

A Curated Dataset on the Acute *In Vivo* Ecotoxicity of Metallic Nanomaterials from Published Literature

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Abstract

Metallic engineered nanomaterials (ENMs) have enormous technological potential and are increasingly applied across different fields and products. However, substances (including ENMs) can be detrimental to the environment and human health, thus requiring systematic testing to uncover potential hazardous effects (in compliance with REACH). Although hazard testing traditionally involves the use of animal experiments, recent years have seen a shift towards *in silico* modeling. High-quality data is required for *in silico* modeling, which is frequently not readily available for ENMs. Vast amounts of data have been published in literature but they are unstructured and scattered across numerous sources. To mitigate the limitations in data availability, we have compiled and created a nanotoxicity dataset based on published literature. The compiled dataset focuses mainly on acute *in vivo* endpoints conducted in a laboratory setting using metallic nanomaterials. The data extracted from literature include material information, physico-chemical properties, experimental conditions, endpoint information, and literary meta-data. The dataset presented here is useful for meta-analysis or *in silico* modeling purposes.

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1. Summary

Engineered nanomaterials (ENMs) with a metal basis are ubiquitously applied across various products (e.g., paint, sunscreen, catalyzers) and fields (e.g., healthcare, agriculture, electronics) [1,2]. By altering their physico-chemical properties, ENMs with different functionalities can easily be constructed, which makes them desirable materials for various use cases [3]. However, substances (including ENMs) can have detrimental human health and environmental effects, thereby making systematic testing to uncover potential hazardous effects crucial (in compliance with REACH) [4,5].

Advanced technology allows for the easy manipulation of material physico-chemical properties and results in a high diversity of manufactured ENMs [6]. Each modification applied to ENMs gives rise to novel functionalities or behavior and hence hazard testing must be conducted on a case-by-case basis [7,8]. The fate and behavior of ENMs are strongly

driven by an interplay of their composition, physico-chemical properties, and the surrounding environmental conditions [9]. Upon entry into aquatic matrices, ENMs undergo rapid transformations that alter their behavior and bioavailability towards species [10,11].

The complexity of ENMs and the diversity of materials with distinct functionalities make case-by-case hazard testing challenging and infeasible [7,8]. As a result, traditional hazard assessment has shifted more towards the use of *in silico* methods in recent years. The successful application of *in silico* modeling requires high-quality data [12], but is frequently obstructed by limited data availability in the case of ENMs [7,13–15]. One reason for the lack of available data is that nanotoxicological data is largely scattered across literature [7,16]. Over the past two decades, numerous studies have investigated the effects of ENMs, thereby generating considerable amounts of response data [3,16,17]. Although data has been compiled into various datasets or databases in the past (e.g., NanoE-Tox and eNanomapper), it may not always be suitable for modeling purposes [18]. The stored data may contain several data gaps or be heterogeneous (e.g., variables that are measured at different timepoints and/or media). This is not necessarily an issue of the databases themselves, but rather stems from unstandardized methods used within studies and a lack of proper reporting [19].

In the past, published nanotoxicological data from literature has been compiled into NanoE-Tox [20] or the dataset of Gakis et al., 2023 [21]. The importance of such curated and openly available data is that literary data can be readily reused and form part of secondary analyses (e.g., meta-analyses or *in silico* modeling). While NanoE-Tox is a comprehensive dataset, it is limited in the amount of features it collected from studies and considerably more data has become available since its release in 2015. To aid with limited data availability, we have compiled and created a nanotoxicity dataset based on published literature. The compiled dataset focuses mainly on acute *in vivo* endpoints conducted in a laboratory setting using metallic nanomaterials. Careful curation was applied to the collected data to facilitate its reuse for secondary analyses but also to give users the freedom to manipulate the data in whichever way they see fit. A key difference between published ENM datasets and the dataset presented here is that the timepoints and media used for measurements were considered and harmonized as much as possible. This dataset is, to the best of our knowledge, the largest dataset currently available for metallic ENMs containing acute *in vivo* EC₅₀ ecotoxicity data on a wide range of aquatic species.

2. Data Description

2.1. Data Structure

The nanotoxicity dataset is compiled of (raw) data collected from literature and presented in the form of Comma-Separated Value (CSV) spreadsheets. Two .csv files are presented, one containing the nanotoxicity dataset (“nanotox_database_raw.csv”) and the second one containing variable descriptions (“variable_descriptions.csv”). The variable descriptions give a short description of the extracted variables and default units. Default units correspond to numeric variables in the dataset where entries are expressed in the described unit (such entries have no explicitly mentioned units entered behind values). Values may also contain explicitly mentioned units behind values, thereby indicating that they differ from the variable’s default unit and require conversion for harmonization.

2.2. Dataset Overview

The compiled dataset contains 2851 ecotoxicological datapoints on acute *in vivo* nanotoxicity based on 59 metallic nanomaterials and 149 aquatic species. Data was extracted from 474 literary papers published between 2006 and 2022. The dataset comprises aquatic species which can be categorized into the following species groups: crustacea, algae, fish, diatoms,

protozoa, (aquatic) plants, cyanobacteria, nematoda, rotifera, gastropoda, cnidaria, insecta, and annelida.

Below follows a brief summary analysis of the dataset which gives insight into the extracted data and its diversity. The majority of the dataset is made up of observations for Ag (26.1%), ZnO (16.6%), TiO₂ (16.2%), CuO (6.4%), SiO₂ (6.4%), and CeO₂ (6.3%) (Figure 1).

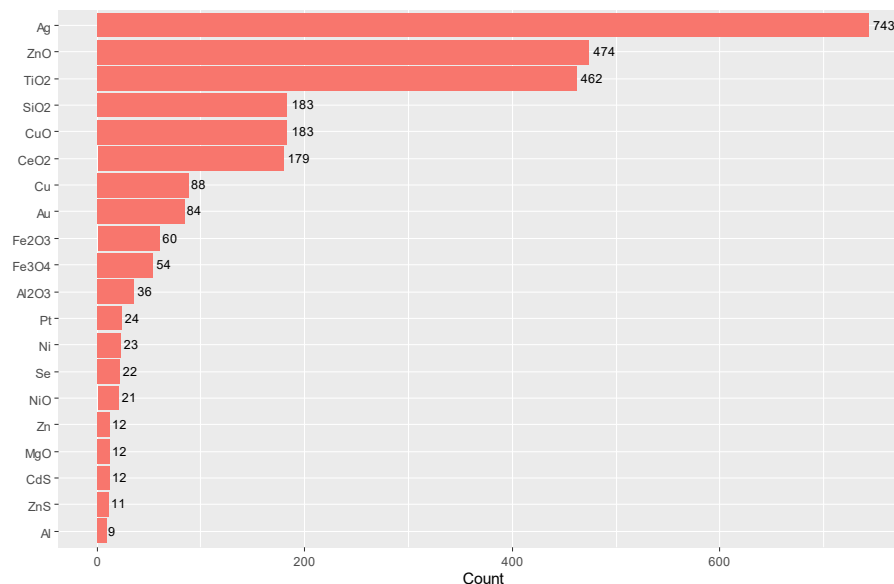


Figure 1. Number of observations for the most abundant 20 ENMs within the dataset. Values on the right of the bars represent the counts for the corresponding material.

Data for the remaining 53 ENMs make up 22% of the observations. Similarly, observations for *Daphnia magna* (24.8%), *Danio rerio* (15.1%), and *Raphidocelis subcapitata* (10.6%) make up the majority of the dataset (Figure 2).

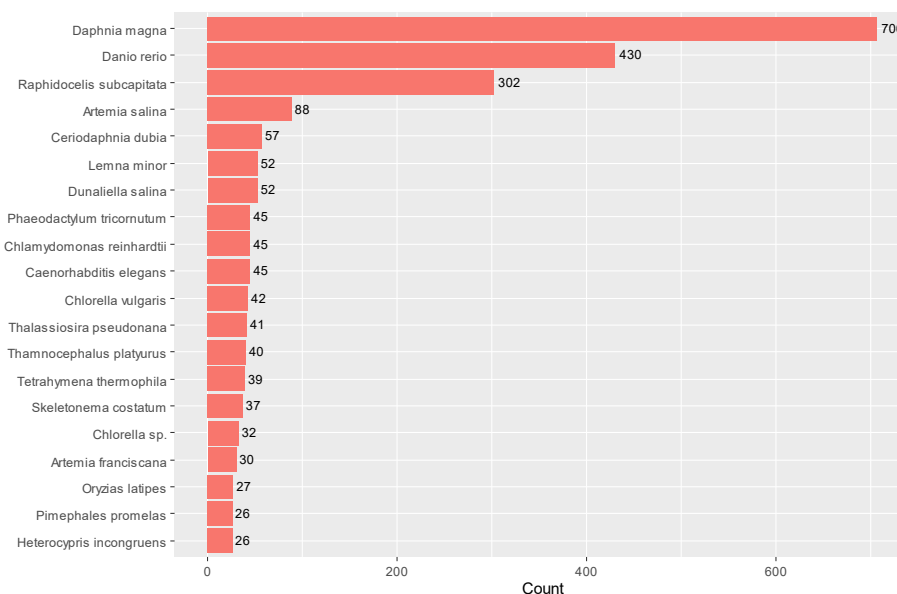


Figure 2. Number of observations for the most abundant 20 species within the dataset. Values on the right of the bars represent the counts for the corresponding material.

Studies may not always report data for all variables, resulting in gaps within the dataset (Figure 3). Figure 3 displays the completeness of the dataset which indicates the availability of information for a given variable. Completion rates of >50% were observed for the majority of variables with the exception of surface area, crystallinity, hydrodynamic

size (measured at the end of exposure), polydispersity index, method of dispersion, pre-illumination, and water quality information (water hardness, conductivity, ionic strength, alkalinity, dissolved oxygen).

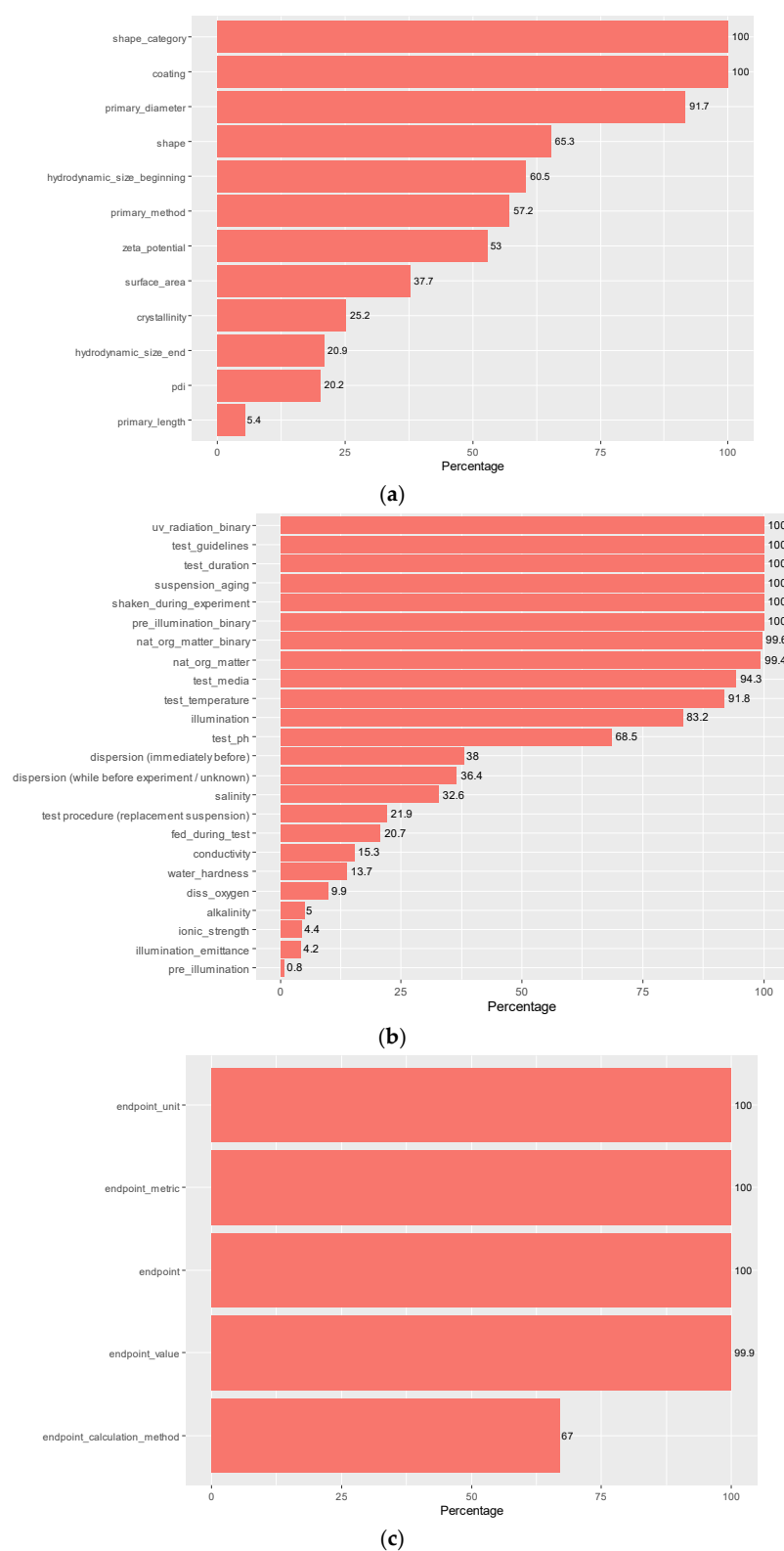


Figure 3. (a): Percentage completeness for variables related to the physico-chemical properties of ENMs within the dataset. (b): Percentage completeness for variables related to the experimental conditions of nanotoxicological experiments within the dataset. (c): Percentage completeness for variables related to the toxicological outcomes of nanotoxicological experiments within the dataset.

Due to its similarity to NanoE-Tox [20], we would like to highlight the key differences between NanoE-Tox and the dataset presented here. Both datasets are similar in their structure and setup, containing largely similar variables extracted from literature. A key difference here is that data from more recent publications are available (after the release of NanoE-Tox and until 2022). Additional differences between NanoE-Tox and our dataset can be found below:

- NanoE-Tox contains data on metallic ENMs, fullerenes, and carbon nanotubes. Our dataset focuses solely on metallic nanomaterials and EC₅₀ values. Additionally, yeast and bacteria are absent from our dataset as they were not species groups focused on during our literature search.
- ENM shape data was harmonized using a categorical scheme (more detail in Section 3), compared to the unharmonized shape information presented within NanoE-Tox.
- Impurity was not collected due to a lack of information within the studies and unstandardized reporting within the studies.
- “Characterization in test environment” from NanoE-Tox does not consider the timepoints at which measurements were performed. Our dataset considers the timepoints and media used to conduct measurements as they strongly affect measurements [22].
- Our dataset does not include the mechanism of toxicity but contains additional experimental detail such as dispersion methods, aging/weathering, UV radiation, and water quality information.

3. Methods

3.1. Data Collection

Research papers containing nanotoxicity data were collected between September 2021 and July 2022 through Web of Science’s advanced search feature. The general focus of the search was laboratory-based ecotoxicological experiments conducted using metallic nanomaterials and aquatic species. Search strings were constructed as follows:

(KEYWORDS RELATED TO SPECIES GROUP AND/OR SPECIES NAME) AND (nanopar* OR nanomat* OR enm) AND (toxic* OR ecotox* OR acute OR mortal* OR lethal* OR vivo OR ec50* OR lc50*) NOT (clay* OR plastic* OR fiber*)

Material-specific keywords were substituted depending on the material and species of interest during the search process. Studies were collected for the following species groups and ENMs:

- ENMs: Zn, Ag, Au, ZnO, TiO₂, CuO, CdO, Bi₂O₃, CeO₂, Cu, Fe₃O₄, Co₃O₄, WO₃, MgO, Sb₂O₃, Pd, Mn₃O₄, Al₂O₃, SiO₂, NiO, La₂O₃, Gd_{0.97}CoO₃, La₂NiO₄, (La_{0.6}Sr_{0.4})_{0.95}CoO₃, Ce_{0.9}Gd_{0.1}O₂, LaCoO₃, LaFeO₃, (La_{0.5}Sr_{0.5})_{0.99}MnO₃, Ce_{0.8}Pr_{0.2}O₂, Co, Se, Fe₂O₃, SnO₂, CuFe₂O₄, CoFe₂O₄, NiFe₂O₄, Al, Ni, Mn₂O₃, Pt, ZrO₂, PbS, Al₂O₃.TiO₂, BaFe₁₂O₁₉, Cr₂O₃, CuZnFe₄O₄, Mg(OH)₂, Sn, W, CdS, Ag-Au, Cr, In₂O₃, ZnS, BaTiO₃, B, Ag₂S, Ag₂O, Y₂O₃.
- Species groups: algae, diatom, cyanobacteria, protozoa, aquatic plants, cnidaria, crustacea (amphipoda, anostraca, cladocera, copepoda, ostracoda), fish, mollusca, rotifera, annelida, nematoda, insecta.

Such a wide range of search terms were necessary to ensure a thorough literature search, allowing for the inclusion of as much ecotoxicity data as possible. Each publications was initially screened on its quality, whereby papers lacking basic material characterization (size and material composition information) were immediately discarded. Data extracted from the studies included information regarding material information and meta-data, physico-chemical properties, experimental conditions, endpoint information and literary meta-data. The majority of collected variables are in line with the requirements of REACH

for the registration of nanoforms [23] as well as reporting checklists such as MIRIBEL [24], nanoCRED [25], and sciRAPnano [26]. It should be noted that there is currently no consensus on which parameters should be reported within studies [27], thus causing significant differences in the level of detail of data across papers. Although the previously mentioned reporting checklists are comprehensive in their requirements, the majority of studies do not report all information. Therefore, the set of variables extracted here were chosen because they were broadly reported across the majority of papers and also generally used within *in silico* models or meta-analyses. Furthermore, while the solubility and purity are considered crucial physico-chemical parameters, these were not collected here due to general underreporting and severely unstandardized measurement of both features.

It is important to recognize that studies may reuse previously published data which can result in duplicates if extracted. Therefore, each paper was carefully examined to ensure the extracted data did not originate from another publication. If data was duplicated across multiple publications, then this data was extracted from only one publication. Additionally, to mitigate human error during data extraction, entries were rechecked multiple times.

3.2. Data Harmonization

Integrating data from different sources requires significant data curation in order to create harmonized data. Therefore, the following steps were taken:

- ENM shape data was harmonized by utilizing the TEM/SEM images in studies (if available). This was performed using the shape classes as described in the Supplementary Materials (Table S3.1.1) of Balraadjasing et al. 2022 [13], with some modifications. Changes made to this classification include the merging of “spherical” and “nearly spherical” classes into “spheroid” due to the large variety of images where no clear distinction could be made between both classes. Furthermore, the new category “triangular” was added for ENMs that resemble triangular shapes. When no images were present, then the shape as described by the authors was used.
- When the coating was not disclosed within a study then the ENM was assumed to be uncoated.
- Exposure conditions (e.g., temperature, illumination, pH) that were not clearly disclosed in the underlying publications were assumed to be the same as the culturing conditions (if this was reported).
- Due to species names changing over time (e.g., *Raphidocelis subcapitata*), their (current) taxonomic information was assessed and harmonized using WoRMS (<https://www.marinespecies.org/> (accessed on 1 September 2022)), algaebase (<https://www.algaebase.org/> (accessed on 1 September 2022)), and fishbase (www.fishbase.org (accessed on 1 September 2022)).

3.3. Filling in Data Gaps

Due to the large gaps from underreported information within studies or unstandardized experiments, we opted to fill in gaps where possible as follows:

- Gaps related to the physico-chemical parameters of well-characterized JRC ENMs (e.g., NM-100, NM-101, etc.) were filled in where possible. This was performed using the following resources: [28–31].
- When the culturing conditions were not reported, then the standardized guidelines were used to fill in the exposure conditions.
- Literature was consulted to fill in water-quality gaps or to identify the composition of the media:
 - SAN PIN tap water: SanPin 2.1.4.1074-01 protocol.
 - (Synthetic) seawater: [32,33].

- Deionized/ultrapure/distilled/double distilled water/MilliQ: ASTM D1193-06(2018) protocol [34], ISO 3696:1987 protocol [35], Millipore (<http://www.merckmillipore.com> (accessed on 1 October 2022)).
- Class I and Class V natural waters: [36].
- Dechlorinated tap water: salinity filled in as 0‰ because in theory no chlorine should be present.
- Composition for remaining standardized and frequently used media: [37].

4. User Notes

Due to the differences in reporting formats across the papers and the unstandardized experiments, the dataset is presented in its raw form and requires further processing and curation before conducting formal analyses. This gives users the opportunity to manipulate and harmonize the data in a manner that suits their analysis. Furthermore, the lack of standardization also resulted in incomplete data being collected for specific variables. This was the case for the hydrodynamic size and zeta potential, wherein the timepoints and media in which they were measured varied significantly across papers, along with the methods used for measurements and reporting of data. Instead, it is noted that data is available for the entry, allowing users to further collect the data should they wish to do so. Additionally, while the primary length was largely incomplete, the gaps can be readily filled in by assuming that the primary length was 0 for spherical particles, should the user wish to do so. Length is not a relevant dimension for spherical particles.

Assessment schemes may be used to assess the quality and reliability of ecotoxicity studies such as nanoCRED [25] or SciRAPNano [26]. While this could give crucial insight into the quality of each datapoint, such an assessment was not conducted here. Such assessment schemes are highly detailed and are time-consuming to complete, which was beyond the scope of this dataset. For quick evaluation of study quality, users may calculate completion scores to assess whether studies reported all necessary experimental and material information.

We would like to note that this dataset represents a fixed snapshot of ecotoxicity literature for metallic nanomaterials until 2022. No updates will be made to this dataset in the future by us, but other users are welcome to do so.

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