proposed collections feature could be used to hold two or more distinct collections – reflecting the iterative AOP filtering approach that was employed. Showcasing the original 19 AOPs in a collection separate from the 10 AOPs used in the first network draft can benefit the wider AOP community. For one, inclusion within a collection could trigger a notification to the AOP authors included in the collection, which might incentivize submission of their work for inclusion in the OECD AOP program. Alternatively, others in the research community such as systems biology tool developers, might be interested in making contributions, which could make the 9 excluded AOPs ready for an OECD program later in the future.

Collaborative efforts like the Mystery of ROS consortium and CIAO have already been working with AOP groupings and a collections feature would allow them to make the groupings visible within the AOP-Wiki, which could facilitate contributions and collaborations between parallel efforts. The proposed collections feature would allow groupings to be defined thematically in ways that align with the subject matter expertise needed to support expanded KOS annotation efforts. By establishing a direct way of linking AOP community leaders to AOP-Wiki content, the proposed collections feature can then serve as thematic units to organize community engagement in the transition of content to AOP-Wiki 3.0.

4 Conclusions

In conclusion, the Society for the Advancement of AOPs Knowledgebase Interest Group (SKIG) has successfully addressed a wide array of topics crucial to the evolution of the AOP framework. Over the course of its meetings in 2023 and 2024, SKIG has examined key issues such as ontology-based harmonization, the application of AI tools in AOP development, the integration of omics data, the incorporation of temporal aspects, and automated access to AOP-Wiki contents, and many others. These discussions have not only clarified numerous complex issues but have also directly contributed to practical, hands-on changes to the AOP-Wiki, enhancing its usability and accuracy.

Through its dynamic and collaborative approach, SKIG has emerged as the leading global community for AOP-Wiki and broader AOP framework issues. This esteemed group of more than 40 international experts continues to provide invaluable insights and guidance, reinforcing its role as the go-to resource for scientists, regulators, and policymakers navigating the intricacies of the AOP domain. As SKIG moves forward into 2025 and beyond, it remains committed to offering competent advice and fostering the development and application of AOPs, thereby supporting safer and more sustainable chemical assessments in line with the European Commission's and other international organisations' policy shift towards non-animal testing.

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List of abbreviations and definitions

Abbreviations	Definitions
AI	Artificial Intelligence
AI4AOP	Artificial Intelligence for Adverse Outcome Pathways
ANT	Adult Neurotoxicity
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
AOP-KB	Adverse Outcome Pathway Knowledge Base
AOP-KB CG	Adverse Outcome Pathway Knowledge Base Co-ordinaton Group
API	Application Programming Interface
ARE	Antioxidant Response Element
CIAO	Modelling the Pathogenesis of COVID 19 using the Adverse Outcome Pathway Framework
COVID	Coronavirus Disease
DNA	Deoxyribonucleic Acid
DNT	Developmental Neurotoxicity
DR	Dose-Response
DRF	Data Reporting Format
ECCC	Environment and Climate Change Canada
EFSA	European Food Safety Authority
EMSA	Electrophoretic Mobility Shift Assay
ESCA	OECD Advisory Group on Emerging Science in Chemicals Assessment

Abbreviations	Definitions
FAIR	Findable, Accessible, Interoperable, Re-usable
FHAIVE	Finnish Hub for Development and Validation of Integrated Approaches
GDIT	General Dynamics Information Technology
GPT	Generative Pre-Trained Transformer
IATA	Integrated Approaches for Testing and Assessment
JRC	Joint Research Centre
JSON	JavaScript Object Notation
KE	Key Event
KER	Key Event Relationship
KOS	Knowledge Organization Systems
LV	Left Ventricle
MIE	Molecular Initiating Event
MoR	Mystery of ROS
MINERVA	Molecular Interaction NEtwoRk VisuAlization
mRNA	Messenger RNA
NAM	New Approach Methodology
NGRA	Next Generation Risk Assessment
NIVA	Norwegian Institute for Water Research
NLP	Natural Language Processing
NMBU	Norwegian University of Life Sciences
NRF2	Nuclear Factor Erythroid 2-Related Factor 2

Abbreviations	Definitions
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
ORCID	Open Researcher and Contributor ID
PARC	Partnership for the Assessment of Risks from Chemicals
PCR	Polymerase Chain Reaction
PPR	Plant Protection Products and their Residues
QSAR	Quantitative Structure-Activity Relationship
RAG	Retrieval-Augmented Generation
RDF	Resource Description Framework
REST	Representational State Transfer
RNA	Ribonucleic Acid
RoB	Risk of Bias
ROS	Reactive Oxygen Species
SAAOP	Society for the Advancement of AOPs
SARS	Severe Acute Respiratory Syndrome
SBGN	Systems Biology Graphical Notation
SBML	Systems Biology Markup Language
SKIG	SAAOP Knowledgebase Interest Group
SOP	Standard Operating Procedure
SPARQL	SPARQL Protocol and RDF Query Language
SSbD	Safe and Sustainable by Design

Abbreviations	Definitions
UI	User Interface
US EPA	U.S. Environmental Protection Agency
WoE	Weight of Evidence
WPHA	OECD Working Party on Hazard Assessment
XML	Extensible Markup Language

List of figures and tables

Annexes

How AI was used to create the heat map in section 2:

A local JRC installation of ChatGPT 4.o was used. The then current version of this manuscript was uploaded and the following prompt submitted:

```
The attached document contains 21 articles covering presentations given in the
SKIG group:
2.01  - Opportunities and challenges of integrating non-chemical stressors in
        AOPs
2.02  - Ontology-based harmonisation of ROS-related KEs
2.03  - Ontology-based KE components as enablers of Umbrella KEs
2.04  - AOP-Wiki content curation
2.05  - AOP-helpFinder 2.0
2.06  - AOP-KB 3.0 workshop discussion about Umbrella KEs
2.07  - How to enter temporal aspects into the AOP-Wiki
2.08  - An approach for applying ChatGPT in AOP development, and umbrella KEs
        related to the Mystery of ROS
2.09  - Applying a systematic approach to the development of evidence-based
        adverse outcome pathways (AOPs) for regulatory use
2.10  - Update on "Guide for Coaches"
2.11  - An SOP developed to translate literature AOPs into AOPs in the AOP-
        Wiki
2.12  - Adverse Outcome Pathways for Safe and Sustainable by Design approaches
2.13  - Automated access to AOP-Wiki contents through knowledge graph and API
2.14  - The SCAHT AOP_HUB
2.15  - When to report an AOP network
2.16  - Omics2AOPs
2.17  - Tools and strategies used in the development of an AOP network leading
        to permanent DNA damage
2.18  - Multi-endpoint Data Reporting Format (DRF) and dedicated user-
        interface (UI) for dose response-data
2.19  - AI4AOP
2.20  - ChatAOP: A chat interface for the AOP-Wiki
2.21  - Physiological maps (and how to use them to establish or assess
        biological relevance in AOPs)
Please review the whole text and especially the 21 articles and identify 8
categories according to which the articles could be categorized. Categories
could be "scientific", "technical", or similar.

Then, please make a table: 21 lines for the articles and 8 columns for the
categories and classify the articles to which extent they fall into one of the
categories, using classifications like 5 for very strong relationship between
article and category and 0 for no relationship at all.
```

The resulting table was imported into Excel and colour coding was inserted with the Excel "conditional formatting" function. All presenters were invited to manually correct the scores if they felt that the AI result did not properly reflect reality.

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