WP1: Develop Criteria & Tools for Grouping / Classification

Introduction

Grouping and read-across has become an important part of chemicals regulation worldwide. Moreover, combining structural data and in vitro data has proven to be extremely valuable for predicting human toxicity by the Tox21 joint project of the US EPA, NIH, and FDA (Huang et al., Nat. Commun. 2016). The novelty of nanomaterials (NMs) and the resultant smaller data base do not allow for such an approach for NMs so far.
Currently, a number of different concepts for grouping NMs are being discussed and have been published. These are based largely on the physicochemical properties of NMs while the toxicological mode-of-action plays only a subordinate role. Some grouping schemes, such as the ones published by the German Federal Institute for Occupational Safety and Health (BAuA) and the US National Institute for Occupational Safety and Health (NIOSH), do consider toxicological mechanisms of action (Committee on Hazardous Substances 2013; Kuempel et al., J Nanopart Res 2012). The materials life-cycle is another important aspect that is rarely considered. Nevertheless, methods to quantify the release of NMs have been developed and the relevance of product composition has been discussed as a grouping criterion (Kingston et al., Carbon 2014; Wohlleben et al. 2014). Arguably the most comprehensive approach was published by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) ‘Nano Task Force’ (Arts et al., Regul Toxicol Pharmacol 2015).
On this basis a multi-perspective approach to developing a grouping scheme is pursued trying to integrate these different aspects. Various parameters may be applicable for grouping of NMs such as different physicochemical parameters or different toxicological effects. The integration of the different aspects into a comprehensive grouping scheme is currently still a major challenge and is therefore central to this project. An associated objective is the identification of underlying physicochemical and toxicological criteria as well as of possible relationships between parameters of the two areas. The developed grouping concept is tested within the project by experiments that are planned in close cooperation with, and carried out by, the other work packages. A further task of this work package is the selection and supplying of representative NMs.
Work package 1 concludes in May 2017 and most of its tasks merge in to work package 6. Work package 1 is subdivided into four tasks as follows.

Task 1.1 - Defining a panel of representative NM & variations

The selection of NMs for this project takes into consideration available data through other projects to create the greatest possible synergies. Thereby, it is possible to build on existing data and systematically gain new, complementary data. This is an important aspect considering the broad application of omics technologies in NanoToxClass. Moreover, different NM classes and various possible mechanisms of action are represented. Both well-established materials and new and innovative materials are investigated. To address the aspect of life-cycle, composite materials are investigated for aging or release of NMs. Aging will be simulated by thermal treatment, and release by cryo-milling.
Six of the NMs to be examined are defined at the start of the project in this task. Another four NMs are determined during the project to react to findings within the project. Some studies in the project can accommodate more NMs while for others (such as detailed mechanistic, Omics-based in vitro

studies or in vivo studies) a selection will be investigated. To verify the grouping concept in the course of the project two additional NMs will be selected.

Task 1.2 - Defining relevant physicochemical descriptors

Out of the wide range of physico-chemical parameters those showing the highest relevance to grouping of NMs will be determined. Both primary properties such as shape, size, dusting propensity, as well as secondary properties such as interaction with biomolecules, agglomeration, and stability are taken into account. Findings from other projects form the basis at the start of NanoToxClass. It will be systematically investigated which of these parameters are most relevant in relation to the formation of groups. Thereafter, criteria are scrutinized in an iterative process using the data generated in other work packages. This ensures that the theoretically developed criteria are verified or falsified, as well as that correlations between physicochemical parameters and toxicological modes of action are considered.

Task 1.3 - Identifying relevant toxicological endpoints & modes of action

As a further basis for developing a grouping scheme relevant toxicological endpoints and biological modes of action are identified. Data already obtained in other projects are considered together with data from an extensive literature review. Initially selected criteria are reviewed and if necessary adapted in an iterative process using the results derived within this project. Due to the focus of NanoToxClass on Omics technologies, which has not been pursued to this extent in any other project previously, the data base derived from other projects can be expanded and new, complementary knowledge (e.g. new biomarkers, certain key signalling pathways) can be integrated into the grouping scheme.

Task 1.4 - Perform grouping on basis of existing data / identify data gaps

An extensive literature review to compile already published data is performed. Data from other projects is merged with the compilation. A first grouping concept can be based on this dataset and different strategies will be used for analysis, e.g. "leave-one-out" methodology. Moreover, the data compilation will uncover data gaps and inconsistencies; this information forms an important basis for the other work packages in NanoToxClass. The data generated in the other work packages in turn are incorporated directly into the grouping scheme and thus contribute significantly to its development.