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SAbyNA

H2020-NMBP-15-2020

Simple, robust and cost-effective approaches to guide industry in the development of safer nanomaterials and nano-enabled products

Start date of the project: 01/03/2020

Duration 48 months

WP3 D3.1 Identification and selection of existing resources (models, databases, tools, and methods) for assessing human and environmental hazard for the purposes of SbD.

WP	3	Name of the WP		
Dissemination level[1]	PU		Due delivery date	28/02/2021
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[1] Dissemination level: **PU** = Public, **PP** = Restricted to other programme participants (including the JU), **RE** = Restricted to a group specified by the consortium (including the JU), **CO** = Confidential, only for members of the consortium (including the JU)

[2] Nature of the deliverable: **R** = Report, **P** = Prototype, **D** = Demonstrator, **O** = Other

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Deliverable abstract

The aim of Task 3.1 (T3.1) has been to distil existing data, methods, models and tools, relating to hazard assessment. The resources evaluated have included computational models, databases, risk assessment (RA) tools, and hazard assessment methods, each in respect to both human and environmental exposure. Their relevance in terms of context and their purpose in supporting Safe by Design (SbD) approaches for nanotechnology have been considered during the analysis process.

Following the criteria selection in Milestone 3.1, four data resources have been considered appropriate for use in the continuation of WP3, including eNanoMapper, NanoCommons platform, MESOCOSM database and a non-EU data source Pubvinas, and ten RA tools were identified as being useful in assessing and providing hazard information. In general, these RA tools were found to be lacking when considering their usefulness for SbD approaches, and as such it was decided to extract relevant approaches from these tools to further develop the GUIDEnano tool within SAbyNA. From these tools, a number of important hazard descriptors and hazard predicting parameters have been identified (e.g. solubility, oxidative potential, inflammatory reactions and morphology); a number of standardised methodologies have been collected that can assess these hazard descriptors, and it is these that will provide the base for further development in Task 3.2.

Key considerations for development of hazard approaches in WP3 include the reliability of the thresholds used during RA, and how informative these thresholds may be, how to best use read-across to inform in SbD selection processes, and how robust are the selected test methods when considering the materials relevant for the SAbyNA case studies.

Table of Content

0. Abbreviations

ART	Advanced Reach Tool
ASTM	American Society for Testing and Materials
BAMA	British Aerosol Manufacturers Association
BMD20	Benchmark Dose 20%
BSI	British Standards Institution
CB	Control Banding
CEN	European Committee for Standardization
CLP	Classification, Labelling and Packaging
CMAR	Carcinogenetic, Mutagenic, Asthmagenic, Reproductive
COSHH	Control of Substances Hazardous to Health
DIN	Deutsches Institut für Normung e.V.
DNEL	Derived No-Effect Level
DoW	Description of Work
EA	Economic Assessment
ECHA	European Chemical Agency
EHS	Environment, Health and Safety
ENM	Engineered Nanomaterial
ERA	Ecological Risk Assessment
EU	European Union
FAIR	Findable, Accessible, Interoperable and Reusable
GIT	Gastrointestinal Tract
GSH	Glutathione
HARN	High Aspect Ratio Nanoparticles
HB	Hazard Band
HHRA	Human Health Risk Assessment
IATA	Integrated Approaches to Testing and Assessment
IFA	German Institut für Arbeitsschutz
ISO	International Standardization Organization
LCA	Life Cycle Assessment
LCIA	Life Cycle Impact Assessment
LCL	Lower Confidence Limit
LD50/LC50	Lethal Dose 50%/Lethal Concentration 50%
MCDA	Multi Criteria Decision Analysis
MS	Milestone
NEP	Nano-Enabled Product
NF	Nanoform
NIOSH	The National Institute for Occupational Safety and Health

NM	Nanomaterial
OECD	Organisation for Economic Co-Operation and Development
OEL	Occupational Exposure Limit
PEC	Predicted environmental concentration
PNEC	Predicted No-Effect Concentration
POD	Point-of-Departure
QSAR	Quantitative Structure-Activity Relationship
R&D	Research and Development
RA	Risk Assessment
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REDOX	Reduction-Oxidation
RL	Risk Level
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SbD	Safer by Design
SDS	Safety Data Sheets
SEA	Socio-Economic Assessment
SIA	Social Impact Assessment
SME	Small and Medium Enterprise
SOP	Standard Operation Procedure
SPM	Swiss Precautionary Matrix
SSD	Species-sensitivity distribution
STP	Sewage Treatment Plant
UCL	Upper Confidence Limit
WEL	Workplace Exposure Limit
WHO	World Health Organisation
WP	Work Package

1. Introduction

The first step in the SAbyNA project is to map and distil existing resources and establish their relevance in terms of context and their purpose in supporting Safe by Design (SbD) approaches for nanotechnology. In WP3, these resources relate to hazard assessment and will include computational models, databases, risk assessment (RA) tools, and hazard assessment methods.

2. Description of the tasks

This deliverable aims to identify and distil the key elements from available models, databases, hazard testing methods RA tools which can predict human health and environmental hazard and would be useful for a SbD strategy at an early stage in the innovation process.

General tasks:

- **Identify and extract data-sources and tools** available, allowing a database-to-tool landscaping and mapping exercise to help distil key elements, and identifying key connections and workflows between the **tools and Nano-EHS data**;
- Highlight **gaps or barriers to** their usability or performance, the pros and cons of their use and potential for improvement;
- **Assess existing experimental testing methods** for their level of **standardization**, existing evidence on their **predictive value**, availability of **inter-laboratory comparisons**, known **benchmark materials**, and potential for calibrated **decision thresholds**;
- Provide **guidance on a FAIR (Findable, Accessible, Interoperable and Reusable) approach** to data curation.

3. Identification and extraction of key data-sources and tools

3.1 Risk Assessment Models and Tools

3.1.1 Introduction to RA tools and their relevance for SbD

Different nanospecific tools have been developed in the last years for hazard and risk assessment of nanomaterials. These include control banding and risk screening tools that may give qualitative or semi quantitative results and other high tier tools, such as SUNDS or GUIDEnano, which may give quantitative results.[4]

Control banding tools are used to estimate the hazard and exposure potential in work settings in order to determine the level of precaution needed and determine the management measures that should be applied. Examples are CB Nanotool, Nanosafer CB, Stoffenmanager Nano. These tools are simple to use, need few input parameters and give bands of hazards and exposure as outputs (Figure 1).

[4] D. Hristozov, S. Gottardo, E. Semenzin, A. Oomen, P. Bos, W. Peijnenburg, M. van Tongeren, B. Nowack, N. Hunt, A. Brunelli, J. J. Scott-Fordsmand, L. Tran, A. Marcomini," Frameworks and tools for risk assessment of manufactured nanomaterials" Environment International, Volume 95, 2016, Pages 36-53. https://doi.org/10.1016/j.envint.2016.07.016.

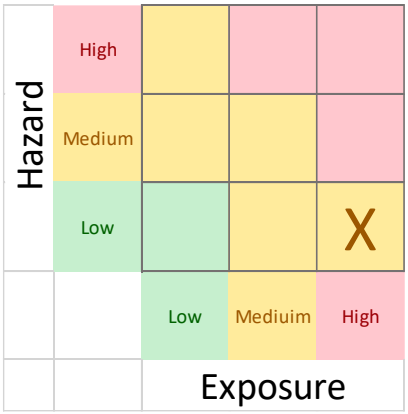

Figure 1 Example of output of a control banding tool

Risk screening tools. Risk screening tools use a similar approach (i.e. easy to use, low data requirements), but are also applicable for consumer and environmental RA. They can cover more than one lifecycle stages (e.g. occupational scenarios in both the production and end-of-life stages, and environmental assessments). An example is the Swiss Precautionary Matrix (SPM) that estimates effects and exposure in workplace, consumer and environmental settings giving as output a precautionary need index. It can also assess uncertainties resulting from knowledge gaps (Figure 2).

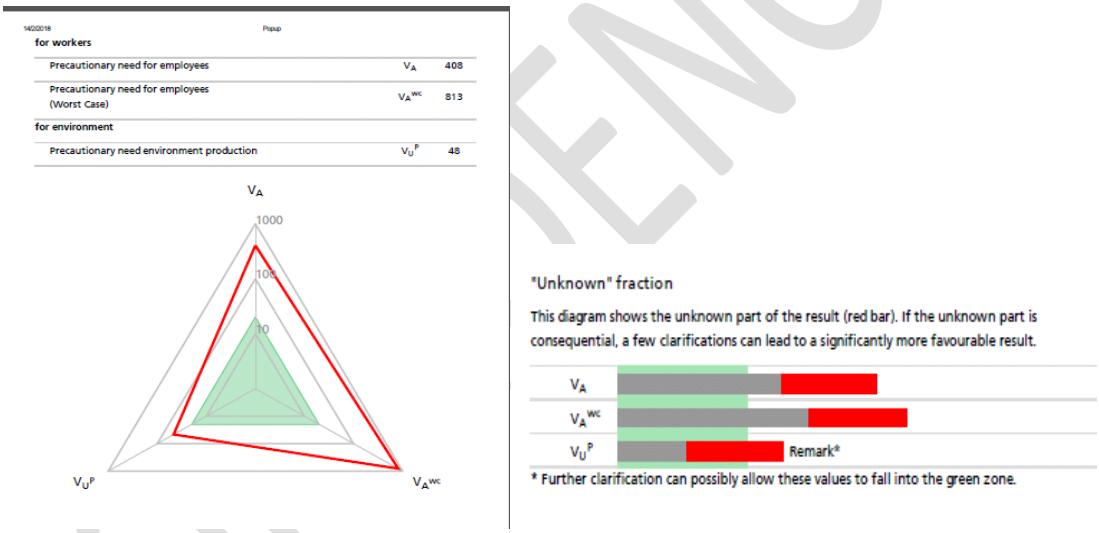

Figure 2 Output of the SPM. Left precautionary need. Right evaluation of uncertainties due to lack of knowledge.

A more complex risk screening tool is LICARA nanoSCAN that has several modules for different aspects including risk benefit, environmental assessment, simplified life cycle assessment (LCA), and human hazard. LICARA nanoSCAN includes several tools for the assessments and gives a comparison between the nanomaterial and the product it substitutes.

Control banding and risk screening tools derive the hazard from a small number of physicochemical parameters (e.g. size, coating, aspect ratio, solubility) and few toxicological endpoints (e.g. reactivity). Most of them can provide default values or worst-case scenarios when information is missing or not available. Due to this and to the easiness of use, they can be used in the first stages of the innovation process.

High tier quantitative tools. These tools require a higher level of expertise and require a greater number of input parameters. They have different modules for environment, hazard assessments and exposure and release assessment and may consider other aspects such as the life cycle of the nanomaterials (NMs). Human hazard prediction is based on DNEL (derived no-effect level) provided by the user or derived from (close to) regulatory studies, while environmental hazard prediction is based on a PNEC (predicted no-effect concentration) or similar endpoint concentration, either provided by the user or derived by the tool. The tool gives a quantitative result

e.g. calculating a RCR (Risk Characterisation Ratio, Figure 3 Output of SUNDS.). An example of this type of tool is: SUNDS.[5] Due to the high number and type of input parameters required, these tools will often require higher level of expertise in human or environmental hazard, and hence are often utilised at later stages of the innovation process.

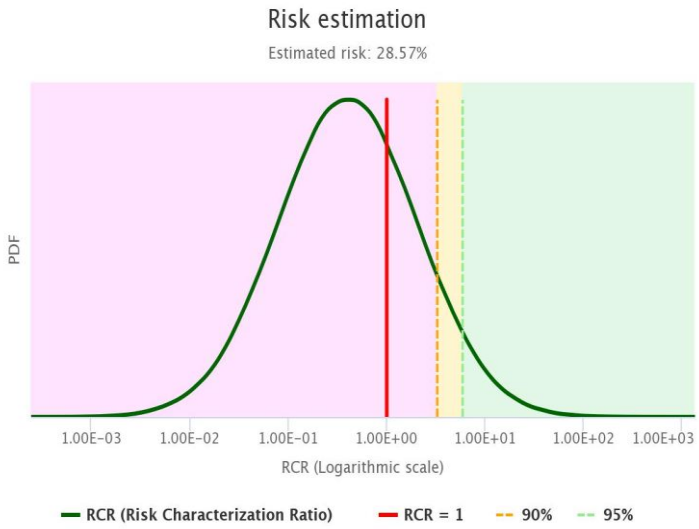

Figure 3 Output of SUNDS.

The form in which risk is predicted and presented by these tools differs dependent on the tool. As stated above, some tools use a DNEL/Point of Departure (PoD) (provided by the user) to perform a risk assessment but do not provide a hazard prediction within the tool.

In order for these RA tools to be useful for implementing SbD, it is key that the property driving the particle toxicity can be identified within the hazard banding assessment. For example, in control banding tools there may be various ways that a particle could be identified as “high” in the hazard band. This could be due to one property which is considered extremely hazardous (e.g. biopersistent fibres based on the HARN paradigm) or it may be a combination of different physicochemical parameters. When developing a SbD approach, these considerations are important as the approach needs to be specific to the hazard driving properties. In the following sections we have extracted what each tool has designated as relevant hazard driving properties, and have assessed these as to whether they are sufficiently addressed, or would require elucidation during the development of the WP3 hazard assessment strategy.

As our aim is to predict hazard, we will first focus on tools that include a prediction of hazard, then a secondary assessment will be utilised to determine usefulness in relation to SbD. In our assessment we have divided tools that include hazard predictions into two broad categories: control banding tools (or low tier assessment) and quantitative tools (or high tier assessment). A divergence in terminology and output strategies should be noted here, control banding is specific to occupational RA, and is not so relevant for environmental RA; this divergence will require clarification during the course of developing the hazard assessment strategy, if these disciplines are required to be reported similarly low tier assessment and high tier assessment may instead be used.

Within the two categories there can be different input-output flows depending on the tools:

- Control banding/ low tier assessment: output is a hazard category
    - Input can be simple: from basic physicochemical information a hazard banding is derived
    - Input can be more complex: experimental testing is needed to derive hazard bands (e.g. dissolution, reactivity, *in vitro* assessment)
- Quantitative/ high tier assessment: output is a DNEL/PNEC

[5] Fadeel, B., Farcal, L., Hardy, B. *et al.* Advanced tools for the safety assessment of nanomaterials. *Nature Nanotech* **13,** 537–543 (2018). https://doi.org/10.1038/s41565-018-0185-0

- Input can be simple: use grouping and read across to derive a DNEL/PNEC
- Input can be more complex: (close to) regulatory studies are needed to derive a DNEL/PNEC

In the next sections various RA tools will be assessed and their relevance and usefulness for SAbyNA will be evaluated.

3.1.2 Distillation of RA tools

For the purposes of WP3, we have assessed various RA tools for their hazard data requirements and use of hazard information; each RA tool also has considerable use and prediction of exposure, these are not considered in any detail here as this is being assessed and reported in WP2 (D2.1).

Initial screening of hazard/RA models and tools

A first screening of the following risk assessment models and tools was performed:

AISE React, ANSES, ART, BAUA SprayExpo 2.3, British Aerosol Manufacturers Association (BAMA) model, CENARIOS, ConsExpo, Consexpo Nano Tool, Consexpo Spray model, Control banding nanotool, DREAM, ECETOC TRA, EGRET2, ENPRA model, Future Nano Needs – Bayesian network (FNN-BNN), GUIDEnano, LICARA nanoSCAN, MEASE, Mend2Nano, One box-Model for accident situations in laboratories, NanoFASE, NanoQSAR, NanoRiskCat, NanoSafer CB, RiskofDerm (exposure model, not toolkit), SB4N, SSWD, Stoffenmanager, Stoffenmanager Nano, Stoffenmanager dermal RA (is based on riskofderm toolkit), SUNDS, Swiss precautionary matrix,[6] Two Box Model.

The first screening assessment included gathering information to determine if, and how, the RA tool assesses hazard; the hazard information required (e.g. DNEL, physicochemical properties, reactivity, CLP classification) was identified as well as how the tool predicts hazard and any rationale behind hazard prediction.

The following tools were excluded as they did not include the use of hazard information for hazard prediction, or are neither hazard nor risk tools: ART, BAUA SprayExpo 2.3, British Aerosol Manufacturers Association (BAMA) model, CENARIOS, ConsExpo, ConsExpo Nano Tool, Consexpo Spray model, DREAM, ENPRA model, Future Nano Needs – Bayesian network (FNN-BNN), Mend2Nano, One box-Model for accident situations in laboratories, NanoFASE, NanoQSAR, RiskofDerm (exposure model, not toolkit), SB4N, Two Box Model.

AISE React, EGRET2, ECETOC-TRA, and MEASE were identified as requiring hazard input but generated no hazard prediction, so were not assessed further.

The original assessment of these RA tools can be viewed here:
https://drive.google.com/drive/folders/1Q0VRsWkocbPsLnAWSeMSf8HW-lLg5vSr?usp=sharing

Based on the criteria generated within MS3.1, the following RA were assessed further in the sections below and in Annex 7.1.

Control banding

- Complex: ANSES, Control banding nanotool, LICARA nanoSCAN, NanoRiskCat, NanoSafer, Swiss precautionary matrix, Stoffenmanager Nano, Stoffenmanager dermal

Quantitative

- Simple: GUIDEnano (option 1 and option 2)
- Complex: GUIDEnano (option 3), SUNDS

[6] SPM is not a risk assessment tool, but a tool to identify key nano-specific properties that require the user to be cautious in their use.

3.2 Usability or performance assessment of distilled risk assessment tools

A critical review of the distilled RA tools is reported here and in subsequent sections, to assess how each may be suitable for SbD approaches, and applicable in the innovation or design stage, on the suitability of data entry requirements and how the hazard decision-making process aligns with experimental outputs (Table 1).

Based on this review, a number of actions have been identified. These include further investigations into the relevance of thresholds identified (see Section 4.2), how read-across (e.g. similar NFs DNELs/OELs or bulk material hazard data) approaches are established into hazard tools, methods for assessing various endpoints (namely reactivity) and available guidance for these methods, guidance on how to fulfil requirements for CLP endpoint assessments *in vitro*, and developing dermal toxicity assessment for NFs. Further discussion on how WP3 will aim to address these actions can be found throughout this deliverable (in particular see Section 5).

Additionally, two main considerations we must address to incorporate hazard assessment in the SAbyNA platform is the ability to identify hazard driving properties within hazard assessment tools and the sensitivity of methods included in our hazard assessment strategy. Generally, the RA tools examined here aim to identify risk based on the hazard associated to a particle, but do not give guidance on how this hazard may be reduced other than indicating that properties such as solubility, particle size or particle shape should be adapted. This is a downfall for all tools considered, as the current level of information given would almost certainly require input from those more experienced in risk reduction to offer advice on how to alter the NF/NEP to reduce the overall hazard score/rating. During the SAbyNA project, WP3 and WP4 will work closely together to address this issue and ensure the identification of hazard driving properties is included in the hazard assessment strategy by relating material properties with technical functions and key hazard outcomes. This work will largely be based on strategies developed in approaches, such as read-across, from projects such as GRACIOUS (see Section 3.5).

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Table 1 Performance assessment of identified RA tools (details are considered in subsequent sections)

Model/Tool	Positives	Limitations	Experimental methods & relevant endpoints	Action required
Control Banding (Complex)/low tier assessment				
ANSES	Specifically for NMs	Expert intervention is required when: 1. There are too many unknown factors concerning the toxicology of the nanomaterial or product. 2. Allocation of a hazard band only considers nanomaterials, whether raw or incorporated in a matrix (liquid or solid), not as emissions from products (as information of emissions from matrix is limited).[7]	• Solubility; • ROS/RNS generation; • Fibre biopersistence; For full hazard assessment – anything that would allow for an identifiable CLP hazard statement – note that these are to be used in early stage innovation tests therefore *in vivo* tests are likely to be limited (if used at all).	No further action required.
CB nanotool	• Specifically for NMs. There is value in using a points system to achieve more sensitivity in ranking – but needs better definition.	The point structure used is questionable, but its place in risk assessment for SbD is thought advantageous.	• Solubility; • ROS/RNS generation; • Characterisation of size and morphology; Assays suitable to assess carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, asthmagenicity – note that these are to be used in early stage innovation tests therefore *in vivo* tests should be limited (if used at all).	Investigate the points structure further and adapt for the needs of the SAbyNA platform.

[7] The applicability of this within SAbyNA WP3 is under discussion and will be considered in the hazard assessment strategy.

Model/Tool	Positives	Limitations	Experimental methods & relevant endpoints	Action required
LICARA nanoSCAN	Nano-specific. Includes a Multi Criteria Decision Analysis (MCDA), allowing a user interpretation of what aspects carry most weight specifically to their particular case. The only control banding tool to include life cycle analysis (LCA). Considers environmental benefits along different life cycle stages. Can be used in assessment of dermal, oral, and inhalation exposure. Simple scoring system attributing equal weights to each underlying question (I.e. not the case for SPM).	Assessment based on other RA tools including SPM and Stoffenmanager-nano. Therefore, similarly to these RA tools, LICARA nanoSCAN is unlikely to distinguish between small difference in NMs and hence would not be suitable for SbD. Currently unavailable online as awaiting re-launch (March 2021).	• Reactivity; • CLP endpoints; • Life cycle impacts of energy and material use, waste generation and treatment and emissions to air and water of pollutants and hazardous substances.	Extract methods to estimate whether the nanoproduct performs better, equal or worse than its conventional alternative regarding environmental benefits.
NanoRiskCat	Provides details of laboratory methodology and considerations, as well as endpoints and cut off values that are critical for key hazard parameters that WP3 should consider. Provides a useful approach for applying existing knowledge of bulk (also possibly analogous) material. It has a database (Nanodatabase) of products that contain NM categorised with the tool.	There is no margin of sensitivity identified, as thresholds of effect have not been identified, options are yes, no or maybe, with no data ranges provided. Fibres always given highest banding. It evaluates risk associated with a specific application of a NM. Based on scientific expert judgment and a holistic assessment of the evidence (i.e. literature review), so relatively low potential for use by non-experts. Uses exposure related parameters in environmental hazard assessment, which is not logical, but could be modified to exclude this. Cannot discriminate between NM with small changes.	• Morphology (i.e. HARN); • Acute toxicity; • CLP health hazards (bulk material and nanomaterial): such acute toxicity, as germ cell mutagenicity, carcinogenicity, reproductive toxicity, skin corrosion/irritation, specific target organ toxicity • Toxicity to environmental species and potential for ecosystem effects • Novelty of the NMs	Extract methods and endpoints for development as guidance or practical methods within later tasks. Evaluate similarity assessment with bulk form – these may be useful for assessment of SbD measures such as substitution.
NanoSafer	• Easy to use • Nano-specific	• The tool is applied to pristine powders only (as may be the case for others also), • A high aspect ratio will always generate the highest score, • Surface coating will also generate a high score, independent of other toxicity information.	• Guidance is required for evaluating, or testing the impact of, aspect ratio, solubility, surface coating • Guidance is required for evaluating risk and hazard statements.	Potentially provide guidance on key toxicity paradigms e.g. HARN, and further elucidate the scoring system of NanoSafer.

Model/Tool	Positives	Limitations	Experimental methods & relevant endpoints	Action required
Swiss Precautionary Matrix (SPM)	• Detailed assessment of for reactivity and dissolution. • The included reactivity parameters for consideration by SPM are based on the known mechanism of action employed by nanomaterials in causing toxicity, and in comparison of acellular and cellular *in vitro* tests, and how these compare and align with *in vivo* work. • LCA based approach for different endpoints • Can be used even in data-poor cases using worst-case reasoning.	• Using only these criteria is not enough for assessing hazard (i.e. no consideration of chemical composition or NM structure), however the elements identified here may be useful to incorporate and include guidance. • No hazard banding is provided, instead a score (complex scoring system) ultimately provides a Y/N for taking precautions. Unlikely to discriminate between NM with small changes.	• Dissolution (stability) in the body and in environmental conditions; • Reactivity (e.g. redox activity, photocatalytical activity, biological oxidation damage, induction of mediators of inflammation, ROS, GSH reduction, protein carbonylation). • Nano-relevance (Size distribution, Specific surface area, Type of NMs) • Status of information (characterisation, functionalisation and impurities)	Assessments for reactivity and dissolution to be developed further. Extract methods for reactivity and stability NMs. Evaluate cut off values of relevant parameters. Evaluated overall scoring system.
Stoffenmanager-nano	Nano-specific	Input information is supposed to be obtained from SDS and technical data sheets, and if information is lacking a "worst case" should be assumed – the precautionary approach; the highest risk categories are applied which are discerned only on the CMAR status of the bulk material, or upon expert judgement. This is likely to be unattainable for most nanoforms, and not useful for SbD.	• Solubility; • Morphology (i.e. fibre or not, particle size <50nm); • CLP endpoints	Investigate size threshold value further. Develop guidance on how to fulfil requirements for CLP endpoint assessments *in vitro*.
Stoffenmanager dermal	Dedicated dermal toxicity tool	Only incorporates hazard assessments covered in CLP. Not nano-specific.	Dermal toxicity	Develop nano-specific dermal toxicity further.
Quantitative (simple)/high tier assessment				
GUIDEnano Option 1 – using read-across DNEL/OEL from similar materials.	Nano-specific. Easy for the user if a similar NF with data exists. Allows a quantitative risk assessment to be performed.	The approach, descriptors and criteria for similarity assessment could not be refined. The approach and descriptors could be aligned to that used in ECETOC nanoApp[8] or in GRACIOUS. The criteria would need to be tailored to the purpose of Safe-by-Design. Might not be able to discriminate between small variations in NFs.	• Will depend on the revisions of the similarity analysis. At the moment, basic phys-chem data.	The similarity assessment part is by itself highly valuable in SAbyNA, as that can support read-across of either hazard thresholds such as OELs/DNELs or toxicity study results. The actual properties and thresholds considered in the similarity assessment need revision. Details are in Park *et al.*, 2018.[9]

[8] Janer, Gemma, Robert Landsiedel, and Wendel Wohlleben. "Rationale and decision rules behind the ECETOC NanoApp to support registration of sets of similar nanoforms within REACH." *Nanotoxicology* (2020): 1-22.

[9] Park, Margriet VDZ, et al. "Development of a systematic method to assess similarity between nanomaterials for human hazard evaluation purposes–lessons learnt." *Nanotoxicology* 12.7 (2018): 652-676.

Model/Tool	Positives	Limitations	Experimental methods & relevant endpoints	Action required
GUIDEnano Option 2 - nanomaterial categorization for generic default hazard threshold values for RA	Specific for nanomaterials. Requires relatively simple data for implementation. Therefore plausible at a SbD level.	The system was developed within the GUIDEnano project, but was never fully implemented into the GUIDEnano Tool. There as a 'placeholder' for such an approach within the hazard derivation strategy. Implementation of such an approach would require revision of the methodology and criteria for dissolution and reactivity. Hazard limit values that have been specifically derived for nanomaterials in the last 5 years, and consistency with this approach should also be considered.	• Morphology • Dissolution • Reactivity	Is concluded to be useful in the framework of SAbyNA, revision of methodologies and thresholds, and implementation within GUIDEnano.
Quantitative (complex)/high tier assessment				
GUIDEnano Option 3 - GUIDEnano (regulatory-like) derivation of DNEL values/CLP conclusions	Might be able to discriminate between NF modifications currently not possible to address in other tools. The approach can be refined within SAbyNA as most of the partners involved in the GUIDEnano tool development (including ThinkWorks) are also partners of SAbyNA. The GRACIOUS framework test-environment and the ECETOC nanoApp are implemented/developed by ThinkWorks and based upon the same object-oriented approach and tooling.	The system was developed within the GUIDEnano project, but was never fully implemented into the GUIDEnano Tool. It is now updated for inhalation, but not for other exposure routes. The rational had been developed for genotoxicity[10] and some other hazard endpoints, but not sure if implemented into the tool. The criteria for similarity assessment could now be refined. This could be aligned to that used in ECETOC nanoApp or in GRACIOUS. Requires high tier type of toxicological data that may not be available at early stages of development.	Long list of high tier toxicology assays, depending on the hazard endpoint that is addressed.	The similarity assessment part is by itself highly valuable in SAbyNA, as that can support read-across of either hazard thresholds such as OELs/DNELs or toxicity study results. The actual properties and thresholds considered in the similarity assessment need revision. Details are in Park *et al.*, 2018.
SSWD	Has the facility to allow weighting of data according to taxonomic group. Generates a threshold concentration (HCx) by either a probabilistic or deterministic approach.	• Relatively onerous data requirements relative to control banding tools: typically, for construction of a robust SSD, a minimum of endpoint data for six species is required.	Acute and chronic toxicity Species sensitivity distributions	Evaluate methods for acute and chronic toxicity. Evaluate the potential for the approach to distinguish among small differences in NMs.
SUNDS	Use of DNEL values allows for better sensitivity and hence will be suitable to SbD. The second assessment tier is based on an adaptation of the authorisation process currently in operation within the EU REACH regulation.	• SUNDS tier 2 assessment requires use of *in vivo* data, • for Tier 2 assessment, expertise in relative background is required, • there are significant data requirements. • Environmental assessment utilises LICARA Nanoscan as a first tier and either nSSWD or pSSD (Gottschalk et al., 2013), which are all discussed separately. The pSSD tool provides a similar approach to SSWD but uses a more complex approach to handling data for a single species in the distribution.	Point of departure, NOAEC or LOAEL from one or several of the following: Skin corrosion, Skin irritation, Eye damage, Eye irritation, Dermal repeated exposure, Respiratory tract corrosion, Respiratory tract irritation, Skin sensitisation, Respiratory sensitisation, Acute toxicity, Repeated dose toxicity, Mutagenicity, carcinogenicity	Currently requires *in vivo* data, further development may involve guidance on how to incorporate *in vitro* assessment to make it suitable for early stage innovation.

[10] Catalán, Julia, Helene Stockmann-Juvala, and Hannu Norppa. "A theoretical approach for a weighted assessment of the mutagenic potential of nanomaterials." *Nanotoxicology* 11.8 (2017): 964-977.

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3.3 Experimental testing methods and/or required guidance for SAbyNA WP3

3.3.1 Key input requirements of assessed RA tools

To help direct the assessment of experimental models useful within RA the key hazard considerations of each RA tool is provided in Table 2. There is notable overlap in the hazard information requirements of the tools described above; as would be expected, numerous tools require the same parameters to enable their hazard prediction. However, the use and interpretation can vary considerably. In ranking the frequency of which certain parameters have appeared when assessing the distilled RA tools, key parameters are collected in Table 2 (the details of how these parameters are processed by each RA tool follows in Section 5):

Table 2 Hazard information requirements of the assessed RA tools, as specifically defined by these RA tools

		ANSES	CB nanotool	GUIDEnano	LICARA	NanoSafer	SPM	Stoffenmanager dermal	Stoffenmanager Nano	SSWD	SUNDS	NanoRiskCat
Solubility/Dissolution rate		x	x	x	x	x	x		x		x	
Fibre paradigm*		x		x	x	x			x		x	x
Reactivity**		x	x	x	x		x				x	
Known severe toxicity***			x	x	x				x		x	x
Dermal toxicity			x	x		x		x				x
Inflammatory reactions/potency				x	x		x				x	
R-phrases, hazard statement ****		x		x	x	x						x
Diameter			x	x	x		x		x		x	
Acute toxicity				x	x					x		x
Chronic toxicity				x	x					x		x
Surface coating				x		x						
OEL (or other)			x (parent)	x		x						
DNEL, PoD*****				x					x	x	x	
Environmental compartments	**Aquatic**			x	x				x	x		x
	Terrestrial			x	x				x	x		x
	Sediments			x								
	Sewage treatment effluent				x				x	x		x

*may include consideration of solubility; **the definition of reactivity differs within different tools; ***often specifically mention to carcinogenicity, reproductive toxicity, mutagenicity dermal toxicity, asthmagenicity, organ specific accumulation; ****Reference to CLP R-phrases and hazard statements, often of bulk or analogous material; *****likely derived from previously mentioned endpoints.

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3.4 Data repositories

This section describes the distillation and assessment of existing data sources for the human and environmental hazard data. This process involved the selection of data sources based on the criteria presented in Milestone 3.1 and the results of the assessment performed for the distillation process.

3.4.1 Criteria for the selection of data repositories

To select which data sources will be considered for the assessment, we identified and prepared an extensive list of data sources available from many completed and ongoing EU sponsored (FP7 & H2020) and Non-EU projects. Full list of data sources is provided in the Annex 7.2. The list contains data platforms which include data from multiple nano projects, individual project databases and knowledge base resources. This work benefited from the related work done in the PROSAFE and GRACIOUS projects where similar resource sheets were produced. To distil the data sources, a number of include and exclude rules were devised for the assessment. If data sources did not meet these criteria, they were not carried forward to the assessment process.

Included for further evaluation were data sources which include relevant data for human and environmental hazard. A comprehensive list of data sources was established from various available EU sponsored and Non-EU projects, comprising data for multiple nano domains including phys-chem characterisation, toxicology, ecotoxicology, release, fate and exposure. Only the relevant data sources which include human and environmental hazard data for the WP3 assessment were selected.

A further critical consideration was database presence. It was essential that, from the project website or known through the working knowledge of the involved partners, human and environmental hazard data generated is made available and stored in a properly structured and normalised database.

Many projects and data sources were excluded from the list due to not having the hazard data required for the WP3 assessment, or if it was not certain that a proper database has been produced by a project then it was excluded from the list.

Based on the initial selection criteria provided above, we short-listed the following data sources which were further assessed and distilled (Table 3).

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Table 3 Data sources selected for further assessment

Project Title	Website	Comments
eNanoMapper	https://enanomapper.net/	Platform for EHS data repository; being adopted and used in GRACIOUS and other projects (CALIBRATE, NanoReg 1 & 2, etc.)
MARINA	http://www.marina-fp7.eu/	Phys-Chem 14 materials; *in vitro* (8 cells types, 10 assay types, 209 Tests); *in vivo* (8 Tests); Eco-tox data (40 Tests); Omics (Proteomics: 52 (substance x cell type x timepoint combinations), Metabolomics: 52, Transcriptomics: 24); Exposure Scenario Data (Workers: 55, Service Life: 4); Most MARINA data transferred to e-NM instance (share with CALIBRATE & Nanoreg2);
NANoREG	http://www.nanoreg.eu/	NanoReg 1 data publicly avail in e-NM DB; phys-chem, *in vitro* & *in vivo* tox., ecotox., exposure; phys-chem and tox. templates
NanoReg2	http://www.nanoreg2.eu/	NanoReg 2 data accumulating in e-NM DB instance; phys-chem, *in vitro* & *in vivo* tox., ecotox., exposure;
ENPRA	http://www.enpra.eu/	Phys-Chem 12 materials; *in vitro* (24 cells, 58 assay types, 650 Tests); *in vivo* (17 Tests); IVIVE (83 Tests); Toxico-Kinetic Data (Yes) ; Exposure Modelling Data (Yes);
NANOMUNNE	http://www.safenano.org/research/nanommune/	Phys-Chem 50+ materials; *in vitro* (8 cells, 12 assay types, 123 Tests);
CALIBRATE	http://www.nanocalibrate.eu	Develop, integrate and validate models; sourcing data from other projects (e.g. MARINA); Modelling and Exposure information; continue to relate and possible data sharing agreements (DSAs)
PATROLS	https://www.patrols-h2020.eu/	Started Jan 2018; ENM Phys-chem, *in vitro*, *in vivo*, ecotox.; tox. modelling; establish relations and poss DSAs
BIORIMA	https://www.biorima.eu/	Started Noc 2017; ENM Phys-chem, *in vitro*, *in vivo*, ecotox.; tox. modelling; RMM toolbox establish relations and poss DSAs
NanoCommons	https://cordis.europa.eu/project/rcn/212586_en.html	Research and Innovation action to support networking & development: Joint Research Activities will integrate existing resources and organise efficient curation, preservation and facilitate access to data/models.
NanoMILE	http://www.nanomile.eu-vri.eu/	MNM screening platform; ENM properties Knowledge Base; Phys-chem; *in vitro*, *in vivo*; omics;
NanoPUZZLES	http://www.nanopuzzles.eu/	Modelling, analysis (QSAR & co); database & data standardisation info
NANOSOLUTIONS	www.nanosolutionsfp7.com	Phys chem re 31 treated and untreated ENMs; Life cycle analysis; ENMs and BioMedia BioCorona; *in vitro* cell models, with HTS; cross-species and environment; disease and translocation studies; OMICs (mRNA, RNAseq; proteomics on beas2b, ecoli; closed proj,
SUN	http://www.sun-fp7.eu	Phys-Chem 8 materials; *in vitro* (2 cells, 4 assay types, 17 Tests); *in vivo* (9 Tests); Ecotox. data (205 Tests); OMICs (Yes); Exposure Scenario Data (96 from NECID); Environment, Release Exposure (28 Datasets);
SANOWORK	http://www.sanowork.eu	Limited *in vitro* results

Project Title	Website	Comments
Dana	http://www.nanoobjects.info/en/nanoinfo/knowledge-base	KB - Searchable for nanomaterials containing products
Nanohub (US/NSF)	https://nanohub.org/	KB on risk assessment & standards etc.; 320+ simulation tools/models ; some example nano DBs and datasets; nano education tools
MODERN	http://modern-fp7.biocenit.cat/index.html	KB re ENM QNPR modelling; database/data repository design; SbD
NanoMiner (FP7 NANOMMUNE project)	http://compbio.uta.fi/estools/nanommune/index.php/	Optimised repository of OMICS data generated by NANOMMUNE project (see entry)
ITS-Nano (Intelligent Testing Strategy for ENMs)	https://www.safenano.org/research/its-nano/	KB re enm testing strategies; Ref - Stone V, Pozzi-Mucelli S, Tran L, Ashberger K, et al. 2014, "ITS-NANO - Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy," Particle and Fibre Toxicology, 11(9), 1-11.
ModNanoTox	http://www.birmingham.ac.uk/generic/modnanotox/index.aspx	Re nano-tox. modelling
OECD Database on Research into the Safety of Manufactured NMs	http://www.oecd.org/env/ehs/nanosafety/publications-series-safety-manufactured-nanomaterials.htm	Publications in the Series on the Safety of Manufactured Nanomaterials
PubVinas	http://www.pubvinas.com/	A web-based nanomaterial database (developed in US) by big data curation and modelling friendly nanostructure annotations. contains 705 unique nanomaterials covering 11 material types. Each nanomaterial has up to six physicochemical properties and/or bioactivities, resulting in more than ten endpoints in the database.
MESOCOSM	https://aliayadi.github.io/MESOCOSM-database/	The first centralized mesocosm database management system for environmental nanosafety containing experimental data collected from mesocosm experiments suited for understanding and quantifying both the environmental hazard and exposure.

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Data sources that met the initial selection criteria were further assessed to determine which go forward for the optimisation Task 3.2. The assessment criteria in this process was more focussed than the initial selection criteria and had the goal of determining which data sources and elements of data sources will be suitable for the assessment and optimisation purpose.

The following considerations were made while performing the further assessment of data sources:

- **Data accessibility:** Data sources which are available online through cloud platforms and easily accessible with clear guidance documentation to use the database (e.g., how to search, filter, link, extract and analyse the data). We also included sources which could be used with the help of SAbyNA partners who are already familiar or were involved in the development of database.
- **Published data:** Data sources that includes publicly and freely available data, which could be openly accessed without any data embargoes or restrictions.
- **Standardised Data:** Data sources that contains data which has been collected and stored in some sort of standardised and harmonised fashion with the use of ontologies or standard terminologies.
- **Curated Data:** Data sources which contains data that has been somewhat curated with the known source, meta data and classifications.
- **FAIR Data:** Data that has been made available (or in process of making) using the FAIR principles by making it findable, accessible, interoperable and reusable
- **Applicability to SAbyNA case studies:** Data that is relevant for SAbyNA case studies
- **Availability through SAbyNA partners:** Data that is available through the SAbyNA partner connections; who already have access to such data or have connections with the data providers.
- **Data from data platforms:** Data in process of publication or already available on other data platforms e.g. NANOFASE through NanoCommons platform.

We excluded the data sources which:

- Did not have relevant data for the SAbyNA Platform
- Are not easily accessible, not published or currently are under data embargos and restrictions
- Does not include data that has been standardised with the use of ontologies
- Does not include data that has been properly curated (i.e. the data has gone through a process where their validity has been checked with those who generated the data, is free of errors and properly organised in a readable format).

Considering the above selection criteria, we further short-listed the data sources as follow:

Table 4 Short-listed data sources

Project Title	Website	Database Platform
eNanoMapper	https://enanomapper.net/	eNanoMapper

Project Title	Website	Database Platform
MARINA	http://www.marina-fp7.eu/	eNanoMapper
NANoREG	http://www.nanoreg.eu/	eNanoMapper
GRACIOUS	https://www.h2020gracious.eu/	eNanoMapper
ENPRA	http://www.enpra.eu/	eNanoMapper
NANOMUNNE	http://www.safenano.org/research/nanommune/	eNanoMapper
CALIBRATE	http://www.nanocalibrate.eu	eNanoMapper
PATROLS	https://www.patrols-h2020.eu/	eNanoMapper
BIORIMA	https://www.biorima.eu/	eNanoMapper
NanoCommons	https://cordis.europa.eu/project/rcn/212586_en.html	NanoCommons
NanoMILE	http://www.nanomile.eu-vri.eu/	NanoCommons
NanoPUZZLES	http://www.nanopuzzles.eu/	NanoCommons
NANOSOLUTIONS	www.nanosolutionsfp7.com	NanoCommons
SUN	http://www.sun-fp7.eu	NanoCommons
SANOWORK	http://www.sanowork.eu	NanoCommons
PubVinas	http://www.pubvinas.com/	PubVinas
MESOCOSM	https://aliayadi.github.io/MESOCOSM-database/	GitHub

Many of the data sources which were selected based on the further include criteria are either hosting their data with eNanoMapper system (e.g. NanoReg, MARINA, PATROLS) or in process of linking/publishing the data through NanoCommons platforms (e.g. NANOFASE, NanoMile, etc). Hence, we will mainly focus on testing the SAbyNA case studies and providing suggestions for optimisation on these two platforms; which will cover most of the data sources selected for the assessment. Some projects data on both of these platforms is publicly available, and some is under embargo. However, we will hugely benefit by having access to the eNanoMapper database and NanoCommons platform through previous projects involvement, working knowledge, open access to some data and good connections with the SAbyNA partners. Other data sources selected for assessment were the MESOCOSM database and non-EU data source Pubvinas. For the case studies testing, we will follow the entire process flow (from start to end) to assess each aspect of the data source, highlighting any encountered issues, reporting potentials constraints and providing suggestions for improvement and optimisation.

3.4.2 Assessment of data repositories

This task aimed to assess the current usability of distilled data sources. The information presented here is not meant to be used for directly accessing the available data, but as a compass for understanding the nature of the data sources/platforms available and their features. The assessment was synchronised with release, fate and exposure assessment task in WP2, to avoid duplicating the efforts where there were overlaps.

Following parameters were assessed for the data source evaluation:

- Project Title/Data Source
- Web Access
- Supported Data Types
- Guidance Documentation Available

- Publicly Available
- Standardised/Use of Ontology
- Data Collection Methods (e.g. standardised templates)
- Data Output Formats/Visualisation (e.g. excel, xml, json etc.)
- Support for Analysis/Modelling
- Allows API (e.g. link to models or Jupyter)
- Data Quality Criteria
- Is Data Curated (e.g. annotated)
- Is Data FAIR (findable, accessible, interoperable and reusable)
- Any Limitations Noted

Assessment findings of the selected data platforms/sources (eNanoMapper, NanoCommons, Pubvinas and MESOCOSM) are given below:

Project Title/Data Source: eNanoMapper system

eNanoMapper system was developed as part of EU sponsored FP7 project to establish a community agreed ontology, databases system and modelling platform to support various domains of nanotechnology. Its use is being effectively mandated by the EU Project Officers to help standardise and harmonise ongoing Nano-EHS research data storage efforts.

Database Setup: eNanoMapper system has in the last few years become the de-facto principal data repository for many recently completed and currently ongoing nano projects. Each project is set up as a database "instance" that encapsulates the project's users and their permissions to access one or more project datasets. eNanoMapper platform facilities (via its interfaces) federated search and retrieval capabilities across different projects throughout the platform. Each contributing project's dataset forms a discrete unit of data, and access to (one or more) projects is granted on a project by project basis, depending possibly on data-sharing agreements, etc.

Web Access: https://search.data.enanomapper.net/

Supported Data Types: eNanoMapper system supports storage of nanomaterials characterization data, biological and toxicological information. More recently, advancements have been made to store the human exposure and environmental release, fate, exposure and OMICS data.

Guidance Documentation Available: eNanoMapper system provides a user-friendly HTML user guidance interface, as well as tailored interface to each database instance.

Publicly Available: Data from many closed projects is available from eNanoMapper, either fully public (e.g. after the project has closed and all data embargos or other restrictions have lapsed) or possibly only to selected users by arrangement. Running projects, can access such older project data, and also can add completely new data as it arises within the project to its own data instance. In general, access to new data is confined to that project when still "live". Hence, current running project X may have access to (and can search across) the dataset of previous projects A, B, C, and its own data instance X; whilst current project Y data may have access to (and search across) the dataset of previous projects C, D, E and its own data instance Y.

Standardised/Use of Ontology: The eNanoMapper team has developed an ontology; which includes and defines common vocabulary terms used in nano-safety research with a classification hierarchy and other relationships. In the GRACIOUS project a framework has been developed for unique endpoint descriptors; for physicochemical parameters, toxicity, release, exposure and activity endpoints. This endpoint descriptor framework has been adapted by eNanoMapper and goes beyond the level of details normally covered by the current ontologies. This descriptor framework will also be adapted by other new projects e.g. SbD4Nano and within the nano-safety cluster where Green Decision will be making it available for further extension through the 'terminology harmonizer' tool developed in GRACIOUS project. GUIDEnano tool has also developed an interface to make a link with the eNanoMapper tool.

Data Collection Methods (e.g. standardised templates): The eNanoMapper team has developed an online template generator tool which supports standardised data collection templates aligned with the eNanoMapper ontology. The tool adapted OECD derived tox and eco-tox templates format that were used in many previous nano project e.g. ENPRA, MARINA, NANOSOLUTIONS and more recently in GRACIOUS, PATROLS and NanoInformatix. This tool has also adapted and supports phys-chem characterisation templates published by JRC in Nanoreg.

Data Output Formats/Visualisation (e.g. Excel, XML, JSON etc): Users can download data from the user interface in a variety of formats and through a predefined selection of queries or the combination of queries, and data categories. Once the selection is made, this can be downloaded in 5 different formats: JSON, CSV, TXT, XML, or XLSX.

Support for Data Analysis and Modelling: In terms of extracting data for analysis and modelling purposes; eNanoMapper offers a widened variety of formats for downloading data, or its extraction via the API. The web application JaqPotQuattro allows building QSAR models and using them for predictions. As an input, JaqPotQuattro uses data available from data.enanomapper.net. After pre-processing, transforming and preparing the datasets, the various modelling algorithms can be applied. eNanoMapper database user interface also allows the export functionality, to save selected data in one of the five available formats, i.e. json, txt, csv, xml or xslx. The exact options offered depend on the complexity of the retrieval and structure of the resulting dataset. For example, a simple table in a “flat” csv dataset may meet certain report and output format needs, but not the more complex dataset structures requiring the much greater flexibility of XML or JSON. The JSON format is always available and is the recommend format to be used for data analysis purposes.

Allows API (e.g. link to models or Jupyter): Application Programming Interface (API), which can be used to directly retrieve data into other packages and systems, e.g. to retrieve data into processing and analysis by Jupyter, or to feed data into a running model, etc. Beyond the collation of useful information regarding experiments undertaken by the studies collected, eNanoMapper offers the means to browse and compare data availability, for example across related experiments. The special “for developers” section gives the opportunity to search and download a series of information by using programming tools (via AMBIT REST web services with free text & faceted search API), for general analysis, data embedding or visualisation purposes, as well as to build data pipelines to meet the needs of more specific modelling. In addition, programmatic data access is critical for true data interoperability and is one of the most important FAIR requirements. It needs to be accompanied by adequate metadata that facilitates accurate and efficient interrogation of the data: The first FAIR criteria, Findable, requires metadata and data to be easy to find for both humans and computers. Machine-readable metadata are essential for the automated discovery of datasets and services, and the eNanoMapper REST API is an essential component of the FAIR data resource.

Data Quality Criteria: Progress is being made on the data quality assurance/quality control (QA/QC) and the adequacy of current metadata for analysis and modelling. More recently, the quality criteria have been developed and implemented within the data collection procedures to ensure the QA and QC.

Is Data Curated (e.g. Annotated): In relation to the database, in some of the older datasets there are some shortcomings and gaps in currently available metadata for some datasets, mainly due to way that the data was captured in the past. eNanoMapper itself fully supports the proper data annotation and storage of metadata information. More recently, advancements have been made with the development of template generator tool to ensure that data is properly annotated using the metadata info and cross platform operability of the data in various formats.

Is Data FAIR (findable, accessible, interoperable and reusable): Database is currently being actively developed to ensure conformance with the guiding principles of FAIR data (https://www.go-fair.org/fair-principles/), and also to enhance its data curation and data stewardship processes for FAIR data. Together with some overlapping work in other ongoing projects (principally Gov4Nano and GRACIOUS,) great progress has been made through the production of FAIR data collection templates for nano-ehs data and their parsing and upload to the database, with necessary meta-data; and other case studies are being run in order to develop

better methods to screen and test the "FAIRness" of data, and better associated curation processes. The first FAIR criteria, Findable, requires metadata and data to be easy to find for both humans and computers. Machine-readable metadata are essential for the automated discovery of datasets and services.

Any Limitations Noted: The further population of the eNanoMapper database is ongoing and will gather more data from many different ongoing nano projects (e.g. PATROLS, GRACIOUS, BIORIMA, caLIBRAte, NanoInformatix, Gov4Nano, RiskGone, SABYDOMA, SbD4Nano). In some of the older data there are some shortcomings and gaps in currently available metadata for some datasets, mainly due to way that the data was captured in the past. These gaps in the older (especially, but not exclusively) data (many of them captured far less formally or systematically five or more years ago, for example) can retard analysis or modelling through doubts on quality or lack of confidence in the accuracy or precision of data (e.g. inadequately described measurement method). Other issue noted is the public accessibility of the available data. Most project data is under embargos. However great progress has been made in terms of data standardisation and to make it FAIR. Further assessment will be done in Task 3.2 to test the SAbyNA case studies and suggestions for the improvement of usability will be provided.

Project Title/ Data Source: NanoCommons platform

NanoCommons is creating an e-infrastructure for enhancing data integration and to promote cross-field cooperation. It has developed a sustainable and openly accessible nano-informatics framework (knowledgebase, integrated computational tools, data interpretation systems etc.) for assessment of the risks of NMs, their products and their formulations.

NanoCommons project aims to provide an integrating platform for the nano-safety community in Europe and globally. It is creating a FAIR data ecosystem guiding the users to the most appropriate solution that fits their needs, promotes data integration, sharing, enrichment and full data exploitation from their original source and ownership. It facilitates integrated approaches combining experiments, modelling and simulation processes.

Web Access: https://ssl.biomax.de/nanocommons/cgi/login_bioxm_portal.cgi

Supported Data Types: NanoCommons system supports storage of nanomaterials characterization data, toxicological information, ecotoxicity, release, fate, exposure and OMICS datasets.

Guidance Documentation Available: NanoCommons system provides a user-friendly web-based user guidance interface to browse the knowledge infrastructure. It also provides an online user guidance handbook for nano-informatics, data management, ontologies and workflows.

Publicly Available: Some data from the closed projects is openly available. Other datasets are still under embargo and require to have data access and analysis agreement with the corresponding data owner.

Standardised/Use of Ontology: NanoCommons platform supports many ontologies e.g. eNanoMapper, NanoParticle, NCI, Gene etc. It has the intention to make a link with the ontology entries for the physicochemical parameters, toxicity, release, exposure and activity endpoints. This endpoint descriptors framework has been developed in the GRACIOUS project and will be made available to wider nano community using the 'terminology harmoniser' tool.

Data Collection Methods (e.g. standardised templates): NanoCommons aims to integrate the different resources so it supports integration with the standardised templates developed by other nano projects e.g. JRC NanoReg1 templates and eNanoMapper *in vitro*, *in vivo* and eco-tox. templates.

Data Output Formats/Visualisation (e.g. Excel, XMl, JSON etc): NanoCommons database platform provides different formats for data analysis e.g. expression analysis, corona analysis and image analysis of the existing data. Summarised results could be printed in PDF format.

Support for Analysis and Modelling: NanoCommons supports integration and federation of existing NMs data, interaction mechanisms, and knowledgebase and underpinning ontologies. It has developed a user-

friendly interface for a suite of computational tools for mechanistic and statistical modelling, read-across, grouping, safe-by-design, life cycle assessment and bench-marking of their predictive power.

Data Quality Criteria: This system supports and provides protocols for the quality assurance criteria.

Data Curated (e.g. Annotated): It supports and provides guidance for data annotation, meta-data and general data management and curation aspects. It aims to integrate different resources (database, models and tools etc.) to promote interoperability and mechanism for SbD approaches.

Is Data FAIR (findable, accessible, interoperable and reusable): NanoCommons infrastructure creates a FAIR data ecosystem guiding the users to the most appropriate solution that fits their needs; promotes data integration, sharing, enrichment and full data exploitation from their original source and ownership; facilitates integrated approaches combining experiment, modelling and simulation processes.

Any Limitations Noted: NanoCommons infrastructure aims to provide knowledge and tools for nano community including characterisation and control of data quality and uncertainty, development of data templates and workflow management tools, repository of protocols and associated metadata templates, knowledge infrastructure, data management guidance, analysis and modelling tools, tool integration for risk assessment, grouping, read-across and risk assessment/decision tools. These are great advancements for SbD, however main limitation noted is the open accessibility of data, which is mostly under embargos. Further assessment will be done in Task 3.2 to test the SAbyNA case studies and suggestions for the improvement of usability will be provided.

Project Title/ Data Source: Pubvinas

A web-based nanomaterial database (developed in US) by big data curation and modelling friendly nanostructure annotations. Database contains large set of nanomaterial data containing annotated nanostructures suited for modelling purposes. The database public access is provided through the URL http://www.pubvinas.com/, contains 705 unique nanomaterials covering 11 material types. Each nanomaterial has up to six physicochemical properties and/or bioactivities, resulting in more than ten endpoints in the database.

Web Access: http://www.pubvinas.com/

Supported Data Types: A variety of *in vitro* and *in vivo* assays evaluating their potential environmental and human health effects generated in vast quantities of experimental data.

Guidance Documentation Available: It also provides an online tutorial for searching, browsing and use of the system.

Publically Available: Structural annotated data is publicly available to all, through online database portal (http://www.pubvinas.com/) that currently can be used to visualize the nanostructures and upload new data.

Data Output Formats (e.g. Excel, XML, JSON etc): PubVinas supports the PDB format (Protein Data Bank), which provides three-dimensional structures of biological macromolecules (e.g. proteins and nucleic acids). The first part of the file contains the basic information on the structure of the nanomaterial (e.g., the form, shape and size); the second part contains information about the atoms (e.g., atom type and coordinates); and the third part includes information on the bond/connection between atoms. Each PDB file of the nanomaterials can be downloaded by clicking the dropdown bars with their corresponding classification (e.g. gold nanoparticles, silver nanoparticles, and platinum nanoparticles). Users can view the nanostructure online from the corresponding PDB file and open the downloaded PDB file using well-known cheminformatics software (e.g., VMD, RasMol, and MOE).

Support for Analysis and Modelling: This database provides a public resource for data-driven nano-informatics modelling research aimed at rational nanomaterial design and other areas of modern computational nanotechnology. The PDB files can be used to generate nano-descriptors, which could be further used for

predictive models of various nanomaterials using machine learning and deep learning (deep neural network) approaches.

Data Curated (e.g. Annotated): This nanomaterials database contains annotated nanostructures of diverse nanomaterials suitable for immediate modelling research. All experimentally obtained information on the structure of the nanomaterials, such as form, size, shape, and surface were annotated and stored as PDB files, which are downloadable from the web portal.

Is Data FAIR (findable, accessible, interoperable and reusable): NanoCommons infrastructure creates a FAIR data ecosystem guiding the users to the most appropriate solution that fits their needs; promotes data integration, sharing, enrichment and full data exploitation from their original source and ownership; facilitates integrated approaches combining experiment, modelling and simulation.

Any Limitations Noted: This is the largest and the only nanomaterial database that contains nanostructure annotations to support nanomaterial modelling and rational nanomaterial design. Furthermore, the predictive models developed from this database can be used to predict three critical properties and bioactivity (i.e., logP, zeta potentials, and cellular uptake) of new nanomaterials. Recently, attempted was made to access the database through online portal but failed. Further assessment and suggestions for optimisation will be provided in Task 3.2.

Project Title/Data Source: MESOCOSM

The first centralized mesocosm database management system for environmental nano-safety containing experimental data collected from mesocosm experiments suited for understanding and quantifying both the environmental hazard and exposure. These entities are divided into different groups i.e. physicochemical properties of ENMs, environmental, exposure and hazard endpoints, and other general information about the mesocosm testing, resulting in more than forty parameters in the database. MESOCOSM aims to predict and explain ENMs behaviour and fate in different ecosystems as well as their potential impacts on the environment at different stages of the nano-products lifecycle. MESOCOSM is expected to benefit the nano-safety community by providing a continuous source of critical information and additional characterisation factors for predicting ENMs interactions with the environment and their risks.

Database Setup: The database is available to all on GitHub repository. Scientists and industries can visualize the totality or a part of the dataset, download the SQL database file and manipulate it with any database management system (Oracle, Postgres, MySQL, DB2 etc.), remotely interact with the database via an application program interface (API), or also download it with its application (graphical user interface) for local usage (on the local computer or server).

Web Access: https://aliayadi.github.io/MESOCOSM-database/

Supported Data Types: Contains 5200 entities covering tens of unique experiments investigating Ag, CeO_2, CuO, TiO_2-based ENMs as well as nano-enabled products. These entities are divided into different groups i.e. physicochemical properties of ENMs, environmental, exposure and hazard endpoints, and other general information about the mesocosm testing, resulting in more than forty parameters in the database.

Guidance Documentation Available: The MESOCOSM database provides its schema and logical database design online on the portal. It also provides online guidance on how to install and use the application.

Publicly Available: MESOCOSM database and application is free to use under the licence agreement Creative Commons Share alike. The default license Creative Commons (CC-BY 4) allows users to download, modify and reuse the data without restriction, but attribution of the source must accompany the reuse. MESOCOSM is also available as downloadable SQL file for free under an Open Database License v1.0.

Data Collection Methods (e.g. standardised templates): MESOCOSM is a distinct database resource providing thousands of experiment data obtained in tens of unique experiments investigating different

nanomaterials based on results obtained within the H2020 projects. It standardises the storage of environmental exposure and hazard data generated in database.

Data Output Formats/Visualisation (e.g. Excel, XML, JSON etc): The MESOCOSM database is equipped with a powerful application, consisting of a graphical user interface (GUI), allowing users to manage and search data using complex queries without relying on programmers. A JAVA Graphical User Interface (GUI) is freely available to provide direct managing, searching and browsing. The web portal of the MESOCOSM database was built in HTML, CSS, JavaScript and JQuery to make it more attractive and user-friendly.

Allows API (e.g. link to models or Jupyter): It allows to remotely interact with the database via an application program interface (API), or also download it with its application (graphical user interface) for local usage (on the local computer or server).

Is Data FAIR (findable, accessible, interoperable and reusable): MESOCOSM was designed from the outset with maximum adherence to the FAIR principles that promote finding, accessing, interoperating, and reusing shared data (Wilkinson *et al.*, 2016). MESOCOSM database system for environmental nano-safety was designed following the FAIR data vision to optimise environmental data sharing and reuse by humans and machines. The proposed database system considers also the principles of linked data mentioned in the works of Bizer *et al.* (Bizer *et al.*, 2011; Pommier *et al.*, 2019) demonstrating that each entity must be correctly identified with a persistent unique identifier (PID), described in a semantic format, and linked with other resources so that they can be located unambiguously by machines, and easily findable by humans through the richness of the metadata used to describe them. Moreover, the database content is registered and archived in different websites (the CEREGE website, GitHub repository and Perma web archiving service2) so that it can be easily found and accessible. Regardless of what may happen to the original source, the archived record will always be available through the cited websites. Similarly, all datasets have been published and are identified with unique DOI identifiers. To make the MESOCOSM database content interoperable and FAIR for the machines, semantic format Resource Description Framework (RDF) have been adopted. All the entities in the database are linked to other resources through an ontology especially designed to support the development of the MESOCOSM database system for environmental nano-safety. The RDF format makes the MESOCOSM data accessible in the semantic web through the powerful SPARQL query mechanism and rich metadata indexing. The adoption of the four FAIR characteristics, makes MESOCOSM a modern database making environmental exposure and hazard data valuable to a wide range of users, including manufacturers, researchers, and government agencies.

Findings/Gaps:

Data Curation/Annotation/Standardisation:

A variety of experimental data for *in vitro* and *in vivo* assays evaluating their potential environmental and human health effects have been generated in vast quantities by many nano projects; requiring data extraction, analysis, and sharing for purpose of SbD guidance of the manufacturing and use of nanomaterials. Great progress has been made to gather the data from experimentalists into structured databases that can be used by computational modellers to predict nanomaterial properties, exposure and hazard values that will support regulatory actions. However, challenges are continuing on how the data is curated, managed, standardised, annotated and made FAIR for sharing with the modellers and regulators. E.g. nanomaterial entities (composition, physicochemical properties, and biological activities of the nanomaterials) in some of these databases exist as text outputs extracted directly from publications, ignoring nanostructure annotations that are critical for modelling studies. As a result, variables (e.g. physicochemical properties) used in previous modelling studies were mostly experimentally generated. Without nanostructure annotations, diverse structural information for predictive modelling and other research such as nanostructure analysis and visualization cannot be performed. The key to building such FAIR datasets of nanomaterials is nanostructure annotation with the meta-data; a computer friendly format for encoding the information. Which could be stored and interpreted by different tools and resources in a standardised fashion. Different standard formats (e.g. JSON and ISA-TAB-Nano) are specially designed in several nanomaterial databases, such as eNanomapper and NanoReg, to store

and manage the curated nanomaterial data. Suggestions will be provided in Task 3.2 for the use of standardised tools and data sources to make data standardised and annotated with meta-data.

Data Quality for modelling & risk assessment:

Data quality and data integrity is essential for any data to be useful for analysis and modelling. There have been some shortcomings in the quality of the data of some of the older projects due the way data was collected and not standardised. Work is ongoing to implement the data quality criteria at the time of data collection to assess the quality of the collected datasets. Quality scoring mechanisms are being implemented for existing and newly collected datasets in the data resources to inform the data quality for SbD purposes. Suggestions will be provided in the Task 3.2 for the implementation and use of quality criteria for the newly generated and existing datasets.

Data Accessibility:

Another critical issue is proprietary data and embargos of the datasets generated by the nano projects. Accessible means that data is always available and obtainable. Even if the data is restricted, the metadata should be open. Data should be made accessible by ensuring that it is retrievable online using standardised protocols and has restrictions in place if necessary. It is important to note that not all data has to be made open. Data needs to be as open as possible for allowing data exchange and reusability between researchers, institutions, organisations and countries.

FAIR Data:

FAIR principles provide guidance to scientific community for data management and stewardship of the data. These principles emphasise on machine action-ability (i.e. ability of computational systems to find, access, interoperate, and reuse data with none or minimal human intervention). We will provide general suggestions in Task 3.2 for making data FAIR and re-useable both by humans and machines.

3.5 Models (*in silico* and grouping/read-across approaches)

A shift away from the use of traditional animal models for practical as well as ethical reasons has found governing bodies such as ECHA encouraging applicants to use other means before deciding on any testing on vertebrate animals. The approaches utilised to achieve this goal are the use of read-across, data sharing, QSAR (largely for bulk materials) and dissemination of alternative non-animal testing methods. REACH identifies grouping as a reasonable approach where technically and scientifically justified, and additional guidance on QSAR and grouping as well as nanoforms applicable to this guidance are available from ECHA[11,12] and the OECD.[13,14] The guidance provided by ECHA states read-across is *a technique for predicting endpoint specific information for one nanoform (or set of nanoforms), by using data on the same endpoint from another form of the substance*. Effectively, the aim of read-across is to establish a correlation between a trend in the physicochemical properties of a set of nanoforms and a trend in hazard endpoint responses. It is worth noting that although read-across approaches must be justified for each endpoint assessed, the same justification may be used for various endpoints. A stepwise approach to grouping and read-across, as outlined in Figure 4, is

[11] ECHA, "Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals," [Online]. Available: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9 (Accessed Feb 2021)

[12] ECHA, "Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals" Available at: https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf/71ad76f0-ab4c-fb04-acba-074cf045eaaa (Accessed Feb 2021)

[13] OECD, "OECD Series on Testing and Assessment, No. 194. Guidance on grouping of chemicals, second edition ENV/JM/MONO(2014)4"," 2014. [Online]. Available: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en (Accessed Feb 2021)

[14] OECD, "OECD Series on the Safety of Manufactured Nanomaterials, No. 76. Grouping and read-across for the hazard assessment of manufactured nanomaterials, Report from the expert meeting ENV/JM/MONO(2016)59"," 2016. [Online]. Available: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)59&doclanguage=en (Accessed Feb 2021)

recommended by ECHA. Relevant to Step 2 of the ECHA stepwise strategy, the following parameters were identified as important for the grouping of nanoforms:

- Solubility (including dissolution rate)
- Hydrophobicity
- Zeta potential
- Dispersibility
- Dustiness (most relevant for dry powders)
- Biological (re)activity (e.g. redox potential, radical formation)
- Photoreactivity

However, it is identified that physicochemical parameters would also play in important role here, an example given was for fibre-like materials, where rigidity and hardness of the material would be important considerations for an inhalation hazard assessment. Some grouping approaches, such as NanoReg2, identify calculated descriptors of nanoform characterisation as important considerations when determining "where they go" and hence, subsequently "what they do" (i.e. fate/toxicokinetics and hazard, respectively). To do this, nano-QSAR models are utilised, with various examples given in the paper by Giusti *et al.* (2019).[15]

A brief review by Pikula *et al.* (2020)[16] outlined the bioinformatics and computational approaches currently being developed and optimised for use in nanotoxicology prediction. These include neural networks, random forest, Nano-QSAR, Bayesian networks, k-nearest neighbour, linear regression, and Support vector machines. Although these techniques are promising, one major drawback is data quality and reliability, hence the success of incorporating bioinformatics and computational approaches into the safety assessment of nanoforms largely relies on development of data repositories to validate the findings. In 2018, Haase *et al.* developed the EU US Roadmap Nanoinformatics 2030, which discusses many of the challenges and future work required in the field.[17]

Much work has been, and is continually being conducted to facilitate the use of grouping and read-across in the risk assessment and classification of nanoforms, including contributions from EU projects such as NanoReg2[18] and GRACIOUS[19] in grouping strategies, and PATROLS,[20] NanoInformaTIX,[21] SmartNanoTox[22] and NanoSolveIT[23] which either include or are specifically for the development of *in silico* approaches. SAbyNA can build upon the approaches used in projects such as GRACIOUS by using the rationale and IATAs (Stone *et al.* 2020)[24] to assess whether NFs can be grouped to allow hazard predictions based on limited experimental data (e.g. through read-across of DNEL, PNEC values, Section 5.11), and will need to account for the outcomes of these projects and carefully consider their up-to-date approaches when appropriate for the SAbyNA hazard assessment strategy.

For example, the GRACIOUS framework guides the user through a set of pre-defined hypothesis covering the full life-cycle of a nanoform, information on physicochemical properties, human and environmental release, fate, exposure and hazard. This information informs then the environmental compartment specific IATAs, e.g. dissolution of a nanoform allowing for read across to the ionic form if it is a fast dissolving NF or flagging potential

[15] Giusti, Anna & Atluri, Rambabu & Tsekovska, Rositsa & Gajewicz-Skretna, Agnieszka & Apostolova, Margarita & Battistelli, Chiara & Bleeker, Eric A. J. & Bossa, Cecilia & Bouillard, Jacques & Dusinska, Maria & Gómez-Fernández, Paloma & Grafström, Roland & Gromelski, Maciej & Handzhiyski, Yordan & Jacobsen, Nicklas & Jantunen, Paula & Jensen, Keld & Mech, Agnieszka & Navas, Maria & Haase, Andrea. (2019). Nanomaterial grouping: Existing approaches and future recommendations.

[16] Pikula, Konstantin, et al. "Risk assessments in nanotoxicology: Bioinformatics and computational approaches." *Current Opinion in Toxicology* 19 (2020): 1-6.

[17] Haase A, Klaessig F: EU US roadmap Nanoinformatics 2030. 2018. released 15 November 2018, https://doi.org/10.5281/zenodo.1486012

[18] NanoReg2, European Union's Horizon 2020 research and innovation program, Grant agreement No 646221, http://www.nanoreg2.eu/

[19] GRACIOUS, European Union's Horizon 2020 research and innovation program, Grant agreement No 760840, https://www.h2020gracious.eu/

[20] PATROLS, European Union's Horizon 2020 research and innovation program, Grant agreement No 760813, https://www.h2020gracious.eu/

[21] NanoInformaTIX, European Union's Horizon 2020 research and innovation program, Grant agreement No 814426, https://www.nanoinformatix.eu/

[22] SmartNanoTox, European Union's Horizon 2020 research and innovation program, Grant agreement No 686098, http://www.smartnanotox.eu/

[23] NanoSolveIT, European Union's Horizon 2020 research and innovation program, Grant agreement No 814572, https://nanosolveit.eu/

[24] Stone, Vicki, et al. "A framework for grouping and read-across of nanomaterials-supporting innovation and risk assessment." *Nano Today* 35 (2020): 100941.

biopersistency in case of a very slowly dissolving NF (e.g. in aquatic media) potentially leading to long-term toxicity in sediments and hence suggesting potential for read across from data of other NFs on accumulation and toxicity in benthic species but not aquatic pelagic organisms. It is the aim of GRACIOUS to provide guidance for integrating relevant parts of the GRACIOUS framework into other tools, including relevant structures, rules/decision logic, endpoints, assays used etc. This will be extremely useful within development of the SAbyNA project.

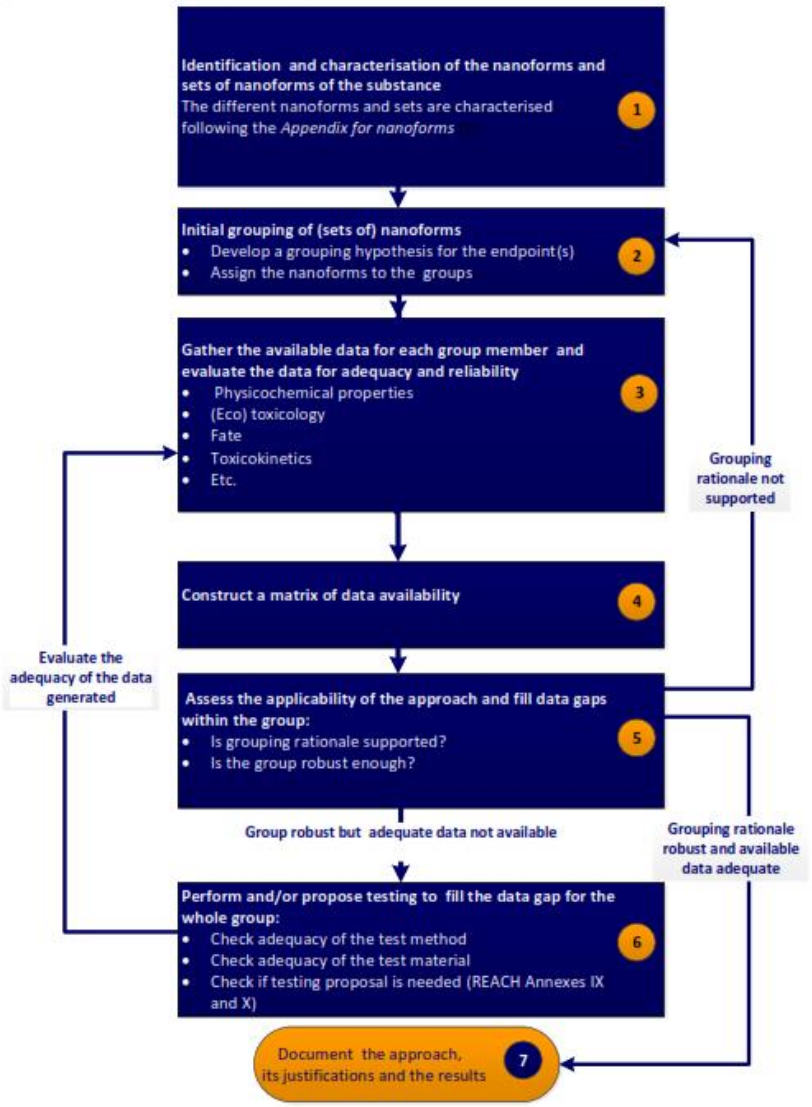

Figure 4 ECHA Scheme of the stepwise strategy for grouping of (sets of) nanoforms

4. Identification of gaps or barriers to the usability or performance of selected resources

4.1 Considerations of uncertainty

There is a high level of variability and inconsistency in much of the hazard related data surrounding engineered nanomaterials, largely due to it being an emerging technology and its multitude of forms, this leads to a high uncertainty in how to correctly interpret information and predict an effect. This is considerably important for the RA approach used in SbD application, and there may be different requirements for the assessment of uncertainty dependent on the needs of the RA process. For example, in the stage-to-gate analysis by the

caLIBRAte project, the requirements for uncertainty characterization were considered. Scoping (or preliminary assessment) requiring none, building a business case required qualitative, R&D (or development) requires qualitative in assessing the decision outcome, but quantitative when considering gaps in input parameters, testing, validation and product launch require quantitative.[25]

The purpose in using an 'uncertainty analysis' is to identify which uncertainty or variation in the information being processed should be acknowledged and communicated (Hristozov 2016).[26] Incorporating uncertainty helps in not underestimating a hazard, and is specifically required to help RA tools satisfy regulatory needs of ENM, as is suggested by Hristozov[27] who used multi-criteria decision analysis (MCDA) and weight of evidence (WoE) to reduce the uncertainty in decision making. There are various methods used for 'uncertainty analysis' already used in RA of nanomaterial, including quantitative uncertainty assessment such as probabilistic Monte Carlo[28] sensitivity and uncertainty analyses, which has also been incorporated into a stochastic multicriteria acceptability analysis by Tervonen *et al.* (2009)[29].

Of the tools we have selected for their applied hazard prediction, only a few address uncertainty. ANSES applies additional hazard increments based on the uncertainty of using a bulk material in place of its nanoform, when using CLP criteria. CB nanotool applies an additional hazard band when information is missing, and "unknown" must be entered (a precautionary approach), as is the case for LICARA NanoSCAN and SPM (partially based on Tervonen *et al.* (2009)) (by increasing scores based on completely or partially lacking information). GUIDEnano uses uncertainty as a factor, which incorporates scoring to provide a scaling factor to ensure that risk assignment is conservative. When assessing already existing individual toxicity studies from literature the hazard assessment strategy of GUIDEnano involves the establishment of these scores to inform on quality (i.e. how good and reliable a study and its reporting is), relevance (i.e. how relevant the NM study is for the respective environmental compartment or human pathway/endpoint) and similarity (i.e. how well does the NM exposure in the study to be used reflect the exposure relevant form of nanomaterial which is being assessed). These scores are used to select studies that can be included in the process to derive safety limit values. For example, a scoring system related to test design and reporting considerations was developed following the principles of the Klimisch score (K scores, which make distinctions of i) reliable without restrictions, ii) reliable with restrictions, or iii) not reliable). Three lists of questions were specifically addressed for *in vivo*, *in vitro*, and for environment (terrestrial, marine and freshwater compartments, marine and freshwater sediments as well as sewage treatment plant environments[30]). While SUNDS applies probabilistic models to account for uncertainty, as described by Pizzol *et al*. 2018. It is unclear what steps, if any, NanoSafer takes against uncertainty. In the case of SSWD, the limited statistical stability for the lower limit computations at 50% confidence interval produced unrealistic values below zero which indicate the poor consistency of the model input data, and which may reflect poor experimental performance or poor material characterization and preparation. Unfortunately, neither the predicted environmental concentrations nor the species sensitivity distributions reflect validated results (Gottschalk et al.[31]).

When considering the approaches used by these tools, whether quantitative or qualitative, it is unclear how this will be sufficient for SbD, as a lack of data may be provide a great contribution to uncertainty for early stage innovation and when gaps in data are found, or data found to be unreliable, we cannot assume a worst case scenario. Therefore, it will be important to suggest the user fills this gap, or at least provide the knowledge of how to do so; a well-structured hazard assessment strategy will ensure that sufficient information/advice can be provided to lower this uncertainty to allow enough sensitivity for SbD.

[25] Franken, Remy, et al. "Ranking of human risk assessment models for manufactured nanomaterials along the Cooper stage-gate innovation funnel using stakeholder criteria." *NanoImpact* 17 (2020): 100191.
[26] Danail Hristozov, 2016. Frameworks and tools for risk assessment of manufactured nanomaterials. Environment International, 95.
[27] Hristozov 2012. Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology, 6(8):880–898
[28] Lisa Pizzol, Danail Hristozov, Alex Zabeo, Gianpietro Basei, Wendel Wohlleben, Antti Joonas Koivisto, Keld Alstrup Jensen, Wouter Fransman, Vicki Stone, Antonio Marcomini,SUNDS probabilistic human health risk assessment methodology and its application to organic pigment used in the automotive industry, NanoImpact, Volume 13, 2019, Pages 26-36
[29] Tervonen, T., Linkov, I., Figueira, J.R. *et al.* Risk-based classification system of nanomaterials. *J Nanopart Res* **11,** 757–766 (2009). https://doi.org/10.1007/s11051-008-9546-1
[30] Environ. Sci.: Nano, 2018, 5, 381
[31] Environmental Toxicology and Chemistry, Vol. 32, No. 6, pp. 1278–1287, 2013

4.2 Thresholds and sensitivity

It is the goal of WP3 to be able to distinguish between, at times, small changes in a nanoform or NEP, which in turn may only present small changes in the biological response. The crucial factor when interpreting these changes will be how meaningful the change is, i.e. what is the extent of change required to incur a change in overall hazard prediction. This raises the question of what thresholds are in place within the current RA tools, in terms of what constitutes a move to a higher hazard integer, as well as what level of hazard is represented by specific levels observed in individual endpoints, and how these can impact on the overall sensitivity of the assessment.

There is a clear distinction in i) tools which provide abrupt thresholds, and ii) tools which build an overall score. For i), thresholds are abruptly crossed, whereby inviting a decision to move to a particular hazard band or continue through a hazard assessment, this provides clarity in decision making; in ii), tools build an overall score based on cumulative assessment incorporating numerous input parameters.

i) Tools which provide clear “cut-off” parameters include ANSES, NanoRiskCat, Stoffenmanager nano, Stoffenmanager Dermal,
ii) Cumulative scoring is utilised in CB Nanotool, NanoSafer, and Swiss Precautionary Matrix

4.2.1 Thresholds used in evaluated RA tools

For the tools identified in the distillation analysis, further investigation of the thresholds utilised in their hazard assessments is detailed in Table 5.

Similarities were identified in the descriptors used in the various RA tools, whereby the most common properties of interest to the tools included identification of fibrous material, solubility, reactivity, and assignment of toxicity with regards to CLP classification. Many of the thresholds utilised are based on previously established risks to human health (e.g. WHO fibre classification, HARN paradigm, COSHH). However, in some instances, thresholds are based on less well-defined or even arbitrary values. Determining the relevance of the various threshold values will be a focus for WP3 and further discussion of this with regards to each descriptor/particle physicochemical property is included in Section 4.2.1.

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Table 5 Thresholds or sensitivities identified for each assessed RA tool and the respective justification given

Tool	Descriptor	Threshold(s) identified	Justification
ANSES	Biopersistent fibre	No threshold, all considered highest hazard band	It is highlighted that although the definition is qualitative, it is very important as the occupational health literature seems to suggest that all respirable and biopersistent fibres should be treated as asbestos unless evidence to the contrary is obtained. Hence, they state the full hazard assessment required.
	Particle dissolution	>1 hr = +1 hazard band	This is based on evidence that some insoluble nanoparticles may penetrate in the epithelial cells and deeper in lung tissues within one hour of exposure. Inhalation was identified as the major route of unwanted exposure in this tool.
	Reactivity (surface chemistry, ROS, RNS)	Higher than bulk material or analogous material	Deemed important because a material with a higher specific surface area is expected to have a higher reactivity than a material of the same chemical composition but with a lower specific surface area
	CLP Hazard banding	See Figure 5	Hazard group allocation of the e-COSHH Essentials tool.
CB Nanotool	Surface Chemistry	High: 10 pts, Medium: 5 pts, Low: 0 pts, Unknown: 7.5 pts	No guidance on how to distinguish between high/medium/low.
	Particle Shape	Tubular or fibrous: 10 pts, Anisotropic: 5 pts, Compact or spherical: 0 pts, Unknown: 7.5 pts	Tubular structures, like carbon nanotubes, have also been shown to cause inflammation and lesions in rat lungs. Based on this information, the highest severity score is given to fibrous or tubular-shaped particles. Particles with irregular shapes (other than tubular or fibrous) are given a medium severity score because they typically have higher surface areas relative to isotropic (e.g. compact or spherical) particles.
	Particle Diameter	1-10 nm: 10 pts, 11-40 nm: 5 pts, <41-100 nm: 0 pts, Unknown: 7.5 pts	Based on the ICRP curve i.e. chance of depositing in the lungs (regardless of the region of deposition) and the fact that smaller particles have a higher overall surface area compared to larger particles for a given mass concentration.
	Solubility	Insoluble: 10 pts, Soluble: 5 pts, Unknown: 7.5 pts	No guidance on the cut-off of soluble/insoluble.

Tool	Descriptor	Threshold(s) identified	Justification
	Carcinogenicity	Yes: 6 pts, No: 0 pts, Unknown: 4.5 pts	No guidance on these descriptors but presumably based on CLP regulation.
	Reproductive Toxicity	Yes: 6 pts, No: 0 pts, Unknown: 4.5 pts	
	Mutagenicity	Yes: 6 pts, No: 0 pts, Unknown: 4.5 pts	
	Dermal Toxicity	Yes: 6 pts, No: 0 pts, Unknown: 4.5 pts	
	Asthmagenicity	Yes: 6 pts, No: 0 pts, Unknown: 4.5 pts	
LICARA nanoSCAN	**Hazard assessment utilises SPM, Stoffenmanager-nano and NanoRiskCat, which are all discussed separately.**		
ECETOC-TRA tool	**ECETOC-TRA tool is excluded from further consideration, although it does provide input of data we classified in MS3.1, its use of such information is limited and better represented in other tools.**		
MEASE	**MEASE tool is excluded from further consideration, as does not provide hazard prediction, only exposure.**		
NanoRiskCat	HARN	Length to diameter aspect ratio greater than 10 to 1. The diameter of the fibres must be thin enough pass ciliated airways; The length must be long enough to initiate the onset of e.g. frustrated phagocytosis and other inflammatory pathways; The nanomaterials must be biopersistent.	Justification is whether material fulfils HARN paradigm.[32]

[32] CL Tran, SM Hankin, B Ross, RJ Aitken, AD Jones, K Donaldson, V Stone, R Tantra. An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. Report on Project CB0406. August 13, 2008

Tool	Descriptor	Threshold(s) identified	Justification
	CLP regulation	Level A if bulk form of NM is: 1. Acute toxicity category 1-4 2. Germ cell mutagenicity category 1A, 1B or 2 3. Carcinogenicity category 1A, 1B or 2 4. Reproductive toxicity category 1 A, 1B or 2 5. Specific target organ toxicity - single exposure category 1 or 2 6. Specific target organ toxicity - repeated exposure and category 1 or 2 7. Aspiration toxicity category 1 8. Skin corrosion/irritation category 1A, 1B or 1C 9. Serious eye damage/irritation category 1 10. Respiratory and skin sensitization category 1 Level B if bulk form of NM is classified to: 1. Skin corrosion/irritation category 2 2. Specific target organ toxicity-single exposure category 3 3. Serious eye damage/irritation category 2	Level A: Based on knowledge of whether the bulk form of the nanomaterial known to cause or may cause serious damaging effects, i.e. is the bulk form classified according to CLP regulation with regard to one or more serious health hazards (irreversible effects). Level B: As above but for other less severe adverse effects according to the CLP (reversible effects). CLP Legislation (ECHA 2013). This read-across approach is assuming that nanomaterials will not be less hazardous than the bulk form.
	Acute tox	For oral and dermal acute toxicity estimates (based on LD50/LC50 when available), the acute toxicity cut-off has been chosen to be 2000 mg/kg. For dusts and mists (solid particles and liquid droplets in a gas) the acute toxicity estimate cut-off has been set to 5 mg/kg.	The cut-off values chosen to determine the toxicity of a nanomaterial are similar to the acute toxicity hazard category 4 in CLP (EP and CEU 2008).
	Genotoxic-, mutagenic, carcinogenic, respiratory, cardiovascular, neurotoxic or reproductive effects	As a general rule the answer would be "yes" if there are indications from epidemiological- and/or *in vivo* studies that indicate or confirm one or more of these effects. The answer would be “maybe” in cases where there is conflicting evidence and no reasonable explanations for why studies differ. The alternate case is if the bulk form of the nanomaterial has a classified as a: • Skin corrosion/irritation CLP category 2 • Specific target organ toxicity-single exposure CLP category 3 • Serious eye damage/irritation CLP category 2	The tool highlights that the process by which the colour code (hazard band/score) is assigned to human hazards associated with the nanoform of a given material is based primarily on scientific expert judgment and a holistic assessment of the evidence of mutagenicity, carcinogenicity, respiratory toxicity, etc. As expert interpretation of scientific literature vary, so can the conclusion reached and the human hazard colour code assigned to nanomaterial.
	Toxicity to environmental species	For environmental species toxicity (based on LC50/EC50 when available), the toxicity cut-off have been chosen to be 10 mg/L and 100 mg/L.	Toxic (1–10 mg/L) or very toxic (<1 mg/Ll) to aquatic organisms (ECHA 2013).
	Ecosystem effects	The answer would be ‘yes’ if there are indications of potential ecosystem effects (e.g., through oxygen, depletion, effects on nutrient balance, shifts in populations) or effects on global scale (e.g., ozone depletion, or global warming potential).	There is historical evidence that the mere fact that a substance or material is novel is a good indicator of potential harm that has yet to become discovered (EEA 2001). Novel would in this case be defined as materials that humans and environment have not previously been exposed to at any significant extent (RCEP 2008).
	Novelty of NM.	the answer would be "yes" if the NM is defined as materials that humans and environment have not previously been exposed to at any significant extent (RCEP 2008); new material, new form, new application, new pathway.	There is historical evidence that the mere fact that a substance or material is novel is a good indicator of potential harm that has yet to become discovered (EEA 2001).
NanoSafer	“Nanorelevance”	Insoluble (<1 g/L)/Soluble	No justification found for the value of 1 g/L for solubility.

Tool	Descriptor	Threshold(s) identified	Justification
	Is the NM a HARN?	Length of > 5 µm, diameter of < 3 µm and length/diameter > 3 If yes then maximum hazard score (H_{tot} = 1.00)	No justification given but likely to be based on WHO classification and fibre toxicity paradigm.
	Is the material chemically surface-modified (coated / functionalized)?	If yes then additional scoring to hazard band (0.45)	Given higher risk as higher uncertainty in toxicological effect.
	Is the OEL for the analogue bulk material <1mg/m^3 OR Nanospecific OEL (OELnano)	<1mg/m^3 then H_{OEL} = 0.25 >1mg/m^3 then H_{OEL} = 0.20	No justification for this threshold could be found.
	Risk sentences OR GHS/CLP hazard statements	Risk sentences/hazard statements are ticked but no indication if these are weighted.	Based on classical COSHH and CLP regulation.
Swiss Precautionary Matrix (SPM)	Nano relevance	The primary particles (in the free or bound state, as an aggregate or agglomerate) will be considered by the tool if at least 50% of number size distribution is in nanosize (one or more exterior dimensions) or specific surface/volume of primary particles if over 60 m/cm^3. Fullerenes, graphene flakes and single wall carbon nanotubes even with dimensions of less than 1 nm are also included. Stability of agglomerates or aggregates over >500 nm is evaluated in the body or under environmental conditions, with a potential inhalation hazard for agglomerates between 500nm and 10µm.	Integration of EU's proposed definition (now integrated in REACH) and precautionary approach. A nanoparticle's stability in the body is important for assessing the need for precautionary measures to protect health, while stability under ambient conditions is important for assessing the need for precautionary measures for the environment.
	Reactivity	Acellular reactivity, biological oxidative damage, redox-activity*, photocatalytic activity, cellular reactivity: induction of inflammatory reactions, oxygen radical formation, GSH depletion, protein carbonylation;100% was assigned to the highest measured value in a particular study. X=obtained values. X≤10%= low reactivity/toxicity= 1 pt 10%≤X≤60%=medium reactivity/toxicity= 5 pts X≥60% = high. Reactivity/toxicity=9 pts *redox-activity of metal oxides NM; conduction band energy overlaps with the redox potentials of cells (-4.12 to -4.84 eV) were regarded as "high" reactive (5 points), all other ones as "low" reactive (1 point)	Chosen on the basis of various studied that compared different effects of nanomaterials measured using cell-free or cellular methods with their *in vivo* effects. The parameters listed were also chosen in the light of the currently known mechanisms of action that are relevant for the adverse effects of nanomaterials on organisms.[33] A differentiation ought to be made between the stability in biotic and abiotic systems when stability in environmental matrices is evaluated. Coating stability should be considered at different life stages of NMs; coating stable= only coated NM should be evaluated, or coating that totally dissolved rapidly= only uncoated NM should be evaluated, or partial dissolution of coating= both coated and uncoated NM should be evaluated.
	Stability in human body	Hours = 1 pt Days-weeks = 5 pts Months = 9 pts	

[33] A. Nel et al.: Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high-throughput screening; Accounts of chemical research (2013), 3, 607-621

Tool	Descriptor	Threshold(s) identified	Justification
	Stability under environmental conditions (half-life)	Hours = 1 pt Days-weeks = 5 pts Months = 9 pts	
Stoffenmanager-nano	Nano relevance	<0.1 g /L	Particles with high water solubility (> 0.1 g/L) are not considered in this tool because their nano-specific properties are considered lost when particles are in solution.[34]
Stoffenmanager-nano	Fibres/fibre like particles?	If fibre then increased hazard band. No guidance given on the classification of a fibre within the tool. However, in the corresponding article it is stated that these are defined as (insoluble) nanofibers exceeding a length of 5000 nm, with the other two dimensions in the nano-size range.[35]	Due to the uncertainty with regards to nanofibres, this classification is based on the paradigm that all insoluble fibres thinner than 3 µm and longer than 20 µm are biopersistent in the lungs and therefore highly hazardous.[36]
	Inhalation hazard	Mutagenic (and possibly carcinogenic) and/or sensitizing = Band E, Carcinogenic (not mutagenic) reprotoxic and/or very toxic = Band D, toxic corrosive and/or respiratory allergens = Band C, harmful and/or irritating = Band B, non-hazardous = Band A	Based on the classical Stoffenmanager tool, however the literature identifies that currently this information is unlikely to be available and therefore the user is directed to the next descriptor.
	Hazard of most widely used MNO's	If listed then assigned based on available data (this is largely based on the size threshold of 50 nm or the crystallinity of silica). A list of widely used MNOs has been published,[37] which was based on lists of MNOs reported by the Organisation for Economic Co-operation and Development (OECD) and Borm *et al.* (2008).[38]	The cut-off of 50 nm was chosen as an arbitrary—size criterion and was introduced to take into account the likelihood of nano-specific health effects. Particles approaching "upper non-nano-size range", i.e. >50 nm are thought to have reactivity analogous to their bulk counterparts, whereas smaller particles sizes would have increased reactivity. Additionally, lung deposition was considered higher, and hence more important for smaller particles.
	Risk phrases of parent material	Classified with one or more of the following R-phrases: R40, R42, R43, R45, R46, R49, R68 Yes = Hazard band E No = Hazard band D	Precautionary principle based on parent material.
GUIDEnano	Fibres	Length of > 5 µm, diameter of < 3 µm and a length-to-diameter ratio of > 3:1	WHO classification. No additional justification given but likely to be based on fibre toxicity paradigm.
	Solubility	Soluble = > 100 mg/L, slightly soluble = 1 – 100 mg/L, and insoluble = <1 mg/L	
	Reactivity	Reactive or non-reactive (no additional information given although presumably this is CMAR and based on BSI classification of materials?).	
SSWD	Acute and chronic toxicity	No effect levels were considered chronic from an experiment duration of ; 72 h and more for algal	Chronic toxicity for aquatic environments is defined as reflecting exposure that covers at least 1 complete life cycle or at least 1 sensitive life stage of

[34] Oberdörster G. (2002) Toxicokinetics and effects of fibrous and nonfibrous particles. *Inhal Toxicol*; 14: 29–56. Review. PubMed PMID: 12122559
[35] Birgit Van Duuren-Stuurman, Stefan R. Vink, Koen J. M. Verbist, Henri G. A. Heussen, Derk H. Brouwer, Dinant E. D. Kroese, Maikel F. J. Van Niftrik, Erik Tielemans, Wouter Fransman, Stoffenmanager Nano Version 1.0: A Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects, *The Annals of Occupational Hygiene*, Volume 56, Issue 5, July 2012, Pages 525
[36] Donaldson K. (2009) The inhalation toxicology of p-aramid fibrils. Crit Rev Toxicol; 39: 487–500
[37] Rijksinstituut voor Volksgezondheid en Milieu. (2010) Tijdelijke nano-referentiewaarden Bruikbaarheid van het concept en van de gepubliceerde methoden. RIVM Rapport 601044001/2010. Bilthoven, The Netherlands.
[38] Borm P, Houba en R, Linker F. (2008) Omgaan met nanodeeltjes op de werkvloer: survey naar goede praktijken in omgaan met nanomaterialen in de Nederlandse industrie en kennisinstellingen. Heerlen, Netherlands: Hogeschool Zuyd.

Tool	Descriptor	Threshold(s) identified	Justification
		21d and more for vertebrates and invertebrates. 28 d and more when based on reproduction; and 21-d and more when only cocoon production was tested for earthworms. 24 h and more for bacteria.	the tested organisms[39]. For soils, long-term limits adapted to Organization for Economic Cooperation Development guidelines were defined[40].
	Species sensitivity distributions	The collected raw data on biological responses (i.e. Mortality, inhibition of growth, reproduction) are converted into species sensitivity values, beneath which long-time negative effects for the organisms may be excluded. The uncertainty of the short- to long-term effect of extrapolation was reflected by a factor of 10. Tools extrapolates from various ENM effects to the standardized no-observed-effect concentrations; Factor of 10 was used to derive no-observed-effect concentrations from L(E)C50, highest-observed-no-effect concentrations, and minimum inhibitory concentrations. Factor of 2 was used to derive the lowest-observed-effect concentration and lethal concentration (L[E]C10–20).	Brix et al., 2001[41] reviewed acute to chronic ratios for different taxonomic groups and aquatic species sensitivity distributions and found (when neglecting extreme outliers) an average of around 12, which supports the REACH recommendation (2008) of a factor of 10[42]. REACH recommendation (2008) Some studies confirm factors in this range for several organisms; see ref 41to 46 from Gottschalk et al., 2013[43].
SUNDS	**Tier one is based on LICARA Nanoscan and SSWD which are discussed separately**		
	Tier two assessment of DNEL or POD	LCL or UCL value determined using APROBA and the DNEL value you insert or compute in the tool.	

[39] European Commission. 2011. Common implementation strategy for the water framework directive. 2000/60/EC. London, UK.
[40] Organization for Economic Cooperation Development. 2000. OECD guidelines for the testing of chemicals. Guidelines 208, 216, 217, 222. Paris, France.
[41] Brix KV, DeForest DK, Adams WJ. 2001. Assessing acute and chroniccopper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. Environ Toxicol Chem 20:1846–1856.
[42] European Chemicals Agency. 2008. Guidance on information requirements and chemical safety assessment. Helsinki, Finland.
[43] Environmental Toxicology and Chemistry, Vol. 32, No. 6, pp. 1278–1287, 2013

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4.2.1 Interpretation of sensitivity of evaluated RA tools

When sufficient background information has been provided within RA tools for certain descriptions, it is possible to identify what level of sensitivity can be expected.

Particle Shape

With the exception of the SPM, all evaluated RA tools incorporate an assessment of fibres in the hazard scoring. These vary within the tools from simply stating if the material is a fibre (Stoffenmanager-nano, CB nanotool), if it can be classified as a HARN (Nanosafer, NanoRiskCat, GUIDEnano) or if it is a biopersistent fibre (ANSES). The difference between these classifications is important when considering sensitivity in SbD. On closer inspection of the tools, many state that by 'HARN' they are specifying biopersistent fibres (e.g. Stoffenmanager-nano), however clarity of this distinction within the tools is lacking. Incorporation of 'HARN' descriptors in the tools is commonly in reference to the HARN paradigm. An in-depth report on this by Lang *et al.* (2008) was referenced in the corresponding literature of Stoffenmanager-nano (see Table 5). This report highlights that additional to length and aspect ratio, the durability of fibres is of great importance for this paradigm as any fibre breakage would ultimately lead to the same clearance mechanism as short fibres, in which the paradigm no longer holds true. Therefore, to provide the required sensitivity for this descriptor, users must be properly informed on what constitutes as HARN and what are the best methods to follow (e.g. choice of suitable simulated body fluids for animal-free assays, length of test, suitable measurement techniques). As pathogenicity is already linked to long biopersistent fibres, it would be sufficient for SbD approaches to regard all long biopersistent fibres as highly hazardous, but we need to ensure that suitable thresholds for persistence are selected to allow for distinction.

The CB nanotool also incorporates other particle shapes in their scoring; assigning particles with irregular shapes (other than tubular or fibrous) a higher severity score than isotropic (e.g. compact or spherical) particles because they typically have higher surface areas. This justification would suggest that a better descriptor to use would be surface area itself, rather than inferring this from the typical particle shape. However, using surface area to infer surface-driven effects can cause some issues, particularly when particles are porous, as porosity would increase the surface area dramatically but may have little to no effect on toxicity. Hence, one would suggest that both surface area and porosity should be considered to allow a deeper understanding of the driving effect of particle reactivity and dissolution. This information would allow us to determine the SbD strategy required to reduce particle toxicity.

Particle size

Particle size is assessed in CB nanotool, Stoffenmanager-nano, SPM, LICARA (as per SPM) and SUNDS (in tier 1 as per LICARA). The CB nanotool identifies three distinct categories 1-10 nm, 11-40 nm, and <41-100 nm in which the smaller particle sizes incur a greater hazard score. This classification of particle size is adapted from the ICRP curve i.e. chance of depositing in the lungs (regardless of the region of deposition) and the fact that smaller particles have a higher overall surface area compared to larger particles for a given mass concentration. Stoffenmanager-nano, however, uses the threshold of 50 nm when classifying the most commonly used nanomaterials. To justify this arbitrary value, the creators describe that as well as increased lung deposition, particles > 50 nm ("upper non-nano-size range") are thought to have reactivity analogous to their bulk counterparts, whereas smaller particles sizes would have increased reactivity. In SPM, LICARA (as per SPM) and SUNDS (in tier 1 as per LICARA), potential effects in the lungs are associated to the nanomaterials forming agglomerates in the range between 500 nm and 10 µm. A nano-specific hazardous threshold of 500 nm is thus considered for the agglomerate stability evaluation in both humans and for the environment, although there is no clear justification for applying this limit to the environment. Using particle sizes for descriptors in this way is regarded as useful as long as the values used are justified. Therefore, one may suggest that the values utilised in the CB nanotool would be more relevant, particularly for SbD, and that the

use of >2 options allow for better sensitivity when comparing two or more nanoforms. To improve the usefulness of this descriptor for SbD purposes, we must ensure that the threshold values are relevant for all possible exposure routes (i.e. not just based on risk of inhalation exposure).

Particle Dissolution

Dissolution and/or solubility descriptors were included in a number of RA tools evaluated. The dissolution threshold utilised in ANSES is 1 hour and was chosen to reflect the risk of particle penetration into lung tissues[44] and in the SPM, stability of a material is assessed in both the human body and in environmental conditions, with half-life thresholds of hours, days-weeks and months determined using the results of cell-free studies compared to *in vivo* effects. GUIDEnano investigates the solubility of a material by placing materials into three distinct groups of soluble, slightly soluble or insoluble (>100 mg/L, 1-100 mg/L or <1 mg/L respectively),[45] similarly Stoffenmanager-nano discriminates between soluble and non-soluble using the threshold of 0.1 g/L,[46] whereas the CB nanotool simply assesses solubility and does not give guidance on how to determine if a material is soluble and in which media. These tools take a very different approach in the use of dissolution data, which will undoubtedly impact the sensitivity of each analysis in regards to SbD.

One concern which is not addressed in these tools is the mode of toxicity. For each tool, short dissolution rates are considered less hazardous (i.e. lower scoring for shorter dissolution times), however in some instances the leaching of ions is the mechanism of toxicity of highest concern. Hence, when this is the case, hazard banding would be lower than it should be. In fact, it has been postulated that there are three distinct classes of soluble particles; of which suspension reactivity may depend on either the particle reactivity alone (slow ion release or lower reactivity of ions), the ion reactivity alone (higher concentration of ions than particles) or both particle and ions contribute significantly to reactivity.[47] This work also highlights an important consideration that the contribution of particles and ions to reactivity can vary depending on what assay is utilised, due to the conditions used (i.e. time and dose-range); hence, this may also impact on the sensitivity of reactivity assessment. Therefore, it is likely that, regardless of the tool and thresholds used, guidance will be required on what assay is most appropriate and which specific conditions need to be followed to gain an accurate representation of particle reactivity in order to be relevant to toxic effects.

It would seem that in order for this information to be useful, and to be sensitive enough for SbD, tools should include combined solubility and dissolution descriptors. Solubility is useful for inclusion to assess nano-relevance (i.e. whether toxicity mechanisms are particle-derived and hence, require nano-specific classification), and dissolution to inform on particle persistence in relevant biological compartments. A lack of guidance exists on assessing solubility of nanomaterials, and in fact ECHA highlight that common OECD protocols for determining solubility may not be applicable for nanomaterials as distinguishing between dispersion and dissolved particles can be difficult.[48] Hence, measurement of dissolution rate may be the most appropriate assessment for nanomaterials, as this would encompass both descriptors; although the dissolution rate constant would likely be a more descriptive unit to use as some materials may never reach full dissolution within the experimental parameters. Furthermore, minor differences in materials may only result in an incremental change in dissolution time (in this case, time to reach saturation rather than 100% dissolution), for example in work by ECOTOC on synthetic amorphous silica,[49] all materials reached saturation within 15 hours, however their respective saturation concentrations and rate constants varied from 1.91 - 2.51 mmol/L and 0.37 - 7.93 x10^{-9} mol/m²·s respectively.

[44] Geiser, M., Kreyling, W.G. Deposition and biokinetics of inhaled nanoparticles. *Part Fibre Toxicol* **7,** 2 (2010). https://doi.org/10.1186/1743-8977-7-2

[45] Values adapted from: Arts, J., M. Hadi, M.A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, et al., 2015. "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (DF4nanoGrouping)." Regulatory Toxicology and Pharmacology 71 (2): S1–S27. doi:10.1016/j.yrtph.2015. 03.007.

[46] Oberdörster, Günter. "Toxicokinetics and effects of fibrous and nonfibrous particles." *Inhalation toxicology* 14.1 (2002): 29-56.

[47] Peijnenburg, W.J.G.M.; Ruggiero, E.; Boyles, M.; Murphy, F.; Stone, V.; Elam, D.A.; Werle, K.; Wohlleben, W. A Method to Assess the Relevance of Nanomaterial Dissolution during Reactivity Testing. *Materials* **2020**, *13*, 2235. https://doi.org/10.3390/ma13102235

[48] ECHA Guidance on information requirements and chemical safety assessment, Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance, Draft (Public) Version 2.0, December 2016

[49] ECETOC. 2006. Synthetic Amorphous Silica. Joint Assessment of Commodity Chemicals (JACC) report No 51. Available at: https://www.ecetoc.org/wp-content/uploads/2014/08/JACC-051.pdf (Accessed Feb 2021)

Taking all these points into consideration, solubility is not yet considered at a level that would be appropriate for SbD approaches, as not only are the thresholds ill-defined (in terms of hazard effect) and as such a level of sensitivity should be defined according to the outcome you wish to predict, and it may be required that this is material-specific. As such, SAbyNA will need to devise a decision making strategy for solubility that i) identifies which solubility paradigm is of concern (e.g. toxicity of soluble ions or biopersistence), ii) which are suitable thresholds to place on solubility, and iii) the correct testing strategy to use, whether by read-across, laboratory testing etc.

Surface chemistry/reactivity

The reactivity of the material assessed is investigated in five RA tools; ANSES, CB nanotool, GUIDEnano, Nanosafer and SPM. In most cases the thresholds used are somewhat open to interpretation (as is the interpretation of reactivity, which is discussed in subsequent sections). In ANSES, the reactivity is compared to either the bulk or an analogous material, in CB nanotool it is simply assigned 'high', 'medium' or 'low' with no guidance on how to distinguish between these, similarly in GUIDEnano materials are classified as either reactive or non-reactive, and in SPM the reactivity is classified with respect to the highest reactivity measured in a particular study (whereby values <10% were classified as low, values ≥10% and <60% were classified as medium, and values ≥60% were classified as high). It seems likely that SAbyNA would need to adopt this level of sensitivity, incorporating multiple thresholds within any descriptor assessment, and with correct guidance on choosing/performing biological assays and how to distinguish between threshold levels, the sensitivity of this approach could well be suitable for the needs to SbD, in particular the CB nanotool and SPM, where 3 threshold options are available, allowing greater distinction between different nanoforms.

In one instance, the presence of surface coating is determined. If a surface coating is present, Nanosafer adds additional points to the hazard score. The justification for this is that the presence of a coating results in higher risk as there is higher uncertainty in the toxicological effect. However, in the SbD process, it is the impact of a surface coating which would require definition, and as such is not a useful descriptor to use in SAbyNA.

CLP regulation

Many of the tools assessed incorporate CLP regulation in their scoring systems (ANSES, CB nanotool, NanoRiskCat, NanoSafer, Stoffenmanager-nano), which is most likely taken from the more traditional COSHH RA. Although this approach would allow tools to have greater regulatory readiness, there is a concern that for nanomaterials the information regarding many of the necessary endpoints (i.e. carcinogenicity, mutagenicity, reproductive toxicity, etc.) is not currently available and users may be penalised either for not having this information or alternatively for having the information, this completely depends upon the scoring system utilised in the tool. To highlight this point, tools such as the CB nanotool would score a material 4.5 points per endpoint if this information is unknown, whereas if information is available the material would either be scored 6 or 0 points for a yes or a no answer respectively. For other tools such as NanoSafer, risk sentences/hazard statements are ticked by the user if relevant for the material, but there is no indication if these are weighted, therefore it is possible that the more classifications are known the greater the hazard score given. Unless SbD approaches can completely eradicate any seriously damaging effects (e.g. carcinogenicity, mutagenicity), it is unlikely that the use of such scoring systems would be sensitive enough for our purposes. One approach may be to assign methods that could be used to infer an effect relevant to CLP hazard statements, ensure that they are robust, and to provide guidance on how to conduct these tests.

Exposure limits or estimations

Both OEL and exposure limit estimations (i.e. LD50/LC50, BMD20) have been used in the tools assessed. OEL values are used as descriptors in NanoSafer, whereby users can choose to insert OEL values for the bulk material or an OELnano can be utilised, if available. The guidance given with the NanoSafer tool outlines a threshold value of 1 mg/m^3 for bulk OEL, however it is not clear if this is also the value used for OELnano's. In either case, there is limited justification given for this value, however one may assume this is based on traditional COSHH assessment tools. BSI recommendations vary from 0.5 x bulk WEL for soluble nanomaterial, 0.1 x bulk

WEL for CMAR nanomaterials to 0.066 x bulk WEL for insoluble nanomaterials.[50] Therefore, if we take the value of 4 mg/m^3 for respirable dust WEL,[51] the only way a material could be above the threshold of 1 mg/m^3 is if the material is soluble (i.e. behaves analogous to the bulk). Hence, nanomaterials will only be below this threshold if they are proven to be non-toxic, which is not suitably sensitive for the needs of SbD. In SUNDS, an LCL or UCL is computed in APROBA using a DNEL value (inserted by user or estimated using PoD values and Proast software). This methodology seems to allow the greatest level of sensitivity; however, the tool requires quite a high level of involvement and may be most suitable for a professional user unless well described guidance can be provided.

Threshold concentrations for environmental effects - species sensitivity distributions (SSD)

The species sensitivity distribution (SSD) approach is used in the nSSWD tool and in the SUNDS Tier 2 environmental exposure assessment (using the pSSD approach[52]). The SSD approach is widely used to derive environmental effects thresholds for substances for which ecotoxicity endpoints for a sufficient number of species points are available, although the minimum number of species for which data are required varies according to study/regulatory regime (see e.g.[53]). Ecotoxicity data are assessed for reliability before inclusion in the SSD dataset, with the highest reliability typically assigned to standardised endpoints related to, for example, mortality, growth and reproduction. Depending on the specific protection goal, requirements may exist for the ecological depth of the required ecotoxicity data; for example, data may be required to be drawn from a minimum number of species from each of a minimum number of taxonomic groups. The approach may be applied to either acute or chronic data, though it is not recommended to mix acute and chronic endpoints within a single SSD. Conversion the variety of effects (NOEC, LOEC, LCx, ECx) observed in either short or long terms to the standardized no-observed-effect concentrations may be conducted through the application of extrapolation factor following REACH recommendations (2008)[54]. More sophisticated methods such as the acute to chronic ratio approach or the acute to chronic transformation[55,56], this may allow a greater sensitivity in regards to SbD approach developed in SAbyNA project. Typically, the ecotoxicity data are processed to provide a dataset for the SSD comprising one endpoint concentration per species. Where multiple measurements of an endpoint are available for a species, these can be averaged to give a single value, or weighted (as in nSSWD). Where multiple endpoint types are available for a species (e.g. growth and reproduction), typically only the most sensitive is used.

A variety of parametric and non-parametric approaches can be used to derive a threshold concentration for the SSD dataset. These approaches can be generalised respectively as (i) fitting of a parametric statistical distribution to the data and taking a chosen percentile of that distribution as the threshold, and (ii) generation of a threshold as the mean of percentiles obtained by repeated sampling of the distribution dataset (bootstrapping). The nSSWD tool uses both approaches. The pSSD tool uses a non-parametric approach and furthermore constructs a statistical distribution of the endpoint concentration for each species, rather than using point values.

Approaches to computing the uncertainty in the threshold concentration may be applied when using either parametric or non-parametric approaches to its calculation. Parametric approaches have been proposed based on both classical and Bayesian statistics (e.g.[57]). An approach has also been proposed for ecotoxicity data

[50] British Standards Institution, 2007. British Standards Nanotechnologies – Part 2 Guide to safe handling and disposal of manufactured nanomaterials

[51] Health and Safety Executive (HSE), EH40/2005 Workplace exposure limits, January 2020. Available at: https://www.hse.gov.uk/pubns/priced/eh40.pdf (Accessed Feb 2021)

[52] F. Gottschalk, B. Nowack. "A probabilistic method for species sensitivity distributions taking into account the inherent uncertainty and variability of effects to estimate environmental risk". Integrated Environmental Assessment and Management, Volume 9, 2013; pages 79-86. https://dx.doi.org/10.1002/ieam.1334

[53] G.W. Suter, T.P. Traas, L. Posthuma "Issues and Practices in the Derivation and Use of Species Sensitivity Distributions". In: "Species Sensitivity Distributions in Ecotoxicology", L. Posthuma, G.W. Suter, T.P. Traas, pp.437–474, 2002. Lewis Publishers, Boca Raton, FL, U.S.A.

[54] European Chemicals Agency. 2008. Guidance on information requirements and chemical safety assessment. Helsinki, Finland.

[55] Duboudin C, Ciffroy P, Magaud H. 2004. Acute-to-chronic species sensitivity distribution extrapolation. Environ Toxicol Chem 23:1774–1785

[56] Brix KV, DeForest DK, Adams WJ. 2001. Assessing acute and chroniccopper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. Environ Toxicol Chem 20:1846–1856.

[57] T. Aldenberg, J.S. Jaworska. "Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions". Ecotoxicology and Environmental Safety, Volume 46, 2000, Pages 1-18.

based on QSARs, to deal with the uncertainty in such data[58]. Confidence intervals can thus be computed for threshold concentrations, which may be used to determine whether or different substances have significantly different thresholds.

In principle, given sufficient ecotoxicity endpoint data for multiple nanoforms of the same substance, the SSD approach could be applied to determine whether and to what extent the threshold effects concentrations for such nanoforms differ, and whether such differences are statistically significant. However, this imposes onerous data requirements given the need to generate sufficient ecotoxicity data for each nanoforms to fulfil the requirements of regulatory regimes, e.g. REACH[59]. If QSAR-type relationships were available, for example, to predict nanoform toxicity on the basis of factors such as particle composition and surface modification[60], these could be employed to gap-fill, however the gap between case-by-case studies (expensive and time-consuming) and sophisticated nano-QSAR models (not suitable for small datasets) requires the development of family-specific models[61]. Therefore, using the SSD approach for distinguishing small differences in environmental toxicity among nanoforms is likely to be severely limited by the data requirements, and more targeted assessment approaches may be appropriate for development or use in SAbyNA.

4.3 Outcome of RA tool considerations

From the assessment so far, although there is a strong justification of the considerations of many of these tools, individually they would not serve as suitable for the SAbyNA platform, and that our most pragmatic approach would be use the key elements identified above, and adapt them within the existing GUIDEnano tool to effectively address SbD needs.

In general, these adaptions would include the assignment of appropriate hazard descriptors with better specificity (e.g. the behaviour of a nanoform responsible for inducing a hazard response, or the induced response e.g. dissolution, oxidation potential, inflammatory reactions), with approaches designed to provide adequate sensitivity and appropriate thresholds (as discussed above). A point-based system is considered advantageous for SbD requirements, but not in the form presented by the RA tools thus far; efforts are required to better define the criteria used, establish realistic weighting between different descriptors, and to define a system which links separate hazard descriptors effectively.

We also have to consider that the approach utilised in the SAbyNA platform must contain information to allow determination of the hazard driving properties so that an appropriate SbD strategy can be suggested. As discussed previously, this will involve collaboration with WP4 and incorporation of approaches such as read-across.

GUIDEnano already contains a strong hazard assessment strategy which can be useful for SAbyNA. Including a similarity assessment, and strategies relevant to skin sensitization and corrosion, acute and chronic toxicity and mutagenicity & carcinogenicity; these can provide a good starting point for SAbyNA.

In the environment, the dissolution rate, reactivity, and size distribution of NFs or NEPs are main determinants of risks. They allow both the identification of receptacle environments (among freshwater, marine, terrestrial, WWTP systems) and the estimation of potential nano-specific effects in the living organisms. Acute and chronic toxicity descriptors are also determinant factors of environmental risks. They are included in fully quantitative approach to hazard prediction using a standard approach – species sensitivity distribution – widely used in environmental risk assessment to generate a threshold concentration.

[58] T. Aldenberg, E. Rorije. “Species Sensitivity Distribution Estimation from Uncertain (QSAR-based) Effects Data”. ATLA-Alternative to Laboratory Animals. Volume 41, 2013, Pages 19-31.
[59] European Chemicals Agency. 2008. Guidance on information requirements and chemical safety assessment. Helsinki, Finland.
[60] Ehret, J., Vijver, M., & Peijnenburg, W. (2014). The application of QSAR approaches to nanoparticles. Alternatives to Laboratory Animals, 42(1), 43-50.
[61] Forest, V., Hochepied, J. F., Leclerc, L., Trouvé, A., Abdelkebir, K., Sarry, G., ... & Pourchez, J. (2019). Towards an alternative to nano-QSAR for nanoparticle toxicity ranking in case of small datasets. Journal of Nanoparticle Research, 21(5), 1-14.

5. Experimental testing methods and/or required guidance for SAbyNA WP3

The experimental testing methods we wish to utilise within SAbyNA should address endpoints which are key to assess human hazard concerns, and may relate to the toxicological and physicochemical properties of NFs and NEPs. A preliminary list was included in MS3.1: generation of reactive species/surface reactivity, immunotoxicity/inflammation, carcinogenicity, reproductive toxicity, oral toxicity, inhalation toxicity, genotoxicity (mutagenicity), sensitization, dermal toxicity, corrosivity, irritating potential (skin/eye), general toxicity/cytotoxicity/cell viability, frustrated phagocytosis, measurements of toxicokinetics, cellular uptake, neurotoxicity, skin absorption, phototoxicity, and dissolution. For environmental hazard concerns MS3.1 identified standardised test systems (OECD, ISO, EPA) required for environmental hazard assessment under REACH. Experimental testing methods available within the project cover aquatic, terrestrial and wastewater treatment plant (WWTP) systems, cover acute toxicity endpoints such as growth inhibition or mortality or chronic effects such as reproduction inhibition or effects on full organism life cycles or can analyse biodegradability potential. As REACH allows for use of non-standardised tests as well, as long as certain quality criteria of the data are fulfilled, environmental partners within WP3 suggested additional tests to be used to supplement the SbD-informing testing strategy. These standard as well as non-standard protocols, some of them already modified for the use in testing nanomaterials, have been listed with details in MS 3.1 and therefore are not listed again.

From this, SAbyNA should prioritise those already required by existing RA tools, those which address the main hazard concerns of nanomaterials and those which are relevant for the SAbyNA case studies. Further prioritisation should be based on ‘simple’ and predictive methods which could be used for a wide range of materials and/or already exist in reliable test guidelines, standards or well-tested and validated SOPs.

MS3.1 included examples of methods and guidelines related to the identified hazard endpoints. This list was used as a starting point for method evaluation in T3.1. During this task, experimental methods have been identified from various sources including previous nano projects (e.g. NanoValid, and Nanoreg), national standards setting organisations (ISO, OECD) and research articles. Once identified, methods have been screened for nanorelevance and assigned to specific descriptors and endpoints identified as required by RA tools (**¡Error! No se encuentra el origen de la referencia.**). In general, many of the validated standard protocols are yet made to be specific to nanomaterials, although this may not always be necessary. This has been a focus area in many previous and on-going nano projects (e.g. Nanoreg2 and Nanoharmony); therefore, it is recognised that new nano-specific protocols will become available as the SAbyNA project progresses and so this list will remain under review.

A current list of experimental methods is included in Annex 7.3. Many of the non-specific methods have been utilised for nanomaterials in literature, although the validity is yet to be determined. In addition, the existing rationale and interpretation of hazard descriptors used in the reviewed RA tools is provided below, and how these relate to specific endpoints and methods; when test guidelines, standards or SOPs are available these are also provided. This provides a useful start to defining how SAbyNA will address hazard testing methods within Task 3.2 to facilitate the development of the hazard assessment strategy and subsequent guidance for SAbyNA platform users.

5.1 Assessment of solubility/dissolution rate

The following tools incorporate assessment of solubility or dissolution: ANSES, CBnanotool, LICARA nanoSCAN (as per SPM and Stoffenmanager Nano), NanoSafer, SPM, Stoffenmanager Nano, SUNDS (as per LICARA nanoSCAN).

For the impact of solubility on hazard score, ANSES identify poorly soluble particles as requiring higher hazard rating due to translocation and biopersistence, while highly soluble particles require no additional consideration. Nanosafer concur with this, however a low solubility does not provide a specific hazard rating, but only that the

nanoform enters the nano-specific RA, and soluble nanoform enter traditional RA, which is an approach mirrored by Stoffenmanager nano. ANSES provide a threshold that to be considered soluble a nanoform must fully dissolve within one hour in water or a simulated lung lining fluid is identified, no units are suggested but an OECD test guideline TG105 has been identified as the methodology to follow for solubility. NanoSafer suggest a solubility of <1 g/L is required to be considered as an insoluble nanoform, and Stoffenmanager-nano use a threshold of a water solubility of >0.1 g/L, as this is the value stipulated within SDS.

CB nanotool acknowledges that both soluble and poorly soluble nanoforms require a potential hazard consideration, with poorly soluble receiving the highest hazard score; the evaluation of solubility appears subjective in CB nanotool. Using the term stability, SPM consider solubility alongside other chemical and physical transformation (sintering, sorption, agglomeration/aggregation, degradation or conversion), each is suggested to be considered under physiological conditions. Examples/suggestions of appropriate media to use is provided by SPM.[62]

Development within SAbyNA and considerations for the hazard assessment strategy: this is undoubtedly a key aspect that will require consideration by WP3, and already various methodology can be extracted from suggested sources from ISO, OECD and ECHA (e.g. OECD TG105, ISO/TR 19057:2017, ECHA Appendix R7-1). However, the tools available are either in conflict in the approach to solubility that they use, or address it too simply. For SAbyNA we will be required to consider solubility in the context of biopersistence of NFs where poorly soluble particles biopersistence can induce toxicity after chronic exposure, as well as solubility in the context of toxicity in relation to the release of ions. It is likely that we will require more complex categorisation than the existing tools use (as is being done in existing projects such as GRACIOUS), and the development of thresholds under specific conditions; there have already been suggested approaches for assessing the response to NM and their released ions[63]. It will be important to link these approaches to solubility with other endpoints and/or methodologies to provide a more robust interpretation (or prediction) of effects, such as physicochemical characterisation (e.g. morphology), biological endpoints relating to key pathology concerns such as frustrated phagocytosis, or *in silico* tools (to allow a better depiction of fate/biodistribution).

5.2 Fibre (or HARN) paradigm

HARN or fibre paradigm is considered by ANSES, CBnanotool, LICARA nanoSCAN (as per NanoRiskCat and Stoffenmanager Nano), NanoRiskCat, NanoSafer, Stoffenmanager Nano, SUNDS (as per LICARA).

ANSES asks whether substance is a biopersistent fibre, and define biopersistence when the lung defensive mechanisms (removal by mucociliary escalator or alveolar macrophages, dissolution, and disintegration) cannot adequately remove the fibre, while no definition of fibres is provided. NanoRiskCat proposal for allocation of the HARN paradigms provides more detail, and is based on published work of Tran (2008), Meldrum (1996) and WHO definitions (Meldrum 1996, BSI 2007).[64] To be described as HARN, an aspect ratio greater than 10:1 is required, the diameter must be thin enough pass ciliated airways, and length must be long enough to induce frustrated phagocytosis and other inflammatory pathways, and the material must be biopersistent. CB nanotool base this solely on morphology, with fibres receiving the highest hazard score, and spherical the lowest, an intermediate is irregular shaped particles, as does NanoSafer, who identify critical geometric measurements of diameter <3 µm, length >5 µm, and aspect ratio of at least 3:1. Stoffenmanager-nano also apply only a geometric size assessment, and also base this on the BSI proposal of diameter <3 µm, length >5 µm, and aspect ratio of at least 3:1.

Development within SAbyNA and considerations for the hazard assessment strategy: as with solubility, to address the response to fibres will also be a key consideration for WP3. With an adopted (or adapted)

[62] Utembe W et al. Particle and Fibre Tox. 2015, 12:11. Pelfrêne A et al. Int. J. Environ. Res. Public Health 2017, 14, 112. Arts J, Irfan MA et al. Regulatory Toxicology and Pharmacology 76 (2016) 234-261

[63] Peijnenburg et al. Materials 2020, 13(10), 2235; https://doi.org/10.3390/ma13102235

[64] Tran CL, SM Hankin, B Ross, RJ Aitken, AD Jones, K Donaldson, V Stone, R Tantra, 2008, 'An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres', Report on DEFRA project CB0406. BSI 2007b. Terminology for Nanomaterials. PAS 136:2007. London: British Standards Institution. Meldrum, M. 1996. Review of Fibre Toxicology (OELs). Health& Safety Executives.Sudbury, Suffolk; Health& Safety Executives.

definition of “nanofiber” from previous tools and literature, a scoring system can be devised to consider all aspects of the fibre pathogenicity paradigm, including the above mentioned solubility, morphology and biological outputs such as (frustrated) phagocytosis of such materials when studied in exposures of nanofibers to macrophages *in vitro*.

5.3 Reactivity

This is addressed by ANSES, CBnanotool, LICARA nanoSCAN (as per SPM), SPM, SUNDS (as per LICARA).

ANSES only relates this parameter against a bulk or analogous material and requires evidence that the nanoform can generate (directly or indirectly) more ROS or RNS than its parent material. CB nanotool ranking of reactivity directly relates to surface formation of free radicals and is scored as per a consultation of research literature.

SPM considers this parameter in far greater detail and highlights both acellular and cellular *in vitro* tests that predict reactivity, including various measures of acellular redox activity (energy band gap estimation and biological oxidative damage), and various cellular assays including formation of ROS, reduction in GSH, and protein carbonylation; as suggested by Nel (2013)[65] (and others[66,67,68,69,70]). SPM combined these redox related reactivity endpoints/outputs alongside photocatalytic activity and inflammatory response, these are considered within one entry of the tool. Here we have identified inflammatory mediators as a separate parameter. SPM justify this extensive list by using examples of when nanomaterials have demonstrated toxicity when using one parameter and not in another, with differing patterns of effect observed for different nanoforms; using a precautionary approach SPM suggest inclusion of as much of this ‘reactivity’ data as possible and using the most sensitive outcome for the hazard classification.

Development within SAbyNA and considerations for the hazard assessment strategy: Reactivity is an important NF property that will be included in the SAbyNA platform. Different assays are available to test NF reactivity: acellular, cellular and *in vivo*. For SAbyNA, it is important to know the applicability domain, simplicity/complexity, distinctiveness, and the predictive value of the assays. Based on these aspects, reactivity assays will be placed in the hazard assessment strategy of SAbyNA. For the cases in which the NFs induce high or low reactivity, hazard assessment may be straightforward. However, the SAbyNA tool should also consider a hazard assessment methodology for cases in which a trend towards increased reactivity is observed (borderline cases).

In addition, it will be beneficial to dissect and better define what has been identified as reactivity, where reactivity can relate to the ability for NF to generate ROS and the biological impact of these ROS and induction of oxidative stress (such as changes a cells’ redox activities, or the interaction with macromolecules such as proteins or lipids) are considered as different tiers with a different weighting of impact associated. There are already well-described methods for testing reactivity, such as the FRAS assay[71], and from projects such as GRACIOUS it will be possible to adopt validated SOPs for detection of ROS by DCFH-DA, for example. As for the use of immunotoxicity, SAbyNA would consider this as separate to the NF reactivity, as would other important endpoint measurements such as genotoxicity and cytotoxicity.

[65] A. Nel et al.: Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and highthroughput screening; Accounts of chemical research (2013), 3, 607-621
[66] M. Auffan et al.: Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective; Nature nanotech. 4, 634-641 (2009)
[67] H. Zhang et al.: Use of Metal Oxide Nanoparticle Band Gap to Develop a Predictive Paradigm for Oxidative Stress and Acute Pulmonary Inflammation; acsnano, 6(5), 4349-4368, 2012.
[68] NA Lee et al.: Development of multiplexed analysis for the photocatalytic activities of nanoparticles in aqueous suspensions; Photochem Photobiol Sci, 2011, 10(12), 1979-82
[69] B.A. van Driel et al.: A quick assessment of the photocatalytic activity of TiO2 pigments – From lab to conservation studio!, Microchemical Journal 126 (2016) 162-171
[70] S.-F. Hsieh. et al.: Mapping the Biological Oxidative Damage of Engineered Nanomaterials, Small 2013, 9, 9-10
[71] rnaud Gandon et al 2017 J. Phys.: Conf. Ser. 838 012033

5.4 Known severe toxicity of nanoform

This is addressed in CB nanotool, LICARA nanoSCAN (as per NanoRiskCat and Stoffenmanager Nano), NanoRiskCat, Nanosafer, Stoffenmanager Nano, SUNDS (as per LICARA).

CB nanotool requires hazard points if the nanoform is known to cause carcinogenicity, reproductive toxicity, mutagenicity, or is an asthmagen; no advice of how to assess these, of where to source this information, or of which specific endpoints can justify this conclusion is given. NanoRiskCat also identifies this as the following endpoints: genotoxicity, mutagenicity, carcinogenicity, and respiratory, cardiovascular neurotoxic or reproductive effects, or has organ-specific accumulation. Nanosafer uses risk sentences or CLP for the scoring of hazard. Stoffenmanager-nano identifies higher hazard bands for nanoforms which are shown to be carcinogenic, mutagenic, or cause sensitisation, but acknowledge that information such as this may be lacking for nanomaterials and assessment is still mostly performed using the bulk materials. NanoRiskCat states that these must have been observed in response to the nanoform in humans and/or laboratory animals, and use of *in vitro* evidence can only define a 'maybe' entry in the tool as predictivity of *in vitro* tests may not be accurate (CCA 2008).[72]

A drawback of comparing nanoforms based on 'known severe toxicity' (often synonym of some specific CLP classifications) is that very often nanoforms will not be compared at equal conditions. In other words, lack of a known severe toxicity may just imply that this has not been evaluated yet. In addition, in the specific case of insoluble nanoforms, controversy on unspecific carcinogenic effects exist. Titanium dioxide (nano) has now a harmonized classification as suspect of causing cancer by inhalation (H351). Tools that rely on CLP classifications to rank hazard potential would conclude on a higher concern for titanium dioxide nanoforms than nanoforms of other substances that are likely to be similarly or more toxic.

Development within SAbyNA and considerations for the hazard assessment strategy: Within SAbyNA this information will probably not be available for the NFs of concern and will also not be tested. We will not demand a 2-year carcinogenicity assay within a SbD platform. However, the rationale of the hazard assessment strategy will be based on known severe toxicity for benchmark materials. The severe toxicity will be linked to NF properties and effects that can be more easily measured. For example biopersistent reactive NFs (e.g. DQ12) might induce cancer upon chronic exposure. Within SAbyNA, biopersistency and reactivity will be used to predict potential severe toxicity.

For genotoxicity, assays can be included in the SAbyNA hazard assessment strategy. For this, available *in vitro* assays can be used. GTTC gives recommendations which assays to use (Elespuru *et al.* 2018[73]). Also, for these assays the applicability domain, simplicity/complexity, distinctiveness, and the predictive value need to be investigated.

5.5 Dermal toxicity

Dermal toxicity is assessed in CB nanotool, NanoRiskCat, NanoSafer, and Stoffenmanager dermal.

CB nanotool requires hazard points if the nanoform is known to cause dermal toxicity; no advice of how to assess this, of where to gain this information, or of which specific endpoints can justify this conclusion is given. Dermal toxicity is defined in NanoRiskCat according to CLP for bulk or analogous materials, and according to ECHA (2008)[74] for acute nanoform toxicity. In Nanosafer it is defined according to Risk sentences or CLP and Stoffenmanager dermal assesses hazard based on toxicological data (e.g. lethal doses, allergenic potency, skin irritation), and R-phrases and hazard statements.

Development within SAbyNA and considerations for the hazard assessment strategy: Compared to other exposure routes (e.g. inhalation), the skin is considered less permeable to NFs and therefore the risk perception may be somehow lower. The literature data on the skin penetration of NFs remains controversial due to the use

[72] CCA. 2008. Small Is Different: A Science Perspective On The Regulatory Challenges of the Nanoscale. Ottawa: The Council of Canadian Academies.
[73] https://doi.org/10.1093/toxsci/kfy100
[74] ECHA 2008. Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance.

of different models and techniques to address this issue. However, it is known that small (<600 Da) and lipophilic molecules can penetrate the skin passively. Also, NF with a diameter below 10 nm seem to be able to penetrate the skin barrier, whereas above this threshold, the penetration seems to be significantly limited (see Crosera *et al.* 2009[75]). For deceased or damaged skin, NP penetration may significantly increase, and a testing strategy is required for this, which may mean penetration studies precede toxicity studies. The SAbyNA tool should minimally consider the NF coating and its size to try to predict the skin penetration of the NF or some of its components. Since penetration does not necessarily mean toxicity, cellular assays with human skin cell lines could complement the dermal hazard assessment strategy.

5.6 Inflammatory reactions

Assessment of inflammatory reactions is identified within LICARA (as per SPM), SPM, SUNDS (in tier 1 as per LICARA). SPM suggests the release of mediators of inflammation such as cytokines and chemokines (as suggested in Nel (2013)[76])

Development within SAbyNA and considerations for the hazard assessment strategy: Inflammation potency is an important property of NFs as inflammation is the most frequently reported effect of ENMs. Inflammation potency can be measured in many ways, including simple *in vitro* assays (submerged exposure using cell-lines), complex *in vitro* assays (air-liquid exposure (in case of inhalation of NFs) using co-cultures of primary cells) and *in vivo* studies. Many *in vitro* assays are still under development to better predict the *in vivo* outcomes. As mentioned above, for these assays, we need to know their applicability domain, simplicity/complexity, distinctiveness, and their predictive value to place them in the SAbyNA hazard assessment strategy.

The inflammatory response is a sequential process in which different biomarkers peak at different time points. Therefore, for a proper assessment, a special focus should be placed on selecting the appropriate biomarkers for each administration route.

5.7 General reference to CLP R-phrases, hazard statement

CLP R-phrases or hazard statements are utilised in the hazard assessment of ANSES, NanoRiskCat, NanoSafer.

NanoSafer applies additional hazard scoring during its assessment as per CLP hazard statements, by which the user is asked to tick all statements that are known for this material. This appears to be related to the nanoform itself; there indication of how much the hazard score is influenced by the affirmation of these.

ANSES and NanoRiskCat both also use CLP regulation,[77] but only when a suitable analogous or bulk material has received adequate CLP documentation. ANSES refers specifically to CLP hazard statements align to hazard bands HB1 through HB5, where:

- HB1 – eye irrit. 2, skin irrit. 2;
- HB2 – acute tox. 4, STOT-SE 2;
- HB3 – acute tox. 3, STOT-RE 2, skin Corr. 1, eye dam. 1, skin sens. 1, STOT-SE 3 (resp, irritant);
- HB4 – acute tox. 1-2, STOT-SE 1, STOT-RE 1, repro. Tox 1A-1B, carc. 2, repro. 2;
- HB5 – resp. sens. 1, carc. 1A-1B, muta. 1A, 1B and 2.

NanoRiskCat makes a distinction between those classified as **serious health hazards** such as:

- Acute toxicity category 1-4

[75] Crosera, M., Bovenzi, M., Maina, G. et al. Nanoparticle dermal absorption and toxicity: a review of the literature. Int Arch Occup Environ Health 82, 1043–1055 (2009). https://doi.org/10.1007/s00420-009-0458-x

[76] A. Nel et al.: Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and highthroughput screening; Accounts of chemical research (2013), 3, 607-621

[77] Regulation (EC) No 1272/2008 of the European Parliament and of the Councul of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

- Germ cell mutagenicity category 1A, 1B or 2
- Carcinogenicity category 1A, 1B or 2
- Reproductive toxicity category 1 A, 1B or 2
- Specific target organ toxicity - single exposure category 1 or 2
- Specific target organ toxicity - repeated exposure and category 1 or 2
- Aspiration toxicity category 1
- Skin corrosion/irritation category 1A, 1B or 1C
- Serious eye damage/irritation category 1
- Respiratory and skin sensitization category 1,

and **less severe effects** (according to CLP), including skin corrosion/irritation category 2, specific target organ toxicity-single exposure category, and serious eye damage/irritation category 2.

Development within SAbyNA and considerations for the hazard assessment strategy: Inclusion of CLP hazard statements will be required within the SAbyNA platform, if available for NF but more likely as a means to compare and read-across from bulk materials. Alongside this, it will be useful for SAbyNA to provide guidance on which methods can be recommended to the user when CLP categories are not defined, or to choose the best classification based on the methods already used; already within GUIDEnano there are 'relevance decision trees' which enable judgement of method relevance for different endpoints, and will provide a starting point here.

5.8 Particle diameter, and assessment of agglomerates

Particle diameter is used by CB nanotool and Stoffenmanager-nano.

The CB nanotool identify a potential hazard dependent on particle diameter based on the likelihood of lung deposition, with the smallest (1-10 nm) having the greatest chance of deposition and therefore receiving the highest score, followed by particles of 10-40 nm, and then 41-100 nm with the lowest score.

Stoffenmanager-nano instead provide a cut off of 50 nm, where nanoforms of <50 nm is given a higher hazard band than those >50 nm; it is acknowledged that this size cut off is arbitrary, and is given solely to account for the expected higher reactivity due to the decrease in size and increase in surface area.

Size distribution of particles is used by SPM, LICARA (as per SPM), SUNDS (in tier 1 as per LICARA) which identify a potential inhalation hazard for agglomerates or aggregates between 500 nm and 10 µm. Disintegration of aggregates or agglomerates into primary particles or smaller agglomerates (<500 nm) under ambient conditions (i.e. in the body or the environment) is also considered by these tools as an important factor for assessing the need for precautionary measures to protect humans and the environment.

Development/use within SAbyNA and considerations for the hazard assessment strategy: The interpretation of particle size within SAbyNA will rely on already established methodology. The consideration of size by RA tools has been largely in relation to inhalation, it will also be important to provide similar considerations for other exposure routes (e.g. dermal exposure, in both healthy and damaged skin). Methods to characterize the size distribution evolution in complex matrices should also be further considered within SAbyNA.

5.9 Acute toxicity

Acute toxicity is noted as a descriptor in NanoRiskCat, and is defined by NanoRiskCat as per guidance (ECHA 2008, United Nations 2009)[78] as "adverse effects resulting from an oral or dermal administration of a single dose or multiple doses within 24 hours to a nanomaterial or an inhalation exposure of 4 hours". This can include clinical signs of toxicity (abnormal body weight, organs/tissues pathological, lethality), but also irritation of GIT,

[78] ECHA 2008. Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance. UNEP 2009. Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Third revised edition United Nations, 2009.

skin or respiratory tract. Cellular level acute toxicity is also noted, as cytotoxicity and cell-specific function toxicity.

In nSSWD as per guidance (EC, 2011[79], OECD guidelines 208[80]) no observed effect levels were considered acute from an experiment duration less than 24 hours for bacteria, 72 hours for algae, 21 days for vertebrates and invertebrates (28 days for earthworm reproduction). These include individual or populational endpoints (i.e. mortality, growth inhibition and reproduction) for which the sensitivity may be lower than key cellular and molecular endpoints (e.g. identified through the development of 'omics' analysis and AOP approach).

Development within SAbyNA and considerations for the hazard assessment strategy: Acute toxicity including different forms of toxicity related to different properties of NFs. Similar to the 'known severe toxicity' the acute toxicity will be used to build the SAbyNA hazard assessment strategy, using properties and assays that can predict whether NFs might induce acute toxicity based on for example ion release or reactivity.

A recurrent issue regarding acute toxicity is the establishment of a threshold to define whether a NF is toxic or not. For instance, cell viability tests give cell viability values ranging between 0% and 100%, and sometimes (e.g. 70-80% viability) it is certainly difficult to rule out toxicity or confirm biocompatibility. This could be partially solved by comparing the IC50 of the NFs, although sometimes this is not possible in the range of concentrations tested in the assay; in such cases it would be advisable to provide PoD assessment such as BMD analysis to define whether a NF alteration effectively reduces toxicity.

5.10 Surface coating.

NanoSafer is the only tool which directly assigns a hazard score relating to surface coating, and assigns a higher hazard if a nanoform has been coated/functionalised. This, however, is rudimentary and only acknowledges the uncertainty in potential hazard caused by having a surface coating.

Although not used as a marker which impacts a hazard score, SPM provides a useful interpretation of how best to consider particle coating/functionalisation. This may include:

- The consideration of whether the coating itself is highly or poorly soluble, and how best to consider the nanoform by either of these situations,
- But also, the potential for highly soluble nanoforms to become more bioavailable with an insoluble coating, and how this impacts on hazard should this coating (and particle) access environment where the coating is no longer insoluble (and therefore removed).

Development within SAbyNA and considerations for the hazard assessment strategy: the approach by Nanosafer is unlikely to be helpful to SAbyNA. Although this approach aligns very well with the precautionary approach, conversely, the addition of a surface coating may just as likely reduce a hazard as it would induce a hazard. We are unlikely to use this as a function in our assessment, users of the platform would instead require ways to interpret what effect their functionalisation may have, and this would be done using the other descriptors mentioned.

A coating can alter the functionalization and the hazard of NFs. For SAbyNA, we would like to know whether a specific type of coating and the amount of coating influences toxicity. For instance, positively charged NFs are likely to interact more with human tissues, which have a net negative charged. By using the assays for reactivity, inflammation and genotoxicity, the effect of the coating can be assessed, or when possible, a similarity approach can be used on existing information. To this end, SAbyNA should provide up-to-date guidance on which coatings are potentially problematic.

5.11 Exposure limit values

Occupational exposure limits (OELs) are used in NanoSafer and CB nanotool.

[79] European Commission. 2011. Common implementation strategy for the water framework directive. 2000/60/EC. London, UK.
[80] Organization for Economic Cooperation Development. 2000. OECD guidelines for the testing of chemicals. Guidelines 208, 216, 217, 222. Paris, France.

NanoSafer uses existing OELs when available for suitable analogous materials, for bulk material, or for the nanoform itself. The tool uses a threshold of 1 mg/m^3 to assign relevant hazard scores associated with a material, based on analogous or bulk material; a scaling factor is used to derive the nano-specific OEL. CB Nanotool assigns points to the OEL for the parent material.

Development within SAbyNA: this should be included as is already done in RA tools, ensuring the scaling approach used is current.

DNEL or PoD values are used in SUNDS and GUIDEnano.

GUIDEnano and SUNDS are the only fully quantitative RA tools. SUNDS uses dose–response assessment and intra/inter-species extrapolations to perform either a deterministic risk assessment – providing a risk characterisation ratio of exposure dose with derived no-effect level (DNEL), or a probabilistic risk assessment (WHO's APROBA model) – using an exposure assessment with a point-of-departure (PoD) or benchmark dose assessment; the SUNDS tier 2 approach is well documented by Pizzol *et al.* (2019).[81]

Development within SAbyNA and considerations for the hazard assessment strategy: In case a DNEL or other PoD is available for a NF, this can be used for risk assessment. For other NFs, grouping and read-across approaches can be used to assess whether the DNEL of one NF can be used also for another NF (with similar characteristics). It is likely that SAbyNA will need to provide guidance on how to perform intra/inter-species extrapolations and conduct PoD analysis.

5.12 Outcome of experimental methods considerations

From the assessment provided above, and in bridging of these to the list provided in Annex 7.3, it has been confirmed that for many of the endpoints previously identified, there are already existing robust experimental approaches. During development of the hazard assessment strategy these methods will be optimised to ensure they are appropriate for the SAbyNA platform. This is not limited to, but will certainly initially focus on the varied expertise of WP3 participants; the methods which are currently performed by WP3 partners include:

- **For human hazard -** cell uptake, phagocytic activity, barrier translocation, barrier integrity (by TEER and FD translocation), imaging (confocal, TEM), cytotoxicity, surface reactivity and ROS generation (FRAS, ESR, AA depletion, DTT consumption, DCFH, RBC haemolysis), oxidative stress (GSH activity, gene expression), inflammation (secreted proteins and gene expression), air-liquid interface exposures, genotoxicity, dermal toxicity (corrosion, irritation, sensitization, penetration), ocular irritation, phototoxicity, endocrine disruption, material dissolution and ion release (for biopersistence or bioavailability (flow-through, static)), *in vivo* (pharyngeal aspiration).
- **Environmental hazard -** there is a wide range of standardised tests (OECD, EPA, ISO) available which can be used (sometimes with slight modifications) for NF and NEP testing. Available tests cover all environmental compartments: freshwater, marine, terrestrial, WWTP the latter deliberately included in the list because a lot of released materials enter the environment through this pathway and undergo substantial transformation prior to their environmental release. The selection of suitable test systems for ecotoxicological evaluation is therefore very much dependent on the environmental release scenarios and fate predictions of the materials in the respective environmental compartment to be able to select the right testing methods in the right environmental compartment. Environmental WP3 partners therefore rely on this information to be able to finalise the testing strategy. The available tests cover acute as well as chronic elements aligned to REACH requirements with rapid screening tests available but also long-term testing (e.g. whole or even multi-generation life cycles of organisms). Specifically, for the testing of potential biodegradability advances were made within the GRACIOUS project where a shorter, 96 well version for readily biodegradability tests (OECD 301) were developed and are available within the SAbyNA project through the project partner UK CEH. Another point to consider is

[81] Pizzol, Lisa, et al. "SUNDS probabilistic human health risk assessment methodology and its application to organic pigment used in the automotive industry." *NanoImpact* 13 (2019): 26-36.

available quantities of the NFs/NEPs to test because different test systems have different requirements of the amounts of materials needed – this typically ranges from a few mg in rapid/high-throughput 96 well set ups to several 100 grams in mesocosm set ups. Between the different environmental partners in SAbyNA we cover aquatic (bacteria, algae, daphnids, nematodes for soil pore water, MT2 test for biodegradation) and terrestrial testing (enchytraeids, plants, earthworms) as well as simulated complex ecological systems (mesocosms), used in mesocosm based RA[82].

5.13 Benchmark materials

For the assays that are available within WP3, Table 6 represents known positive control treatments (both nanomaterials and chemicals) used by the consortium for human hazard assessment. From this list it is clear that for particle-derived effects suitable controls are not already obvious. As such, the hazard assessment strategy will need to provide suggestions for suitable controls, through already existing literature, or through testing. These considerations will take into account NMs associated with the sector-specific case studies; the particles identified in D7.1 as relevant to the SAbyNA case studies have been compared to Table 6 for relevance in our assay selection, and is presented in Table 7.

Ecotoxicological standard test guidelines always offer information on positive controls to be used to ensure reproducible data quality which are routinely included in the test set ups applied by the partners. Because the selection of test systems is not finalised yet (see 5.12 for reasons on information demands on release scenarios and release forms next to the pristine forms, but also available quantities of the test materials) we do not list every potential positive control from the standard tests here but will updated this accordingly when the experimental testing strategy is developed. Test-system-dependent very-well-characterised Zn, Cu or Ag based nanomaterials can be used as a positive control due to the high amount of available literature data to compare to but also because of 10 years of ecotoxicological experience with these materials of the partners involved here. We therefore aim to select known particles from other projects (such as NanoFATE, GUIDEnano, NanoFASE, GRACIOUS, caLIBRAte) where we can access a large data set for different environmental compartments as a reference. A list of well characterized nanomaterials from NM-series of representative manufactured nanomaterial is also available in the European Commission Joint Research Centre (JRC), including cerium dioxide NMs, zinc oxide NMs, synthetic amorphous silicon dioxide NMs, titanium dioxide NMs, and multi-walled carbon nanotubes. However, their use as Benchmark materials is limited to few of the key hazard descriptors identified above (e.g. photocatalytic activities and Ce NM-212[83]).

Table 6 Positive control treatments used by WP3 partners, potential for benchmark treatment

Endpoints/assays	Known/used benchmark material - particle	Known/used benchmark material - other
Cell uptake	Fluorescent polysterene (PS) nanoparticles, electron dense (e.g. metal) NPs	SDS
Cytotoxicity	ZnO*, TiO_2*, and SiO_2* nanoparticles, Aminated Polystyrene (50 nm) Sigma-Aldrich L0780	2-aminoantracene; 2-nitrofluorene; sodium azide; 9-aminoacridine; MMS, Benzalkonium chloride, 0.1% Triton X-100, DMSO, camptothesin, *Staurosporine, SDS, Tert-butyl hydroperoxide (TBHP), Doxorrubicin hydrochloride*

[82] Auffan, Mélanie, Armand Masion, Catherine Mouneyrac, Camille de Garidel-Thoron, Christine Ogilvie Hendren, Alain Thiery, Catherine Santaella, et al. « Contribution of mesocosm testing to a single-step and exposure-driven environmental risk assessment of engineered nanomaterials ». NanoImpact 13 (1 janvier 2019): 66 69. https://doi.org/10.1016/j.impact.2018.12.005

[83] Singh, C., Friedrichs, S., Ceccone, G., Gibson, N., Jensen, K. A., Levin, M., & Rasmussen, K. (2014). Cerium Dioxide, NM-211, NM-212, NM-213. Characterisation and test item preparation., JRC Repository: NM-series of Representative Manufactured Nanomaterials. Ispra, Italy: European Commission Joint Research Centre Institute for Health and Consumer Protection.

Endpoints/assays	Known/used benchmark material - particle	Known/used benchmark material - other
Reactivity and oxidative stress (FRAS, ESR, AA depletion, DTT consumption, DCFH)	DOFA (Diesel exhaust particles extracted from filter) CuO*; Mn_2O_3; ZnO* NPs, CB*, Mn_2O_3, Aminated Polystyrene (60 nm) Oxidative reactivity: SiO2 NM-201-202-203*, MWCNT-400-401-402*	Sin-1 (linsidomine), Rotenone, tBHP, H_2O_2, $KBrO_3$
Inflammation (cytokines)	DQ12, CB*, CuO*, TiO_2*, SiO_2* (several NFs)	Lypolysacharide (LPS), other proinflammatory cytokines
ALI	DQ12, TiO_2*	LPS (inflammation)
Genotoxicity	(CoO)(NiO) (<150 nm) Sigma-Aldrich 634360	H_2O_2, mitomycin C, etoposide, mitomycin c, methane methylsulfonate, MMS, EMS, $KBrO_3$, , 4-nitroquinoline-N-oxide or 2-aminoanthracene; depending on the assay
Dermal corrosion/irritation/sensitization		SDS (irritation) corrosion (KOH), sensitization (DNBC), caffeine (dermal penetration)
Ocular irritation		Methylacetate
Phototoxicity	Anatase NMs (Anatase Nanopowder (TiO2), Sigma),Ce NM-212	CPZ (phototoxic) SDS (cytotoxic but not phototoxic)
Endocrine disruption		Corticosterone, 17-alpha-estradiol and 17-Beta-estradiol (as agonists); and Tamoxifen and flutamide (as antagonist)
Phagocytic activity	Fluorescent nanoparticles	Cytochalasin D, PMA
Dissolution	Highly soluble: Ag NPs*, ZnO (NM110)*, Cu* Slightly soluble: Ce NM-211, 212, 213 Low solubility: TiO_2*	MMVF
Barrier translocation		Lucifer Yellow, dextran blue
in vivo (Pharyngeal aspiration)	Mitsui-7*, carbon black*	Mitomycin C (depending on the assay and time point)

*Also relevant particles for SAbyNA case studies.

6. FAIR (Findable, Accessible, Interoperable and Reusable) approach to data curation

Numerous amounts of data are continuously generated, stored and provided on cloud platforms which means that data needs to be preserved and made available in view of life span. Providing the research and regulatory community with access to FAIR data facilitates knowledge discovery, improves transparency and helps make better decisions.

FAIR principles provide guidance to scientific community for data management and stewardship of the data. Humans increasingly rely on computational support to deal with data as a result of the increase in volume, complexity, and creation speed. These principles emphasise on machine action-ability (i.e. ability of computational systems to find, access, interoperate, and reuse data with none or minimal human intervention). They describe how research outputs should be organised so they can be more easily accessed, understood,

exchanged and reused both by humans and machines. Major funding bodies, including the European Commission, promote FAIR data to maximise the integrity and impact of their research investment.

Four Basics of FAIR data are:

Findable:
Findable means making sure that data is findable by ensuring it is (1) identifiable and locatable by means of a standard identification mechanism (a persistent identifier); (2) discoverable with metadata; (3) and is searchable online

Persistent identifiers (PIDs) are essential because they unambiguously identify the data and facilitate data citation e.g. Digital Object Identifier (DOI). When data is published to a repository, it should be made sure that selected repository assigns a persistent identifier to the dataset (e.g. Zenodo).

Metadata is the data that provides information about other data (e.g. descriptive metadata, structural metadata, administrative metadata, reference metadata and statistical metadata). Rich metadata provides important context for the interpretation of the data. The metadata description supports findability, citation and reuse and makes it easier for machines to conduct automated analysis.

Searchable means being able to find data online. Identifiers and rich metadata descriptions alone will not ensure 'findability' on the internet. Perfectly good data resources may go unused simply because no one knows they exist. If the availability of a digital resource such as a dataset, service or repository is not known, then nobody (and no machine) can discover it. There are many different ways to make your data searchable e.g. by indexing the data for search engines, publishing data on OpenAire or putting it on the registries of research data (e.g. https://www.re3data.org/).

Accessible:
Accessible means that data is always available and obtainable. Even if the data is restricted, the metadata should be open. Data should be made accessible by ensuring that it is retrievable online using standardised protocols and has restrictions in place if necessary. It is important to note that not all data has to be made open. Data can be restricted (as open as possible and as closed as necessary) and still be FAIR. However, if access is allowed, data should be retrievable without the need for specialised protocols. If the full content is not made openly available, the data must be as findable as possible. If possible, it should be stored on the repositories that support keeping it safe for the long-term, describe the appropriate metadata information, gives licensing information and provides necessary authentication and authorisation mechanisms.

Interoperable:
Interoperable data means it can be integrated with other data, applications and workflows. The data usually need to be integrated with other datasets. It should not be created with proprietary software and should be store/made available in commonly used open formats. Community agreed schemas, controlled vocabularies, keywords, or ontologies should be used where possible. In addition, the data needs to interoperate with applications or workflows for analysis, storage, processing, allowing data exchange and reusability between researchers, institutions, organisations and countries.

Reusable
The fourth goal of FAIR is to optimise the reuse of data. To achieve this, metadata and data should be well-described so that they can be replicated and/or combined in different settings i.e. sufficiently described and shared with the least restrictive licences, allowing the widest reuse possible and the least cumbersome integration with other data sources. While planning the data management it should be clearly specified:

- How the data will be licensed to permit the widest reuse possible.
- When the data will be made available for re-use. If applicable, should specify why and for what

period a data embargo is needed.
- Whether the data produced and/or used in the project is useable by third parties, in particular after the end of the project? If the re-use of data some data is restricted, then it should be explained why.
- The length of time for which the data will remain re-usable.

7. Appendices

7.1 Information Requirements of Tools

7.1.1 Control Banding Tools

ANSES

ANSES is a peer-reviewed control banding tool for nanomaterials and is designed to assess mid phase (indicator) and late phase (demonstrator) of the innovation process, is used for **worker assessment**, assesses **risks from exposures** by the **dermal, oral, and inhalation** routes of exposure, and has **qualitative and semi-quantitative outputs**. Currently ANSES is noted as applicable within the following domains: biocide products, chemical substances, cosmetic products, drugs, food contact materials, food labelling, and medical devices.

ANSES **hazard bands identify** as:

- HB1: Very low: No significant risk to health;
- HB2: Low: slight hazard – slightly toxic effects rarely requiring medical follow-up;
- HB3: Moderate: Moderate to significant health effects requiring specific medical follow-up;
- HB4: High: Unknown health effects or serious hazard: material highly toxic, sensitising, or with unknown effects on health or the environment. Emission or exposure in the environment requires a specific survey;
- HB5: Very high: Severe hazard requiring a full hazard assessment by an expert.

The **toxicological information collected** on the nanomaterial (or product containing the NM) used for ANSES hazard banding is used as follows:

After establishing if NMs are present, pre-existing classification is used to immediately identify a hazard band, as shown in Figure 5a. In the absence of existing information, the following approach is taken:

- Establish if NM is a biopersistent fibre.
- If no, identify bulk or analogous NM, available CLP classification (Figure 5b) is used to attain hazard band.
- Additional increments are assigned (Figure 5c) when analogous NM is used (to address uncertainty), substance water solubility half-time is above 1 hour, and when there is evidence of NM having higher reactivity than the parent or analogous material (defined as the ability to generate ROS and RNS).
- If there is no bulk or analogous substance, immediately assign HB5: full hazard assessment.

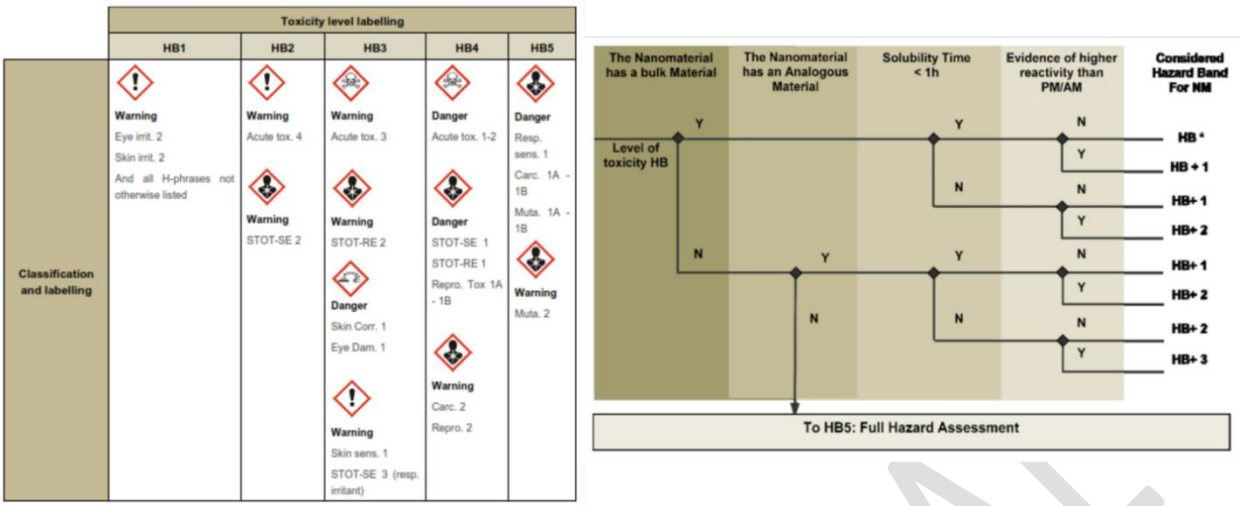

Figure 5 Diagram showing how a nanomaterial is allocated to a hazard band according to the level of knowledge of the nanomaterial

Control banding nanotool

CB nanotool is a 'market-ready' control banding tool, and is a simplified approach for experts and non-experts to determine the potential exposure of employees to nanomaterials. The CB nanotool allocates 4 bands for hazard (severity score) and 4 bands for exposure (probability score) and 4 risk level control bands (Figure 2). The overall level of risk and corresponding control band is determined by a matrix arranged with the probability scores in the columns and the severity scores in the rows. It has been quantitatively validated with some case studies. It is used for **worker assessment**, assesses **risks from exposures** by **dermal and inhalation** routes of exposure, and has a **qualitative or semi quantitative approach**. An ISO standard, providing technical specification on the use of CB for managing inhalation risk from engineered nanomaterials (ISO, 2014), described CB nanotool as proactive assessment (note that Stoffenmanager Nano is described as retroactive risk assessment).

Probability

Severity	Extremely Unlikely (0-25)	Less Likely (26-50)	Likely (51-75)	Probable (76-100)
Very High (76-100)	RL 3	RL 3	RL 4	RL 4
High (51-75)	RL 2	RL 2	RL 3	RL 4
Medium (26-50)	RL 1	RL 1	RL 2	RL 3
Low (0-25)	RL 1	RL 1	RL 1	RL 2

RL 1: General Ventilation
RL 2: Fume hoods or local exhaust ventilation
RL 3: Containment
RL 4: Seek specialist advice

RL = Risk Level

Figure 6 Risk levels assigned by CBnanotool, when combining severity of hazard and probability of exposure.

CB nanotool **hazard bands contribute to a risk level value, and are obtained via a scoring system identified** as:

- A nanomaterial severity score – 70% contribution to score based on assessment of surface chemistry, particle shape, particle diameter, solubility, carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, asthmagenicity;

- A parent material severity score – 30% contribution to score based on assessment of OEL, carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, asthmagenicity.

The **toxicological and physicochemical information collected** on the nanomaterial (or parent material) is used for CB nanotool hazard banding by amassing scores from different severity factors as follows:

Surface chemistry (nanomaterial) – input high (10pts), medium (5 pts), low (0 pts), unknown (7.5 pts);

  - Particle surface free radical activity is judged according to available research articles
- **Particle Shape** (nanomaterial) – input tubular or fibrous (10pts), anisotropic/irregular (5pts), compact or spherical (0pts), unknown (7.5pts);
  - Irregular is given higher score than spherical as expected higher surface area; highest severity score is given to fibrous or tubular-shaped particles.
- **Particle Diameter** (nanomaterial) – 1-10 nm (10 pts), 11-40 nm (5 pts), <41-100 nm (0 pts), Unknown (7.5 pts);
  - Based on probability of increased lung deposition of smaller particles, and the increased surface area of smaller particles.
- **Solubility** (nanomaterial) – Insoluble (10 pts), soluble (5 pts), unknown (7.5 pts);
  - Based on studies which show poorly soluble inhaled nanoparticles can cause oxidative stress, inflammation, fibrosis, or cancer. Soluble nanoparticles also cause adverse effects through dissolution in the blood therefore severity points are still assigned.
- **Carcinogenicity/Reproductive Toxicity/Mutagenicity/Dermal Toxicity/Asthmagenicity**
  - Yes (6 pts), No (0 pts), unknown (4.5 pts)
- **Parent material** assessed for OEL, carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, asthmagenicity.

NanoRiskCat

NanoRiskCat is not a control banding tool, but a guidance document to assist in assessing, ranking and communicating risks of nanomaterials, and is applied with **professional end-users, consumers and the environment** in mind, considers both **exposure and hazard**, and addresses **inhalation, dermal and oral exposure** routes.

In relation to **human hazard**, key considerations identified in NanoRiskCat include:

- Does the nanomaterial fulfil the HARN paradigm?
- Does the bulk form of the nanomaterial cause serious effects, e.g. CLP health hazards: such as germ cell mutagenicity, carcinogenicity, reproductive toxicity, skin corrosion/irritation, specific target organ toxicity.
- Is the specific nanomaterial known to be acute toxic?
- Is the nanomaterials known to be genotoxic, mutagenic, carcinogenic, cause respiratory, cardiovascular, neurotoxic or reproductive effects in humans and/or laboratory animals or has organ-specific accumulation been documented?

The **toxicological information suggested** by NanoRiskCat predict a potential hazardous material, identified as coloured dots to signal whether a material has a high (red), medium (yellow), low (green), or unknown (grey) hazard. These considerations are staged in a decision tree (Figure 7).

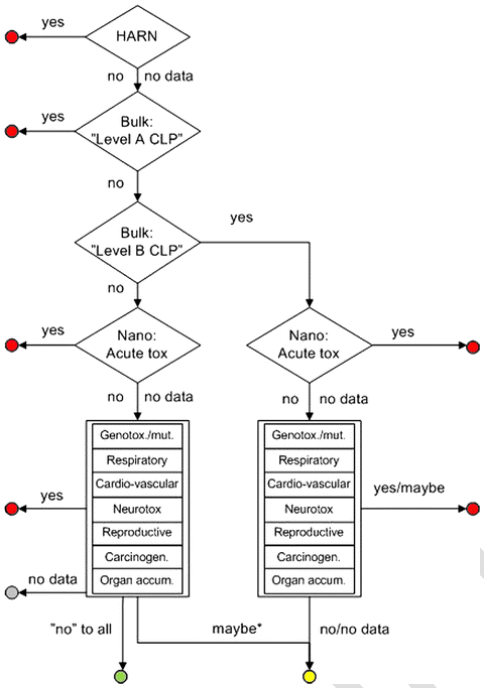

Figure 7 Road-map for assigning a human hazard colour code in NanoRiskCat. Red, yellow and green indicate high, medium and low indication of effect whereas grey indicates too limited data to make an assessment.

NanoSafer

NanoSafer is control banding and risk management tool, which generates **quantitative scores** and **bands for risk**. It is for use by **SME** on the production and use of nanomaterials, for **worker assessment**, and in relation to **dermal, oral, and inhalation routes of exposure**.

NanoSafer **risk bands,** as with ECETOC-TRA, are based on risk quotients (i.e. the ratio of exposure against toxicity thresholds). With thresholds based on a score given between 0.2-1.0 (where 1 means high potential hazard), which is generated from **toxicological information collected** on material characteristics, or known hazard parameters, including:

- aspect ratio (>1:3 hazard level=1),
- solubility in water (as greater or less than 1 g/L),
- presence of surface coating (yes/no),
- An OEL for the nanomaterial (and if not available, for an analogous material) (as greater or less than 1 mg/m^3),
- SDS risk statements and hazard statements.

Swiss Precautionary Matrix

Swiss Precautionary Matrix (SPM) is a tool designed to advise precautionary needs relating to nano-specific risk properties, and can be applied to synthetic nanomaterials. It also provides the basis for early decision-making. It is intended for use in the RA of **consumer, environment and general population** exposures, by the **dermal, oral, and inhalation** routes of exposure, and addresses various stages along a nanomaterial's life cycle, including during research and development, production, use, recycling and disposal.

SPM **hazard bands identify** as:

- Hazard Bands (1 to 3, as Low-Medium-High),
- Ultimately leading to two classifications: "Class A": need for action is low and does not need further clarification, or "Class B": Nanospecific action is needed.

The **toxicological information collected** on the nanomaterial (or product containing the NM) used for SPM hazard banding is on two parameters, reactivity and stability:

- Reactivity – acellular by band gap analysis, photocatalytic activity or biological/oxidative damage, cellular reactivity by inflammatory response, induction of GSH reduction, protein carbonylation. (Low-Medium-High). The systems gives the score of the highest effect.
- Stability – dissolution in physiological conditions and dissolution in environmental conditions; Low-Medium-High).

Stoffenmanager-nano

As with SPM, Stoffenmanager-nano is also designed to take the precautionary approach to RA, and like many other tools is primarily based on exposure control. Stoffenmanager-nano assesses workplace risk to the inhalation of manufactured nanomaterials, through assessment of available hazard information alongside inhalatory exposure estimate, and is primarily for use by SMEs.

Stoffenmanager-nano **risk banding** is based on certain nanoform **physicochemical properties** and **hazardous properties**, and identifies that the R-phrases of bulk materials is insufficient to accurately predict the nano-hazard, and the use of certain properties is required. It is acknowledged by Stoffenmanager-nano that a weighted approach to categorising the response to certain parameters can produce a semi-quantitative hazard banding approach, however Stoffenmanager-nano have not opted for this as the scoring would be relatively arbitrary.

The interpretation of necessary **toxicological information** used by Stoffenmanager-nano is based on BSI and IFA benchmark considerations, including fibrous morphology and solubility, and can be broken down into three decision steps (Figure 5), which include:

- After defining a nanomaterial, the **first stage is solubility**, if biopersistence can be predicted the materials moves on to further evaluation, if soluble it is suggested the standard Stoffenmanager should be applied; solubility is inferred by a water solubility of > 0.1 g/L.
- If biopersistent, a high aspect ratio (> 3:1) **fibre morphology** with > 5 µm length (it is assumed that diameter is in the nanoscale) automatically receives the highest hazard category.
    - Hazard band E
- If not a biopersistent fibre intrinsic hazard properties of the nanomaterial is considered:
    - Hazard band E – Is a carcinogenic, mutagenic, or causes sensitisation;
    - Hazard band D – Carcinogenic (but not mutagenic), reprotoxic, very toxic;
    - Hazard band C – Toxic, causes burns, causes irritation;
    - Hazard band B – Harmful/irritant;
    - Hazard band A – practically non-hazardous;
    - A general distinction is made by a size threshold of 50 nm, with < 50 nm receiving a higher hazard band.

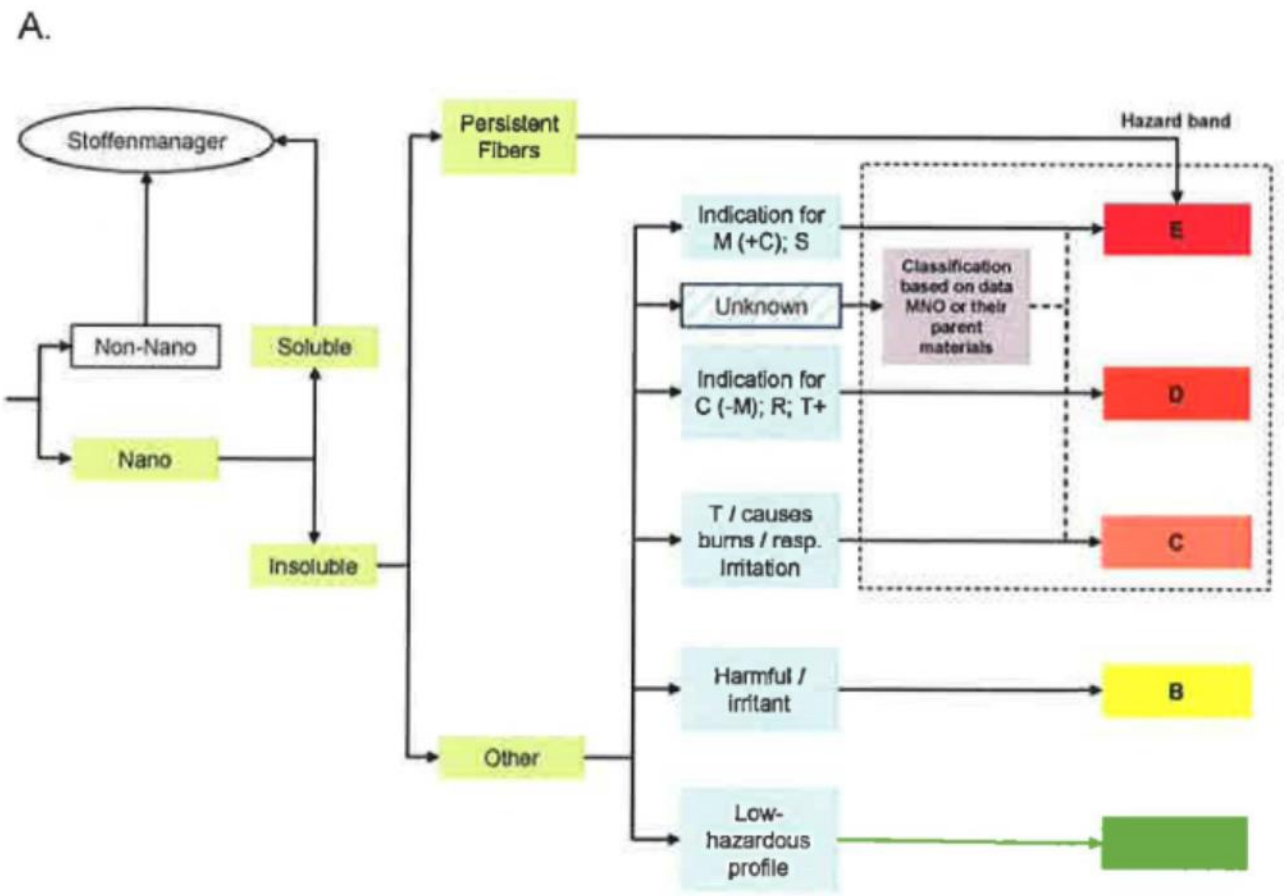

Figure 8 Stoffenmanager-nano hazard classification. Five hazard bands (A-E) for MNOs based on water solubility, structure (i.e. fibre-like or not), fibre length and hazard of the MNO itself.

Stoffenmanager dermal

Stoffenmanager dermal is a RA tool specifically for dermal exposure, and is fundamentally based on Riskofderm and is not a tool specific for nanomaterials. It addresses both exposure and hazard, and is **worker and task** orientated, with **qualitative outputs**.

Stoffenmanager dermal **hazard bands identify** as A to E (low, average, high, very high, extreme), which are considered separately for local and systemic effects (after uptake through the skin), by assessing:

- toxicological data (e.g. lethal doses, allergenic potency, skin irritation), and/or
  - R-phrases, hazard statements.

LICARA nanoSCAN

LICARA nanoSCAN is a tool that implements the EU FP7 project LICARA Guidelines: for the sustainable competitiveness. The tool, in general, is used to provide a nanoproduct assessment for decisions on whether to continue, adjust, or abandon use, for which a balance of high potential benefits must outweigh potential risks, with a high confidence level. If a positive assessment is not reached, the tool suggests steps to e.g. improve benefits if found lacking, or lessen nano-related risks if risks were found to high. These details are presented as a Multi Criteria Decision Analysis (MCDA), allowing a user interpretation of what aspects carry most weight specifically to their particular case.

In terms of risk, LICARA nanoSCAN provides assessment of consumer, general and occupational populations, as well as the environment, and is the only control banding tool to include life cycle analysis (LCA), and can be used in assessment of **dermal, oral, and inhalation** exposure. It is structured within modules which are largely based on existing tools: module 4: public health and environmental risks, on Swiss Precautionary Matrix (see Section 0); module 5: occupational health risks to nano, on Stoffenmanager-nano (see Section 0); module 6 consumer health risks to nano, on Stoffenmanager-nano and NanoRiskCat (see Section 0).

Hazard info used/requested; for **public health and environmental risks**, is based on SPM, and excluding exposure related questions, the tool requests the following information:

- Whether the "nanoform causes redox activity, catalytic activity or have a potential for oxygen radical formation or to induce inflammation reactions" – generalised within one question.

Hazard info used/requested; for **occupational and consumer health risks**, the tool requests the hazard class classification from Stoffenmanager-nano and NanoRiskCat, respectively (discussed in more detail in Sections 0 and 0).

LICARA nanoSCAN **hazard scaling**:

- For each separate category (public health, environmental risks, occupational health risks, consumer health risks) a scale between 0 (no risk from nanomaterials) and 1 (indicates the highest possible risk from nanomaterials) (Figure 9a). In addition, the tool weighs benefits against risk to derive a potential decision (Figure 9b).

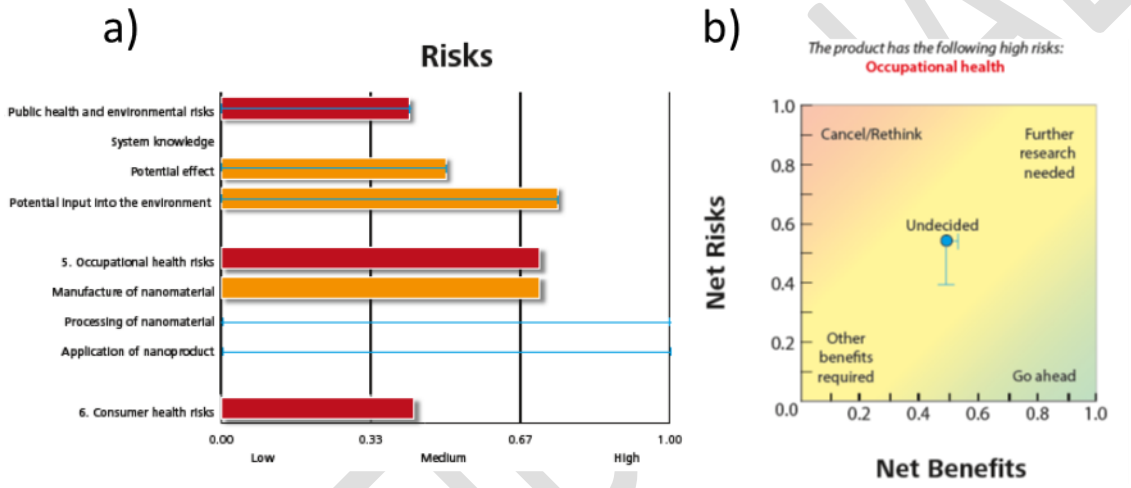

Figure 9 LICARA nanoSCAN hazard scaling, a) risks classified for each category are scaled between 0 and 1, b) risk-benefit analysis

NanoCommons Risk Assessment Tool

The NanoCommons platform offers a number of tools to predict toxicity of nanomaterials including:-

Enalos Cloud Platform, developed by NovaMechanics Ltd, is an online, freely available cheminformatics and nanoinformatics cloud platform, that hosts predictive models released as web services, which aim to address the need to reduce the amount of time and cost spent on experimental testing during the drug discovery and the risk assessment procedures for small molecules and nanomaterials (NMs). Several predictive models, based on open source, in-house algorithms and software, are already available within the Enalos Cloud platform, including models for NMs toxicity, biological activity and properties evaluation.

NanoCommons Molecular Initiating Events prediction tool

Using published data from *in vivo* and *in vitro* experimental studies of nanomaterials toxicity and different signaling and functional databases, the NanoCommons Molecular Initiating Events (MIE) gene set database (NanoCommons GS-MIE DB) captures gene signatures (GS) of MIEs by integrating knowledge from KEGG, REACTOME, GO, WikiPathways public databases and custom gene sets from published data. To date, 132 gene sets representing three different types of MIE actions have been manually collected and via a user-friendly interface can be used to calculate a prioritized list of MIEs with identified biological processes triggered by exposure to nanomaterials.

GUIDEnano

The hazard assessment strategy in GUIDEnano which consists of three options:

1) consider if existing hazard thresholds exist for a similar nanoform, and read-across to these;

2) default hazard thresholds;

3) hazard threshold derivation based on high tier hazard data, with consideration of quality, relevance and similarity.

Option 1 – using read-across DNEL/OEL from similar materials.

If a similar NF exists with OEL or DNEL values, use these. Similarity assessment as described in Park *et al.*, 2018.[84]

Option 2 - Guidenano nanomaterial categorization for generic default hazard threshold values for RA

The GUIDEnano project had proposed a nanomaterial categorization scheme to assign generic and conservative default hazard threshold values to groups of nanomaterials. Such scheme focused only on **risks from exposures** by the **dermal, oral, and inhalation** route of exposure, and had a **quantitative output**.

Such defaults were as it follows:

The **toxicological information collected** on the nanomaterial (or product containing the NM) used for GUIDEnano to derive such default hazard threshold values were: morphology, dissolution and reactivity. A scoring system for reactivity, based on multiple assays and parameters had been developed. Dissolution was considered only in water. The thresholds for reactive and non-reactive materials were suggested considering the distribution of the current existing OELs for respirable dusts. At that point, around 5 years ago, only beryllium, nickel, and cadmium compounds had OEL values below the default hazard threshold that we suggest for reactive nanomaterials. Note that in any case, if the bulk OEL for a nanoform divided by ten would be lower than the generic threshold, this one would apply.

7.1.2 Quantitative Human health prediction models

GUIDEnano

Option 3 - GUIDEnano (regulatory-like) derivation of DNEL values/CLP conclusions

In cases where no available OEL/DNEL for similar NFs exist, and general defaults (as explained above) lead to risk characterization ratios approaching or exceeding 1, GUIDEnano supports the user on the derivation of DNEL values (or CLP conclusions) based on toxicological studies available for nanoforms similar to the exposure-relevant materials.

The tool enables evaluation of each study using criteria related to:

a) similarity between the exposure-relevant NM and the already tested material

b) quality of the data,

c) relevance of the study for each given endpoint, making use of all available information, regardless of its compliance with test guidelines.

Thereafter, the derivation of the DNEL value or CLP statements follows regulatory guidance, with the addition of an uncertainty factor linked to the similarity score.

[84] Park, Margriet VDZ, et al. "Development of a systematic method to assess similarity between nanomaterials for human hazard evaluation purposes–lessons learnt." *Nanotoxicology* 12.7 (2018): 652-676.

SUNDS

SUNDS is described as "cloud-based nano-product sustainability assessment decision support system". It uses a two tiered assessment[85] which, dependent on information supplied, provides qualitative or quantitative results for **worker, consumer, environment** risk assessment. Its design was planned to provide aid in two routes to REACH authorisation: by demonstrating adequate control of risk through risk management or substance substitution or by demonstrating that benefits outweigh costs. Its use is intended for use by SME with only a capacity for low data/information requirements, and large industry who have the capacity or need for more advanced RA tools, and can be used in assessment of **dermal, oral, and inhalation exposure**. SUNDS has since been separated to include modules for assessment of consumer products and modules for assessment of nanobiomaterials used in medical devices, this latter module is being developed by the ongoing Biorima project.

The first tier assessment by SUNDS is based on LICARA Nanoscan, and as such provides a balanced assessment between risks and benefits. The SUNDS second tier is based on an authorisation process currently in operation within the EU REACH regulation and provides an opportunity to validate risk control measures (based on the CENARIOS standard), as well as further comparison of how benefits may outweigh risks, in this case using Socio-economic Assessment (SEA). The Tier 2 assessment is further separated into modules of ecological risk assessment (ERA), public health risk assessment, occupational and consumer human health risk assessment (HHRA), life cycle impact assessment (LCIA), economic assessment (EA) and social impact assessment (SIA).

Human hazard information is pertinent to public health risk assessment and HHRA modules. Both of these require exposure related data to input, and the **toxicological information** is derived from dose–response assessment and intra/inter-species extrapolations, providing **hazard outputs** of either:

- Deterministic risk assessment – providing a risk characterisation ratio of exposure dose with derived no-effect level (DNEL), or
- Probabilistic risk assessment (WHO's APROBA model) – using an exposure assessment with a point-of-departure (POD) of benchmark dose assessment.

7.1.3 Quantitative environmental prediction models

SUNDS

SUNDS is described in general in Section 7.1.2. The environmental risk assessment module at Tier 2 allows the user to perform a quantitative environmental exposure and hazard assessment at multiple stages of the product life cycle (synthesis, product manufacturing, use, end of life) and for multiple environmental compartments (soil, water), and provides built-in tools to allow this to be done.

In exposure assessment, for each combination of life stage and environmental compartment, predicted environmental concentrations (PECs) can be directly input (deterministic PEC) or computed (probabilistic PEC). A material flow-based model, PMFA[86], is used for these calculations. If a probabilistic PEC is required, the user is required to input information relating to the total mass of material input to the system at the relevant life stage, the proportion of that input due to the user's activities, the volumes of the receiving environmental compartments (air, water, sediment and soil – default volumes can be used) and transformation and transport factors relating to (i) the fate of the material within technical and natural compartments, and (ii) the transport of the material into other compartments. A probabilistic PEC is expressed as either a mean and standard deviation, or as upper

[85] Note – It had been noted in a Workshop held in Utrecht in 2014 that there should be a mid-level tier of SUNDS, this would allow read across and grouping approaches to be incorporated. The workshop, however, concluded that this would be done within Guidenano and not in SUNDS.

[86] F. Gottschalk, R.W. Scholz, B. Nowack. "Probabilistic material flow modeling for assessing the environmental exposure to compounds: methodology and an application to engineered nano-TiO2 particles". Environmental Modelling and Software, Volume 25,. 2010; page320-332. https://dx.doi.org/10.1016/j.envsoft.2009.08.011.

and lower confidence intervals. SUNDS outputs a quantitative estimate of risk for each environmental compartment (Figure 10 shows example output).

Effects thresholds (PNECs) are required for each environmental compartment of concern. They can either be entered directly or computed by one of two species-sensitivity distribution (SSD) based tools (pSSD or nSSWD). The pSSD tool, which has not been evaluated separately, constructs an SSD based on probabilistic distributions of threshold concentrations of individual species. The nSSWD tool, which was evaluated separately, is described in the following section.

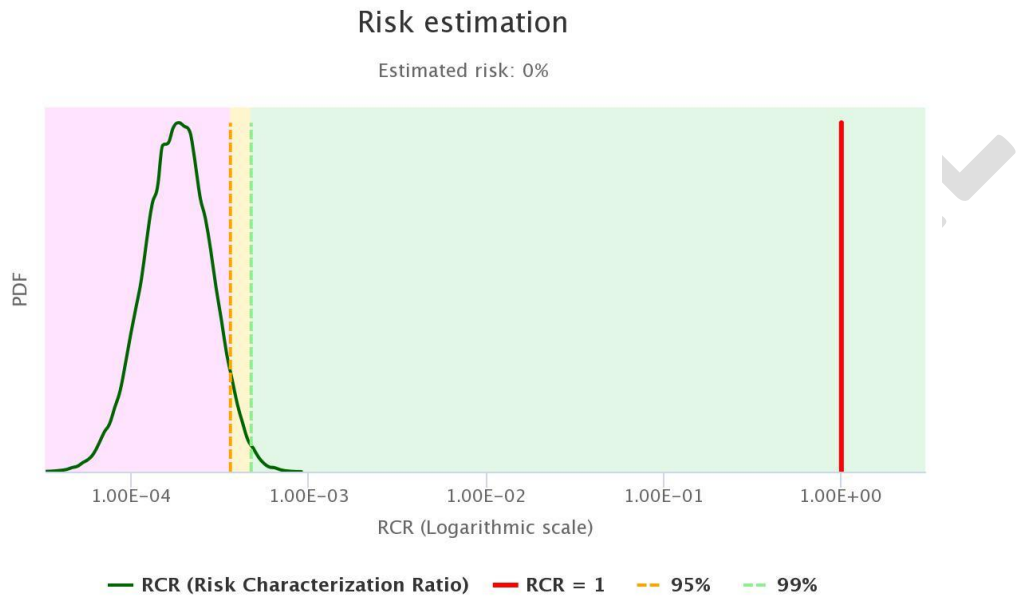

Figure 10 Example environmental risk output of SUNDS, showing a predicted probabilistic distribution of PEC (green line) compared to the threshold of potential risk (red line).

nSSWD

The nSSWD[87] tool predicts PNECs for nanomaterials using a modified version of the species sensitivity distribution approach conventionally used for deriving PNECs for data-rich chemicals. The tool allows the user to weight individual endpoint concentrations according to the number of points available per species, trophic level abundance, and data reliability. The tool description3 provides example applications assuming either a lognormal or empirical distribution of the data; however, it is not clear from the online version of the tool (https://shinyapps.greendecision.eu/nsswd/) what options are actually implemented. As noted in the previous section, the tool is implemented within SUNDS as an option for the derivation of environmental PNECs, where the available functionality and options are similar to those in the standalone tool.

[87] E. Semenzin, E. Lanzellotto, D. Hristozov, A. Critto, A. Zabeo, E. Giubilato, A. Marcomini. “Species sensitivity weighted distribution for ecological risk assessment of engineered nanomaterials: The n-TiO2 case study”. Environmental Toxicology and Chemistry, Volume 34, 2015, Pages 2644-2659. https://dx.doi.org/ 10.1002/etc.3103

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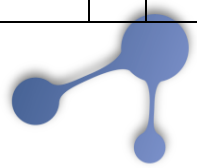
7.2 Excel template of list of data sources

Table 7 List of data sources

Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
GUIDEnano	www.guidenano.eu	Y		Y	Y		Certain	Exposure Scenario Data (Workers: 196, Service Life: 4); Web-based Risk Assessment Tool; ecotox. data?
MARINA	http://www.marina-fp7.eu/	Y	Y	Y	Y		Certain	Phys-Chem 14 materials; *in vitro* (8 cells types, 10 assay types, 209 Tests); *in vivo* (8 Tests); Ecotox. data (40 Tests); OMICs (Proteomics: 52 (substance x cell type x timepoint combinations), Metabolomics: 52 , Transcriptomics: 24); Exposure Scenario Data (Workers: 55, Service Life: 4); Most MARINA data transferred to e-NM instance (share w CALIBRATE & Nanoreg2);
NanoDefine	www.nanodefine.eu	Y					Unknown	Explores and develops conceptual and technical tools for the classification of materials. The NanoDefiner e-tool, a decision support framework for the characterisation of potential nanomaterials.
NanoFase	http://nanofase.eu/	Y		Y	Y		Likely	Phys-Chem, ecotox., exposure; Models; Poss data sharing? - DSA req;
NanoMICEX	http://www.nanomicex.eu	Y		Y	Y		Likely	Poss phys-chem, some ecotox. & exposure data;
NanoMILE	http://www.nanomile.eu-vri.eu/	Y	Y	Y			Certain	MNM screening platform; ENM properties Knowledge Base; Phys-chem; *in vitro*, *in vivo*; OMICs;
NanoPUZZLES	http://www.nanopuzzles.eu/	Y	Y				Certain	Modelling, analysis (QSAR & co); database & data standardisation info
NANoREG	http://www.nanoreg.eu/	Y	Y	Y	Y		Certain	NanoReg 1 data publicly avail in e-NM DB; phys-chem, *in vitro* & *in vivo* tox., ecotox., exposure; phys-chem and tox templates
NanoReg2	http://www.nanoreg2.eu/	Y	Y	Y	Y		Certain	NanoReg 2 data accumulating in e-NM DB instance; phys-chem, *in vitro* & *in vivo* tox., ecotox., exposure;

Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
GRACIOUS	https://www.h2020gracious.eu/							eNanoMapper DB established and data population ongoing;
NANOSOLUTIONS	www.nanosolutionsfp7.com	Y	Y	Y			Certain	Phys-chem re 31 treated and untreated ENMs; Life cycle analysis; ENMs and BioMedia BioCorona; *in vitro* cell models, with HTS; cross-species and environment ; disease and translocation studies; OMICs (mRNA, RNAseq; proteomics on beas2b, ecoli; closed proj,
SANOWORK	http://www.sanowork.eu	Y	Y		Y		Certain	Limited *in vitro* results
SUN	http://www.sun-fp7.eu	Y	Y	Y	Y		Certain	Phys-Chem 8 materials; *in vitro* (2 cells, 4 assay types, 17 Tests); *in vivo* (9 Tests); Ecotox. data (205 Tests); OMICs (Yes); Exposure Scenario Data (96 from NECID); Environment, Release Exposure (28 Datasets);
Nanomaterial Biological Interactions Knowledgebase	http://nbi.oregonstate.edu/	Y		Y			Certain	Knowledgebase (KB) on Nano-Bio interactions and NanoMaterial phys-chem Library
Dana	http://www.nanoobjects.info/en/nanoinfo/knowledge-base	Y	Y	Y	Y		Certain	KB - Searchable for nanomaterials containing products
CaNanoLab - Cancer Nanotechnology Laboratory	https://cananolab.nci.nih.gov/caNanoLab/#/	Y					Certain	KB & Poss data source on materials in US database
Nanohub (US/NSF)	https://nanohub.org/	Y	Y				Certain (likely for phys-chem)	KB on risk assessment & standards etc; 320+ simulation tools/models ; some example nano DBs and datasets; nano education tools
MODERN	http://modern-fp7.biocenit.cat/index.html	Y	Y	Y			Certain	KB re EMN QNPR modelling; database/data repository design; SbD
NanoMiner (FP7 NANOMMUNE project)	http://compbio.uta.fi/estools/nanommune/index.php/		Y				Certain	Optimised repository of OMICs data generated by NANOMMUNE project (see entry)
Nanoparticle Information Library	http://nanoparticlelibrary.net/	Y					Certain	KB by US NIOSH; Research database on emerging nanoparticles and their potential health effects
ITS-Nano (Intelligent Testing Strategy for ENMs)	https://www.safenano.org/research/its-nano/	Y	Y				Absent	KB re enm testing strategies; Ref - Stone V, Pozzi-Mucelli S, Tran L, Ashberger K, et al. 2014, "ITS-NANO - Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy," Particle and Fibre Toxicology, 11(9), 1-11.

Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
ModNanoTox	http://www.birmingham.ac.uk/generic/modnanotox/index.aspx		Y				Likely	Re nano-tox. modelling
OECD Database on Research into the Safety of Manufactured NMs	http://www.oecd.org/env/ehs/nanosafety/publications-series-safety-manufactured-nanomaterials.htm	Y	Y	Y	Y		Likely (Certain for ecotox)	Publications in the Series on the Safety of Manufactured Nanomaterials
NECID	http://www.perosh.eu/research-projects/perosh-projects/necid/				Y		Certain	NECID (Nano Exposure and Contextual Information Database) by PEROSH group Database system for detailed nano-exposure data and measurements; potential tool for use and as source of data from other projects
S2NANO	http://portal.s2nano.org/						Unknown	KB on modelling; links with OpenTox, NanoBank & NanoWiki
Serenade	http://www.labex-serenade.fr/labex-serenade						Unknown	KB and networking platform on ENMs Exposure and RA modelling
ENPRA	http://www.enpra.eu/	Y	Y		Y		Certain	Phys-Chem 12 materials; *in vitro* (24 cells, 58 assay types, 650 Tests); *in vivo* (17 Tests); IVIVE (83 Tests); Toxico-Kinetic Data (Yes) ; Exposure Modelling Data (Yes);
NANOMUNNE	http://www.safenano.org/research/nanommune/	Y	Y				Certain	Phys-Chem 50+ materials; *in vitro* (8 cells, 12 assay types, 123 Tests);
CERASAFE	http://www.cerasafe.eu/						Unknown	KB on NM in relation to Ceramics production; Phys-chem characterisation, tox. & Exposure tools development
eNanoMapper	https://enanomapper.net/	Y	Y	Y			Certain	Platform for EHS data repository; being adopted and used in Gracious and other projects (CALIBRATE, NanoReg 1 & 2, etc)
HSEnano	http://www.hsenano.com/en/						Unknown	KB on risk assessment & standards etc
NANOTEST	http://www.nanotest-fp7.eu/						Certain	Uploaded to NanoReg 2 (eNM); IOM *in vitro* DB; poss sharing via DSA
CALIBRATE	http://www.nanocalibrate.eu	Y	Y		Y		Certain	Develop, integrate and validate models; sourcing data from other projects (eg MARINA); Modelling and Exposure information; continue to relate with possible data sharing agreements (DSAs)



Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
PATROLS	https://www.patrols-h2020.eu/	Y	Y	Y			Certain	Started Jan 2018; ENM Phys-chem, *in vitro*, *in vivo*, ecotox.; tox. modelling; establish relations and poss DSAs
BIORIMA	https://www.biorima.eu/	Y	Y		Y		Certain	Started Noc 2017; ENM Phys-chem, *in vitro*, *in vivo*, ecotox.; tox. modelling; RMM toolbox establish relations and poss DSAs
ACENANO	http://www.acenano-project.eu/						Likely	Tools & data repository developments
IUCLID 6	https://iuclid6.echa.europa.eu/							Knowledge and Guidance on use & application of IUCLID 6 Standard/Format
NanoCommons	https://cordis.europa.eu/project/rcn/212586_en.html	Y	Y	Y	Y		Certain	Research and Innovation action to support networking & development: Joint Research Activities will integrate existing resources and organise efficient curation, preservation and facilitate access to data/models.
EC4SafeNano	http://www.ec4safenano.eu/						Unknown	EC4SafeNano aims to build an open collaborative network gathering expertise in risk management of nanotechnologies.
Hisents	https://hisents.eu/						Unknown	HISENTS aims to deliver an advanced nanosafety platform capable of providing high-throughput toxicity screening for the risk assessment of novel nanomaterials.
NanoGenTools	https://www3.ubu.es/nanogentools/						Unknown	NANOGENTOOLS combines toxicogenomics, proteomics, biophysics, molecular modeling, chemistry, bio/chemoinformatics to develop fast *in vitro* high throughput (HTS) assays, with molecular based computational models for nanotoxicity.
NanoStreeM	http://www.nanostreem.eu/						Unknown	The goal of the NanoStreeM project is to promote good practices by identifying and implementing standards, identify gaps in methodologies and directions for further investigations in order to support governance of the occupational risk induced by the use of nanomaterials in semiconductor industry.

Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
Pandora	https://www.pandora-h2020.eu/						Unknown	Project will compare the effects of a selected number of NP of wide application (iron, titanium and cerium oxide) on the immune response of several earth and marine organisms in parallel to human. The highly conserved system of innate immunity/stress response/inflammation will be the focus of PANDORA
SmartNanoTox	http://www.smartnanotox.eu/						Unknown	SMART TOOLS FOR GAUGING NANO HAZARDS
NICK	http://nikc.egr.duke.edu/#!/home						Certain	Largely literature data, but also NanoFASE project data being incorporated too??
BIOMAX							Certain	Used to store data from NanoFASE project
NanoSolveIT knowledge base	Not publically available yet, though a publication is in progress. Sam has access (to some of the data, at least)						Likely	NanoSolveIT WP1 will create a knowledge base using NM "fingerprints" to enable read-across and grouping. Draws in pre-existing data and gap-filling done with the project (WP2 experimental, WP3-5 modelling). Data will be stored in eNanoMapper.
TNO spatial release database	-						Likely	Spatial NM release database for all EU developed as part of NanoFASE project. Spatial database developed by TNO, using MFA outputs from EMPA.
Literature data								Data available in literature but not stored on any specific database.
LEITAT release data							Certain	Release data generated by LEITAT, e.g. TiO_2 road coating release rates. Most data will be held in different databases depending on the project (e.g. Biomax for NanoFASE, eNanoMapper for GRACIOUS).
NanoHarmony	https://nanoinfo.org/nanodatabank						Likely	NanoHarmony project, which has just started, aims to drive forward development of nano-specific guidance. Will include gap analysis and targeted experimental work. Includes tox. (human and eco) and physchem characterisation.
NanoDataBank							Likely	UCLA database associated with MendNano and LearNano models.

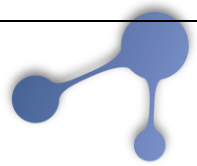
Project Title	Website	Phys-chem	Toxicology	Ecotox	Exposure	Other	Database presence	Comments
Pubvinas	http://www.pubvinas.com/						Certain	A web-based nanomaterial database (developed in US) by big data curation and modelling friendly nanostructure annotations. contains 705 unique nanomaterials covering 11 material types. Each nanomaterial has up to six physicochemical properties and/or bioactivities, resulting in more than ten endpoints in the database.
MESOCOSM	https://aliayadi.github.io/MESOCOSM-database/						Certain	The first centralized mesocosm database management system for environmental nanosafety containing experimental data collected from mesocosm experiments suited for understanding and quantifying both the environmental hazard and exposure.

7.3 Standardised methodology for human hazard assessment

Table 8. Human hazard methodology with associated guidelines and SOPs

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Acute toxicity	Cytotoxicity and viability	ATP assay with murine fibroblasts.	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	ATP cell viability assay for silver (Ag) NP-treated A549 cells	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Caspase 3/7 activity detection assay	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Measurement of IL-1β and TNF-α secretion by ELISA	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Measurement of intra cellular Reactive Oxygen Species (ROS)	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	MTS cell viability assay for gold (Au) NP-treated A549 cells	Nanovalid Project	Validated SOP	Yes	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Acute toxicity	Cytotoxicity and viability	MTS cell viability assay for silver (Ag) NP-treated A549 cells	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	MTS cell viability assay for Copper Oxide (CuO) NP-treated Caco-2 cells	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	MTS cell viability assay for Copper Oxide (CuO) NP-treated HepG2 cells	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	MTS cell viability assay for silver (Ag) NPs-treated THP-1 cells	Nanovalid Project	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	MTT with mesenchymal stem cells	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	PI assay with mesenchymal stem cells	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	PI assay with murine fibroblasts	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	WST1 assay with murine fibroblasts	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Oral toxicity	Acute oral testing (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	
Acute toxicity	Cytotoxicity and viability	LDH assay	Nanoreg	Validated SOP	Yes	
Acute toxicity	Apoptosis/necrosis	Apoptosis/Necrosis Analysis	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Real-time label-free impedance-based nanotoxicity assessment	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	SOP for High Throughput Cell Impedance Measurement	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Label-free nanotoxicity assessment by impedance-based flow cytometry	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	Viability protocol by using a Cell Counter	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	HEL16T008 AlamarBlue Assay	Nanoreg	Validated SOP	Yes	
Acute toxicity	Cytotoxicity and viability	HEL17T010 Colony forming efficiency assay	Nanoreg	Validated SOP	Yes	The CFE assay is a label-free method for assessment of basal cytotoxicity. Being non-colorimetric and nonfluorescent the method avoids possible interferences of NMs with toxicity assessments. It has been optimized and standardized for NMs testing by the JRC's Nanobiosciences Unit and validated



Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
						in the interlaboratory comparison study of the Colony Forming Efficiency assay for assessing cytotoxicity of nanomaterials (Kinsner-Ovaskainen A and Ponti J,2014).
Acute toxicity	Inhalation toxicity (subacute)	Test No. 412: Subacute Inhalation Toxicity: 28-Day Study	OECD	Validated Test Guideline	Yes	
Acute toxicity	Inhalation toxicity	Test No. 403: Acute Inhalation Toxicity	OECD	Validated Test Guideline	No	
Acute toxicity	Inhalation toxicity	Test No. 433: Acute Inhalation Toxicity: Fixed Concentration Procedure	OECD	Validated Test Guideline	No	
Acute toxicity	Inhalation toxicity	Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method	OECD	Validated Test Guideline	No	
Acute toxicity	Oral toxicity	Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents	OECD	Validated Test Guideline	No	
Acute toxicity	Oral toxicity	Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure	OECD	Validated Test Guideline	No	
Acute toxicity	Oral toxicity	Test No. 423: Acute Oral toxicity - Acute Toxic Class Method	OECD	Validated Test Guideline	No	
Acute toxicity	Oral toxicity	Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure	OECD	Validated Test Guideline	No	
Acute toxicity	Cytotoxicity and viability	ISO 19007:2018 - Nanotechnologies — In vitro MTS assay for measuring the cytotoxic effect of nanoparticles	ISO	Validated Test Guideline	Yes	"ISO 19007:2018 specifies a method for evaluating the effects of nano-objects and their aggregates and agglomerates (NOAA) on cellular viability using the MTS assay. The assay design includes performance requirements and control experiments to identify and manage variability in the assay results. ISO 19007:2018 is applicable to the use of a 96-well plate."
Acute toxicity	Cytotoxicity and viability	ISO/DTR 22455 - High throughput screening method for nanoparticles toxicity using 3D cells	ISO	Draft/Literature Method	Yes	Under development
Acute toxicity	Cytotoxicity and viability	ISO/TR 16197:2014 - Nanotechnologies — Compilation and description of toxicological screening methods for manufactured nanomaterials	ISO	Validated Test Guideline	Yes	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Acute toxicity	Various	In vitro Exposures at the air-liquid interface to aerosols of NM	Loret et al. Particle and Fibre Toxicology (2016) 13:58, DOI 10.1186/s12989-016-0171-3	Validated SOP	Yes	
CLP	Genotoxicity	Genotoxicity assessment of ENMs by Comet assay (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	Pre-validated
CLP	Genotoxicity	HEL11T005 HTS Comet Assay with and without FPG - 12 wells	Nanoreg	Validated SOP	Yes	
CLP	Genotoxicity	HTS Comet Assay with and without FPG - 20 wells	Nanoreg	Validated SOP	Yes	
CLP	Genotoxicity	HEL11T007 Mammalian in vitro HPRT Mutation test	Nanoreg	Validated SOP	Yes	
CLP	Genotoxicity	HEL16T009 Mouse Lymphoma Assay in vitro	Nanoreg	Validated SOP	Yes	
CLP	Genotoxicity	Micronucleus Assay	Nanoreg	Validated SOP	Yes	
CLP	Genotoxicity	Micronucleus assay using Flow Cytometry	Nanoreg	Validated SOP	Yes	
CLP	Carcinogenicity	Test No. 451: Carcinogenicity Studies	OECD	Validated Test Guideline	No	
CLP	Carcinogenicity	Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies	OECD	Validated Test Guideline	No	
CLP	Carcinogenicity	Human lung cell transformation assay	OECD	Draft/Literature Method		
CLP	Carcinogenicity	Bhas 42 Cell Transformation Assay	OECD	Draft/Literature Method		
CLP	Reproductive	Test No. 414: Prenatal Developmental Toxicity Study	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 415: One-Generation Reproduction Toxicity Study	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 416: Two-Generation Reproduction Toxicity	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 421: Reproduction/Developmental Toxicity Screening Test	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 422: Combined Repeated Dose Toxicity Study with the	OECD	Validated Test Guideline	No	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
		Reproduction/Developmental Toxicity Screening Test				
CLP	Reproductive	Test No. 440: Uterotrophic Bioassay in Rodents	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 441: Hershberger Bioassay in Rats	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 443: Extended One-Generation Reproductive Toxicity Study	OECD	Validated Test Guideline	No	
CLP	Reproductive	Test No. 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 471: Bacterial Reverse Mutation Test	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 473: In Vitro Mammalian Chromosomal Aberration Test	OECD	Validated Test Guideline	No	TG noted "For manufactured nanomaterials, specific adaptations of this Test Guideline may be needed but are not described in this Test Guideline."
CLP	Genotoxicity	Test No. 474: Mammalian Erythrocyte Micronucleus Test	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 478: Rodent Dominant Lethal Test	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 483: Mammalian Spermatogonial Chromosomal Aberration Test	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 487: In Vitro Mammalian Cell Micronucleus Test	OECD	Validated Test Guideline	No	TG noted "For Manufactured Nanomaterials, specific adaptations of this

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
						Test Guideline are needed but they are not described in this Test Guideline."
CLP	Genotoxicity	Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 489: In Vivo Mammalian Alkaline Comet Assay	OECD	Validated Test Guideline	No	
CLP	Genotoxicity	Test No. 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene	OECD	Validated Test Guideline	No	TG noted "For manufactured nanomaterials, specific adaptations of this Test Guideline may be needed but are not described in this Test Guideline"
CLP	Skin Sensitisation	Test No. 406: Skin Sensitisation	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 429: Skin Sensitisation	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 442A: Skin Sensitization	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 442B: Skin Sensitization	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 442C: In Chemico Skin Sensitisation	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 442D: In Vitro Skin Sensitisation	OECD	Validated Test Guideline	No	
CLP	Skin Sensitisation	Test No. 442E: In Vitro Skin Sensitisation	OECD	Validated Test Guideline	No	
CLP	Corrosivity/Irritation	Test No. 404: Acute Dermal Irritation/Corrosion	OECD	Validated Test Guideline	No	
CLP	Corrosivity/Irritation	Test No. 405: Acute Eye Irritation/Corrosion	OECD	Validated Test Guideline	No	
CLP	Corrosivity	Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)	OECD	Validated Test Guideline	No	
CLP	Corrosivity	Test No. 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method	OECD	Validated Test Guideline	No	This Test Guideline describes an in vitro procedure allowing the identification of non-corrosive and corrosive substances and mixtures, based on three-dimensional human skin model. Not rigorously tested with NMs although some studies done in OECD programme. Some critical factors for

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
						the evaluation of MNMs induced skin corrosion were not addressed in detail and might need to be further investigated. These include the optimum exposure duration and time of evaluation, choice of the exposure concentrations (i.e. stable dispersions without material aggregation should be used), evaluation of the cellular uptake, surface area of exposure, compatibility of the receptor fluid for MNMs) (OECD Evaluation of in vitro methods for human hazard assessment applied in the OECD Testing Programme for the Safety of Manufactured Nanomaterials)
CLP	Corrosivity	Test No. 435: In Vitro Membrane Barrier Test Method for Skin Corrosion	OECD	Validated Test Guideline	No	The test method utilizes an artificial membrane designed to respond to corrosive substances in a manner similar to animal skin in situ. It may be used to test solids, liquids (aqueous substances with a pH in the range of 4.5 to 8.5 often do not qualify for testing) and emulsions. It allows the identification of corrosive chemical substances and mixtures and allows the subcategorisation of corrosive substances as permitted in the GHS. Not rigorously tested with NMs
CLP	Corrosivity/Irritation	Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	OECD	Validated Test Guideline	No	
CLP	Corrosivity/Irritation	Test No. 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	OECD	Validated Test Guideline	No	
CLP	Irritation	Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method	OECD	Validated Test Guideline	No	Reconstructed human epidermis method for hazard identification and labelling, to comply with a range of legislation including EU REACH and the CLP Regulation. The output of this test is a binary classification (Irritant or Non-Irritant). Not rigorously tested with NMs

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CLP	Irritation	Test No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	OECD	Validated Test Guideline	No	
CLP	Irritation	Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage	OECD	Validated Test Guideline	No	Not tested with NMs
CLP	Irritation	Test No. 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	OECD	Validated Test Guideline	No	
CLP	Irritation	Test No. 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	OECD	Validated Test Guideline	No	
CLP	Neurotoxicity	Test No. 418: Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure	OECD	Validated Test Guideline	No	
CLP	Neurotoxicity	Test No. 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study	OECD	Validated Test Guideline	No	
CLP	Neurotoxicity	Test No. 424: Neurotoxicity Study in Rodents	OECD	Validated Test Guideline	No	
CLP	Neurotoxicity	Test No. 426: Developmental Neurotoxicity Study	OECD	Validated Test Guideline	No	
Dermal toxicity	Dermal toxicity	Acute dermal testing	Nanovalid Project	Draft/Literature Method	Yes	
Dermal toxicity	Dermal toxicity	Nose model protocol for ENMs	Nanovalid Project	Draft/Literature Method	Yes	
Dermal toxicity	Dermal toxicity	Subchronic dermal testing	Nanovalid Project	Draft/Literature Method	Yes	
Dermal toxicity	Acute dermal toxicity	Test No. 402: Acute Dermal Toxicity	OECD	Validated Test Guideline	No	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Dermal toxicity	Repeated dose dermal toxicity	Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study	OECD	Validated Test Guideline	No	
Dermal toxicity	Subchronic dermal toxicity	Test No. 411: Subchronic Dermal Toxicity: 90-day Study	OECD	Validated Test Guideline	No	
Dermal toxicity	Absorption	Test No. 427: Skin Absorption: In Vivo Method	OECD	Validated Test Guideline	No	
Dermal toxicity	Absorption	Test No. 428: Skin Absorption: In Vitro Method	OECD	Validated Test Guideline	No	
Fibre/HARN	PSD and Fibre length	Test No. 110: Particle Size Distribution/ Fibre Length and Diameter Distributions	OECD	Validated Test Guideline	No	
Inflammatory reactions	Inflammation	Production of pro-inflammatory cytokines	Nanoreg	Validated SOP	Yes	Nanommune Handbook/NCL method ITA10. Determination of IL-12, IL-1β and TNF-α production by ELISA. Exposure may be with ALI system, submerged, Different cell lines or cell models (e.g pulmonary barrier)
Inflammatory reactions	Cytokine secretion	Whole blood cell assay for cytokine production	Nanoreg	Validated SOP	Yes	
Inflammatory reactions	Haemolytic properties	ASTM E2524 - 08(2013) - Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles.	BSI	Validated Test Guideline	Yes	
Inflammatory reactions	Chemoattractant Capacity	ASTM E3238 – 20 - Standard Test Method for Quantitative Measurement of the Chemoattractant Capacity of a Nanoparticulate Material in vitro.	BSI	Validated Test Guideline	Yes	
Inflammatory reactions	Cytokine secretion	Measurement of inflammatory cytokine secretion by ELISA	Bartosh TJ, Ylostalo JH. Macrophage Inflammatory Assay. Bio Protoc. 2014;4(14):e1180. doi:10.21769/bioprotoc.1180			Kits available for this assessment commercially (e.g. from R&D Systems). Various cytokines possible, for inflammation tumor necrosis factor alpha (TNF-α) and interleukin-8 and -10 (IL-8, IL-10) commonly tested.
OELs	OEL	ISO/TR 18637:2016 - Nanotechnologies — Overview of available frameworks for the development of occupational exposure limits and bands for nano-	ISO	Validated Test Guideline	Yes	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
		objects and their aggregates and agglomerates (NOAAs)				
Particle diameter	PSD	WP5-DLS analysis of ENMs	Nanovalid Project	Validated SOP	Yes	
Particle diameter	PSD	WP5-TEM analysis of ENMs	Nanovalid Project	Validated SOP	Yes	
Particle diameter	Surface area	WP5-BET analysis of ENMs	Nanovalid Project	Validated SOP	Yes	
Particle diameter	PSD	For measurement of hydrodynamic Size-Distribution and Dispersion Stability by DLS	Nanoreg	Validated SOP	Yes	
Particle diameter	PSD	For particle size determination of a given MNM by the CLS technique	Nanoreg	Validated SOP	Yes	
Reactivity	Surface reactivity	For determination of LDH, IL-6, IL-8 adsorption onto MNM	Nanoreg	Validated SOP	Yes	
Reactivity	Surface reactivity	Determination of ROS by DCFH-DA	GRACIOUS	Validated SOP	Yes	Under development
Reactivity	Barrier Integrity	Evaluation of NMs impact on Caco-2 cell barrier model	Nanoreg	Validated SOP	Yes	
Reactivity	Oxidative stress	Reaction Oxygen Species Detection	Nanoreg	Validated SOP	Yes	DCFH assay (cellular): "Measurements of oxidation of dichlorofluorescin (H2DCF), dihydrorhodamine 123 (DHR) or hydroethidine (DHE), Exposure may be with ALI system, submerged
Reactivity	Toxicokinetics	ISO/TR 22019:2019 - Nanotechnologies — Considerations for performing toxicokinetic studies with nanomaterials	ISO	Validated Test Guideline	Yes	
Reactivity	Toxicokinetics	Test No. 417: Toxicokinetics	OECD	Validated Test Guideline	No	A report on preliminary review of OECD Test Guidelines for their applicability to nanomaterials indicates that TG 417 may not apply to nanomaterials. See OECD (2009) Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials, Series on the Safety of Manufactured Nanomaterials No. 15, ENV/JM/MONO(2009)21, OECD, Paris
Reactivity	Phototoxicity	Test No. 432: In Vitro 3T3 NRU Phototoxicity Test	OECD	Validated Test Guideline	No	The reliability and relevance of the in vitro 3T3 NRU Test has not been specifically validated for NMs (Spielmann et al. 1998). In some instances neutral red may interfere with NMs (Lanone et al., 2009; Guadagini et al., 2015)

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Reactivity	Phototoxicity	ICH Guidance S10 on Photosafety Evaluation of Pharmaceuticals	ICH	Validated Test Guideline	No	Not rigorously tested with NMs
Reactivity	Oxidative stress	FRAS protocol	Gracious	Validated SOP	Yes	
Reactivity	Oxidative stress	ISO/TS 19006:2016 - Nanotechnologies — 5-(and 6)-Chloromethyl-2',7' Dichloro-dihydrofluorescein diacetate (CM-H2DCF-DA) assay for evaluating nanoparticle-induced intracellular reactive oxygen species (ROS) production in RAW 264.7 macrophage cell line	ISO	Validated Test Guideline	Yes	
Reactivity	Oxidative stress	Test No. 495: Ros (Reactive Oxygen Species) Assay for Photoreactivity	OECD	Validated Test Guideline	No	
Reactivity	Oxidative stress	ISO/TS 18827:2017 - Nanotechnologies — Electron spin resonance (ESR) as a method for measuring reactive oxygen species (ROS) generated by metal oxide nanomaterials	ISO	Validated Test Guideline	Yes	
Reactivity	Phototoxicity	ISO 20814:2019 - Nanotechnologies — Testing the photocatalytic activity of nanoparticles for NADH oxidation	ISO	Validated Test Guideline	Yes	
Reactivity	Endocrine disruption	Test No. 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals	OECD	Validated Test Guideline	No	
Reactivity	Oxidative stress	Commercially available ROS detection kits	Müller, Loretta, et al. Journal of the Royal Society Interface 7.suppl_1 (2010): S27-S40.			e.g. Image-iT LIVE Green Reactive Oxygen Species Detection Kit, Molecular Probes, Invitrogen AG (used in study by Müller et al. 2010 doi:10.1098/rsif.2009.0161.focus)
Severe toxicity	Oral toxicity	Subchronic oral testing (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	
Severe toxicity	Biodistribution	ENP biodistribution assessment in organs and tissues by PIXE (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	Pre-validated
Severe toxicity	Biodistribution	Quantitative Imaging of nanoparticle uptake and distribution in environmental organisms by LA-ICP-MS	Nanovalid Project	Draft/Literature Method	Yes	

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Severe toxicity	Specific organ toxicity	Kidney model (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	
Severe toxicity	Translocation	MRI study on impact of ENPs on rat ear barriers to simulate skin, mucosa, and brain biological barriers (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	
Severe toxicity	Translocation	Multifunctional rat ear model for evaluating impact of ENMs on skin, mucosa, and nerve system (in vivo)	Nanovalid Project	Draft/Literature Method	Yes	
Severe toxicity	Inhalation toxicity (subchronic)	Test No. 413: Subchronic Inhalation Toxicity: 90-day Study	OECD	Validated Test Guideline	Yes	
Severe toxicity	Oral toxicity	Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents	OECD	Validated Test Guideline	No	
Severe toxicity	Oral toxicity	Test No. 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	OECD	Validated Test Guideline	No	
Severe toxicity	Chronic toxicity	Test No. 452: Chronic Toxicity Studies	OECD	Validated Test Guideline	No	
Solubility/dissolution	Solubility	Test No. 105: Water Solubility	OECD	Validated Test Guideline	No	Two methods outlined: column and flask methods. Not rigorously tested with NMs, need to determine if NMs are efficiently removed from final sample for analysis. Xu et al. (2013) have reported the incomplete removal of nanomaterial from the supernatant after exhaustive centrifugation.
Solubility/dissolution	Solubility	Filtration and centrifugation	Fabricius et al., 2014	Draft/Literature Method	No	Similar to TG105, use of physical filters (as in the flask method) has been used in the characterisation of nanomaterial solubility. Again, thought that centrifugation does not remove NMs (Xu et al (2013)
Solubility/dissolution	Solubility	Equilibrium Dialysis	Fabrega et al., 2012; Fabricius et al., 2014	Draft/Literature Method	Yes	
Solubility/dissolution	Solubility	Ultrafiltration (UF)	Fabricius et al., 2014	Draft/Literature Method	Yes	One limitation in UF relates to potential interactions of the membrane not only with the nanomaterial but also with the dissolved species. The choice of membrane is thus crucial.
Solubility/dissolution	Solubility	Ion exchange technology (IET)	Hadioui et al., 2013, 2014	Draft/Literature Method	Yes	Despite the promising features of this IET, only a few studies have been carried out in relation to the measurement of nanomaterial dissolution/solubility.

Descriptor	Endpoint	Method	Source	Type	Nano-relevant?	Comment
Solubility/dissolution	Dissolution	For characterizing MNM fate in biological media and digestive fluids by multi-technique based method	Nanoreg	Validated SOP	Yes	
Solubility/dissolution	Reactivity and dissolution	For hydrochemical reactivity and biodurability testing using an Atmosphere-Temperature-pH-controlled SBR	Nanoreg	Validated SOP	Yes	
Solubility/dissolution	Reactivity and dissolution	For SDR analyses of MNM hydrochemical reactivity and dissolution in in vitro medium	Nanoreg	Validated SOP	Yes	Only works with pH between 5 and 9
Solubility/dissolution	Solubility	Protocol for the measurement of water solubility	Nanoreg	Validated SOP	Yes	Protocol includes 1) Determination of NM dissolved fraction by filtration/centrifugation followed by ICP-MS of the filtrate, or 2) Determination of NM dissolved fraction by ISE measurement (NM-300K, Ag NM)
Solubility/dissolution	Dissolution	ISO/TR 19057:2017 Nanotechnologies — Use and application of acellular in vitro tests and methodologies to assess nanomaterial biodurability	ISO	Validated Test Guideline	Yes	
		LAL Assay for Nanoparticles	Nanoreg	Validated SOP	Yes	
		Human Lung Cell transformation assay. Long term chronic experiment	Nanoreg	Validated SOP	Yes	
		TaqMan real-time Reverse Transcription PCR	Nanoreg	Validated SOP	Yes	
		High Content Analysis-based nanotoxicity assessment	Nanoreg	Validated SOP	Yes	
	Cellular Uptake	ISO/CD TS 23034 - Method to estimate cellular uptake of carbon nanomaterials using optical absorption	ISO	Draft/Literature Method	Yes	Under development
	Cellular Uptake	Flow cytometry	Nanoreg	Draft/Literature Method	Yes	Experimental system was human pulmonary barrier. Cellular uptake determined by flow cytometry. Exposure may be with ALI system, submerged,

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