Molecular mechanisms and potency for ENM grouping

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1. Introduction

Engineered nanomaterials (ENMs) have unique physicochemical properties that have led to a widespread interest for diverse industrial applications. The variability of the produced ENMs pose challenges for their efficient hazard assessment. Grouping along with read-across strategies have been proposed to overcome this obstacle to allow for generalizability of hazard for existing and emerging ENMs.[1] However, present grouping approaches often lack sufficient consideration of the impact of individual studies, not encompassing a wide range of experimental conditions and materials tested. Furthermore, existing strategies have been largely centered around chemical aspects and use of apical endpoints. Toxicogenomics, on the other hand, offer a system level understanding. It has been used to characterize early molecular mechanisms of action (MOA) in the context of adverse outcome pathway (AOP) framework.[2] This framework has also been allied with benchmark dose (BMD) modelling[3] to determine dose-dependent effects elicited by ENM exposure.[4] It is known that direct effects typically exhibit a dose-dependent trend. Transcriptional derived BMD values has been linked to ENM potential of triggering a response. Here, we hypothesized that AOP-based direct molecular effects and ENM potency[5] derived from BMD modelling could be used to group ENMs.

2. Statement of contribution and methods

In this work, we applied an AOP-based strategy for ENM grouping. A collection of genome-wide expression data of ENM exposures with 51 experimental conditions, defined by the combination of GEO accession number, material tested, and exposure time was used. We focused on direct MOA triggered by ENMs represented by differentially expressed genes that respond in a dose-dependent manner. Before the grouping, we investigated the influence of time and dose of the exposure in the biological response. To achieve this, we analysed how often a molecular initiating event, or an adverse outcome is enriched within the AOPs. Additionally, we investigated enrichment frequency of different levels of biological organization, such as molecular, cellular, and tissue, are enriched key events (KE). This included examining the effects of

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both low and high doses. Furthermore, we examined the distribution of BMD values, all analyses being conducted over time. After determining relevant aspects to be considered in the grouping, hierarchical clustering was performed considering direct MOA induced by ENMs within the AOP framework and their potency based on transcriptional BMD modelling to group ENMs with similar hazard profiles at different exposure ranges. Distinct similarity metrics were used to obtain a consensus grouping. Then, a decision-tree based ensemble classifier was used to predict the pre-defined clusters enabling to determine exposure features that contributed to the grouping.

3. Results & Discussion

Mechanistic knowledge was successfully incorporated into the ENM-induced MOA through key event-AOP enrichment analysis. BMD modelling of toxicogenomic data allowed to determine direct MOA of ENM exposures. Time wise analysis of biological response and ENM potency highlighted relevant KEs within the AOP framework. Our results demonstrate that there are time and dose-dependent mechanisms triggered by distinct exposures. These findings suggest that exposure time should be considered to capture common mechanisms across distinct exposures. Genotoxicity related KEs were highlighted in longer exposures. In contrast, higher potency was found particularly associated with less advanced phase of the biological response. The opposite was observed for lower potency, which suggests that system complexity may play a role on the attenuation. Considering the influence of time, biological response and ENM potency in a consensus hierarchical clustering, 5 and 3 clusters were identified at shorter and longer exposures, respectively, indicating that common molecular responses could be identified by this approach. Relationship between exposure characteristics, such as exposed system and material tested, were considered major drivers of the clustering.

4. Conclusions

Our study suggests that ENM potency and time are important factors to distinguish shared mechanisms induced by distinct ENM exposures. The former was related to the responsiveness and the latter was associated with the type of response. We conclude that the similarity between ENM exposures considering mechanistic insight and potency at different timescales can be an effective way to form a consistent grouping that accounts for ENM complexity, given commonalities in attributes such as ENM features, biological system, and biological response. By exploring the relationship between dose of exposure and mechanisms, we open possibilities for the development of quantitative AOPs. This approach could work as a proxy for ENM grouping in safety assessment to support decision makers.

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6. References

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