Deliverable 2.1 – Overview of relevant AOPs to inform NAM choice of KEs

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Public Summary

Adverse Outcome Pathways (AOPs) are a conceptual framework developed for organizing and presenting scientific data related to toxicological processes. An AOPs is a linear depiction of a toxicological process beginning with a molecular initiating event (MIE), in which a stressor first interacts with a biological target, pathway or process, leading to a series of intermediate or key events (KEs), triggering the adverse outcome (AO). The AOP framework is designed with the aim of both providing knowledge for use in risk assessment, and for identification of key uncertainties and research priorities.

The main objective of T2.1 is to provide an overview of AOPs for engineered nanomaterial (ENM) induced AOs relevant for human health and ecotoxicity for further development. Based on this, KEs useful as predictive markers of the AO were identified and this information will be fed to WP2.2 and WP3 for correlation analyses and NAM development.

Nano-relevant AOPs were identified and compiled from other EU projects (e.g. SmartNanoTox and PATROLs), through different approaches in the AOPwiki, and by other approaches. This resulted in a list of 25 ENM-relevant AOPs for human toxicology and 7 ENM-relevant AOPs for ecotoxicology. When possible, AO-focused networks were created based on the selected ENM-relevant AOPs, with the aim of visualizing relationships and nodes, and to identify key KEs and subsequently, potential predictive in vitro biomarkers/assays.

Following a thorough search based on the listed search criteria, 25 AOPs relevant for human toxicology and 19 AOPs relevant for ecotoxicology were identified. HARMLESS members decided to include only AOPs for human toxicology focusing on pulmonary or oral exposure, and to exclude AOs not targeting lung or liver. One exception was the AOP targeting cardiovascular disease, as it heavily driven by KEs located in the lung and liver. It was also concluded that no exclusion based on developmental status was to be conducted. Based on these decisions, the list of AOPs relevant for human toxicology was shortened to 21 AOPs.

Four networks for human toxicology based on AOs (lung fibrosis, lung cancer/mesothelioma, decreased lung function and liver AOPs) were created to visualize the interplay between AOPs and to identify common and potentially predictive KEs. Each identified KE node was briefly assessed for its use as a potentially predictive marker for the AO for further use in WP3 and WP4. For lung fibrosis, KE nodes could be divided into early and late KE nodes. The early KE nodes were related to inflammation and later KE nodes were related to ECM, in particular collagen, deposition. The lung cancer/mesothelioma network was highly intertwined, with several major KE nodes. Inflammation and oxidative stress was identified as the central initial KEs, leading to the node KEs related to oxidative DNA damage and mutations, and subsequently to increased cell proliferation. In contrast, no KE nodes were identified for decreased lung function. The AOPs in the liver network were also relatively

detached, but common node KEs were identified for liver fibrosis-related events, such as inflammation, stellate cell activation and collagen deposition.

Seven AOPs were evaluated as being of high or medium relevance for ecotoxicity following a ranking and selection process. Of these, 5 were overlapping with AOPs identified as relevant for human toxicology. Ecotoxicology AOPs could not be merged into meaningful groups and networks were therefore not created for these. Ecotoxicity assessment involves a large number of endpoints and species. Therefore, it would require specific considerations for each taxa and species within different environmental compartments when using AOPs for ecotoxicology. There is likely little conservation in AOPs across taxa and in some cases even within taxa, due to organism's unique physiologies. This presents an extra layer of complexity when applying/integrating an AOP framework approach in ecotoxicity assessment.

ENM-relevant AOPs were presented and discussed during the "WP2.1 workshop" organized by NRCWE and BfR the 4th of November, 2021 (online).

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