A common European approach to the regulatory testing of nanomaterials

NANoREG

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Deliverable D 6.05

Database on manufactured nanomaterial physical chemical properties related to (eco)toxicological endpoints

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1 Description of task 6.3

The possibilities for precise design of MNMs and thereby affecting their behaviour has been the starting point for the concept that MNMs can be engineered to be Safe By Design. This means a design maximizing the benefits for functionality while posing minimal risks to human health and the environment. This approach, however, assumes that potential adverse effects can be engineered out through manipulation of physical and chemical properties while the beneficial properties are maintained. Insight in impact of physical and chemical properties on toxicity as well as on functionality is therefore a crucial aspect in this approach. The chemists in this WP differentiate from those in WP2 as they are material scientists rather than analytical chemists. The results of the activities in this task will not only help to prevent marketing of unsafe nanomaterials but will also help identifying characteristics of safety concern for already marketed nanomaterials. The precise design will help to simulate characteristics of the same material as present in bulk, in a product (to be) marketed, etc. thereby supporting extrapolation (WP3) and risk assessment along the value chain (WP1).

The Safe by Design approach will require close collaboration between material experts dealing with functionality and characterization, analytical chemists dealing with characterization and toxicologists/risk assessors for toxicity testing and interpretation of the results. Moreover, liaisons with safe nano design initiatives (e.g. the NIOSH Prevention through Design) should be established in order to get overview of hurdles that block multidisciplinary/multistakeholder collaboration (process driven) as well as getting overview of scientifically driven hurdles, before the Safe by Design concept can be beneficial for all stakeholders. This task is composed exclusively of advanced materials engineering, testing and development, but with links to regulatory requirements. This will require the following activities: Initial development of a decision tree based on the safe window of (eco)tox parameters. Test and validation of the decision tree in a Value Chain Case Study (Task 1.6). Compilation of the decision tree as a tool for the NANoREG tool box.

• Establishing a first set of relationships between certain functionalities and triggers for testing specific (eco)toxicity endpoints, for example by an extensive programme of material development, characterization and testing.

• Inventory of topics of shared interests between certain stakeholders/experts in the safe design process, compilation of the interests to a recommendation as part of the NANoREG tool box.

2 Description of work & main achievements

2.1 Summary

The goal of deliverable 6.5 is to identify a set of physicochemical properties that are related to the (eco)toxicity of nanomaterials. To this end, TNO has developed a relational database that contains information on nanomaterial's physicochemical characteristics and (eco)toxicity, called the WP6 D6.5 literature database. This database is not the same database as the the intermediate form of the NANoREG experimental database as gathered for NANoREG by TNO.

For the development of the WP6 D6.5 literature database various activities were completed. The task started by 'Setting the criteria for the database'. After defining these criteria an assessment of usefulness of other databases and database initiatives was made. This has resulted in an official memo 'Assessment of usefulness of other databases' which was uploaded in CIRCABC (Annex I). At the time none of the reviewed initiatives (ISA-TAB-Nano, The Nanomaterial registry, The Napira Hub and the Modena COST action) seemed appropriate for our purpose. Main reasons for this were the fact that other initiatives were also under development and not ready to use yet, the lack of a prioritization tool for literature data for the purpose of Safe by Design, the availability of data generated by other initiatives and compliance with NANoREG minimum requirements.

Based on the defined criteria the structure for the WP6 D6.5 literature database was developed. The database was filled by assessing peer reviewed literature with all partners in Task 6.3. The first task was to select the appropriate literature. In order to harmonize these activities guidelines for literature selection and a web-based tool for the literature prioritization were developed. Similarly, for the actual data-entry of the data from the selected literature, a web-based tool for data-entry was developed. The tool for prioritization and data entry is still available via https://diamonds.tno.nl/nanoreg/. This tool is not the same tool as the tool used for the data entry of experimental data. For the harmonized use of the data entry tool among partners, 'guidelines for data-entry' were written and a face-to-face training for all relevant partners was organized for the prioritization- and data-entry process. The database, 'guidelines for literature prioritization' and 'guidelines for data-entry' are all collected in an overall guidance document which is uploaded to CIRCABC (Annex II). Terms and conditions on use of the entry tool were formulated and added to the tool.

The inventory of impact of physical chemical properties on (eco)toxicological endpoints has been finalized and near to 1400 studies have been prioritized. The data-entry of 170 of these studies was completed resulting in data on 398 unique nanomaterials (of which data on titanium dioxide, silver, silicium dioxide, multi walled carbon nanotubes and zinc oxide is the most abundant). Physical chemical characteristic of these material are related to 1368 *in vivo* endpoints and 2431 *in vitro* endpoints.

D6.5 is finalized in the form of the database supported with the Guidance document. Additional activities are ongoing. This includes investigation of possibilities for data analysis by Nilu and the Directorate-General of Health in Portugal and collaborations with the FP7 eNanoMapper project, the H2020 NanoReg2 and H2020 Calibrate project. These latter collaboration focus on investigating possibilities to transfer the data to the eNanoMapper database. The transfer to this database will make use of the data for modelling and calibration easier.

2.2 Background of the task

The information in the relational database that contains information on nanomaterial's physicochemical characteristics and (eco)toxicity can be used to identify physicochemical properties related to the fate and toxicity of nanomaterials, to develop structure-activity relationship (SAR) models, to derive grouping principles and to contribute to the development of a Safe by

Design strategy (deliverable 6.6 of the NANoREG project) by defining a “safe window” of (eco)toxicological parameters. The database is designed to store information on both the properties of pristine nanomaterials (e.g. particle’s composition) as well as their interaction with biological and environmental components; this is done to keep track consistently of the particle’s history and identify the influence of biological and environmental conditions on nanomaterial’s toxicity (e.g. the effect of the biomolecular corona on the cellular uptake and toxicity of nanoparticles). The relational database is filled with information retrieved from peer reviewed scientific literature and for the purpose of data entry, a software tool has been developed within the NANoREG project, to enable the data entry process in a consistent manner. The database includes 3 types of data on nanomaterial characteristics and toxicity:

1. parameters that define the intrinsic characteristics of nanomaterials;

2. measurement on nanomaterial properties under specific conditions;

3. *in vitro* and *in vivo* (eco)toxicity endpoints.

Data type 1 stores information on nanoparticle primary characteristics, these are, e.g., properties that define the chemical composition, primary size, crystallinity and CAS registry number of nanoparticles. These type of properties allow the comparison of identical nanoparticles used over different studies. It is well known, however, that the properties of nanomaterials are affected by the biological and environmental matrices and dispersion protocols used during the experiments; the second type of data includes this type of information, e.g.: surface properties (charge, and surface chemistry), agglomeration state and hydrodynamic size. In order to develop structure or property activity relationships (i.e. links between primary and/or secondary physicochemical properties with (eco)toxicological endpoints) the database contains a relevant selection of *in vitro* and *in vivo* endpoints. All information identifying a specific bio-assay is stored in the database, this includes: exposure conditions, species information, assay properties and the way of quantifying the endpoints. For this different activities were scheduled.

2.3 Description of the work carried out.

2.3.1 Criteria for a database structure

The criteria for the database structure were defined by collecting the input of partners active in the task. Within the task experts on physical chemical characterization, human *in vitro and* in vivo toxicity and ecotoxicity. Geochem and the Debye institute delivered input for physical chemical characterization, TNO for in vitro toxicity, Nilu for in vivo toxicity and ENEA for ecotoxicity. The input was collected and merged in different tables containing the most important parameters for the database. A series of teleconferences and videoconference on the content of the tables was organized to finalize these results.

2.3.2 Assessment of usefulness of other databases

An Official Memo on the Assessment of usefulness of other Databases has been delivered and uploaded in CIRCABC in February 2014.

The following databases and initiatives were assessed keeping the previously defined criteria in mind:

- ISA-TAB-Nano (research of the website and publications and contact with Egon Willighagen of the University of Maastricht was made); http://www.enanomapper.net/
- The Nanomaterial registry (research of the website and publications); https://www.nanomaterialregistry.org/

- The Napira Hub (research of the website and publications); http://www.nanohub.eu/ and http://iuclid.echa.europa.eu/index.php?fuseaction=home.project
- Modena COST action (Participation of TNO and ENEA to the COST Modena workshop in Gdansk, Poland in December 2013), http://www.modena-cost.eu/

Based on this assessment it was decided to develop our own relational database.

2.3.3 NANoREG WP6 literature Database and entry tool development

Based on the criteria for the database structure a database and data entry tool were developed by TNO. The database is a relation MySQL database (see Figure 1). For more information regarding the database structure see Annex II.

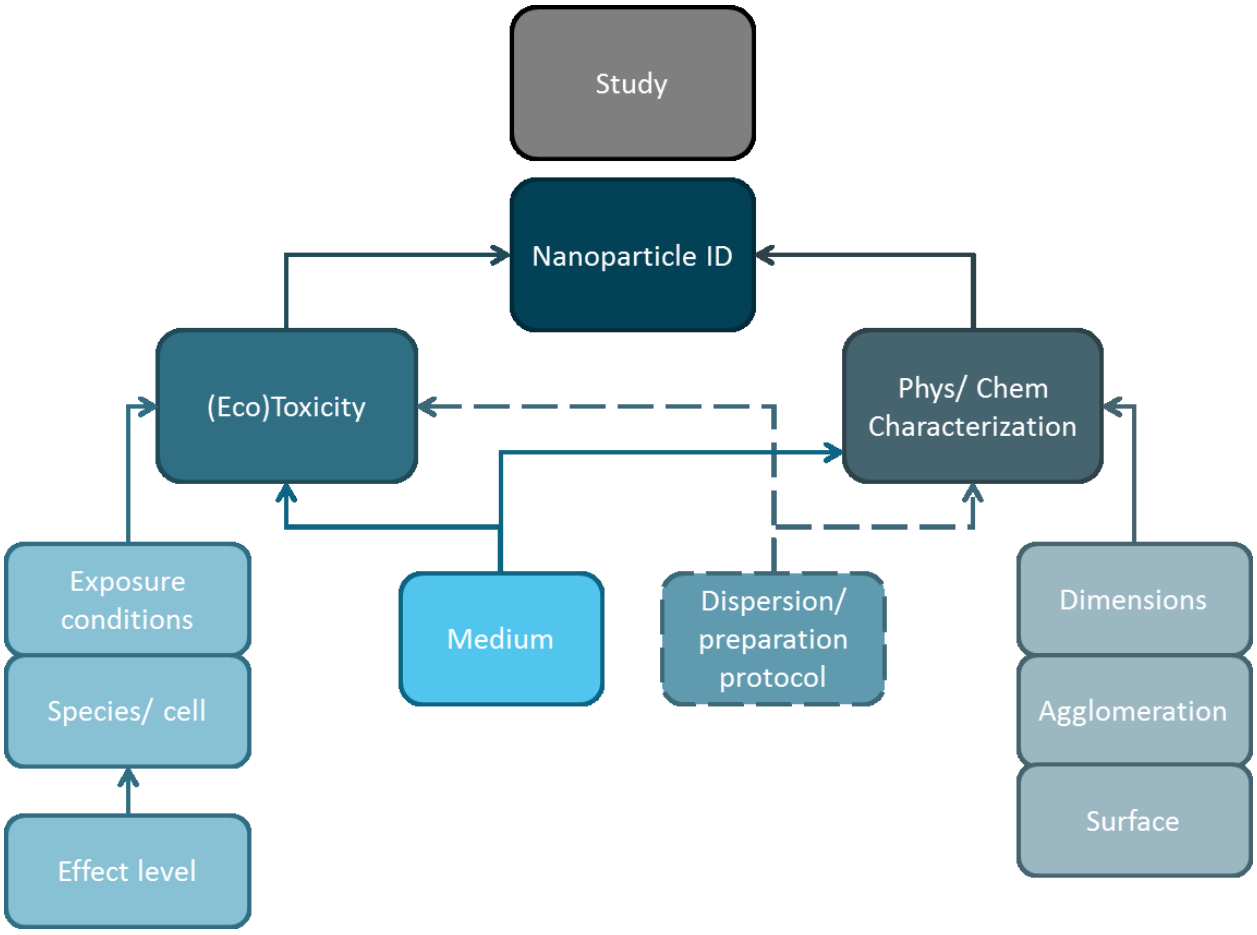


Figure 1: Structure of the relation MySQL database developed for the storage of physical chemical and toxic properties of nanoparticles.

In order to prioritize and enter the data into the database in a structured and harmonized way an entry tool was developed. The flow of that entry tool is shown in Figure 2. For more information regarding the entry tool see Annex II.

2.3.4 Literature Selection & Prioritization

For the development of the Inventory of impact of physical chemical properties on (eco)toxicological endpoints data were collected from peer-reviewed public literature. The literature was selected and prioritized. Then the data from the literature was entered into the database.

Publication searching was carried out in Scopus. For literature papers on toxicity of nanoparticles the following search terms were used:

"(TITLE-ABS-KEY(*nano* *tox*) AND NOT TITLE-ABS-KEY(eco*)) AND DOCTYPE(ar) AND (EXCLUDE(SUBJAREA, "MATE") OR EXCLUDE(SUBJAREA, "CENG") OR EXCLUDE(SUBJAREA, "ENGI") OR EXCLUDE(SUBJAREA, "PHYS")) AND (EXCLUDE(SUBJAREA, "EART")) AND (EXCLUDE(SUBJAREA, "SOCI") OR EXCLUDE(SUBJAREA, "ENER") OR EXCLUDE(SUBJAREA, "MATH") OR EXCLUDE(SUBJAREA, "COMP") OR EXCLUDE(SUBJAREA, "ARTS") OR EXCLUDE(SUBJAREA, "BUSI") OR EXCLUDE(SUBJAREA, "PSYC") OR EXCLUDE(SUBJAREA, "DECI") OR EXCLUDE(SUBJAREA, "ECON") OR EXCLUDE(SUBJAREA, "SOCI") OR EXCLUDE(SUBJAREA, "ENER") OR EXCLUDE(SUBJAREA, "MATH") OR EXCLUDE(SUBJAREA, "COMP") OR EXCLUDE(SUBJAREA, "ARTS") OR EXCLUDE(SUBJAREA, "BUSI") OR EXCLUDE(SUBJAREA, "PSYC") OR EXCLUDE(SUBJAREA, "DECI") OR EXCLUDE(SUBJAREA, "ECON")) AND (LIMIT-TO(LANGUAGE, "English")) AND (LIMITTO(PUBYEAR, "PUBYEAR"))

For literature papers on ecotoxicity of nanoparticles the following search terms were used:

TITLE-ABS-KEY(*nano*) AND TITLE-ABS-KEY(*eco*) AND TITLE-ABS-KEY(*tox*)) AND DOCTYPE(ar) AND (LIMIT-TO(PUBYEAR, "PUBYEAR"))

The results of each search (per publication year) were stored in a .bibtex file including "all available information". These files were uploaded in the NANoREG WP6 literature database in specific queries. After uploading of the .bibtex file the database can extract the reference information and abstract from the .bibtex file and store it in the database.

After the literature was collected, it was further selected and prioritized according to the below criteria by reviewing the reference and abstract:

- The article should be published in English (in search);
- The article should be the primary source of data (manual);
- The article should be presented as a full article (in search);
- The title and abstract are checked for relevance for inclusion based on the following criteria (manual);
  - Is the paper regarding a nanomaterial of interest;
  - Does the paper contain information relevant for biological activity;
- Studies that contain a good physical chemical characterization are assessed with higher priority (manual)

Some of these criteria are already taken into account in the search term (in search) some are automatically checked by the software (automatic) and some need to be assessed by the person doing the prioritization (manual). Duplicate articles will be automatically flagged in the database using the D.O.I.

The articles that were not excluded based on the above criteria were prioritized first according to the number of relevant topics, and the availability of good characterization, secondly on the date of publication, most recent publications first. Prioritization was done by various partners within the task after attending a training. Guidance on the prioritization process is given in the overall guidance document (Annex II). Prioritization was carried out by various partners active in this task, i.e. TNO, NILU and Geochem.

2.3.5 Data Entry

The data entry tool works using a specific methodology for data extraction. This methodology is in compliance with the type of information that should be extracted from the studies. The methodology asks for the entrance of a particle to a given study first. Therefore it was recommended to start making a list of all particles that are investigated in any experimental study. Then the data that are available regarding this particle should be ordered. For each type of data

the medium and the protocol should be defined (unless data is provided just like that in which case they should be entered as “provided”). From the materials and methods and results in any study the preparation method of particles dispersions, and the relevant medium in which the particles were dispersed should be described. (For many characterization methods like TEM and SEM the dispersion medium is dried or removed, still the medium in which the particle was dispersed before sample preparation should be added as the relevant medium). For use in the tool it is easy to add different measurements to a specific combination of a medium and protocol so it makes sense to put the tests done under these condition together. In Table 1 the conceptual idea for the structuring of the data is provided. In Appendix C, an example is worked out. It was also recommended to write down the most important details on the particle, the preparation protocol, the medium type of tests straight away. Data Entry was carried out by various partners that were active in the task: ENEA, Genøk, Veneto nanotech and NILU.

Table 1: Conceptual table for ordering and entry of the data into the database

Particles	Preparation Protocol	Medium	Tests
Particle 1	Provided	Provided	Phys/chem test 1
	Protocol 1	Medium 1	Phys/chem test 1 Phys/chem test 2 Phys/chem test 3
	Protocol 2	Medium 2	Phys/chem test 1 Phys/chem test 1 Phys/chem test 1 Tox 1 Tox 2 Tox 3
	Protocol 3	Medium 3	Ecotox 1
Particle 2	Protocol 1	Medium 2	Phys/chem test 1 Phys/chem test 2 Phys/chem test 3
	Protocol 3	Medium 3	Tox 1 Tox 2 Tox 3
Etc	Etc	Etc	Etc

2.4 Results

The assessment of usefulness of other databases is added to this document in Annex I. The conclusions of the assessment of other initiatives based on the set criteria for our own database are lead to the decision to develop the NANoREG WP6 literature database.

Reasons for this were:

- Time (state of development of the other initiatives)
- The possibility of a clear prioritization of literature data for the purpose of Safe by Design
- The lack of availability of data generated by other initiatives
- Compliance with NANoREG minimum requirements

Important further conclusion that further acknowledge the important of the criteria that we developed for the NANoREG WP6 literature database were:

- TNO initiative on building a database for nanomaterials SARs is in line with other EU and US initiatives and will benefit from already ongoing projects to better define the structure of the database and the literature to be selected.
- All initiatives acknowledge the fact that nanomaterials behavior can change in relation to environmental factors, including time: for this reason it is necessary to record the history of

a nanomaterial from its production to its disposal to identify exposure scenarios, dose forms, etc.. and eventually develop SAR models.

- It follows from point 2 that characterization of nanomaterials (also in biological and environmental matrixes) is a prerequisite for enabling the development of reliable SARs.
- The development of SARs will depend mainly on the quality of the characterization and toxicological tests; it should be noted here, however, that a genuine SAR model should use theoretical descriptors to enable predictions on virtual compounds.

The Overall guidance document is attached as Annex II. Tables 1 and 2 describe the data that is present in the database. The NANoREG WP6 literature database contains 8 types of information that can be extracted from published studies.

- Study information (1)
- Nanoparticle identification parameters (2)
- Physicochemical characteristics of a nanoparticle (4)
- Data of experimental media (3)
- Data of the preparation protocols (3)
- Data of the test subject (4)
- Data of the exposure conditions (4)
- Data on the endpoints (5)

The underlying database structure is a MySQL database, which is a relational database. The database consists of multiple tables. A specific field in a table is defined by its name and the table in which it exists and the relation of that table to others. The tables are built up in a hierarchy. The number in the list of 8 types of information indicate how the hierarchy of tables is built up. A database entry is defined by information on the study from which it is extracted. In a study, multiple particles can be described/investigated, so multiple tables on nanoparticle identification parameters can exist within one study. Of each particle multiple physical chemical characteristics of the nanoparticle can be provided/measured. Within each combination of an exposure/test medium and preparation protocol multiple measurements of physical chemical characteristics multiple and exposure conditions with test subject characteristics can be added to each nanoparticle. Finally multiple (eco)toxicity/ bio assays can be added to each set of exposure conditions and test subject characteristics.

Table 2: Data in the NANoREG WP6 literature database

Number of (2013 & 2014) studies prioritized	1387
Number of Studies in which at least one nanomaterial was identified	170
Number of unique nanomaterials entered	407
Number of in vitro endpoints	2431
Number of in vivo endpoint	1368

Table 3: List and number of Nanomaterials in the database identified by the main chemical component

TiO2	57
Ag	53
SiO2	41
MWCNT	36
ZnO	26
Au	20
Fe	16
CuO	14
Fe3O4	13
CeO2	13
Buckyball	12
SWCNT	11
S	7
Ni	7
Co	7
Al2o3	6
Apatite	6
Si	6
Zn	5
Polystyrene latex (PSL)	5
Fe2o3	4
Cd	4
Ca	4
O	4
Al	4
Tungsten Carbide (WC)	3
Ag2o	3
Graphene	3
Solid lipid nanoparticles (SLN)	3
Pbo	3
Graphite	2
Mn	2
C	2
Cs	1
Nio	1
Mo	1
Carbon Nanohorns (CNHs)	1
Diamond	1

The full database is available on CIRCABC in the form of 2 .zip files. There is no interface included for searching and browsing of the data. The .zip files contain the database dump of the NANoREG literature database in 2 different formats:

- .csv: format for human readability and data processing by software

https://circabc.europa.eu/d/a/workspace/SpacesStore/b290d2e1-3c0c-4fbe-b8cb-a4bffa2b4625/NANoREG%20Deliverable%20D%206.5%20Database.csv.zip

- .sql: can be used to reconstruct the complete database (with data) using mysql on any linux platform

https://circabc.europa.eu/sd/a/9887ad2f-3cd7-42f6-b70a-f793798551a3/NANoREG%20Deliverable%20D%206.5%20Database.sql.zip

The Prioritization and Entry tool is available via: https://diamonds.tno.nl/nanoreg/

2.5 Evaluation and conclusions.

The goal of this deliverable is to deliver a database of physicochemical properties that correlate with (eco)-toxicological endpoints by analysing research studies from literature that report information on both structural characteristics and toxicological profiles of nanomaterials. For this purpose a database structure has been developed and data has been prioritized and collected.

A large amount of data has been collected that could serve this purpose.

Additional activities such on database compatibility are ongoing. In a collaboration with the FP 7 eNanoMapper project the mapping of our database ontology to existing ontologies has been done. Due to small overlap with existing ontologies the eNanoMapper ontology will be expanded and an output report on the mapping will be formed by the eNanoMapper project.

The full database has been shared with NANoREG partners and with the eNanoMapper project and will be made publically available as all other NANoREG data.

The use and direct transfer of the WP6 literature database in the NanoReg2 and Calibrate project is currently under discussion. Focus of the work in these project is the transfer of the experimental data from NANoREG to the eNanoMapper instances. Finalization of this transfer would facilitate the transfer of the WP6 literature data to a large extend due to the strong overlap in database structure. In order to properly transfer all data and allow use of the data in these project the mapping of fields should be completed. Whether this will be done depends on the availability of resources and the expected impact of the literature data on these projects. Decisive conclusions on the use of the literature data in these project will be drawn in the first quarter of 2017.

There are currently no ongoing data analysis activities.

2.6 Experience and lessons learned

Experience in this task has led to the involvement of TNO (task assigned outside NANoREG) in the experimental data collection in NANoREG. The structure developed for this deliverable has formed the basis for the structure applied for the entire NANoREG experimental data collection. Due to these activities the data collected within the NANoREG project can be transferred to other project such as the eNanoMapper project.

The experience and use of the data continues in other European projects such as the H2020 projects NanoReg2 and Calibrate. Within these initiatives new database structures will be developed and the data will be transferred for analysis. The data will support risk assessment and safe innovation decision schemes, tools and methods in these projects.

2.6.1 Database structure

Dispersion protocol and medium conditions

The database structure is meant to store published experimental data on physical chemical characteristics measurements and toxicity of nanomaterials. The published information on these nanomaterial properties consists of measured data. Various assays are used to measure this large extend of different properties and toxicity end-points. It is well known that the results of these measurements are influenced by the medium in which the nanomaterial is dispersed and the way the nanomaterial is dispersed. In fact within the NanoReg project harmonization of these medium conditions and dispersion protocols has been a major task. It was already identified in a very early stage of the database structure development that storing of data on medium conditions and the dispersion protocol in a structured fashion is essential. We have developed the means to store information on the dispersion protocols and medium conditions in a very structured way. The database supports the step by step description of the treatment of the nanomaterials before and during measurements take place. Due to the structured storing of the information these treatments

can be compared to each other. This aspect is essential for the investigation of the relationships between properties that are influenced by the medium and dispersion protocol (such as agglomeration, size distribution and zeta potential) and toxicity.

The data entry of this step by step description of the nanomaterial is very time consuming. There is also no standardized terminology for the various steps in dispersion protocols (for example, incubation, sonication etc.). Development of ontologies on these protocols is advised.

A more efficient approach would be to refer to specific standardized dispersion protocols and standardized media. This experience is shared and used to further develop similar database structures in other projects, such as the NanoReg2 project. In this project there is emphasis on standardized operating procedures (SOPs) and protocols and storing of this information in the database. In experimental settings however the SOPs or media are often customized ,adapted or changed to support the need of a specific experiment in the laboratory. In that case it is hard to refer to a standardized descriptions of the protocol or medium. Scientists publish these adaptions in literature but this does in general not result in standardized protocols. In the quickly adapting world of nanomaterial toxicity testing protocols change continuously. Version control of these protocols and availability of these versions is another issue when referring to SOPs. Therefore it is our belief that the time consuming effort of entering the step by step description of the dispersion protocols in our database will be a necessity in experimental settings.

Assay results

The database structure of the NANoREG WP6 literature database contains different tables for different experimental results. There are specific fields in the database assigned to specific experimental results such as: nanomaterial density, surface reactivity, solubility etc. (see Appendix 2, Chapter 2.2.). The inclusion of separate fields for these experimental results has resulted in a complex database structure. Most experimental results however can be stored in one identical database structure.

This can be done by generating a “results” table that contains general fields such as:

- “description of the measured parameter”,
- “result value”,
- “results unit”
- etc.

This new insight is obtained from initiatives such as the eNanoMapper project and are applied in the intermediate form of the NANoREG experimental database as gathered for NANoREG by TNO and in other initiatives. It simplifies the database structure but require a more fixed ontology to describe the results. Ontologies are further elaborated in chapter 0.

2.6.2 Data entry

Literature interpretation and data entry

For the purpose of a harmonized data entry from peer reviewed literature by all partners a training was organized. The training elaborated on the structure of the database, the entry-tool and the interpretation of fields in the entry tool. Six persons from different WP6 partners institutes participated in this training. During the project the persons receiving the training were not always able to continue the work. It turned out to be difficult to transfer the knowledge of the person that received the training to these new persons. Feedback at the end project showed that each individual should receive proper training for data entry.

With the help of the training and the guidance trained people are able to interpret literature for data entry. It turned out that a high level of expertise in the field of nanomaterial characterization, toxicology or ecotoxicology is necessary to interpret the literature and extract the essential information.

Minimum requirement/ data completeness

It is essential to set the minimum requirements of the level of completeness of the data depending on the purpose of its use. Two factors are important:

- What is the minimum data that is needed to properly interpret the results of a given experiment considering the goal of the data collection.
- Which contextual can be added via a reference document.

For modelling purposes not always all data needs to be stored in a structured fashion. It is worthwhile to investigate beforehand which data are essential to be stored in a harmonized and structured way and which data can be added via a reference document or otherwise. Data entry of the literature was a time consuming process due to the high level of detail that was required in the entry tool.

In current and future projects experts on various assay are asked to provide what the essential data is that is needed to interpret experimental results. The final set of minimum required information should be defined in a dialog between experts on the specific assays and experts on the database structure and purpose of the data collection.

Data quality

In the WP6 literature database the quality of the data is only guaranteed by the fact that all data was retrieved from peer reviewed literature. Experience with this database has resulted in the following two conclusions:

1. Peer reviewed literature should still be assessed for its quality. For this various tools such as the Klimish score[1] exist.
2. Manual data entry should be checked.

The interpretation of the data in the literature differs from person to person and mistakes are made. This can partly be prevented by checking the data entry by at least one other person or by (partly) automating the data entry. The use of strict ontologies further helps in improving data quality.

Ontologies

For the terminology used in the WP6 literature database no existing ontology was used. For the column headers (field names) the WP6 NanoReg terminology was mapped against existing ontologies. 258 terms in the Wp6 NanoReg database needed to be mapped to Uniform Resource Identifiers (URIs). 207 terms were mapped (~80%) but most of the terms were mapped against multiple URIs. For example dispersion protocol was mapped against the Nanoparticle ontology term 1969, dispersion, and the Information Artifact Ontology term 0000301, protocol (NPO_1969 IAO:0000301). 51 terms could not be mapped to URIs at all(~20%). This mapping was done only based on the exact term (not the actual meaning of the term). In future projects it is essential that mapping of ontologies and database fields it done on the meaning and interpretation of the term and field rather than a textual match. For example: “number of different incubation times” was matched to the terms “number”(PATO:0001555), “different”(C46003), “incubation” (npo:NPO_2000) and “times” (PATO:0000165). None of these terms in itself cover the meaning of the term “number of different incubation times”.

The use of existing ontologies for the column headers helps in harmonizing the interpretation of the database fields and the interpretation of the database structure. This facilities for example data analysis and data entry.

In the data-entry tool users are allowed to enter free text fields for various entries. Moreover users can extend and edit pick-list that are used for various fields. By doing this there has been no

[1] http://nsf.kavi.com/apps/group_public/download.php/30992/Money,%20C.,%20Reg%20Toxicol%20Pharmacol,%202013.pdf

control on the terminology that was used to explain various parameters. For example titanium dioxide can be explained by: Titanium dioxide, TiO2, tio2 etc. these are 3 terms meaning the same thing. An important lesson is that data entry should be restricted to specific terminology, preferably linked to a specific existing ontology as much as possible. This guarantees the description of parameters to be both unique and exclusive By doing this the interpretation of a specific term by different experts is fixed.

Existing ontologies can and should be used for: Chemical abbreviations, nanoparticle characterization, cell assays, biological assays, physical conditions, physical chemical characteristics, media etc. Through the eNanoMapper database existing ontologies are used for the NanoReg2 and Calibrate projects.

3 Deviations from work plan

The title of this deliverable was changed from “Inventory of impact of physical chemical properties on (eco)toxicological endpoints” to “Database on manufactured nanomaterial physical chemical properties and (eco)toxicological endpoints. The title describes the content of the deliverable better. It was initially expected that in collaboration with D6.6 of Task 6.3 a Safe by Design decision tree should be facilitated with this database. The key questions on physical chemical characteristics and toxicology in this decision tree should be queried to the database. Thereby the database would facilitate the decisions by providing information from public literature to support key questions for specific materials. It was expected that this database should the need of this decision better than an Inventory of impact of physical chemical properties on (eco)toxicological endpoints.

The purpose of this inventory was to facilitate a Safe design strategy by providing the relevant information on the structure-toxicity relationships of nanomaterials as input for a decision tree (D6.6). Such a decision tree will implement an algorithm for the safe-by-design strategy. The development of such a decision tree in D6.6 appeared overambitious, but good support for information to be gathered will be delivered in D6.6. Partners involved in NanoReg2 have secured that work performed in for this deliverable and for D6.6, will be continued to reach the initial goal under the EU-project NanoReg2.

4 Annexes

Annex I: WP6 D6.5 Activity 2 Memo on the usefulness of other databases

Annex 2: NANoREG WP6 literature Database_Background, Structure and Guidance

Annex I Memo

Annex I

Date:	2014-01-21
From:	TNO
To:	Nanoreg WP6
Topic:	WP6 D6.5 Activity 2 Memo on the usefulness of other databases

Assessment of usefulness of other databases

In NANoREG, TNO is contributing to the development of a Safe by Design strategy, where Structure-Activity Relationship (SAR) models on nanomaterials toxicity will be integrated with information on structure-functionality. In this context, the SAR models constitute the knowledge basis of the safe-by-design strategy; for this reason it is necessary to collect and organize data on nanomaterials structural and toxicological properties in a way that will allow the development of SAR models. The first step, therefore, in the NANoREG project is to develop a database where selected information is collected from literature and other large scale projects to derive the SAR models. Before building the database, we have been studying at other similar initiatives where databases on nanomaterials toxicity were developed to find consensus and possible knowledge gaps. With this respect, we have identified the following activities:

ISA-TAB-Nano

The Nanomaterial registry

The Napira Hub

COST Modena

This report is presenting and discussing these initiatives in the perspective of building a database for SAR models within NANoREG.

ISA-TAB-Nano

One promising format for storing data is the ISA-TAB-Nano, which records data and metadata from studies designed to either characterise nanomaterial structures/physicochemical properties or their effects on biological systems in a standardised fashion – i.e. with a standardised syntax for recording measurements and experimental conditions (and other kinds of metadata) as well as encourages the use of standardised terms from ontologies (or controlled vocabularies) for defining the measurements/metadata

being recorded. The first version of ISA-TAB-Nano is described in Thomas et al 2013 (http://www.biomedcentral.com/1472-6750/13/2). However, the format is evolving; it is currently on version 1.1, although this only differs slightly from version 1.0 described in the paper. The most up to date information on the format can be found here: https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano. The format consists of a linked set of four kinds of spreadsheet files (Investigation, Study, Assay, Material), templates for which can be found on this same webpage. In principle, these files can be populated by hand in Excel – although this is a very time consuming process. The aim of its developers is to adapt the format to ensure compatibility with the ISA-Tools software (http://isa-tools.org/), which would allow files to be checked for errors (validated) and more easily created, as well as converted to other useful formats such as RDF triples which could be used to populate a Triple Store database software platform. Various EU projects are developing software to parse/create ISA-TAB-Nano files. For example, Robert Rallo (robert.rallo@urv.cat) in MODERN and Dominik Mertens (dominik.mertens@genedata.com) in MOD-ENP-TOX.

The Nanomaterial registry

The National Nanotechnology Initiative (NNI), in its Nanotechnology Signature Initiative (NSI) for a Nanotechnology Knowledge Infrastructure (NKI), has recognized the importance of enabling "a robust digital nanotechnology data and information infrastructure to support effective data sharing, collaboration, and innovation across disciplines and applications." The Nanomaterial Registry is a National Institutes of Health (NIH)-funded public tool that has been developed in support of this goal. The Nanomaterial Registry properly archives curated nanomaterial data and has made them available to the nanomaterial community. As multidisciplinary nanomaterial data are archived, they are transformed into information via specific data curation and structured presentation. One goal of the Registry is that researchers can use this information in downstream analyses to elicit the discovery of emergent trends and data gaps. Key concepts—such as a minimal information set for nanomaterials, data compliance scoring, controlled vocabulary, and ontology—are being developed and applied to the data and the data collection process by the Registry.

Studies suggest that nanomaterials interact with their respective environments in unique ways. In order to capture and track these phenomena, the Nanomaterial Registry curates Instance of Characterization (IOC) information within each record. The IOC information defines the “when” or “where” a material was characterized in terms of a time point or environmental condition. A goal of tracking IOCs is not just for the comparison of nanomaterial measurements, but also to identify if two originally identical nanomaterials retain the properties that make them similar over the course of various processing and characterization steps. A second goal is to determine if nanomaterials that share certain physico-chemical characteristics (PCCs) have similar or predictive interactions with biological or environmental systems. To support these goals, substantial importance is placed on the IOC of each nanomaterial entry in the Registry.

Unique IOCs are curated with PCC and study data whenever reported. IOC designations speak to both the dynamic and kinetic state of a nanomaterial (see Figure 1). IOC designations are listed below:

- As Synthesized – used when an investigator has characterized a nanomaterial after synthesis. This IOC often includes a manufacturer name, product name, lot #, and general description of synthesis.

- As Received –used when an investigator has characterized a nanomaterial after receiving it from a manufacturer. This IOC often includes a manufacturer name and product name.

• As Processed – used when an investigator has either received or synthesized a nanomaterial and then subjects it to a thermodynamic change (e.g., suspending a powdered nanomaterial in a media, sonicating a nanomaterial suspension, changing the media in which a nanomaterial is suspended, or changing the pH of a nanomaterial suspension). Each processing step is designated by a letter: for example, “As Processed A” and “As Processed B.”

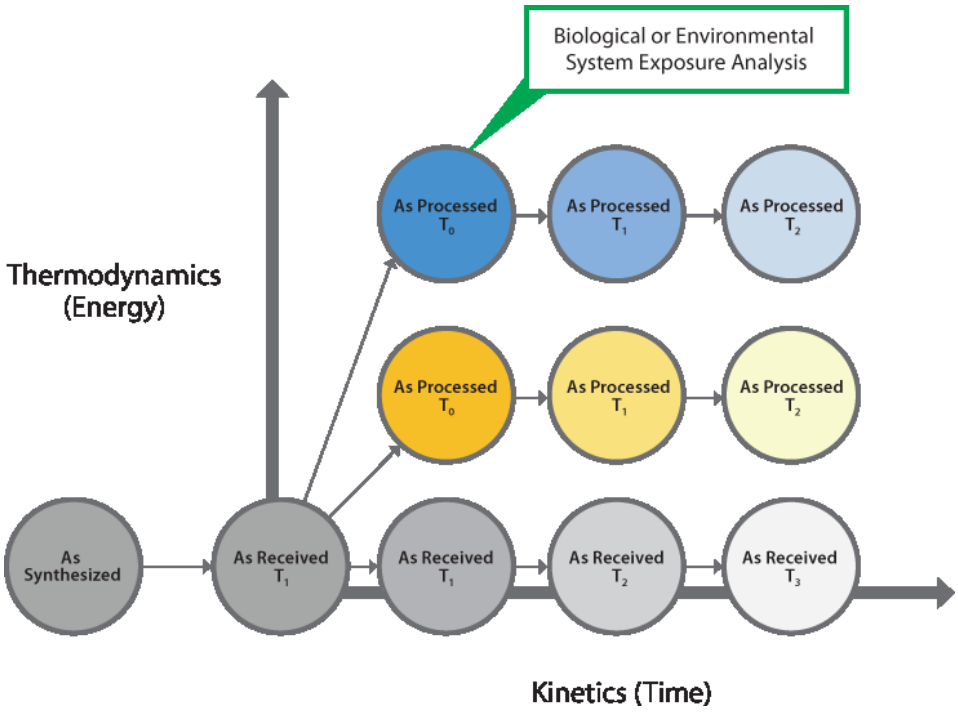


Figure 1. Instances of Characterization of a nanomaterial undergoing dynamic and kinetic changes

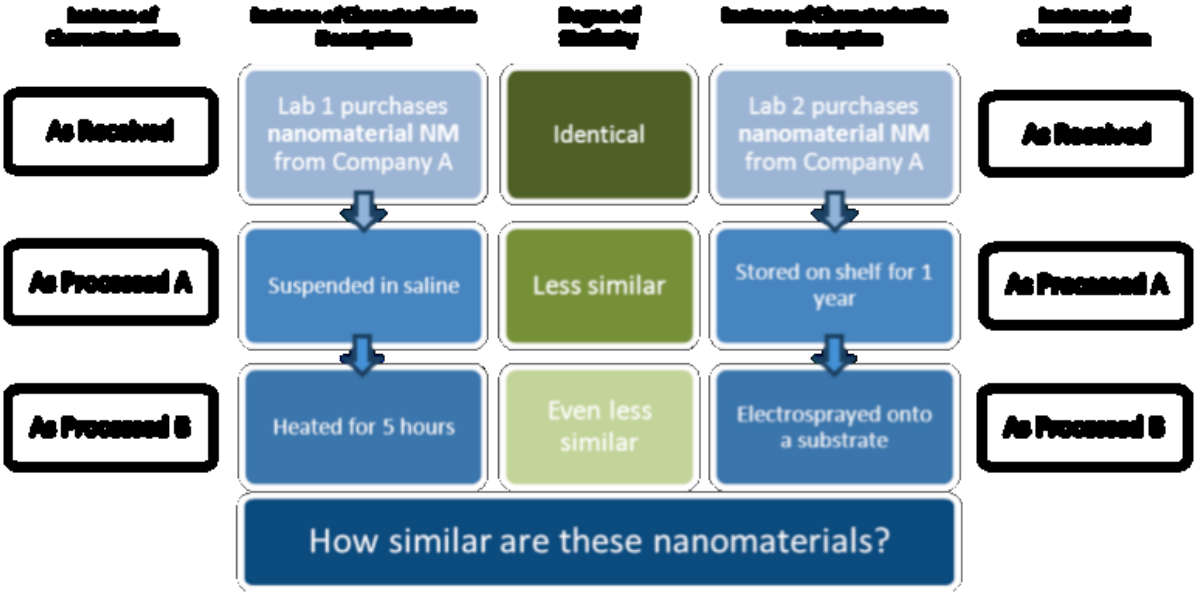
Nanomaterial records in the Registry database may have one or many IOCs. Sometimes, these nanomaterials are characterized in various systems (thermodynamic analysis) or in one system at various times (kinetic analysis). A nanomaterial can be characterized in isolated events, either by measuring different samples of the same batch or by processing a sample in a step-by-step method. A nanomaterial sample can also be characterized in a chronological fashion (measuring the same sample over time). The Registry indicates step-wise and chronological testing by displaying a predecessor IOC in the upper-right-hand corner of the data box. If a predecessor is not listed, the record's IOCs are not step-wise or chronological but, rather, are isolated tests. Because of the differential nature of information within each IOC, the Registry collects specific minimal information for each type of IOC. The collected meta-data is depicted in the IOC Description. By reporting this information (e.g., manufacturer, product name, Digital Object Identifier (DOI) of synthesis procedure, etc.), the Registry can be used to accurately track a nanomaterial sample's life. Table 1 lists several of the categories of minimal information requested for each IOC category.

Table 1. Minimal information curated for each Instance of Characterization category

As Synthesized	Manufacturer or Laboratory Name Product Name Lot Number General Synthesis Description Digital Object Identifier (DOI) Citation of Synthetic Procedure
As Received	Manufacturer Laboratory Name Product Name Lot Number
As Processed	Processing Details (e.g., aerosolized, suspended, dried, milled, heated)

Figure 2 gives an example of how a nanomaterial's degree of similarity can change when it is used by two different laboratories. At this time, the Registry uses simple, rule-based data matching to show the user how similar nanomaterials are to each other. As the depth and breadth of data in the Registry increase and as minimal information standards become more refined, nanomaterial matching will improve.

Figure 2. Examples of chronological Instances of Characterization and their respective descriptions for a purchased nanomaterial being processed in different ways indicate, in part, a decreasing degree of similarity of these nanomaterials over time



The Nanomaterial Registry has developed a minimal information about nanomaterials (MIAN) for the physico-chemical characterization of nanomaterials. The scope of this MIAN includes the most descriptive characteristics of a nanomaterial—the characteristics that govern a nanomaterial's interaction with biological and environmental systems. Nanomaterial PCCs are highly affected by their surroundings, consequently affecting a nanomaterial's behavior in any given system.

The PCC MIAN, while capturing minimal characteristics, is deep in its capacity to capture characteristic data as well as the protocols, parameters, and metadata associated with each measurement. This is done with the understanding that instrument settings can greatly impact the data received during an analysis. This depth will encourage the adoption of

greater reporting in literature and also will promote standards creation about data collection and communication. Within the PCC MIAN, the most relevant measurands, units, techniques, protocols, and instrument parameters have been identified in order to form a controlled vocabulary for use during curation.

Within the PCC MIAN are also best practice questions, which are used for evaluating the quality of the characterization. The best practice questions that are applied to each measurement value are the following:

1) Are raw data provided by the data source?

2) Were proper controls used and reported?

3) Was the instrument within calibration?

4) How many replicates were performed?

5) Was the measurement protocol reported?

6) Was there a citation to the protocol?

7) Were there modifications made to the cited protocol?

The Nanomaterial Registry has also created a MIAN for studies performed on the biological and environmental implications of nanomaterials. The minimal information for studies ensures that the data from studies are curated in a way that summarizes the vital information in the study and helps the user better interpret how the study was performed and what its conclusions were.

The Napira Hub

Another initiative to store data has been developed by the EU's Joint Research Centre. This database is called Napira Hub and is meant to collect information about nanomaterials generated in different European projects. The template which stores the data is based on IUCLID 5.5 format. To enhance the understanding, the format is divided into three sections: The root objects, Endpoint Study Records, Endpoint summaries and Archive. For more information see http://iuclid.echa.europa.eu/index.php?fuseaction=home.project.

Cost Modena

To promote the development of a new generation of ENM that are SAFE-by-DESIGN, an understanding of the relationship between the ENM STRUCTURE and the biological ACTIVITY is needed. In this context, Quantitative Nanostructure-Toxicity Relationships (QNTR) computational modelling technique is an effective alternative to experimental testing since it enables the prediction of (eco)-toxicological effects based on ENM structure only. The construction of QNTR model requires the integration of expertise of nanomaterial scientists, (eco)-toxicologists, and modellers from academia, regulatory agencies and industry. Therefore, a network for trans-disciplinary cooperation is needed. Thus, the MODENACOST Action was proposed to promote and to realise through the coordination of these inter-disciplinary collaborations of different parties with the ultimate aim of producing QNTR models for ENM.

The important benefits from MODENA include:

(i) the development of a new generation of SAFE-by-DESIGN ENM;

(ii) the effective reduction of animal testing and

(iii) The creation of transparent, validated and rigorous QNTR tools for regulatory purposes in the field of nanotoxicology according to OECD principles.

One of the first important tasks in this action is the development of a database to store:

- Data derived from literature
- Data generated in one of the following EU projects.
  - ENPRA
  - Nanomune
  - Nanotest
  - Marina (in vitro part)
  - Cell nanotox

A structure for this database is proposed as well as a method for data extraction. For data extraction an automated tool is designed that prioritized literature based on Number of citations together with the impact factor of the articles that cite.

The following information will be entered in the database:

- Paper, Author, Nanoparticle, Assay-end point, model, figure head, data point
- NP name, Unique code, chemical formulation, manufactured by, synthesis mode (in case not ommercial), crystalline, weight, impurities, coat material, porosity, zeta potential, specific surface area, morphology, size, characterized by
- In vitro: media constitute, serum type, % serum, confluent, non-confluent
- Ex Vivo: media constitue, serum, % ser, mode of exposure……
- Data point : Particle, model, assay, concentration value, exposure duration, exposure unit, numerical results……..

The database structure and tools should be finished around the summer of 2014.

Conclusion

The field of nanotoxicology faces many challenges in the development of standards to support meaningful data submission and information exchange. Nanomaterial characterization requires numerous physico-chemical, in-vitro, and in-vivo assays where measurements mostly depend on non-standardized protocols and diverse technology types. Unfortunately, information describing the nanomaterial, including functionalizing entities and three-dimensional (3D) structure, is often represented in an undisciplined fashion. In addition, there has been no standard way to associate this information with the data and metadata from characterization studies. This lack of standardization has been a significant deterrent to meaningful data sharing across the nanotechnology community; few publications contain sufficient information to enable adequate interpretation of results and successful achievement of experimental reproducibility. Furthermore, there has been very limited

success in using non-standardized data to represent or derive SARs that are critical for understanding the effects of nanomaterial structure on biological activity in nanotoxicology.

After looking at the different initiatives that attempt to build databases for developing SARs on nanomaterials we can conclude that:

1. TNO initiative on building a database for nanomaterials SARs is in line with other EU and US initiatives and will benefit from already ongoing projects to better define the structure of the database and the literature to be selected.

2. All initiatives aknowledge the fact that nanomaterials behavior can change in relation to environmental factors, including time: for this reason it is necessary to record the history of a nanomaterial from its production to its disposal to identify exposure scenarios, dose forms, etc.. and eventually develop SAR models.

3. It follows from point 2 that characterization of nanomaterials (also in biological and environmental matrixes) is a prerequisite for enabling the development of reliable SARs.

4. The development of SARs will depend mainly on the quality of the characterization and toxicological tests; it should be noted here, however, that a genuine SAR model should use theoretical descriptors to enable predictions on virtual compounds.

Annex II

NANoREG WP6 literature Database:

Background, Structure and Guidance

Working Document

If in trouble, please send feed-back to

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Version 1.1

Date: 25 November, 2016

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Annex II Contents

1. Introduction

The goal of deliverable 6.5 is to identify a set of physicochemical properties that are related to the (eco)toxicity of nanomaterials. To this end, TNO has developed a relational database that contains information on nanomaterial's physicochemical characteristics and (eco)toxicity; this information is used to identify physicochemical properties related to the fate and toxicity of nanomaterials, develop structure-activity relationship (SAR) models, derive grouping principles and contribute to the development of a safe by design strategy (deliverable 6.6 of the NANoREG project) by defining a "safe window" of (eco)toxicological parameters. The database is designed to store information on both the properties of pristine nanomaterials (e.g. particle's composition) as well as their interaction with biological and environmental components; this is done to keep track consistently of the particle's history and identify the influence of biological and environmental conditions on nanomaterial's toxicity (e.g. the effect of the biomolecular corona on the cellular uptake and toxicity of nanoparticles).

The relational database is filled with information retrieved from peer reviewed scientific literature and for the purpose of data entry, a software tool has been developed within the NanoReg project, to enable the data entry process in a consistent manner. The database includes 3 types of data on nanomaterial characteristics and toxicity:

1. parameters that define the intrinsic characteristics of nanomaterials;
2. measurement on nanomaterial properties under specific conditions;
3. *in vitro* and *in vivo* (eco)toxicity endpoints.

Data type 1 stores information on nanoparticle primary characteristics, these are, e.g., properties that define the chemical composition, primary size, crystallinity and CAS registry number of nanoparticles. This type of properties will allow the comparison of identical nanoparticles used over different studies. It is well known, however, that the properties of nanomaterials are affected by the biological and environmental matrices and dispersion protocols used during the experiments; the second type of data includes this type of information, e.g.: surface properties (charge, and surface chemistry), agglomeration state and hydrodynamic size. In order to develop structure or property activity relationships (i.e. links between primary and/or secondary physicochemical properties with (eco)toxicological endpoints) the database contains a relevant selection of *in vitro* and *in vivo* endpoints. All information identifying a specific bio-assay is stored in the database, this includes: exposure conditions, species information, assay properties and the way of quantifying the endpoints.

2. NANoREG WP6 literature Database

Upon finalization of the NANoREG project, the NANoREG WP6 literature database will contain a large amount of data (it is foreseen that at least 500 papers will be stored). The next chapter will explain in more details the database structure and how fields in the database are defined; the definition of the fields and their relation to each other is named the ontology of a database.

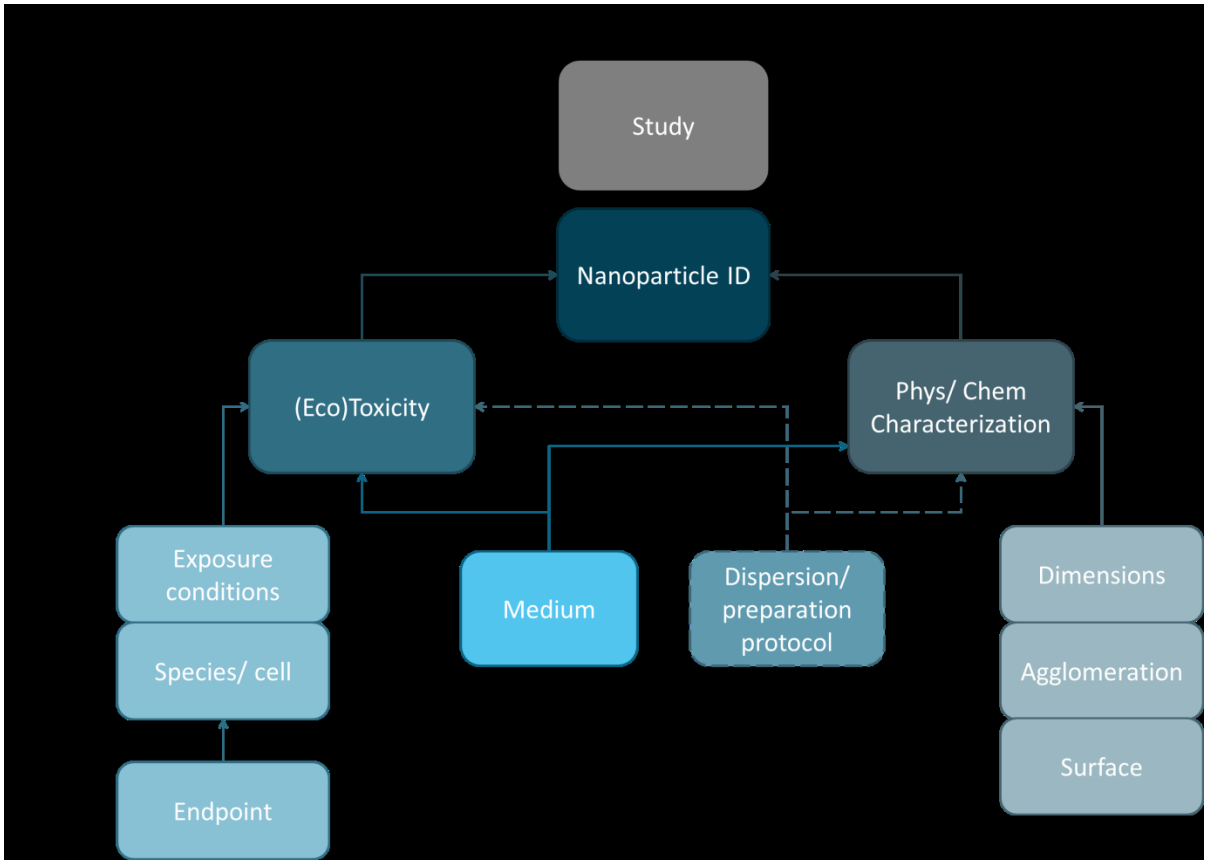
2.1. Database Structure

The NANoREG WP6 literature database contains 8 types of information that can be extracted from published studies.

- Study information (1)
- Nanoparticle identification parameters (2)
- Physicochemical characteristics of a nanoparticle (4)
- Data of experimental media (3)
- Data of the preparation protocols (3)
- Data of the test subject (4)
- Data of the exposure conditions (4)
- Data on the endpoints (5)

The underlying database structure is a MySQL database, which is a relational database. The database consists of multiple tables. A specific field in a table is defined by its name and the table in which it exists and the relation of that table to others. The tables are built up in a hierarchy. The number in the list of 8 types of information indicate how the hierarchy of tables is built up. A database entry is defined by information on the study from which it is extracted. In a study, multiple particles can be described/investigated, so multiple tables on nanoparticle identification parameters can exist within one study. Of each particle multiple physical chemical characteristics of the nanoparticle can be provided/ measured. Within each combination of an exposure/ test medium and preparation protocol multiple measurements of physical chemical characteristics multiple and exposure conditions with test subject characteristics can be added to each nanoparticle. Finally multiple (eco)toxicity/ bio assays can be added to each set of exposure conditions and test subject characteristics. Figure 1 explains more clearly on the structure of the database and the hierarchy.

figure 1: General structure of the database. The study is highest in hierarchy. A study can contain multiple particles, therefore the particle ID is lower in hierarchy. Each measurement (Eco)toxicological or Phys/Chem is defined by the particle ID, The Medium and the dispersion protocol. Multiple measurements can be added to one particle, therefore the (Eco)toxicological or Phys/Chem measurements are lower in hierarchy as the particle.



Two types of measurements can be added to a specific particle: Multiple physical/chemical characterization measurements and (Eco)toxicological measurements can be added. Each of these measurements is defined by the parameters of the measurement itself, the medium in which the measurements are carried out and the preparation protocol of the particle for the specific measurement (the only exception is physical chemical characteristics that have been provided by the manufacturer where the definition of a medium and preparation protocol is not necessary). An infinite number of different measurements can be added to each specific particle. In figure 3 an overview of the complete database structure is displayed. The hierarchy or relations in the database are set-up in such a way as experiments in studies are set up as well. This is done to facilitate the entry of experimental data as it has been reported in literature.

figure 2: Detailed overview of the MySQL database structure.

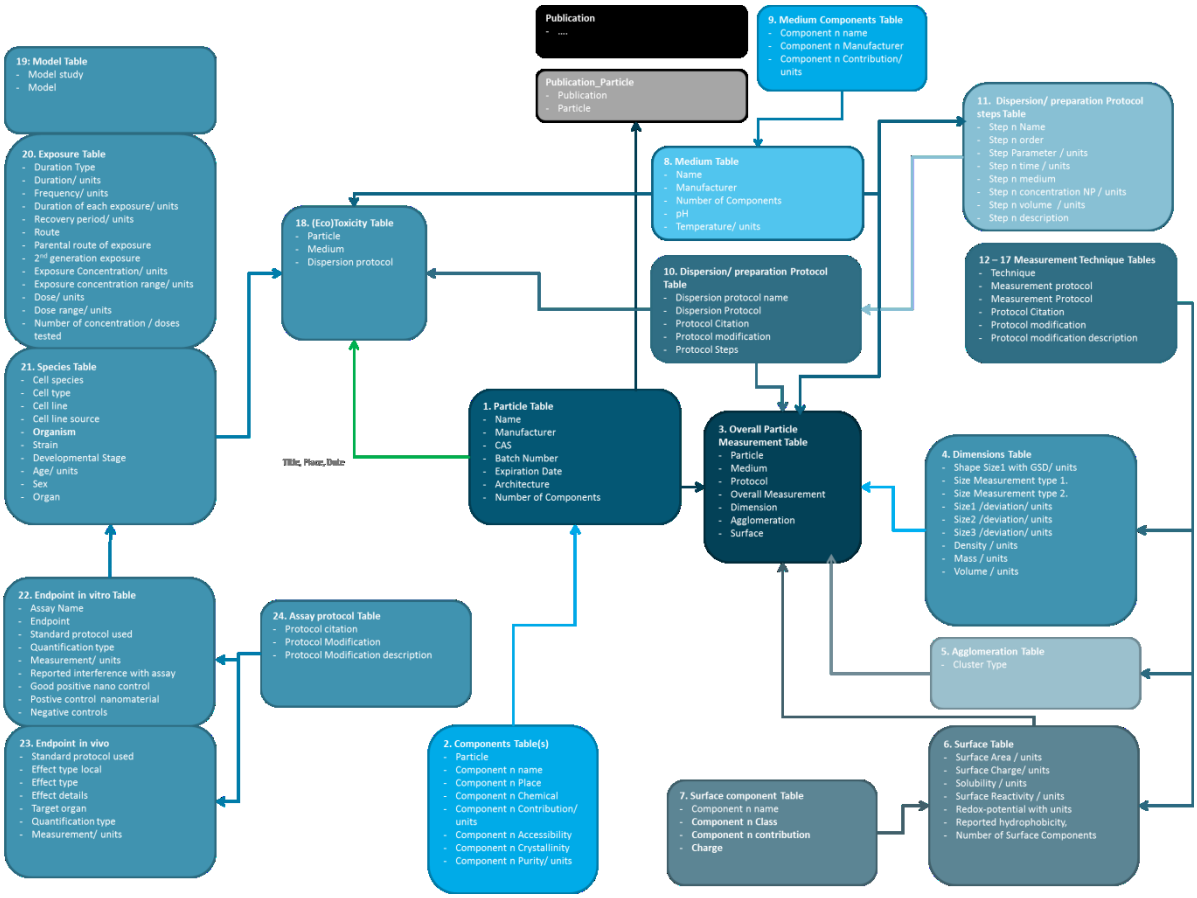


figure 3: Overview of the entire database structure with most important parameters

2.2. Database Ontology

The database ontology describes the fields of a database and the meaning of specific terms in the database. In the tables in this chapter an overview of all the field in the database is given. The fields are ordered according to table. In the figure 3 on overview of the relationships between tables is given. The first column in the tables represent the database field, the second and third column the type of entry that is allowed for these field and the last column a description of the field.

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0	Assay protocol	57

Field	Entry restriction	List options	Remarks
A. Particle Table			
Name	List Choice	Pre-filled reference particles (new entries auto-generated)	This field is generated automatically based on the entries in the database. However during the data-entry it is required to use the name of the nanoparticle as used by the authors in the study.
Manufacturer	List Choice	(to be populated by users)	Enter the manufacturer of the nanoparticle as mentioned by the authors.
Cas	Number xxxxxxxxxx-xx-x	n/a	Up to ten digits in the first part- then two – then 1 (checksum) *DB: Last number is checksum digit (will be checked in database)*
Batch number		n/a	Enter the batch number of the nanoparticle as and if mentioned in the publication
Expiration date	Date yyyy-mm-dd	n/a	Enter the Expiration date of the nanoparticle as and if mentioned in the publication
Sample handling according to NANoREG standard	List Choice	Yes No	Enter whether the smapling vials were treated as described in NANoREG SOPs
Architecture	List Choice	Simple Core/Shell Onion Doped (to be populated by users)	Enter the architecture of the nanoparticle as mentioned in the publication. The Architecture is not the same as shape. The architecture describes the way components are ordered in the nanoparticle. E.g. Simple means one main material like carbon in a carbon nanotube. Onion means multiple layers like in a quantum dot.
Number of components[R]	Number	n/a	The number of component should be set before entering specific data on the specific components. If the number of components is changed during the entry process, all data on the components will be removed. *DB: Connects to Components Table(s) (0)* [R] *For compliance with NANoREG characterization requirements entry of the composition including the number of components as measurement is recommended.*

Components Table(s)			
Component n name	Free text		Write the name of the chemical of which the component consists as stated by the authors.
Component n place	List choice	Core Shell 1 Shell 2 Shell 3 Coating Doping Functionalization Impurity (to be populated by users)	Here the place of the component can be defined. A core component is considered a component of which the core of the particle consists. If the particle consists of only one component this should also be selected as a choice. Shell 1 is considered the first layer around the core, shell 2 the second etc. A coating is considered a non-covalent shell. A functionalization is used if stated by the authors. An impurity is added if clearly defined by the authors. If an impurity is not clearly defined the purity field in this table can be used to enter a purity in %.
Component n chemical type[R]		Elemental Metal Metal oxide Carbon/Fullerene Dendrimer Liposome Polymer Liposome Group IV- non carbon Group III-V Alkaline Earth Alkali Halogen Lanthanide Actinide Biological (To be populated by users)	Indicate the type of chemical component. Based on this the chemical component field is populated with a list of choices. Chose elemental if an elemental analysis of the components is made. *[R] For compliance with NANoREG characterization requirements entry of the composition including the component chemical type as measurement is recommended.*
Component n chemical[R]	List Choice	Elemental *Periodic table* To be added by users	Pick the chemical from the list *[R] For compliance with NANoREG characterization requirements*

		Metal Au Ag Ti Vn Cr Fe Co Ni Cu Zn Hg To be added by users Metal-Oxide *Most metal-oxides* To be added by users Carbon/Fullerene Buckyball SWCNT MWCNT Graphene Graphite To be added by users Dendrimer To be added by users Polymer To be added by users Liposome To be added by users Group IV- non carbon To be added by users	*entry of the composition including the component chemical as measurement is recommended.*

		Group III-V To be added by users Alkaline Earth Be Mg Ca Ba To be added by users Alkali Li Na Ka Ce To be added by users Halogen To be added by users Lanthanide Ce To be added by users Actinide To be added by users Biological (To be populated by users)	
Component n contribution[R]	Number		Add the contribution of the component to the nanoparticle if no further components are described use 100. [R] *For compliance with NANoREG characterization requirements entry of the composition including the component contribution as measurement is recommended.*

Component n contribution units[R]	List Choice	% w/w % v/v	If not further defined by the authors use % w/w as a unit [R] *For compliance with NANoREG characterization requirements entry of the composition including the component contribution units as measurement is recommended .*
Component contribution Measurement[R]	List Choice	Measured Provided	Indicate whether the particle components were measured or provided by the manufacturer *DB: Connects to Component contribution measurement* [R] *For compliance with NANoREG characterization requirements measurement of the composition is recommended*
Component contribution measurement Technique[R]	List Choice	EDX[R] EDS[R] FTIR XRD[R] Raman scattering[R]	Enter the measurement technique with which a possible component analysis has been carried out. [R] *For compliance with NANoREG characterization requirements entry the measurement technique for measurement of the composition is recommended and restricted to EDX/EDS, XRD or Raman Scattering for chemical composition, crystalline phases and CNTs respectively.*
Component contribution Measurement protocol	List Choice	(to be populated by users)	Only indicate if specifically mentioned by authors
Component contribution Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Component contribution Protocol modification	List Choice	Yes No	
Component contribution Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol in free text.
Component contribution Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure

Component n accessibility		Yes outer layer Yes by leaching No Unknown	Indicate if it is mentioned that this component is accessible for biological interaction or not. The outer layer is always considered accessible.
Component n crystallinity[R]	List Choice	Crystalline Amorphous Rutile Anatase (to be populated by users)	Add a specific crystallinity if applicable (e.g. anatase/rutile for TiO2).  *DB: Connects to Component crystallinity measurement* [R] *For compliance with NANoREG characterization requirements the composition including the crystallinity of components .*
Component crystallinity Measurement[R]	List Choice	Measured Provided	Indicate whether the particle components were measured or provided by the manufacturer [R] *For compliance with NANoREG characterization requirements measurement of the crystallinity is recommended*
Component crystallinity measurement Technique[R]	List Choice	XRD[R] Raman Scattering[R]	Enter the measurement technique with which a possible component analysis has been carried out. [R] *For compliance with NANoREG characterization requirements entry the measurement technique for measurement of the crystallinity is recommended and restricted to XRD or Raman Scattering crystalline phases and CNTs respectively.*
Component crystallinity Measurement protocol	List Choice	(to be populated by users)	Only indicate if specifically mentioned by authors
Component crystallinity Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Component crystallinity Protocol modification	List Choice	Yes No	
Component crystallinity Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol in free text.

Component crystallinity Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Purity	Number		Add the purity here if it is clearly stated by the authors and if the impurity is not further specified. Specified impurities can be added as a component with the "place" impurity. .
Purity Units	List Choice	%	
Overall Particle Measurement Table			
Medium[M]	List Choice	(to be populated by users) Add new medium	Indicate in which medium the test was carried out. ⚠ *DB: Connects to Medium table (0)* [M] *For compliance with NANoREG characterization requirements description of medium during particle measurements is mandatory*
Dispersion/ preparation protocol[M]	List Choice	(to be populated by users) Add new protocol	Indicate what the preparation method of the nanoparticle dispersion/ aerosols was before measuring/ testing ⚠ *DB: Connects to Dispersion/ preparation protocol table (0)* [M] *For compliance with NANoREG characterization requirements description of medium during particle measurements is mandatory*
Overall Measurement[M]	List Choice	Measured Provided by manufacturer	[M] *For compliance with NANoREG characterization requirements measurement of various parameters, shape, size and dissolution is mandatory*
Dimensions Table			
Shape [R]	List choice	Sphere (1d) Tube/ cylinder/ fiber (2d) Platelet/ flake (2d) NOCube (1d) Cristal (3d)	⚠ *DB: Connects to Shape Measurement Technique Table (0)* [R] *For compliance with NANoREG characterization requirements measurement of the shape before toxicity testing is recommended*

Size Measurement type1:[M,R]	List Choice	Mean primary particle size Mean hydrodynamic diameter Mean Aerodynamic diameter (to be populated by users)	⚠ *DB: Connects to Size Measurement Technique Table (0)* *[R] For compliance with NANoREG characterization requirements measurement of the size before toxicity testing is recommended* *[M] For compliance with NANoREG characterization requirements measurement of the size in dispersion, in batch, in exposure medium before and after toxicity testing is mandatory*
Size Measurement type2:	List Choice	Single Bimodal Range Bimodal range	
Size 1: diameter/ thickness	Number (positive)	n/a	Always available Enter 1 value for single. Enter 2 values for bimodal Enter 2 values for Range Enter 4 values for bimodal range
Size 1 Deviation:	Number (positive)	n/a	Only if size is Single or Bimodal Enter 1 value for single Enter 2 values for Bimodal
Size 1 Deviation type	List choice	SD GSD PDI	Only if Size is Single or Bimodal
Size 1 units	List Choice	Å nm µm mm cm m (to be populated by users)	
Size 2: length	Number (positive)	n/a	Only for Shape = Tube/ cylinder/ fiber

			Platelet Cristal Enter 1 value for single. Enter 2 values for bimodal Enter 2 values for Range Enter 4 values for bimodal range
Size 2 Deviation:	Number (positive)	n/a	Only if size 2 is available Only if size is Single or Bimodal Enter 1 value for single Enter 2 values for Bimodal
Size 2 Deviation type	List choice	SD GSD PDI	Only if Size is Single or Bimodal
Size 2 units	List Choice	Å nm µm mm cm m (to be populated by users)	
Size 3: width	Number (positive)	n/a	Only for Shape = Tube/ cylinder/ fiber Cristal Enter 1 value for single. Enter 2 values for bimodal Enter 2 values for Range Enter 4 values for bimodal range
Size 3 Deviation:	Number (positive)	n/a	Only if size 3 is available Only if size is Single or Bimodal

			Enter 1 value for single Enter 2 values for Bimodal
Size 3 Deviation type	List choice	SD GSD PDI	Only if Size is Single or Bimodal
Size 3 units	List Choice	Å nm µm mm cm m (to be populated by users)	
Density	Number (positive)	n/a	
Density Unit	List Choice	g/cm^3 (to be populated by users)	
Volume	Number (positive)	n/a	
Volume Unit	List Choice	$Å^3$ nm^3 $µm^3$ mm^3 cm^3 m^3	
Agglomeration Table			
Cluster Type	List Choice	Agglomerate/ Aggregate	Only indicate if specifically mentioned by authors
Surface Table			
Surface Area	Number (positive)	n/a	*DB: Connects to Size Measurement Technique Table (0)*
Surface Area SD	Number (positive)	n/a	Enter surface area standard deviation if provided
Surface Area Units	List Choice	cm^2/g m^2/g (to be populated by users)	
Surface Charge	Number (positive	n/a	*DB: Connects to Surface Charge Measurement Technique*

	and negative)		*Table (0)*
Surface Charge SD	Number (positive)	n/a	
Surface Charge Unit	List Choice	mV V/cm^2 Microequivalents/L $Coulomb/m^2$ (to be populated by users)	
Solubility[R]	Number (positive)	n/a	⚠ *DB: Connects to Solubility Measurement Technique Table (0)* [R] *For compliance with NANoREG characterization requirements measurement of the solubility in the exposure medium is recommended*
Solubility Units	List Choice	Concentration Molarity Molality Mole fraction Ksol	
Surface Reactivity[R, M] (casusticity, redox potential, photocatalysis etc.)	Number	n/a	Chemical reactivity towards specific chemical systems ⚠ *DB: Connects to Surface Reactivity Measurement Technique Table (0)* [R] *For compliance with NANoREG characterization requirements measurement of the redox potential in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements measurement of the redox potential in the exposure medium eco toxicity tests is mandatory*
Surface Reactivity Units[R, M]	List Choicep	(To be populated by users)	[R] *For compliance with NANoREG characterization requirements measurement of the redox potential in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements measurement of the redox potential in the exposure medium eco toxicity tests is mandatory*

Number of Surface components	Number	n/a	Opens the given number of surface component tables *DB: Connects to Size Measurement Technique Table (0)*
Surface Component Table			
Surface Modification n name	Free type	n/a	Indicate whether surface of pristine nanomaterials was treated and/or modified (also by interaction with biological matrices)
Surface component n class	List Choice	Protein Surfactant DOC	Indicate the type of surface components (e.g. protein corona)
Surface component n contribution	Number (positive)	n/a	
Surface component n contribution units	List Choice	% w/w % v/v	
Component n Charge	List Choice	Positive Negative	Indicate the components charge in the given medium
Medium table			
Medium Name	Automatically generated text	n/a	Autogenerated test based on “Med component 1;contibution component 1;Med component 2;contribution component 2;etc;etc)

manufacturer	List Choice	(to be populated by users)	
Number of Components			*DB: Database connects to Medium components table (0)*
pH type	List Choice	Single Range	
pH[R,M]	Number (0-14)	n/a	Enter one number with SD if single, 2 number if range. [R] *For compliance with NANoREG characterization requirements measurement of the pH in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements measurement pH in the exposure medium of eco toxicity tests is mandatory*
pH deviation	Number	n/a	
pH deviation type	List Choice	SD GSD Variance	
Ionic strength, salinity	Number		
Ionic strength, salinity Units	mM M		
Temperature[R, M]	Number		[R] *For compliance with NANoREG characterization requirements measurement of the pH in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements measurement pH in the exposure medium of eco toxicity tests is mandatory*
Temperature units	List Choice	°C K F	
O2 concentration[R, M]	Number		[R] *For compliance with NANoREG characterization requirements measurement of the O2 concentration in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements*

			measurement O2 concentration in the exposure medium of eco toxicity tests is mandatory
O2 concentration units	List Choice		
CO2 concentration[R, M]	Number		[R] *For compliance with NANoREG characterization requirements measurement of the CO2 concentration in the exposure medium of vitro tests is recommended* [M] *For compliance with NANoREG characterization requirements measurement CO2 concentration in the exposure medium of eco toxicity tests is mandatory*
CO2 concentration units	List Choice		
Medium components table			
Name component n	List Choice	DNEM Purified Water MiliQ Water PBS (to be populated by users)	Component should be added according to a standard scheme: Component 1: Main component e.g. water Component 2 : Main component 2 e.g. DNEM/PBS Component 2/3 : Next component with largest contribution e.g. proteins/ DOC/ biological substrates Component 2/3/4: Next Component with largest contribution e.g. proteins/ DOC/ biological substrates/ antibiotics etc.
Manufacturer component n	List Choice	(to be populated by users)	Only indicate if specifically mentioned by authors
Contribution Component n	Number		
Units contribution component n	List Choice	% w/w % v/v g/l µg/l ml/l	

		l/l U/l mol/l M (to be populated by users)	
Dispersion/ preparation protocol table			
Dispersion protocol Name	Automatically generated text	n/a	
Dispersion protocol	List Choice	(to be populated by users)	
Dispersion Citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Dispersion protocol modification	List Choice	Yes No	
Dispersion protocol modification description	Free type		Describe the modification of a standard protocol or a completely new protocol
Dispersion protocol/ preparation steps Table			
Step name n	List Choice	Wetting Centrifugal Mill Jet Mill Ball Mill Stirring High Speed homogeniser High pressure homogeniser Ultrasound sonification bath Ultrasound probe sonification Ultrasonic disruptor De-agglomeration Dispersion Stabilization Mixing Incubation (to be populated by users)	
Step n order	Number		Indicate which step this specific step is in the order of all activities forming the protocol
Step n parameter	Number		Give the important parameter for the used technique.

			E.g. for a centrifuge the RPMs are important to register
Step n parameter units	List Choice	°C Rpm W (to be populated by users)	
Step n instrument	List Choice	(to be populated by users)	
Step n instrument manufacturer	List Choice	(to be populated by users)	
Step n instrument calibrated	List Choice	Yes No	Indicated whether the instrument used in this step of the preparation/ dispersion protocols was calibrated according to a standard calibration method.
Step n time	Number (positive)	n/a	
Step n time units	List Choice	s min hour	
Step n medium	List Choice	Medium Name	Also here new media should be entered when necessay
Step n concentration of nanomaterial	Number (positive)		
Step n concentration of nanomaterial units	List Choice	kDA/l pg/l ng/l µg/l mg/l g/l kg/l mg/g g/g etc.	If the step is the dispersion of a nanomaterial or the dilution of a nanomaterial dispersion indicate only the final concentration of this step.
Step n volume			
Step n volume units	List Choice	µl ml dl l cm^3 m^3	
Step n description	Free Type		Type a clear abstract of the preparation process. What happened

			to the nanoparticle before the test.
Shape Measurement Technique Table			
Technique[R]	List Choice	SEM[R] (Scanning Electron Microscopy) TEM[R] (Transmission Electron Microscopy) (to be populated by users)	[R] *For compliance with NANoREG characterization requirements measurement of shape before toxicity testing with a restriction of SEM/ TEM is recommended*
Instrument	List Choice	(to be populated by users)	
Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	NANoREG SOP CODA CERVA (TEM) BS 2406-4(1993) (SEM) BS ISO 16700 (SEM) BS ISO 29301 (TEM) (to be populated by users)	Only indicate if specifically mentioned by authors
Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Size Measurement Technique Table			
Technique[R, M]	List Choice	SEM[R] (Scanning Electron Microscopy) TEM[R] (Transmission Electron Microscopy) DLS[M] (Dynamic Light Scattering) (to be populated by users)	If DLS is selected the DLS medium should be the same as the selected measurement medium [R] *For compliance with NANoREG characterization requirements measurement of the size before toxicity testing is recommended with a restriction to SEM/ TEM* [M] *For compliance with NANoREG characterization requirements measurement of the size in dispersion, in batch, in exposure medium before and after toxicity testing is mandatorywith a restriction to DLS*
Instrument	List Choice	MALVERN Nano ZS (to be populated by users)	

Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	NANoREG SOP (DLS) NANoREG SOP CODA CERVA (TEM) BS 2406-4(1993) (SEM) BS ISO 16700 (SEM) BS ISO 29301 (TEM) BS ISO 22412 (DLS) (to be populated by users)	Only indicate if specifically mentioned by authors
Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Surface Area Measurement Technique Table			
Technique	List Choice	BET (Brunauer, Emmett and Teller) TEM (Transmission Electron Microscopy) (to be populated by users)	
Instrument	List Choice	(to be populated by users)	
Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	NANoREG SOP CODA CERVA (TEM) ISO 9277 (BET) (to be populated by users)	Only indicate if specifically mentioned by authors
Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text	

		Table Figure	
Surface Charge Measurement Technique Table			
Technique	List Choice	ζ-potential with Doppler Microelectrophoresis Electrophoretic Mobility Isoelectric point Charge Density (to be populated by users)	
Instrument	List Choice	(to be populated by users)	
Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	(to be populated by users)	Only indicate if specifically mentioned by authors
Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type	n/a	Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Solubility Measurement Technique Table			
Technique	List Choice	Conductivity Calometric assay UV-vis spectroscopy Ion exchange chromatography (to be populated by users)	
Instrument	List Choice	(to be populated by users)	
Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	ASTM STP 195 (Ion exchange chromatography) SAC GB/T 19267-2 (UV-vis) (to be populated by users)	Only indicate if specifically mentioned by authors

Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type		Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Surface Reactivity Measurement Technique Table			
Technique (test system)	List Choice	DPRA GSH (to be populated by users)	Indicate the main reactant and/or reaction equation and the technique used
Instrument	List Choice	(to be populated by users)	
Instrument Manufacturer	List Choice	(to be populated by users)	
Measurement protocol	List Choice	(to be populated by users)	Only indicate if specifically mentioned by authors
Protocol citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type		Describe the modification of a standard protocol or a completely new protocol
Protocol modification description	Free type		Describe the modification of a standard protocol or a completely new protocol
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Overall (Eco)Toxicity Measurement Table			
Medium	List Choice	(to be populated by users) Add new medium	Indicate in which medium the test was carried out.
Dispersion/ preparation protocol	List Choice	(to be populated by users) Add new protocol	Indicate what the preparation method of the nanoparticle dispersion/ aerosols was before measuring/ testing

Model Table			
Model study	List Choice	Human Environmental	
Model	List Choice	In Vitro In Vivo Ex Vivo	
Exposure Table			
Duration type	List Choice	Acute Chronic Repeated dose	Indicate whether the study is acute or chronic Human Acute < 48 hr Eco Acute < 96 hr???
Exposure technique			Indicate which instrument was used to deliver the nanomaterials to the animals
Duration	Number (positive)	n/a	Indicate the duration of the exposure during the entire study is.
Duration Units	List Choice	Minutes Hours Days Weeks Months Years	
Frequency of exposure	Number (positive)	n/a	Indicate the frequency of exposure either the total number of exposures (n) or the number of exposures per time unit.
Frequency of exposure units	List choice	n /hour /day /week /year	
Duration of each exposure	Number (positive)	n/a	The duration of a single exposure.
Duration of each exposure units	List Choice	Seconds Minutes Hours Days Weeks	
Recovery Period	Number (Positive)	n/a	Indicate what the recovery period between two exposure was
Recovery Period Units	List Choice	Seconds	

		Minutes Hours Days Weeks Months	
Exposure Route	List Choice	Inhalation (whole body) Inhalation (nose only) Instillation Oral Dermal Intavenous Intraperitoneal Intramusculair parental (To be populated by user)	In vivo only
Parental route of exposure	List Choice	Inhalation (whole body) Inhalation (nose only) Instillation Oral Dermal Intavenous Intraperitoneal Intramusculair (To be populated by users)	For reproductive toxicity only
2nd generation exposure	List choice	Yes No	For 2nd generation reproductive toxicity only
Number of exposure concentrations/ doses tested	Number (Positive)	n/a	Indicate how many doses or concentrations the concentration range consists of. Excluding the negative control.
exposure concentration/ dose units	List Choice	kDA/l pg/l ng/l µg/l mg/l g/l kg/l	

		mg/g g/g ng/ kg bw pg/ kg bw mg/ kg bw g/ kg bw etc.	
Concentration / dose 1	Number (positive)	n/a	
Concentration/ dose n+1	Number (positive)	n/a	
Species Table			
Cell Species	List Choice	Human (To be Populated by user)	Enter terms as indicated by Authors
Cell Type	List Choice	(To be Populated by user)	Enter terms as indicated by Authors
Cell line	Free type	n/a	Enter terms as indicated by Authors
Cell line Source	List Choice	(To be Populated by user)	Enter terms as indicated by Authors
Cell cycle	G0 G1 S G2 M		Indicate the phase of a cell cycle
Organism	List Choice	Rats Mice Hamster Guinea Pig Zebra Fish (To be Populated by user)	Enter terms as indicated by Authors
Strain	List Choice	(To be Populated by user)	
Developmental stage	List Choice	Pup Adult (To be Populated by user)	
Age	Number (positive)		
Age units	List Choice	Day Week Month	

		Year	
Sex	List Choice	Male Female Unknown	
Organ	List Choice	Skin (and oral mucosa) Gastrointestinal tract Liver Pancreas Kidney Cardiovascular (Skeletal) muscle (To be added by users)	Ex vivo only
Endpoint Table in vitro			
Assay Name	List Choice	Micronucleas test Ames bacterial reverse mutation assay Thymidine kinase (Tk) assay Hypoxanthine-guanine phosphoribosyl transferase (Hprt) assay Rodent erythrocyte micronucleas assay Mammalian bone marrow chromosomal aberration assay Mouse spot test RetinoBLast (eye-spot) assay Aprt assay Dlb-1 assay Sister-chromatid exchange assay Unscheduled DNA synthesis assay SHE CTA (Cell transformation assay) Bhas CTA Comet assay – strand breaks Come assay – oxidised DNA bases Double strand breaks (γH2AX) Double strand breaks (by the comet assay) DNA repair (by comet assay)	

		MLA CAT MTT Clonogenic assay (Plating efficiency) Growth activity test (proliferation assay) (To be added by users)	
Endpoint	List choice	Cytotoxicity Oxidative stress Inflammation/immune markers Genotoxicity In vitro carcinogenicity Endocrine disruption	
Assay Manufacturer	List Choice	(To be added by users)	
Standard Protocol Used	List Choice	Yes No Modified	Mention OECD guidelines etc.
Quantification Type	List Choice	NOAEL LOAEL LC50 (To be populated by user)	
Measurement	Number		
Measurement Units	List Choice	kDA/l pg/l ng/l µg/l mg/l g/l	
Reported interference with assay	List Choice	Yes No	Indicate if the nanomaterial is interfering with the assay technique
Good positive nano controls	List Choice	Yes No	
Positive control	Free type		

nanomaterial			
Negative controls	List Choice	Yes No	
Data Provided	List Choice	Text Table Figure	Indicate whether the data is provided in text, a table or a figure
Endpoint Table in vivo			
Standard Protocol Used	List Choice	Yes No Modified	
Effect type local	List Choice	Local Systemic	
Effect type	List Choice	Reproduction toxicology Teratogenicity / embryotoxicity Neurotoxicity Immunotoxicity Endocrine disruption Phototoxicity Inflammation Stress response Mortality Carcinogenicity Chronic toxicology Genotoxicity / mutagenicity Local inhalation Local oral Skin effects (corrosion, irritation) Skin sensitization Eye effects (To be populated by users)	Only enter significant effects as stated by the author
Effect details	List choice	(Tob e populated by users)	Description of specific marker
Target Organ		Respiratory system Skin (and oral mucosa) Gastrointestinal tract	

		Liver Pancreas Kidney Lower urinary tract Cardiovascular (Skeletal) muscle Bones & joints Nervous system Special sense organs Lymphoid organs / immune system Endocrine organs Male reproductive system Female reproductive system (To be populated by users)	
Quantification type	List Choice	NOAEL LD50 (To be populated by users)	
Measurement	Number		
Measurement units	List Choice	Mg/kg bw (To be populated by users)	
Good positive nano controls	List Choice	Yes No	
Positive control nanomaterial	Free type		
Negative controls	List Choice	Yes No	
Stand level (1-3)			
Species/target environment 1-4			
Geographic distribution 1-4			
Sensitivity Dev. Stage 1-4			
Data Provided	List Choice	Text	Indicate whether the data is provided in text, a table or a figure

		Table Figure	
Assay protocol			
Protocol Citation	List Choice	(to be populated by users)	Enter the citation to which the author refer for the protocol
Protocol modification	List Choice	Yes No	
Protocol modification description	Free type		Describe the modification of a standard protocol or a completely new protocol

3. Compliance with the NANoREG goals and database Utility

3.1. Compliance with NANoREG Guidance on minimum Requirements

Only studies carried out using no less than the mandatory dispersion protocols and the specific characterization and reporting requirements are considered of sufficiently quality to be eligible for NANoREG funding. Studies that deviated from these requirement do not comply with the quality criteria for NANoREG studies (but can comply to other quality criteria). It is important that NANoREG WP6 literature database allows for the entry of these minimum reporting requirements and the specific characterization parameters. Moreover the database should allow for the assigning of quality levels to specific studies based on these same requirements and characterization parameters. Studies that have been carried out according to NANoREG minimum requirements are flagged "*according to mandatory NANoREG procedures and characterization*". Eventually data analysis of the data within the NANoREG WP6 literature database could take into account these quality levels in for example assigning a higher weight to this type of data, or select only these studies for specific regulatory purposes. Finally the NANoREG WP6 literature database can assist in assuring experimental studies are according to the required quality criteria by providing a tool and guidance for the logging of experimental data.

3.2. NANoREG Core Nanomaterials

To facilitate the easy data-entry of experimental studies carried out within the NANoREG project, a part of the 19 NANoREG core Nanomaterials (NM101 -103, 200, 203, 110, 111, 212, 220, 300K, 302, 400, 401, 410, NFC Fine, NFC Medium-Coarse, UPM Biofibrils AS, UPM Biofibrils NS, UPM Bleached Birch Pulp) have been added to a preset pick-list. For these particles a number of field in the database has been prefilled. In the tables in Appendix B

Reference material data-entry, the specific fields that are filled are displayed. Upon selection of these particles the following particle parameters in "as provided" particle measurement fields do not require manual data-entry:

- Particle
  - Name
  - Manufacturer
  - CAS number
  - Architecture
  - Number of components
- Components
  - Name
  - Place
  - Chemical type
  - Chemical
  - Accesability
  - Crystallinity
- Particle Measurement
  - Medium
  - Dispersion protocol
  - Measurement
- Measurement info
  - Measurement type
- Size and shape information
  - Shape
  - Size measurement
  - Size measurement type
  - Diameter/ thickness
  - Diameter/ thickness size unit
  - Length
  - Length size unit
  - Surface area
  - Surface area unit

3.3. NANoREG material handling and selected Standard operating procedures

In the NANoREG WP6 literature database a flag option is made to indicate whether materials from the distributor were supplied and handled according to NANoREG standards. In the NANoREG Guidance on Minimum Requirements a clear description is given on how vials and materials should be handled. Flagging the Nanoparticle in the database with the "*Sample handling according to NANoREG standard"* indicates that criteria for sample handling as described in the Guidance on Minimum Requirements have been met (see chapter: 2.2 **Database Ontology**, A Particle Table).

The database is fully compatible with important parameters of standard operator procedures described by the Guidance on Minimum Requirements. A pick list of the NANOGENOTOX, ENPRA and the NOM*-water protocols has been added to the database. Upon selection of one off the prefilled protocols, specific parameters do not require manual data-entry. Other parameters are still available for manual entry. **Appendix C** shows the parameters that have been prefilled in the database upon selection of one of the standard protocols and which one are still available for manual entry. For the Calibration of sonicators a flag button has been added to the database for sonication steps in the preparation protocols. This flag indicates whether the sonicator was calibrated before use (see chapter: 2.2 **Database Ontology**, 0 Dispersion protocol/ preparation steps Table).

3.4. Minimum characterization requirements for dispersions and exposure media

According to the Guidance on Minimum Requirements the hydrodynamic size (-distribution) of nanoparticles in dispersion should be measured in the batch dispersion and in the exposure medium before and after toxicity testing (initial and final respectively). The database allows for data-entry of these measurement by describing the medium and preparation protocol for the hydrodynamic size (-distribution) measurements. For the batch dispersion the batch medium can be described (purified water) as well as the batch dispersion protocol. For the initial hydrodynamic size the exposure medium is described as well as the dispersion protocol for the exposure medium. For the final hydrodynamic size (-distribution) the final step in the dispersion/ preparation protocol should be the specific toxicity assay, or if the dispersion in the exposure medium in which the final measurement have been carried out the final dispersion/ preparation protocol step should be "incubation" for the duration of the toxicity assay. Measurement techniques (like DLS or analytical ultra-centrifuge) as used can be entered into the database.

All the additional characteristics (both initial and final) of the nanomaterial in the exposure medium can be entered in the existing fields in the database. In tables A-0 of chapter 2.2 it is indicated with an (R), (M) or (O) which parameters are Recommended, Mandatory or Optional according to the NANoREG Guidance Document on Minimum Requirements.

3.5. WPs 1-5 Utility

The possible use of our database to other work packages is important for the efficient expense of budgets and collaboration between work packages. This chapter elaborates on the possible/ proposed use of the NANoREG WP6 literature database to the goals and deliverable of other work packages.

It should be noted firsly that the NANoREG WP6 literature database is uses a dynamic ontology, during the project or at least until mid-2015 changes in the database and the database ontology can still be made to fit requirements of other work-packages. The overall structure as shown in figure 2 and figure 3 of this document should be compatible with all possible requirements. Underlying tables are under review and open for changes.

WP1. Scientific answers to regulatory issues

Task 1.5 of work package 1 is the development of a data platform. The NANoREG WP6 literature database is a currently existing data platform that allows the entry and storage of existing data and the documentation of its resources. During the development of the database existing data platforms like, ISATAB nano, JRC-NanoHub and the Nanomaterial registry have been taken into account. Our collaboration with the e-nanomapper consortium will result in a mapping of the ontology of other existing data-platforms compared to the NANoREG WP6 literature database ontology, and ensure compatibility with these other initiatives.

In retrospect all data entered into the database can be analyzed and comparison of different metrics can be made, for now the database allows for entry of all metrics as reported. Task 1.5 c “explore the possibilities to provide guidance on how to convert data based on different metrics” can be facilitated using the data within the NANoREG WP6 literature database. An analysis and comparison of endpoint outcomes with different input metric can be made. This analysis will provided an empirical validation of possible metric conversions.

With minor additions the database will also be appropriated for the storing of new NANoREG data. Indeed the addition of quality criteria and the indication of the mandatory minimum requirements for characterization will ensure proper data-storing and collection.

Finally the data within the NANoREG WP6 literature database could be a good supplyer of data for the NANoREG Toolbox. Queries and analysis of the database can assist in forming rules for decision making tools for regulatory purposes.

WP2. Synthesis, supplying and characterization

Within work packages 2 an important task is the selection of reference nanomaterials. In Chapter 3 of this guidance document it is explained how compatibility of the NANoREG WP6 literature database with these reference nanomaterials is ensured. Likewise of the important characterization requirements for toxicity testing (as explained in the guidance document on minimum requirements) are flagged in the database. This allows for the entry of these data, moreover it gives guidance on users of the database on which parameters are essential to be measured/ provided for good toxicity testing.

Specific other parameters outside the guidance on minimum requirements are covered for data-entry either directly or indirectly, these include:

- Hydro chemical reactivity
- Dispersion protocols
- Interaction with medium
- Interaction with test assay

WP4. Bio kinetics and toxicity testing in vivo

Information on all endpoints can be added to the database. The database can be used to store the information of the experimental studies.

WP3 and 5

For now the NANoREG WP6 literature database database does not seem to contribute to the goals and tasks of WP3 and 5. The database does not facilitate data-entry on exposure scenarios or measurements. Nor does it provided direct guidance or tool that facilitate regulatory decision. However data-quality and availability are important factors in regulatory risk assessment. In the future the database could well be used as a source for data to be used in the developed of tools for regulatory dossier building and assignment of quality criteria. At least it could provide a useful tool for searches and data collection.

4. Data Quality

For the determination of data quality within a certain study, various aspects of the data are regarded. Quality determination of a study will depend on the amount of relevant measured data within a study and the way that these are measured including the contextual information. Information for the development of a system for the determination of data quality is mainly obtained from the “Compliance with NANoREG Guidance on minimum Requirements document” and “Quality criteria for data on engineered nanomaterials for a Decission support system” document developed by the Dutch National institute for public health and the Environment (RIVM, July 2014) within the NanoNextNL program.

Within the NanoReg database quantitative criteria to data-quality have not been assigned yet. Specific fields that are regarded for quality assignment and the categories for labeling for these fields are described in tables 1 to 4. Statistical analysis of the data (after data-entry) will partly determine how the quality criteria will result in a quantitative measure of data quality.

Important input for the categorization of data quality of physical chemical characterization of nanoparticles is derived from the Guidance on minimum requirements. The mandatory information according to this document plays a big role in the assignment of “reliable” data. These considerations are displayed in Table 4 and Table 5.

In the tables 1 to 4 the fields that are considered relevant for determination of data quality are displayed. Depending on the field a label for data quality is automatically assigned to the entry. This can only be done after data-entry. Various aspect that are considered for quality assignment are:

- Amount and type of particle identification criteria available (Table 4).
- Amount and type of physical chemical characterization carried out including criteria for used protocols etc. (Table 5).
- Information reported on the toxicological/ biological assays carried out including criteria for used protocols etc. (Table 6)
- Overall criteria (Table 8)
    - Similarity of the exposure medium and the physical chemical characterization medium (if applicable)

- Similarity of preparation protocols for the exposure medium and the physical chemical characterization medium (if applicable)
- Physical chemical characterization in exposure medium before and after toxicological/ biological testing

Based on these criteria a quality algorithm can be developed after analysis of a considerable amount of studies entered (at least 50). Based on these algorithms a quality can be assigned to specific studies and specific data-points within the study. The measure of quality can finally be used to assign a weight to different data points when analysis the data for QSAR modelling or safe design purposes.

Table 4: Particle Identification (Relevant for parameters in Table A of Chapter 3)						
Field	Description	Quality				
		High	Mid	Low	1 (Unreliable)	n/a
Manufacturer	Enter the manufacturer of the nanoparticle as mentioned by the authors.	Provided			Missing	
CAS	Enter the CAS number as mentioned by the authors	Provided			Missing	
Sample handling according to NANoREG standard[M]	Indicate whether the sample handling during transport and preparation was according to NANoREG Standards as mentioned by the authors	Yes			No	

Table 5: Physical Chemical and component Measurements (Relevant for parameters in Tables (B to G and M to Q of Chapter 3) Properties that are included in the quality assessment: Composition, compound crystallinity, shape, solubility, size, surface charge, surface area, surface reactivity.						
Field	Description	Quality				
		High	Mid	Low	1 (Unreliable)	n/a
Specific property measurement	Indicate whether the property was measured or provided by the manufacturer	Measured		Provided	Missing	Irrelevant property measurement.

Specific property measurement protocol	Only indicate if specifically mentioned by authors	Protocol fit for specific property measurement			Protocol unfit for specific property measurement or no protocol cited	Missing property measurement
Specific property measurement protocol modification	Indicate whether modification to the standard protocol were made	No			Yes	Missing property measurement protocol
Specific property measurement Technique[R]	Enter the measurement technique with which a possible property was measured	Measurement Technique fit for specific property measurement			Other	Missing or provided component contribution measurement
Specific property Data Provided	Indicate whether the data is provided in text, a table or a figure	Text, Table			Figure	Missing or provided component contribution measurement

Table 6: Endpoint (Relevant for parameters in Tables V and W of Chapter 3)						
Field	Description	Quality				
		High	Mid	Low	1 (Unreliable)	n/a
Standard protocol used	Indicate whether a standard protocol for the assay has been used	Yes	Modified		No	
Reported interference with assay	Indicate if the nanomaterial is interfering with the assay technique according to the authors	No			Yes	
Good positive nano controls		Yes			No	
Data Provided	Indicate whether the data is provided in text, a table or a figure	Text, Table			Figure	

Table 7: Overall quality criteria					
Description	Quality				
	High	Mid	Low	1 (Unreliable)	n/a

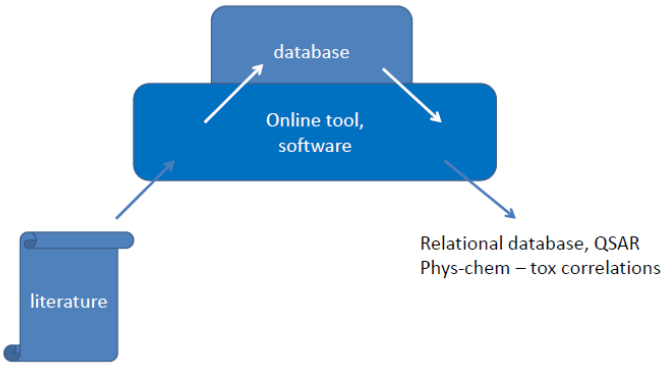
Comparison of the exposure medium to the medium in which physical chemical characterization is carried out	Same for measured characteristics			Different for measured characteristics	No characterization measurements
Comparison of the preparation protocol for toxicity measurement and physical chemical	Same for measured characteristics			Different for measured characteristics	No characterization measurements
Characterization before and after exposure	Yes			No	

5. NANoREG WP6 literature Database literature extraction process

For the development of the Inventory of impact of phys chem properties on (eco)toxicological endpoints data needs to be collected. This data is gathered from peer-reviewed public literature. Before entry of the data into the database can take place the literature from which the data can be extracted needs to be selected and prioritized. The process of prioritization of publications is described in Chapter 5.2. of this document. After literature prioritization, data-entry takes place (the actual entry of relevant information from peer-reviewed literature into the database). To assure consistent data-entry between users of the tool data-checks can be done (see *figure 5* for an overview of the consequetive steps leading from a selected papers to the checked information in the database).

This chapter describes the methodology behind the literature search, exclusion, prioritization and the data-entry of data captured in peer reviewed literature. Chapter 8 explains how to use the web-based tool to prioritize, exclude and enter experimental data from peer reviewed literature into the database (see figure 5) these issues are not described in this chapter. In chapter 5.1 the methodology behind the literature searches is described. In Chapter 5.2 the prioritization criteria and methodology are described.

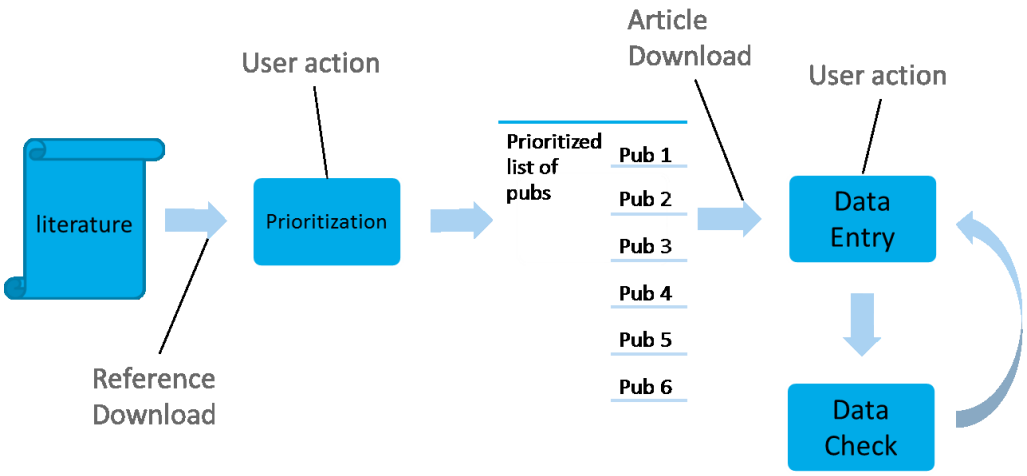
figure 4: Visualisation of the information flow from peer reviewed literature to the database through the web-based tool.



The existing database is a relational database in MySQL. It consists of multiple tables. The way the tool works asks for a specific approach of data-extraction from literature for data-entry. The complicated structure of the database (demonstrated In chapter 2.1 of this guidance) asks for a standardized approach to order data found in public literature. In chapter 7.1 an approach to order the information found in experimental studies in a smart way for database entry is proposed, this is the methodology behind the data-entry that the

tool uses. By using the proposed methodology, data can be entered into the database by use of the tool with maximum efficiency.

figure 5: The reference and abstract of a publication popping up from a literature search is first downloaded into the database. Based on the title and abstract, a prioritization is carried out by users. From the prioritized list of publications the data from most relevant studies are entered into the database first by the users. Finally a data check by a final user can be done.



5.1. Search terms and strategy

Publication searching is carried out in scopus. For papers on toxicity of nanoparticles the following search terms are used:

"(TITLE-ABS-KEY(*nano* *tox*) AND NOT TITLE-ABS-KEY(eco*)) AND DOCTYPE(ar) AND (EXCLUDE(SUBJAREA, "MATE") OR EXCLUDE(SUBJAREA, "CENG") OR EXCLUDE(SUBJAREA, "ENGI") OR EXCLUDE(SUBJAREA, "PHYS")) AND (EXCLUDE(SUBJAREA, "EART")) AND (EXCLUDE(SUBJAREA, "SOCI") OR EXCLUDE(SUBJAREA, "ENER") OR EXCLUDE(SUBJAREA, "MATH") OR EXCLUDE(SUBJAREA, "COMP") OR EXCLUDE(SUBJAREA, "ARTS") OR EXCLUDE(SUBJAREA, "BUSI") OR EXCLUDE(SUBJAREA, "PSYC") OR EXCLUDE(SUBJAREA, "DECI") OR EXCLUDE(SUBJAREA, "ECON") OR EXCLUDE(SUBJAREA, "SOCI") OR EXCLUDE(SUBJAREA, "ENER") OR EXCLUDE(SUBJAREA, "MATH") OR EXCLUDE(SUBJAREA, "COMP") OR EXCLUDE(SUBJAREA, "ARTS") OR EXCLUDE(SUBJAREA, "BUSI") OR EXCLUDE(SUBJAREA, "PSYC") OR EXCLUDE(SUBJAREA, "DECI") OR EXCLUDE(SUBJAREA, "ECON")) AND (LIMIT-TO(LANGUAGE, "English")) AND (LIMIT-TO(PUBYEAR, "PUBYEAR"))

For ecotoxicological papers this is:

TITLE-ABS-KEY(*nano*) AND TITLE-ABS-KEY(*eco*) AND TITLE-ABS-KEY(*tox*)) AND DOCTYPE(ar) AND (LIMIT-TO(PUBYEAR, "PUBYEAR"))

The results of each search (per publication year) or stored in a .bibtex file including "all available information". This files can uploaded in the NANoREG WP6 literature database in specific queries. After uploading of the .bibtex file the database can extract the reference information and abstract from the .bibtex file and store it in the database.

5.2. Prioritization Criteria

After the literature is collected, it is further selected and prioritized according to the following criteria by reviewing the complete reference and if necessary abstract in the database. Some of these criteria are already taken into account in the search term (in search) some are

automatically checked by the software (automatic) and some need to be assessed by the person doing the prioritization (manual).

Duplicate articles will be automatically flagged in the database using the D.O.I. (automatic)

The article should be published in English (in search)

The article should be the primary source of data (manual)

The article should be presented as a full article (in search)

The title and abstract are checked for relevance for inclusion based on the following criteria (manual)

- Is the paper regarding a nanomaterial of interest (Table 8).

  In this field the substance that is investigated in the study is checked. As the question on "the nanomaterial of interest" states, the relevance of the substance is assessed here. Exclusion criteria in this field should only indicate whether the substance is relevant as a nanomaterial of interest for the database.

- Does the paper contain information relevant for biological activity. For this category multiple options can and should be selected. The question gives the opportunity to either asses the priority of the paper based on the amount and type of biological activities described in the paper or excluded the paper if there are no relevant biological interactions described. The prioritization occurs through counting of the amount of relevant topics. See Table 9 for Studies that are considered relevant and Table 10 for Studies that are not considered relevant.

⚠ Important to note is that during the prioritization process more reasons for inclusion and exclusion can be added to the lists. A guidance on how to do this is found in Chapter 8.3.4. These additions will be stored in the database ontology as new markers for prioritization. So only if they are useful as a totally new category, they should be used.

Finally studies that contain a good physical chemical characterization are assessed with higher priority (manual)

The articles that are not excluded based on the above criteria should be prioritized

6. First according to the number of relevant topics, and good characterization
7. Secondly on the date of publication, most recent publications first.

Table 8: A study can be excluded based on the lack of relevant nanomaterials for the following reasons.	
The study concerns a material in which nanoparticles are embedded.	These studies are excluded. Links between physical chemical characteristic of the nanoparticle and biological interactions for embedded nanoparticles are not relevant for the database.

The study concerns a mixture of nanoparticles	From a mixture of nanoparticles it is not possible to link the properties of a specific nanoparticles to the biological activity. Therefor these studies are not relevant for our database.
The study concerns a mixture of nanoparticles with toxic chemicals/solvents	From a mixture of nanoparticles with toxic chemicals it is not possible to link the properties of a specific nanoparticles to the biological activity therefore these studies are not relevant for our database.
The study concern process generated or accidentally released nanoparticles	The database focusses on engineered nanoparticles (interntionally developed or produced nanoparticles. Process generated and accidentally released nanoparticles are too diverse in their properties and composition. Process generated nanoparticles are particle that are generated unintentionally during specific processes and are not uniform in their characteristics. Of these particles it is not possible or very hard to link their phys/chem properties to biological interaction therefor hey will be excluded from the database.
The study concerns no nanoparticles	There is no nanoparticles mentioned in the abstract therefore the paper is not relevant for our database.
The Study is on the efficacy of a medicine with a nanomaterial carrier	The nanomaterial is used to enhance the properties or improve the toxicity profile of another substance. The investigated biological interactions in these studies focus more on the relation between biological effect due to the “other substance” rather than the nanomaterial which is merely used as a carrier.

Table 9: Studies are considered relevant for biological activity when one of the following topics is addressed.	
Interactions with biological matrices or matrix components	The binding of biological matrix components (like surfactants, proteins, lipids etc) in lung surfactant, blood and protein solution etc, on the nanoparticle surface thereby changing the particle characteristics or toxicity.
Interaction with environmental matrices of matrix components	The binding of matrix components (like DOC, surfactants, proteins, lipids etc) in environmental matrices like surface water, seawater, or soil on the nanoparticle surface thereby changing the particle characteristics or toxicity. The studies included studies on Environmental fate.
Nanoparticle aging	The change of nanoparticle characteristics in environmental matrices over time.
Uptake processes	Species, systemic, tissue or cellular uptake processes.
Bioavailability	The uptake of nanoparticles in exobiological compartments (like organisms) compared to the surrounding concentration
Translocation	Studies on the translocation of nanoparticles from the site of entry in an organism (like the lungs) to other sites like organs

Toxicity	Studies containing toxicity data
Eco toxicity	Studies containing eco-toxicity data
Mode of action	Studies on the bio mechanism leading to nanoparticle toxicity.

Table 10: Studies are considered not relevant for biological activity due to one of the following reasons	
It's a study on the efficacy of nano medicines	In these the pharmacological properties of nanoparticles are tuned for maximum efficacy. If studies contain only information the efficacy of nano medicines without taking useful biological activity into account
It's a study on the functionality of nanoparticles	The studies only reports on the functionality/ or application of nanoparticles (like catalysis, structural strengthening, UV absorption, antibacterial properties, environmental remediation, etc. etc.) or how to improve or understand these processes
It's a study on the development of assays	If the studies only reports on the validation of test assays or the development of new assays without providing useful biological activity information.
It's a study on exposure characterization	A study on the characterization of nanoparticle exposure like exposure on the workplace, accidental release characterization etc.
It's a study on the production of nanoparticles	A study on new production or synthesis methods etc.
Conceptual	A study on concepts or theories regarding nanomaterial interactions or risk assessment of nanomaterials. Experimental data in these studies lacks in general.
Characterization method	Studies on the characterization methods of nanoparticles in general data on biological interactions lacks in these studies.

7.1. Guidance on Data-entry

For guidance on the meaning of fields and the ontology during data-entry refer to chapter 2.2 **Database Ontology**. The field in the database are explained in the tables in this chapter.

The software tool that is developed for entry of experimental data into the database is a software tool that is constantly under development. Moreover the tool has been built with limited resources and even though from a scientific point of view the tool is sufficiently detailed, it is not very user friendly. Nevertheless the tool works using a specific methodology for data extraction. This methodology is completely in compliance with the type of information that should be extracted from the studies (as explained in chapter 2**Error! Reference source not found.**). For this reason it is smart to think about the structuring of experimental data according to this methodology in any given study in order to be able to enter data efficiently into the database.

The methodology asks for the addition of a particle to a given study first. Therefore it is recommended to start making a list of all particles that are investigated in any experimental study. Then the data that are available regarding this particle should be ordered. For every

type of data the medium and the protocol should be defined (unless data is provided just like that in which case they should be entered as “provided”). From the materials and methods and results in any study the preparation method of particles dispersions, and the relevant medium in which the particles were dispersed should be described. (for many characterization methods like TEM and SEM the dispersion medium is dried or removed, still the medium in which the particle was dispersed before sample preparation should be added as the relevant medium). For use in the tool it is easy to add different measurements to a specific combination of a medium and protocol so it makes sense to put the tests done under these condition together. In Table 11 the conceptual idea for the structuring of the data is provided. In Appendix C an example is worked out. It is also recommended to write down the most important details on the particle, the preparation protocol, the medium type of tests straight away.

Table 11: Conceptual table for the ordering of experimental data in any give study.

Particles	Preparation Protocol	Medium	Tests
Particle 1	Provided	Provided	Phys/chem test 1
	Protocol 1	Medium 1	Phys/chem test 1 Phys/chem test 2 Phys/chem test 3
	Protocol 2	Medium 2	Phys/chem test 1 Phys/chem test 1 Phys/chem test 1 Tox 1 Tox 2 Tox 3
	Protocol 3	Medium 3	Ecotox 1
Particle 2	Protocol 1	Medium 2	Phys/chem test 1 Phys/chem test 2 Phys/chem test 3
	Protocol 3	Medium 3	Tox 1 Tox 2 Tox 3
Etc	Etc	Etc	Etc

8. Use of the Software

The NANoREG database website is used to prioritize and enter data from articles into the database.

The website can be accessed using the following link:

https://diamonds.tno.nl/nanoreg/

figure 6: The NANoREG WP6 literature Entry Tool Homescreen

To log in, enter your personal username and password at the top of the screen, accept the terms and conditions by ticking the "I accept the terms and conditions" tick box. and press log-in. The screen in should now be visible. There are two small button on the left of this screen and five main buttons in this screen. The two button on the left are the Home screen specific menu, this contains the "Start" button and the "contact" button (which will be give you contact details for issues with the database). The four main buttons on the top are: on the left, the "Personal review status" 2nd from the top left, the "Prioritization Publications" button, 2nd on the right the "Data Entry Particles" button and on the right, the "Browse the NANoREG WP6 literature database" button. These four functions of the entry tool will be explained in the following chapters. Below there is a "Manage the NANoREG WP6 literature database" link, which can only be used by administrators.

figure 7: The Menu which is available in any screen, except the home screen

8.1. The Menu

On the left of the screen in any screen but the home screen you find the menu that is always available (see figure 7). This menu starts with the "start" button which will take you back to the home screen. Next are the "Status", "Prioritization", "Data Entry", "Ontology button", "Query" and the "Help" buttons. In any screen this menu is available (except the home screen) and this

allows you to navigate between the various functions of the entry tool. The “Ontology” button will only will give you information on the ontology without further functions and the “Help” button the contact details to people that can help you with publication reviewing or the software. These functions will not be further explained. The other functions will be explained in the following chapters.

8.2. Personal Review Status

The personal review status screen shows the overview of what has been done and what is going on in the database. Secondly in this screen users can claim papers for data-entry, decline papers for data-entry and download reference information of claimed papers.

8.2.1. Information in this screen

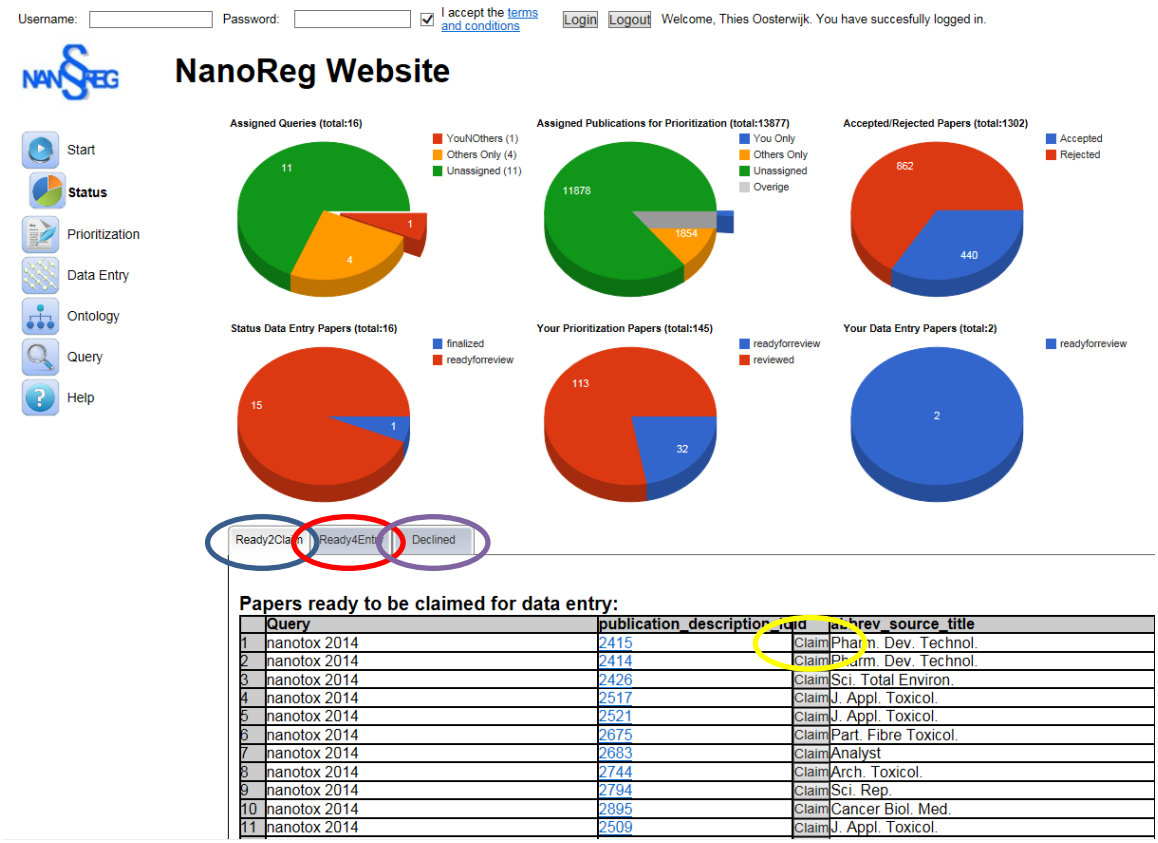
In this screen there are 6 circle diagrams displaying different types of information (see *figure 8*).

1. On the top left there is an overview of the different files (queries) containing reference information and to how many you or all other users are assigned to work on the prioritization of these studies.
2. On top in the middle the diagram displays the total amount of references in the database, how many of these are not assigned to any user, how many are assigned to all users but you, and how many of these studies are assigned to you for prioritization.
3. On the top right there is an overview of how many studies have been prioritized in total, how many are rejected and how many are accepted for data-entry.
4. On the bottom left the diagram displays how many papers are currently being entered into the database and how many are finalized.
5. On the bottom in the middle an overview of the papers that have been assigned to you for prioritization are displayed. In red it shows how many you have prioritized and in blue how many there are currently available for prioritization for you.
6. On the bottom on the right the total amount of papers that you are currently working on (that you claimed) for data entry are shown. In red the papers that you claimed and have not finalized yet are shown and in blue the total amount of papers that are finalized by you.

8.2.2. Claiming papers for data-entry (The Ready2Claim and Ready4Entry tabs)

In the Ready2Claim tab (blue circle in *figure 8*) within the personal review status screen you can also see the list of papers that has already been prioritized by all users. In this screen you can claim papers for data-entry. By claiming a paper the database will assign the paper to be entered by you and other users cannot claim it anymore. Claiming a papers is done by clicking the “claim” button. This button is displayed in yellow in *figure 8*. When a paper is claimed it moves to the “Ready4Entry” tab (red circle in *figure 8*). In the Ready4entry tab there is a button “download publication information” by clicking this, the reference information in the database is exported to an excel file. This file can be used to search the reference and download the full paper.

figure 8: The personal review status screen. Blue circle: the "Ready2Claim" tab, Red Circle: the "Ready4Entry tab", Purple Circle: The "Declined" tab, Yellow circle: the "claim" button.



8.2.3. The declined tab

Under the "declined" tab (purple circle in *figure 8*) all the papers are displayed that you have claimed for data-entry and then declined again. These will be available for all other users in the Ready2Claim tab again but not for you. How to decline papers is explained in chapter 8.4.

8.3. Prioritization Publication

For the prioritization of publications the "prioritization publications" button should be clicked on ones. As soon as you do this the first paper that is ready for review will pop-up on the screen the screen should look as in figure 9.

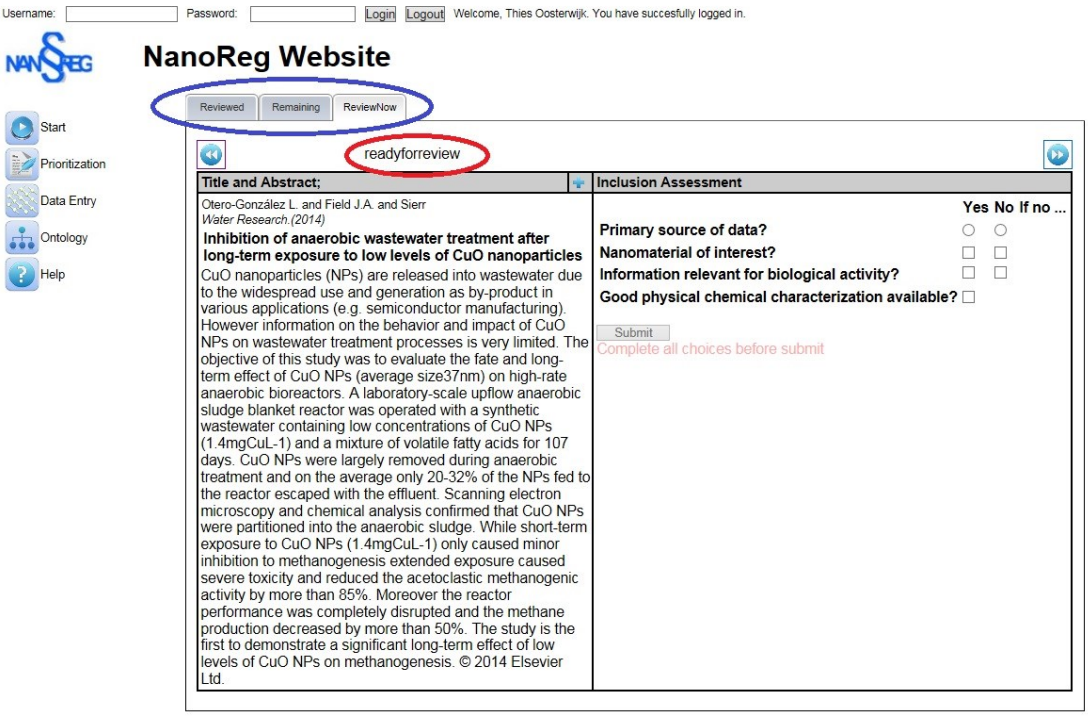
There is some information in this screen and some options.

The text in the red circle in the figure 9 below indicates whether the current publication is ready for review or already reviewed by indicating "readyforreview" or "reviewed" respectively. When no publications have been assigned to you for review yet the current "ReviewNow" screen will be blank.

The arrows on the top left and the top right of the publication box allow you to scroll between publications that have been assigned to you.

On the top of the screen (blue circle) there are 3 tabs. The "Reviewed" tab will show you the publications that have been assigned to you and that are already reviewed. The "Remaining" tab will show you the publications that have been assigned to you and that have not been reviewed yet. The "ReviewNow" tab will allow you to review a publication.

figure 9: The Prioritization screen. In the blue circle: The "Reviewed", "Remaining" and "ReviewNow" tabs, In the red circle: The status of the reference is displayed.



8.3.1. The Reviewed tab

Under the "Reviewed" tab the reference that have already been reviewed for inclusion are displayed. When clicking on a specific reference number, the reference is showed. The inputs of the assessment are showed in the "ReviewNow" tab and you have a choice to overwrite these entries by clicked the overwrite button. In this screen only the reference that you have reviewed yourself are showed.

8.3.2. The Remaining tab

In this tab the references that have been assigned to you are displayed. When clicking on a specific reference number the "ReviewNow" tab for this reference will pop-up.

8.3.3. The ReviewNow tab

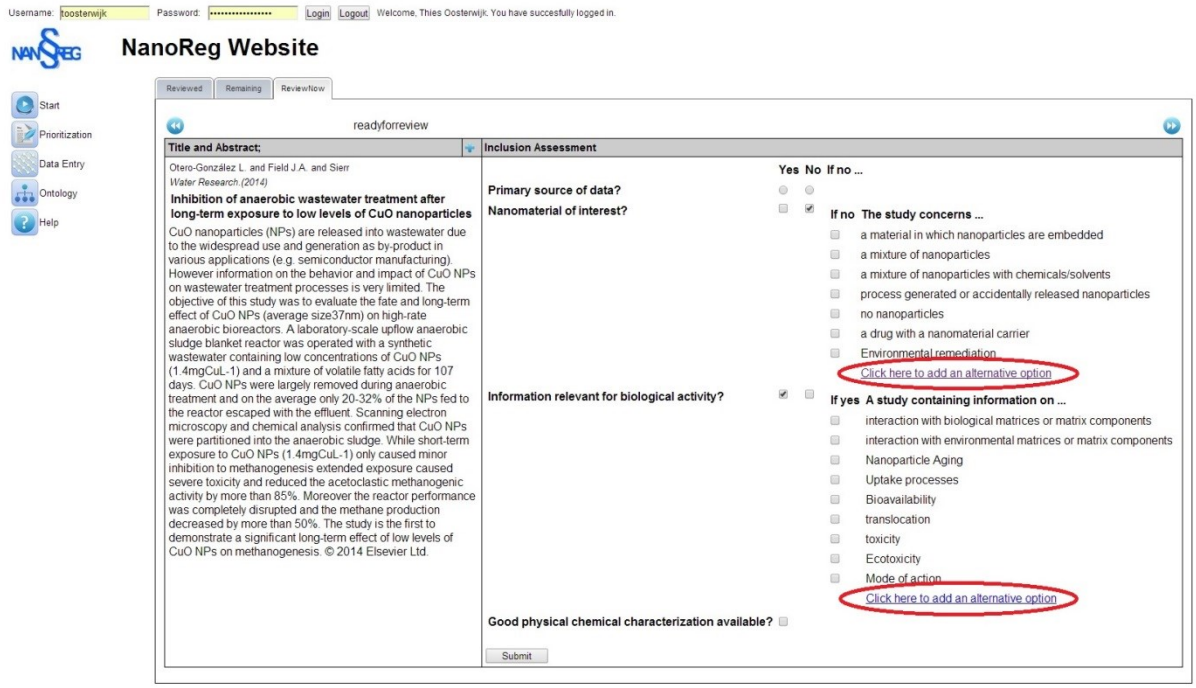
In the ReviewNow tab specific reference that have been assigned to the user are displayed. On the left of the publication box the Title and Abstract are displayed, on the right specific questions for the determination of the relevance of the reference for our database are displayed in the "Inclusion assessment" part. The review process consists of answering every question in the "Inclussion Assessment" part. Answering the questions can be done using the tick boxes. After ticking a box in every category the "submit" button should pop up. When you are confident the reference has been assessed properly the submit button can be clicked and the assessment of the reference will be saved.

After submitting an assessment the specific publication will be flagged as "reviewed" in the underlying database and will become visible in the "Reviewed" tab (blue circle in figure 9) instead of the "Remaining" tab. Also the text in the red circle in figure 9 will changed from "readyforreview" to "reviewed".

8.3.4. Adding Alternative options

During the review process it is possible that the possible terminology and selections for exclusion criteria or relevance as described by Table 8, Table 9 and Table 10 do not suffice. In this case there is the possibility to “add and alternative option” (figure 10).

figure 10: Showing the “add alternative option” in red circles in the ReviewNow Tab.



⚠ Addition to the options will be stored in the database as new markers for prioritization. So only if they are useful as a totally new category, they should be used. In most if not all existing categories should be used.

When using the “add alternative option” link you are linked to the “Add/Edit Ontology Term” screen. Editting an existing ontology term should never be done unless it is the term that you just created yourself (before submitting an paper containing the term). To add a new term (alternative option) change the text in the “short field” and replace italic text in the following term stating replacetextafterminus “no-*replacetextafterminus” or “*yes-*replacetextafterminus”.* If the term you would like to add is “chemical” (which should not be done) then the text in the “short” field should be “no-chemical” or “yes-chemical” depending on to which table the term should be added. When hovering over any question mark in the tables an explanation is also given. After submitting the ontology term it will be added to the option in the prioritization screen (if the format is correct). The term will be available for selection from now on.

8.4. Data Entry

In this chapter not all fields during data entry will be described, these are explained in chapter 2.2. Instead, navigation through the data-entry screen and different functions will be explained.

When opening the data-entry screen in the menu or via the home screen, the “info” tab of the data-entry screen will be shown (see *figure 11*). The info screen allows for the entry of particle information parameters (see Chapter 2.2, tables A and B for more information on these parameters).

The other tabs available in this screen are the “Overview”, “ParticleMeasurement”, the “(Eco)toxicity” and the “Reference” tabs. These as well as the particle info tab will be discussed in this chapter.

8.4.1. The “Select”, “Decline”, and “Finalize”, publication buttons.

Before any data-entry can be done, the publication should be selected. In the “select publication” field, all the publication that you claimed (see chapter 8.2.2 for more info) can be selected. The publication that you intend to work on should be selected here (blue circle in *figure 11*) and the “select” (red circle in *figure 11*) button should be clicked. Next to the select button you find the “reject” button (red circle in *figure 11*). The “reject” button can be used to reject a papers for data-entry, either when you claimed it accidentally or when the papers does not fit you expertise enough to be able to do the data-entry. See chapter 8.2.3 to read more about declining a publication. When a publication is completely finished and ready for data-checking the “finalize” button can be clicked (red circle in *figure 11*). The paper will be stored in the database and is not available to edit or work on anymore. The “Select”, “Decline”, and “Finalize”, publication buttons are always visible during data-entry. In this way you can always check if you are working on the right publication during data-entry.

8.4.2. The “Select”, and “Edit” particle buttons.

The second thing that needs to be selected before any data-entry can be done is a specific nanoparticle. Multiple nanoparticle can be added to one publication, but when doing any data-entry, a specific particle should always be selected. If no particles are assigned to the publication yet, there is only the option to select “Addnewparticle”. By selecting the “addnewparticle” and clicking the “select” button (green circle in *figure 11*) you can add new particle information parameters (described in chapter 2.2, tables A and B) to the database and thereby creating a new particle. Before doing this, always make sure you select “addnewparticle”. To store the information on a new particle, the submit button at the bottom of the page (purple circle in *figure 11*) should be clicked. When the new particle has been added to the database (by clicking “submit”), the particle can be selected in the select particle box. By clicking “Edit” (green circle in *figure 11*) the particle information parameters can be changed. After changing the parameters the “submit” button should be clicked again to store the information in the database. As well as the “Select”, “Decline”, and “Finalize”, publication buttons the “Select” and “Edit” particle buttons are also always visible during data entry. In this way you can always check if you are working on the right particle during data-entry.

figure 11

8.5. Queries

Appendix A

Ordering of experimental data

The software tool that is developed for entry of experimental data into the database is a software tool that is constantly under development. Although from a scientific point of view the tool is sufficiently detailed, it is not yet very user friendly. For this reason it is smart to think about the structuring of experimental data in any given study according to a certain scheme in order to be able to enter data efficiently into the database.

The tool ask for the addition of a particle to a given study first. Therefore it is recommended to start making a list of all particles that are investigated in any experimental study. Then the data that are available regarding this particle should be ordered. For every type of data the medium and the protocol should be defined (unless data is provided just like that in which case they should be entered as "provided"). From the materials and methods and results in any study the preparation method of particles dispersions, and the relevant medium in which the particles were dispersed should be described. (for many characterization methods like TEM and SEM the dispersion medium is dried or removed, still the medium in which the particle was dispersed before sample preparation should be added as the relevant medium). For use in the tool it is easy to add different measurements to a specific combination of a medium and protocol so it makes sence to put the tests done under these condition together. In *Table 11* below the conceptual idea for the structuring of the data is provided. In Table 2 an example is worked out. It is also recommended to write down the most important details on the particle, the preparation protocol, the medium type of tests straight away.

Table 12: Example of overview of experimental conditions for 2 particles (out of 9) from an experimental study to facilitate proper data-entry. Data extracted from: Hamilton Jr., R.F., Wu, Z., Mitra, S., Shaw, P.K., Holian, A. Effect of MWCNT size, carboxylation, and purification on in vitro and in vivo toxicity, inflammation and lung pathology (2013) Particle and Fibre Toxicology, 10 (1), art. no. 57.

Particles	Preparation Protocol	Medium	Tests
MWCNT N/S-O Manufacturer: Cheapptubes inc. Elemental composition measured	Provided	Provided	Size 1: 10-20 nm Size 2: 0.5-2 µm
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Milli-Q water	SEM (shape size)
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Milli-Q water 25°C	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Unknown	Surface area
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	RPMI media	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max	5% infasurf/saline	Size, Zeta-potential

	power, 5mg/ml		
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml 5.mixing, pipette action to 25ug/ml	RPMI media, 10% BSA 50 uM beta-mercapto ethanol, 1mM Na pyruvate 250 ng/ml amphotericin B, 100 U/ml penicillin and streptymicin, 5nM phorbol 12-myristate 13-acetate, 10ng/ml LPS, 37°C	THP-1 cells, human monocytic cell line MTS IL-1ß IL-6 ELISA TNF-α ELISA
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml 5.mixing, pipette action to 25ug/ml	RPMI media, 10% BSA 0.05 mM 2-mercapto ethanol, 1mM Na pyruvate antimyocitic/antibiotic coctail, 20ng/ml LPS, 37°C	Mice alveolar macrophages, C57BL/6 mice MTS IL-1ß
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Suspension culture	Mice alveolar macrophages C57BL/6 mice TEM imaging
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	5% Infasurf/ saline	Mice oropharyngeal aspiration Mouse lung lavage cells IL-1ß IL-6 ELISA TNF-α ELISA Lung Histology Lung particle burden
MWCNT N/S-F Manufacturer: Cheapptubes inc. Elemental composition measured	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Milli-Q water	SEM (shape size)
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Milli-Q water 25°C	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	RPMI media	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	5% infasurf/saline	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	RPMI media	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	5% infasurf/saline	Size, Zeta-potential
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml 5.mixing, pipette action to 25ug/ml	RPMI media, 10% BSA 50 uM beta-mercapto ethanol, 1mM Na pyruvate 250 ng/ml amphotericin B, 100 U/ml penicillin and streptymicin, 5nM phorbol 12-myristate 13-acetate, 10ng/ml LPS, 37°C	THP-1 cells, human monocytic cell line MTS IL-1ß IL-6 ELISA TNF-α ELISA
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml 5.mixing, pipette action to 25ug/ml	RPMI media, 10% BSA 0.05 mM 2-mercapto ethanol, 1mM Na pyruvate antimyocitic/antibiotic coctail, 20ng/ml LPS, 37°C	Mice alveolar macrophages, C57BL/6 mice MTS IL-1ß
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	Suspension culture	Mice alveolar macrophages C57BL/6 mice TEM imaging
	1.Heating, degassing 300°C, 3hr 2.Suspension, 5% Infasurf 3.Dilution, sterile saline 4.Sonification. 2 min half max power, 5mg/ml	5% Infasurf/ saline	Mice oropharyngeal aspiration Mouse lung lavage cells IL-1ß IL-6 ELISA

			TNF-α ELISA Lung Histology Lung particle burden

Appendix B

Reference material data-entry for NM-101, -102, -103, -110, -111, -212, -300K, -400 and -401.

NM-101

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-101*	Select Medium	*none*	Select Medium	*none*
manufacturer		Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Titanium Dioxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*TiO2*	Diameter/thickness, size	38	Diameter/thickness, size	6
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
crystallinity_1	*Anatase*	surface_area	320		

		surface_area_unit	m2/g		

NM-102

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-102*	Select Medium	*none*	Select Medium	*none*
manufacturer		Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Titanium Dioxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*TiO2*	Diameter/thickness, size	132	Diameter/thickness, size	20
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
crystallinity_1	*Anatase*	surface_area	90		
		surface_area_unit	m2/g		

NM-103

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-103*	Select Medium	*none*	Select Medium	*none*
manufacturer		Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Titanium Dioxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*TiO2*	Diameter/thickness, size	186	Diameter/thickness, size	20
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
crystallinity_1	*Anatase*	surface_area	60		
		surface_area_unit	m2/g		

NM-110

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-110*	Select Medium	*none*	Select Medium	*none*
manufacturer	*BASF*	Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Zinc Oxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*ZnO*	Diameter/thickness, size	150	Diameter/thickness, size	20
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
		surface_area	13		
		surface_area_unit	m2/g		

NM-111

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-111*	Select Medium	*none*	Select Medium	*none*
manufacturer	*BASF*	Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*coated*	Measurement info:			
Number_components	*2*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Zinc Oxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*ZnO*	Diameter/thickness, size	140	Diameter/thickness, size	34
accesability_1	*Unknown*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
		surface_area	16		
name_2	*triethoxycaprylsilane*	surface_area_unit	m2/g		
place_1	*Coating*				
chemical_type_1	*Organic Compound*				
chemical_1	*triethoxycaprylylsilane*				
accesability_1	*Yes, outer layer*				

NM-212

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-212*	Select Medium	*none*	Select Medium	*none*
manufacturer		Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Cerium (IV) Oxide*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal-Oxide*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*CeO*	Diameter/thickness, size	28	Diameter/thickness, size	33
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*
crystallinity_1	*precipitated*	surface_area	28		
		surface_area_unit	m2/g		

NM-300K

Field	Value	Field	Value	Field	Value
Particle:		ParticleMeasurement		ParticleMeasurement	
Name	*NM-300K*	Select Medium	*none*	Select Medium	*none*
manufacturer	*RAS GmbH*	Select Dispersion Protocol	*none*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*	Select Measurement	*provided 2*
Architecture	*Simple*	Measurement info:			
Number_components	*1*	measurement_type	*provided*	measurement_type	*provided*
Components:		Size and Shape information:		Size and Shape information:	
name_1	*Silver*	Shape	*Sphere*	Shape	*Sphere*
place_1	*Core*	size_measurement	Mean particle size	size_measurement	Mean primary particle size
chemical_type_1	*Metal*	size_measurement_type	Single	size_measurement_type	Single
chemical_1	*Ag*	Diameter/thickness, size	15	Diameter/thickness, size	15
accesability_1	*Yes, outer layer*	Diameter/thickness, size_unit	*nm*	Diameter/thickness, size_unit	*nm*

NM-400

Field	Value	Field	Value
Particle:		ParticleMeasurement	
Name	*NM-400*	Select Medium	*none*
manufacturer	*Nanocyl*	Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*
Architecture	*Simple*	Measurement info:	
Number_components	*1*	measurement_type	*provided*
Components:		Size and Shape information:	
name_1	*MWCNT*	Shape	*Tube*
place_1	*Core*	size_measurement	*Average Size*
chemical_type_1	*Carbon/fullerene*	size_measurement_type	*Single*
chemical_1	*MWCNT*	Diameter/thickness, size	*9.5*
		Diameter/thickness, size_unit	*nm*
accesability_1	*Yes, outer layer*	Length, size	*1.5*
		Length, size_unit	*microm*
		surface_area	*280*
		surface_area_unit	*m2/g*

NM-401

Field	Value	Field	Value
Particle:		ParticleMeasurement	
Name	*NM-401*	Select Medium	*none*
manufacturer		Select Dispersion Protocol	*none*
cas		Select Measurement	*provided 1*
Architecture	*Simple*	Measurement info:	
Number_components	*1*	measurement_type	*provided*
Components:		Size and Shape information:	
name_1	*MWCNT*	Shape	*Tube*
place_1	*Core*	size_measurement	*Average Size*
chemical_type_1	*Carbon/fullerene*	size_measurement_type	*Range*
chemical_1	*MWCNT*	Diameter/thickness, size 1	*10*
accesability_1	*Yes, outer layer*	Diameter/thickness, size 2	*30*
		Diameter/thickness, size_unit	*nm*
		Length, size 1	*5*
		Length, size 2	*15*
		Length, size_unit	*microm*
		size_unit	*nm*
		surface_area	*300*
		surface_area_unit	*m2/g*

Appendix C

Standardized protocols data entry: ENPRA dispersion protocol for NANoREG, NANOGENTOX dispersion protocol for NANoREG, Prospect dispersion protocol for NANoREG, and the standard operating procedure for the preparation of aqueous dispersion of carbon nanotubes (CNTs).

All prefilled dispersion protocols should be edited to reflect correct nanoparticle amount/ concentrations etc.

If a standard given standard dispersion protocol precedes a toxicity assay in which nanoparticles are re-dispersed in another medium. All preceding steps should be included as well. Therefore, to edit the standard dispersion protocol (in the database) is most simple.

ENPRA dispersion protocol for NANoREG (Example for NM-300K)

dispersion_name	*The ENPRA dispersion protocol for NANoREG for NM-300K*
dispersion_citation	*The ENPRA dispersion protocol for NANoREG for NM-300K, v1.0, 2014JUL11*
step-name_ 1	*Pipetting*
step_order_1	*1*
step_time_1	
step_time_unit_1	
step_medium_1	*NM-300K material medium*
step_amount_np_1	*0.1*
step_amount_np_unit_1	*g/ml*
step_description_1	*Pipetting from the transport vial medium*
step-name_ 2	*Mixing*
step_order_2	*2*
step_time_2	
step_time_unit_2	
step_medium_2	*MilliQ Water-98.0;Bovine Serum-2.0;;Celsius*
step_amount_np_2	*2.56*
step_amount_np_unit_2	*mg/ml*
step_description_2	*Mixing with dispersion medium*
step-name_ 3	*Sonication*
step_order_3	*3*
step_parameter_3	*7.35*
step_ parameter_unit_3	*Watt*
step_ instrument_calibrated_3	*yes*
step_time_3	*16*
step_time_unit_3	*minutes*
step_medium_3	*MilliQ Water-98.0;Bovine Serum-2.0;;Celsius*
step_amount_np_3	*2.56*
step_amount_np_unit_3	*mg/ml*
step_description_3	*Sonication in ice-water*

NANOGENTOX dispersion protocol for NANoREG

dispersion_name	*The NANOGENTOX dispersion protocol for NANoREG*
dispersion_citation	*The NANOGENTOX dispersion protocol for NANoREG, v1.0, 2014JUL11*
step-name_ 1	*Prewetting*
step_order_1	*1*
step_time_1	*1*
step_time_unit_1	*minutes*
step_medium_1	*ethanol*
step_description_1	*Pre-wetting with ethanol*
step-name_ 2	*Prewetting*
step_order_2	*2*
step_medium_2	*MilliQ Water-99.5;BSA-0.05;;Celsius*
step_description_2	*Pre-wetting with 99.5 vol% sterile filtered BSA-water (0.05% w/v)*
step-name_ 3	*Incubation*
step_order_3	*3*
step_parameter_3	*0*
step_ parameter_unit_3	*Celcius*
step_time_3	*5*
step_time_unit_3	*minutes*
step_medium_3	*MilliQ Water-99.5;BSA-0.05;;Celsius*
step_amount_np_3	*2.56*
step_amount_np_unit_3	*mg/ml*
step_description_3	*Incubation on ice*
step-name_ 4	*sonication*
step_order_4	*4*
step_parameter_4	*7.35*
step_ parameter_unit_4	*Watt*
step_ instrument_calibrated_4	*yes*
step_time_4	*16*
step_time_unit_4	*minutes*
step_medium_4	*MilliQ Water-99.5;BSA-0.05;;Celsius*
step_amount_np_2	*2.56*
step_amount_np_unit_2	*mg/ml*
step_description_4	*Sonication*

Prospect dispersion protocol

dispersion_name	*Prospect dispersion protocol*
dispersion_citation	*Prospect dispersion protocol 2010MAY18*
step-name_ 1	*Prewetting*
step_order_1	*1*
step_medium_1	*MilliQ Water-100;;Celsius*
step_description_1	*As recommended by guidelines BS ISO 14887 (2000) ["Sample Preparation – dispersing procedures for powders in liquids"]*
step-name_ 2	*Mixing*
step_order_2	*2*
step_medium_2	*MilliQ Water-100;;Celsius*
step_amount_np_2	*1.0*
step_amount_np_unit_2	*mg/ml*
step_description_2	*Gentle mixing with spatula*
step-name_ 3	*sonication*
step_order_3	*3*
step_parameter_3	*90*
step_ parameter_unit_3	*% Amplitude*
step_ instrument_calibrated_3	*no*
step_time_3	*20*
step_time_unit_3	*seconds*
step_medium_3	*MilliQ Water-100;;Celsius*
step_amount_np_3	*1*
step_amount_np_unit_3	*mg/ml*
step_description_3	*Sonication of the dispersion by*
step-name_ 4	*Mixing*
step_order_4	*2*
step_medium_4	*MilliQ Water-100;;Celsius*
step_description_4	*Gentle mixing to desired concentration*

Standard operating procedure for the preparation of aqueous dispersion of carbon nanotubes (CNTs).

dispersion_name	*SOP, preparation of aqueous dispersion of carbon nanotubes (CNTs).*
dispersion_citation	*SOP, preparation of aqueous dispersion of carbon nanotubes (CNTs), SINTEF 2013*
step-name_ 1	*Mixing*
step_order_1	*1*
step_amount_np_2	*20*
step_amount_np_unit_2	*mg*
volume_medium_1	*50*
volume_medium_unit_1	*ml*
step_description_1	*Adding of aqueous medium to the CNTs*
step-name_ 2	*sonication*
step_order_2	*2*
step_ instrument_calibrated_2	*no*
step_time_2	*15*
step_time_unit_2	*minutes*
step_amount_np_2	*20*
step_amount_np_unit_2	*mg*
volume_medium_2	*50*
volume_medium_unit_2	*ml*
step_description_2	*Sonication of the dispersion*
step-name_ 3	*Mixing*
step_order_3	*3*
step_amount_np_3	*20*
step_amount_np_unit_3	*mg*
volume_medium_3	*100*
volume_medium_unit_3	*ml*
step_description_3	*Adding of aqueous medium to the CNTs*
step-name_ 4	*sonication*
step_order_4	*4*
step_ instrument_calibrated_4	*no*
step_time_4	*15*
step_time_unit_4	*minutes*
step_amount_np_4	*20*
step_amount_np_unit_4	*mg*
volume_medium_4	*100*
volume_medium_unit_4	*ml*
step_description_4	*Sonication of the dispersion*
step-name_ 5	*Mixing*
step_order_5	*5*
step_amount_np_5	*20*
step_amount_np_unit_5	*mg*
volume_medium_5	*150*
volume_medium_unit_5	*ml*
step_description_5	*Adding of aqueous medium to the CNTs*
step-name_ 6	*sonication*

step_order_6	*6*
step_ instrument_calibrated_6	*no*
step_time_6	*15*
step_time_unit_6	*minutes*
step_amount_np_6	*20*
step_amount_np_unit_6	*mg*
volume_medium_6	*150*
volume_medium_unit_6	*ml*
step_description_6	*Sonication of the dispersion*
step-name_ 7	*Mixing*
step_order_7	*7*
step_amount_np_7	*20*
step_amount_np_unit_7	*mg*
volume_medium_7	*200*
volume_medium_unit_7	*ml*
step_description_7	*Adding of aqueous medium to the CNTs*
step-name_ 8	*sonication*
step_order_8	*8*
step_ instrument_calibrated_8	*no*
step_time_8	*15*
step_time_unit_8	*minutes*
step_amount_np_8	*20*
step_amount_np_unit_8	*mg*
volume_medium_8	*200*
volume_medium_unit_8	*ml*
step_description_8	*Sonication of the dispersion*
step-name_ 9	*Mixing*
step_order_9	*9*
step_amount_np_9	*20*
step_amount_np_unit_9	*mg*
volume_medium_9	*10.75*
volume_medium_unit_9	*l*
step_description_9	*Adding of aqueous medium to the CNTs*
step-name_ 9	*Incubation*
step_order_9	*9*
step_amount_np_9	*20*
step_amount_np_unit_9	*mg*
volume_medium_9	*10.75*
volume_medium_unit_9	*l*
step_description_9	*Incubation at room temperature in the dark*