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Analysis of OECD WPMN dossiers regarding the availability of data to evaluate and regulate risk

Full Report

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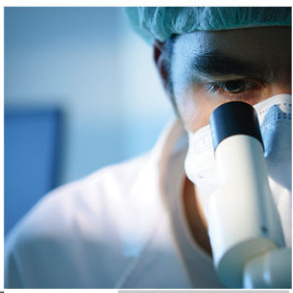

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1 INTRODUCTION

This study was carried out as part of a wider project sponsored by Velux Foundations to support civil society organisations to ensure the adoption of a precaution-based regulatory framework for the responsible development of nanotechnologies in the EU and beyond, based on adequate risk assessment methodologies and risk management tools. Activities focus on key fora where scientific, technical and political discussions influence developments in EU regulatory governance of nanomaterials. These fora include CEN and ISO standardisation technical committees, and the OECD Working Party on Manufactured Nanomaterials (WPMN).

In 2007, the WPMN launched a Sponsorship Programme for the Testing of 11 Manufactured Nanomaterials (MNM), that were in, or close to, commercial use. The resulting dossiers for each of the nanomaterials tested were published[1] in June 2015. An initial screening of the dossiers concluded that that there are obvious disparities on data availability across the various nanomaterials. In particular, the endpoints related to the lifecycle of tested materials and the possible exposures are poorly addressed. Dossiers in relation to human exposure scenarios are also missing, except for gold nanoparticles, multi-walled carbon nanotubes (MWCNTs), silver nanoparticles, nano titanium dioxide (TiO_2), and nano zinc oxide (ZnO).

In addition to disparities in data availability, the sources and quality of data vary: a number of datasets were derived from peer-reviewed scientific publications while others were taken from corporate sources (especially the phys-chem characterisation data). The data quality also seemed to differ depending on the existence and validity of established testing guidelines and standards. For some aspects (e.g. phys-chem characterisation) there were established testing procedures while the testing of toxicological endpoints was more exploratory (this seemed to be the case particularly in relation to hazard assessment).

The dossiers in their current form are a compilation of datasets for each nanomaterial presented in International Uniform Chemical Information Database (IUCLID) format, with annexes of original studies, from which the endpoint-related data were derived. Dossier summaries are still being prepared for most of the 11 types of nanomaterials, and these may serve as the basis for a weight of evidence evaluation of the data quality. The WPMN has not yet drawn conclusions on the data quality and rather suggests that regulators and researchers interpret the data themselves.

The Sponsors approached IOM and requested that they develop a proposal to carry out a dossier analysis of the OECD data.

[1] http://www.oecd.org/chemicalsafety/nanosafety/overview-testing-programme-manufactured-nanomaterials.htm. Last accessed on 24 November 2016

1.1 OBJECTIVE

Very few studies have been published analysing or assessing the dossier information. Most of the studies underway appear to focus on the eco-toxicological and wider environmental impacts. Hence, little apparent scrutiny of the dossiers from a human health perspective has occurred to date. This project aimed to look beyond the raw data and provide preliminary elements of analysis from a civil society perspective, with a particular focus on the human health aspects.

The project aimed to provide a deeper analysis of the dossier data in order to assess its usefulness for the regulatory risk management of MNM. It had a focus on human health aspects, to supplement existing analysis and studies published during the course of the delivery of the work.

The analysis done within this project provides a horizontal assessment of all 11 dossiers and a more detailed assessment of 3 specific nanomaterials.

1.2 METHODS

We carried out a 2-step analysis in this project. In the first step, we scanned all OECD nanomaterial dossiers that were available online on the OECD website to get an understanding of what type of information the dossiers contain. This data analysis step aimed to provide information about whether the endpoints assessed are in principle sufficient to do a risk assessment, or if important elements are missing (e.g. if no ecotoxicological data exists, one cannot directly assess related risks but will have to find such information elsewhere). In the second step, three types of nanomaterials in the OECD Testing Programme were selected for further assessment of the available data in comparison with minimal data requirements established by leading journals in the toxicology and exposure field or other relevant sources defining minimum characterisation requirements. ☐This refined data analysis step aimed to inform whether the data assessed should in principle allow toxicologists and exposure specialists to make an assessment of the nanomaterials. The underlying assumption for both steps was that the methods themselves were conducted correctly and that the results obtained with these methods were valid from a scientific perspective.

2 METHODS

2.1 STRATEGY

In order to assess the completeness of the OECD nanomaterial dossiers, we systematically reviewed the dossier documents of a list of manufactured nanomaterials the OECD's WPMN identified for the Sponsorship Program for the Testing of Manufactured Nanomaterials. The overall strategy of this study is shown in Figure 1.

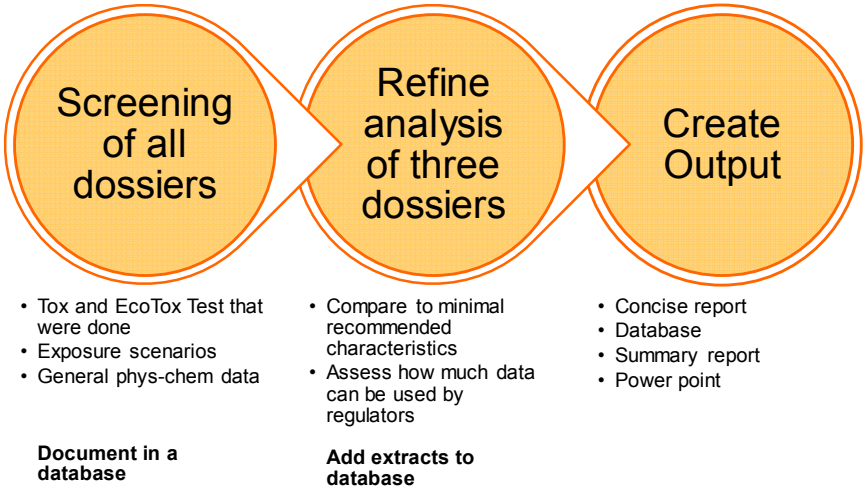


Figure 1. Study strategy

An **initial analysis** provided us with a good understanding of what information was available in the dossiers and whether it seems, in general, sufficient for risk assessment. In an initial analysis, all of the OECD dossier documents as well as the associated annex materials (Testing Programme of Manufactured Nanomaterials - Dossiers and Endpoints[2]) were reviewed with regards to:

- Test guidelines used for characterization of physical and chemical properties
- Environmental fate and pathways assessed, including Endpoints, Nanomaterial, Reliability score, Test guideline adopted, Test system/organism, and a Summary of the results.
- Ecotoxicological/Toxicological information, including Endpoints, Nanomaterial, Reliability score, Test guideline adopted, Route of Administration, Test cell/tissue/organism, Type of data, Exposure and dose information, and Phys-chem characterization of the NM
- Manufacture, Use and exposure of nanomaterials
- Human exposure scenarios

[2] http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm. Last accessed on 24 November 2016

A **refined analysis** was done afterwards by focusing on fullerenes, single-walled carbon nanotubes and zinc oxide. The reason behind the selection is that these nanomaterials are actively researched or widely used in commercial applications while they have received relatively lower attention for risk assessment than mainstream nanomaterials such as silver and titanium dioxide nanoparticles. The analysis assessed in more detail ecotoxicological and toxicological information in comparison to literature recommending minimal reporting characteristics for conducting risk assessments (see section 2.3).

To also understand the general trend of data availability in all the other dossiers, we carried out a refined analysis on a stratified random sample of about 17% of all the Endpoint Study Records (ESRs) with stratification done along dossiers and second-level endpoint study record numbers (e.g. 6.1, 6.2, etc.). We called this approach "sub-sampling" (see section 2.4).

2.2 INITIAL ANALYSIS OF OECD NANOMATERIAL DOSSIERS

To assess the completeness of the OECD nanomaterial dossiers, we systematically reviewed the dossier documents for the manufactured nanomaterials the OECD's WPMN identified for the Sponsorship Program for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). This comprised 11 types of nanomaterials: i) cerium oxide; ii) dendrimers; iii) fullerenes; iv) gold nanoparticles; v) multi-walled carbon nanotubes (MWCNTs); vi) nanoclays; vii) silicon dioxide; viii) silver nanoparticles; ix) single-walled carbon nanotubes (SWCNTs), x) titanium dioxide; and xi) zinc oxide. The assessment was carried out in a 2-step analysis: an initial and a refined analysis.

The **initial analysis** provided a good understanding of what information was available in the dossiers and whether it seems, in general, sufficient for risk assessment. In the initial analysis, all of the dossier documents (under DOSSIER section of Testing Programme of Manufactured Nanomaterials - Dossiers and Endpoints[3] of each type of the nanomaterials) were reviewed. The associated annex materials (under ANNEXES section of Testing Programme of Manufactured Nanomaterials - Dossiers and Endpoints[4] of each type of the nanomaterials) were referred to when necessary (e.g., no meaningful information or insufficient details provided by the dossier documents). This initial analysis was focused on the following aspects, with the extracted information summarized as below.

[3] http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm. Last accessed on 24 November 2016

[4] http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm. Last accessed on 24 November 2016

Data structure used in the dossiers: An overview of data contained in the various dossiers is provided in the "Endpoint Finder Excel Spreadsheet"[5]. The nomenclature and structure corresponds to the IUCLID reporting format. In the following data analysis, the term "Section" refers to the sections of the OECD dossiers, which correspond in title and numbering to the IUCLID reporting sections.

Test guidelines used for characterization of physical and chemical properties: The test guidelines used for characterization of physical and chemical properties of each type of nanomaterials were tabulated against endpoints as listed in Section 4. We documented the findings in an Excel spreadsheet tab named "Phys-chem" as part of the database containing the information extracted from the dossier documents (hereafter the database). The database provides a quick access to some key information of the dossiers for risk assessment.

Environmental fate and pathways: The following information was extracted from Section 5. The results were documented in the Excel spreadsheet tab named "EnvironFate".

- Endpoint
- Nanomaterial
- Nanomaterial ID/characteristics
- Endpoint study record (ESR) title
- Reliability score
- Test guideline adopted: Blue color highlights the test guideline adopted with deviations; if no test guideline was adopted, the result would be recorded as "method other than guideline" *(applied to other "Test guideline adopted" of data record)*
- Test system/organism
- Summary of results
- Remarks

Ecotoxicological/Toxicological information: The following information was extracted from Section 6 and Section 7. The results were documented in the Excel spreadsheet tab named "(Eco)Tox".

- Endpoint
- Nanomaterial

[5] http://www.oecd.org/env/ehs/nanosafety/Testing Programme on Manufactured Nanomaterials Endpoints.xlsx. Last accessed on 24 November 2016

- Nanomaterial ID/characteristics
- ESR title
- Reliability score
- Test guideline adopted
- Route of Administration: inhalation, gavage, intraperitoneal, subcutaneous, intravenous
- Test cell/tissue/organism
- Type of data: raw, derived
- Exposure concentration value and unit
- Dose value and unit
- Number of repetitions of exposure
- Total duration of exposure and its unit
- Remark (exposure)
- EP indicator
- Phys-chem characterization of the NM (feed, dispersion, aerosol)
- References
- Remarks

Manufacture, Use and exposure of nanomaterials: The manufacture & use information was extracted from Section 3. The results were documented in the Excel spreadsheet tab named "Manufacture-Use-Exposure".

Human exposure scenarios: OECD adopted the IUCLID format for reporting data. In this format, human exposure scenarios are reported in Section 3. While OECD did not have the intent to collect such data, the dossiers still prominently list this section header without commenting that there was no intent to collect such data. Indeed, when investigating the dossiers, exposure scenario information is totally missing for all of the 11 types of nanomaterials in Section 3. Some relevant information, such as exposure related observation in humans, health surveillance data and sensitization data, was identified in Section 7.10 for 5 types of nanomaterials (i.e., gold nanoparticles, multi-walled carbon nanotubes, silver nanoparticles, titanium dioxide and zinc oxide) was documented in the Excel spreadsheet named "Exposure" in the database. The fact that the dossiers do not clarify that OECD did not intend to collect this information can lead to misunderstandings amongst readers not familiar with this aspect of the testing programme.

2.3 REFINED ANALYSIS OF THREE DOSSIERS

Fullerenes, single-walled carbon nanotubes and zinc oxide were selected for the refined analysis of ecotoxicological and toxicological information.

The following documents were reviewed to identify the minimal data requirements established by leading peer-reviewed journals in the toxicology and exposure field.

- Join the dialogue, *Nature nanotechnology*, 7, 545 (2012).
- Minimal analytical characterization of engineered nanomaterials needed for hazard assessment in biological matrices, Bouwmeester H. *et al. Nanotoxicology*, 5, 1-11 (2011).
- Limitations and information needs for engineered nanomaterial-specific exposure estimation and scenarios: recommendations for improved reporting practices, Clark K. *et al. Journal of Nanoparticle Research*, 14: 970 (2012).
- Airborne engineered nanomaterials in the workplace—a review of release and worker exposure during nanomaterial production and handling processes, Ding Y. *et al. Journal of Hazardous Materials*, S0304-3894(16)30416-2 (2016).

The minimal requirements identified in these articles includes characteristics for hazard assessment and exposure assessment, as summarized in *Table 1*.

Table 1. Minimal data requirements for hazard assessment and exposure assessment for nanomaterials recommended by the identified peer-reviewed journal articles

	Nat. Nanotechnol.	**Bouwmeester H. *et al.***	**Clark K. *et al.***	**Ding Y. *et al.***
Hazard & Exposure				
Chemical composition	√	√	√	√
Impurities	√			
Degree of aggregation/agglomeration	√	√	√	√
Morphology (including aspect ratio)	√	√	√	√
Particle size / size distribution	√	√	√	√
Particle mass concentration			√	√
Persistence (e.g., solubility, UV-stability, thermal-stability)	√	√	√	√
Surface area	√	√	√	√
Surface chemistry	√	√	√	√
Surface charge		√		
Details on the matrix surrounding the nanomaterials			√	
Exposure				
Physical/chemical form of released/detected NPs			√	
Potential other source of ultrafine and other particles			√	
Production/handling details (e.g., amount, duration and frequency)			√	√
Particle number concentration			√	√
Description of activities leading to exposure in a standardized format			√	
Exposure duration/frequency			√	√
Particle release rate				√
Description of site (e.g., room size, windows)			√	√
Control measures			√	√
Sampling strategy			√	√

We reviewed Section 6 (Ecotoxicology) and Section 7 (Toxicology) of the dossier documents of fullerenes, SWCNTs and zinc oxide to identify presence or absence of the characteristics in *Table 1* for each ESR. Degree of purity was also identified. The results were documented in the 3 Excel spreadsheets named "C60", "SWCNT" and "ZnO" for fullerenes, SWCNTs and zinc oxide in the database, respectively. In addition, the following information was also documented for each endpoint study of the 3 types of nanomaterials.

- Endpoint
- ESR title
- Test guideline adopted
- Klimisch score (reliability score)
- GLP[6] / other quality certificate
- Nanomaterial ID

Presence was recorded as "1" while absence was recorded as blank, except for degree of purity (the actual value provided in the dossiers) and Klimisch score, which was recorded as the actual score (1 – 4) or blank if no score was provided. As to degree of aggregation/agglomeration, morphology, particle size, particle size distribution, surface area, surface charge and particle number concentration, if an ESR clearly indicated that the relevant measurements had been conducted for a characteristic without showing the results, we still recorded this characteristic as presence.

2.4 SUB-SAMPLING

To compare the general trend of data availability of dossiers for refined analysis with that of all of the dossiers, 17% of all of the ESRs of ecotoxicological and toxicological information of 11 types of nanomaterials was randomly selected, reviewed and assessed with the same method as refined analysis.

There were 782 ESRs of ecotoxicological and toxicological information of 11 types of nanomaterials. These records were numbered from 1 to 782 and 100 of them were randomly selected by the sampling function of data analysis in Excel 2013. The first 75 records without duplicates were used for sub-sampling analysis.

After the random sampling, a stratified strategy was applied to ensure that at least 10% of ESRs were sampled for each type of nanomaterials at the

[6] GLP = Good Laboratory Practice, a quality system of management controls for research laboratories

second level of endpoint (i.e., 6.x and 7.x). The sampling function of data analysis in Excel 2013 was used to randomly identify ESR numbers. The selected ESRs were reviewed and assessed with the same method as the refined analysis.

2.5 ASSESSMENT OF DATA COMPLETENESS

To assess whether any study was conducted for each type of nanomaterials at the third level of endpoint (e.g., IUCLID code 6.1.1, *Short-term toxicity to fish*), we counted the number of ESRs for each type of nanomaterials at each of the third-level endpoint. The results were presented in a format of heat map. They were also compared with the availability of ESRs shown in the OECD Endpoint Finder Excel spreadsheet to assess the accuracy of the endpoint finder.

The data completeness was calculated as percentage for each characteristic in *Table 1* at a third-level endpoint. For example, 4 out of the 7 seven ESRs of SWCNTs at "*6.1.1 Short-term toxicity to fish*" mentioned impurities. Therefore, the completeness of "impurities" for endpoint 6.1.1 was 57%. The percentage was calculated for each characteristic at each third-level endpoint based on the results of refined analysis for fullerenes, SWCNTs and zinc oxide, respectively. It was also calculated based on the results of sub-sampling analysis while considering all 11 types of nanomaterials as a whole for each third-level endpoint. The results of completeness were also presented in a format of heat map for refined analysis of fullerenes, SWCNT and zinc oxide, respectively, refined analysis of the 3 types of nanomaterials as a whole and sub-sampling analysis.

2.6 DOCUMENTATION OF ESRS USING NUMBER CONCENTRATION

As particle (or fibre) number concentration is of great importance in determining potential risk for exposure to nanomaterials in some cases, we documented the ESRs using particle number concentration as dose in the Excel spreadsheet named "Number conc." in the database. The recorded information included nanomaterial name, endpoint and ESR title.

3 RESULTS

3.1 TEST GUIDELINE ADOPTED IN THE TESTING PROGRAMME

A total of 131 test guidelines (TGs) were adopted in the testing programme. These are tabulated (Table 8 in the Annex) according to the endpoint study where they were adopted. Within the ESRs, a number of TGs were reported to deviate from the published TG. This concerned only TGs investigating endpoints related to sections Ecotoxicology and Toxicology (section 6 and 7).

Among these 131 TGs identified, 65 were from OECD, approximately 50% of all the adopted TGs. Four of the OECD TGs were in draft stage and identified only with a title but not with a TG-number. It should be noted that, in this report, we considered different methods in the same TG as well as different revisions as different TGs. For example, "*OECD 301* ***C***" and "*OECD 301* ***F***" were considered as two different TGs. It should also be noted that TGs in Table 8 in the annex were tabulated according to endpoint where they were shown. A few TGs were obviously placed in wrong endpoints (e.g., TGs in Section 7.5.3) because the associated ESRs were placed in wrong sections.

The 131 TGs were adopted in 4 first-level endpoints as follows with the number of TGs adopted in each of the first-level endpoints.

- Physical and chemical properties: 26 TGs
- Environmental fate and pathways: 11 TGs
- Ecotoxicological information: 54 TGs
- Toxicological information: 42 TGs

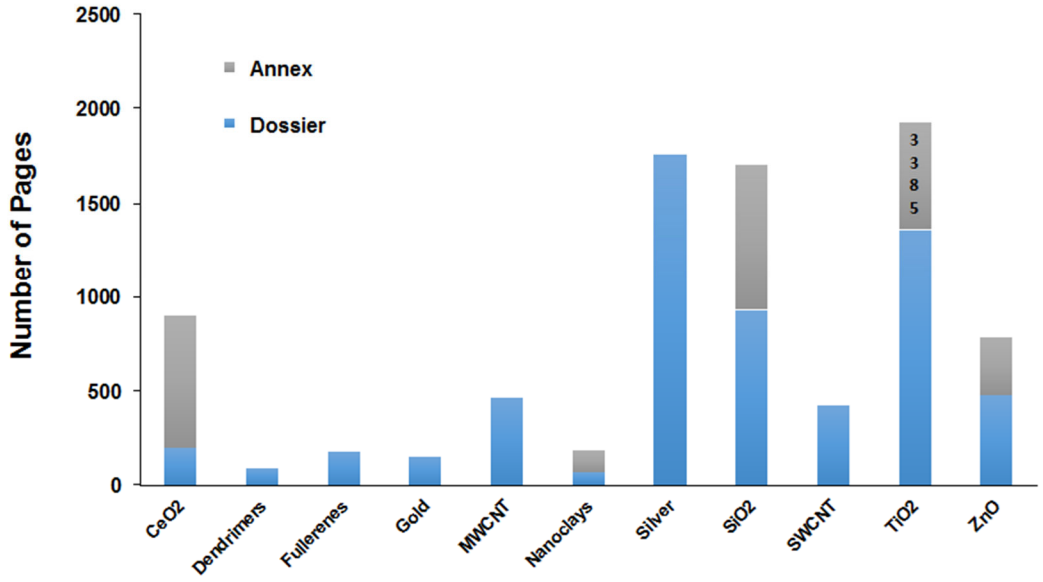
There are 155 third-level endpoints in the Testing Programme. Among them, TGs were adopted in 58 (i.e., 37%) endpoints. The TG adoption rate in the 4 abovementioned first-level endpoints was 55.2%. This means that only about half of the endpoints had been tested at least partly following OECD guidelines. Since a number of third-level endpoints are not testable, such as 1.6 sponsors, 2.1 GHS, and 3.3 sites, the overall actual TG adoption rate should be higher.

A number of TGs were adopted with deviations as claimed by data submitters. In the endpoints under ecotoxicological and toxicological information, 33 TGs (out of 94 TGs, 35%) were adopted with deviations for 144 endpoint studies in tests of 8 types of nanomaterials, except for dendrimers, gold nanoparticles and nanoclays. These TGs and the associated NMs were highlighted in yellow colour in Table 8. Among these TGs, 51 were from OECD, which were adopted for 424 endpoint studies in tests of the same 8 types of nanomaterials.

3.2 INITIAL ANALYSIS OF OECD NANOMATERIAL DOSSIERS

In the initial analysis, we reviewed all the dossier documents, extracted necessary information and compiled it into a number of Excel spreadsheets in the database. *Figure 2* shows the page numbers of the dossier document for each type of nanomaterials. In total, 6075 pages of dossier documents were thoroughly reviewed and 5279 pages of annex documents were referred to when necessary.

Figure 2. Number of pages of dossier and annex documents of the Testing Program



3.2.1 Environmental fate and pathways

Section 5 of all the dossiers was reviewed to identify what key information is contained in the ESRs, and recorded in a database.

Key findings:

- No information of environmental fate and pathways was available for dendrimers, gold nanoparticles and nanoclays.
- There are 112 ESRs for the other 8 types of the nanomaterials in the Testing Programme (see *Table 2*).
- For most of the third-level endpoints with ESRs, data was only available for less than 4 types of nanomaterials.

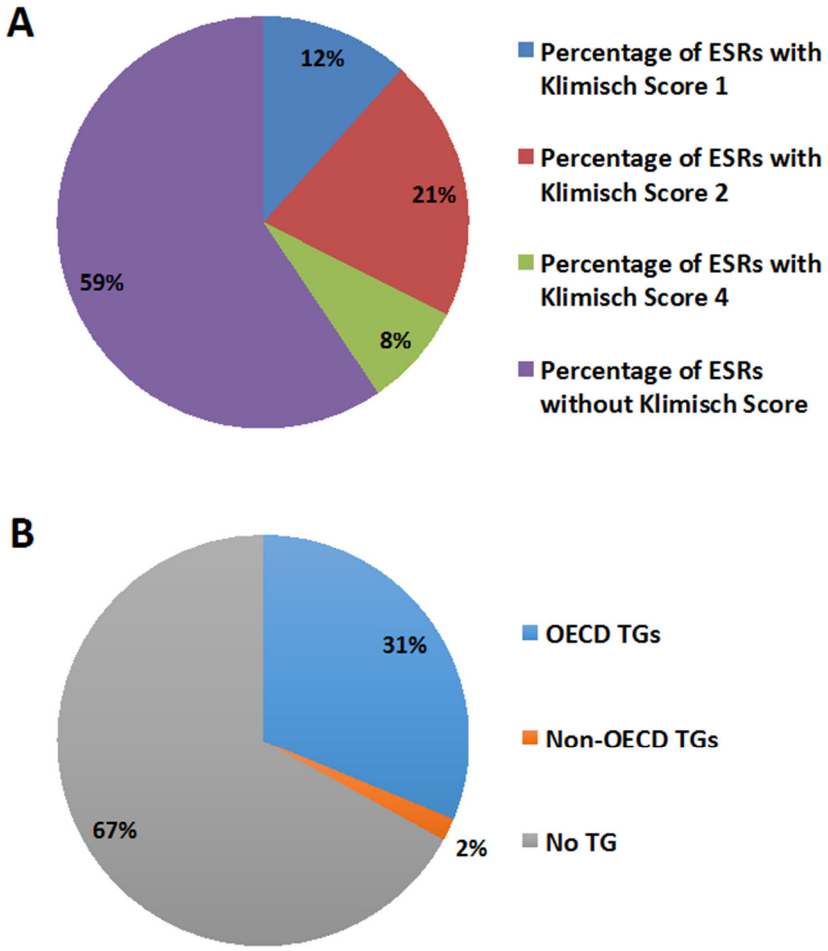
Table 2. General data availability for environmental fate and pathways

EP no.	Endpoint	NM	No. of ESRs available
5.1.1	Phototransformation in air	Silver	1
		SiO_2	4
5.1.2	Hydrolysis	SWCNT	2
5.1.3	Phototransformation in water	Fullerenes	6
		SWCNT	1
5.1.5	Preliminary: Dispersion stability in water	Silver	3
		TiO_2	23
5.1.6	Preliminary: Abiotic degradability and fate	TiO_2	2
5.2.1	Biodegradation in water: screening tests	MWCNT	5
		Silver	2
		SWCNT	6
5.2.2	Biodegradation in water and sediment: simulation tests	CeO_2	1
		TiO_2	4
5.2.3	Biodegradation in soil	Silver	1
5.2.4	Mode of degradation in actual use	Silver	1
5.3.1	Bioaccumulation: aquatic / sediment	CeO_2	2
		Fullerenes	1
		Silver	1
		SWCNT	3
		TiO_2	2
		ZnO	3
5.4.1	Adsorption / desorption	CeO_2	2
		Silver	5
		TiO_2	4
		ZnO	1
5.4.4	Other distribution data	Fullerenes	2
		Silver	3
		TiO_2	7
5.6	Additional information on environmental fate and behaviour	Fullerenes	6
		Silver	5
		TiO_2	3

The Klimisch score was developed to assess the reliability of toxicological studies for regulatory purposes under OECD. Studies rated with a Klimisch score of 1 or 2 are considered to be of direct regulatory relevance, while other scores may still be considered as supporting evidence.

Figure 3 shows the availability of Klimisch scores (*Figure 3*, A) and TGs (*Figure 3*, B) in the ESRs of environmental fate and pathways. Approximately 60% of the ESRs did not have a Klimisch score. Only one third of the endpoint studies were conducted according to TGs.

Figure 3. Availability of Klimisch score (A) and TGs (B) in ESRs of environmental fate and pathways



3.2.2 Ecotoxicological and toxicological information

The initial analysis was focused on Section 6 and Section 7 of the dossier documents, i.e., ecotoxicological and toxicological information, and 25 columns of information. It was summarized in an Excel spreadsheet documenting the information in 3820 rows and 25 columns. The spreadsheet also includes 198 rows of data that OECD had extracted from a summary of a literature review, which was unique for TiO_2.

3.2.2.1 General data availability

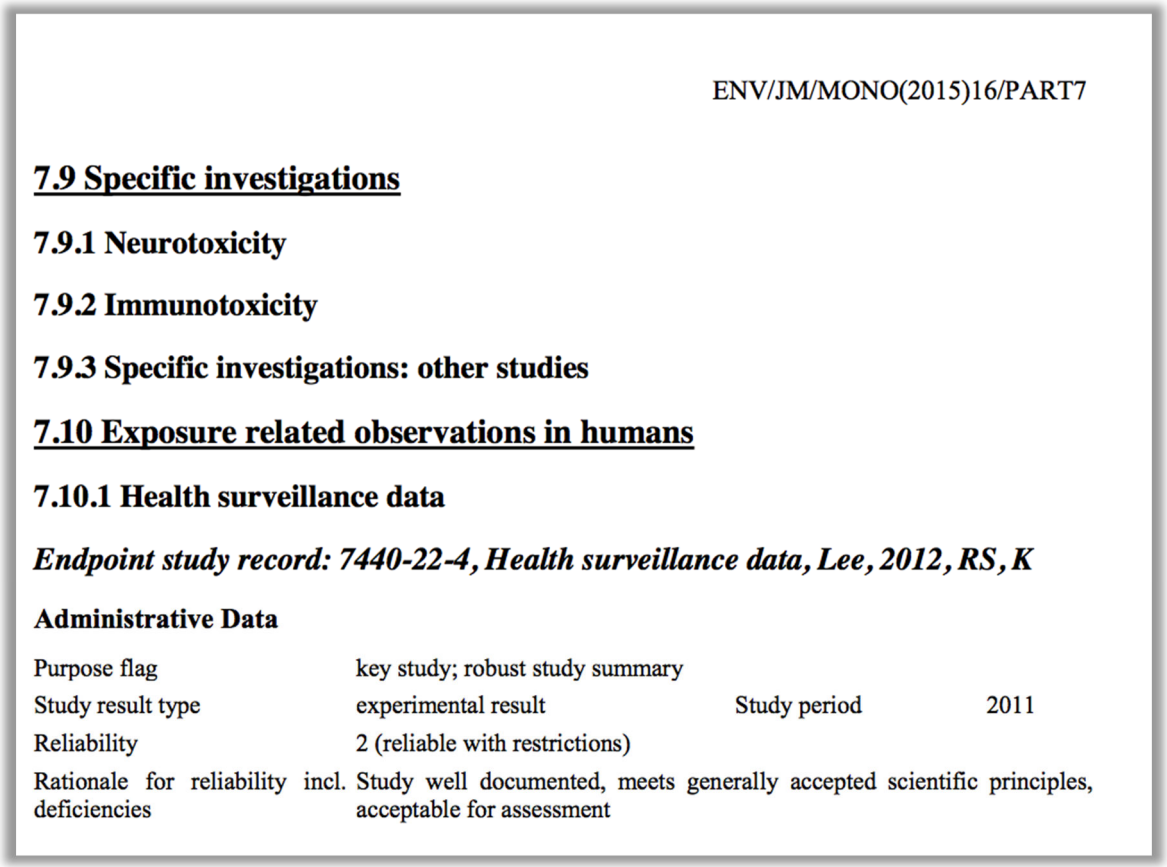
We documented 782 ESRs in the Excel spreadsheet of ecotoxicological and toxicological information. The number of ESRs at each third-level endpoint for each type of nanomaterials was calculated and shown in a heat map (*Figure 5*).

We compared the data availability (presence/absence of ESRs without number of ESRs) obtained from this study with the data availability shown in the OECD Endpoint Finder Excel Spreadsheet (*Table 3*). We found that, in the 50 third-level endpoints (i.e., 550 endpoints for 11 types of nanomaterials) in ecotoxicological and toxicological information, data was available in 178 ESRs for the 11 types of nanomaterials in the Testing Program. In other words, no data was available for 67.6% of all the endpoints for 11 types of nanomaterials. *Figure 6* shows the heat map of comparison of data availability between this study and the OECD Endpoint finder. The endpoints labelled with green colour indicate that the Endpoint Finder failed to show data availability while the ones labelled with red colour indicate false availability shown by the Endpoint Finder. The reasons for false availability are that endpoint titles without content (Figure 4) and endpoints without any information (even the titles) were still considered as effective data.

Table 3. Number of endpoints with data available in this study and in OECD Endpoint Finder Excel Spreadsheet

NM	No. of endpoints with data identified in this study	No. of endpoints with data reported in OECD Endpoint Finder Excel Spreadsheet
CeO_2	7	5
Dendrimers	5	5
Fullerenes	12	9
Gold	13	13
MWCNT	25	25
Nanoclays	4	4
Silver	31	40
SiO_2	14	14
SWCNT	23	22
TiO_2	21	36
ZnO	23	23

Figure 4. Screenshot of incomplete data availability in the OECD dossiers of silver nanoparticles: no actual data is provided. Instead, the dossier only lists endpoint titles for this section. However, the OECD Endpoint Finder reports that the silver dossier contains information about these endpoints.



ENV/JM/MONO(2015)16/PART7

7.9 Specific investigations

7.9.1 Neurotoxicity

7.9.2 Immunotoxicity

7.9.3 Specific investigations: other studies

7.10 Exposure related observations in humans

7.10.1 Health surveillance data

Endpoint study record: 7440-22-4, Health surveillance data, Lee, 2012, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2011
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Study well documented, meets generally accepted scientific principles, acceptable for assessment		

Figure 5. Heat map of data availability of ecotoxicological and toxicological information. The numbers indicated the number of ESRs available for the endpoint.

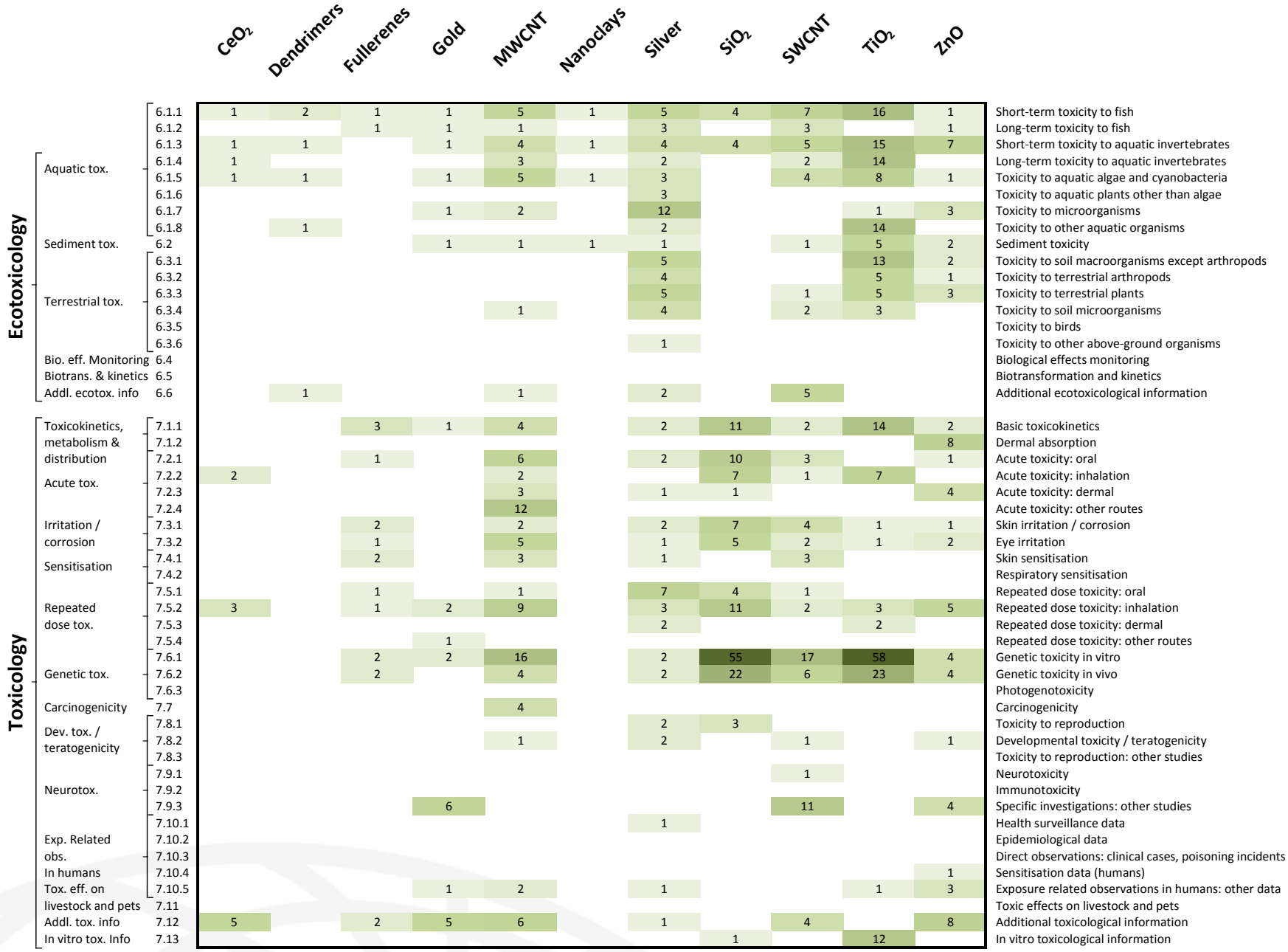
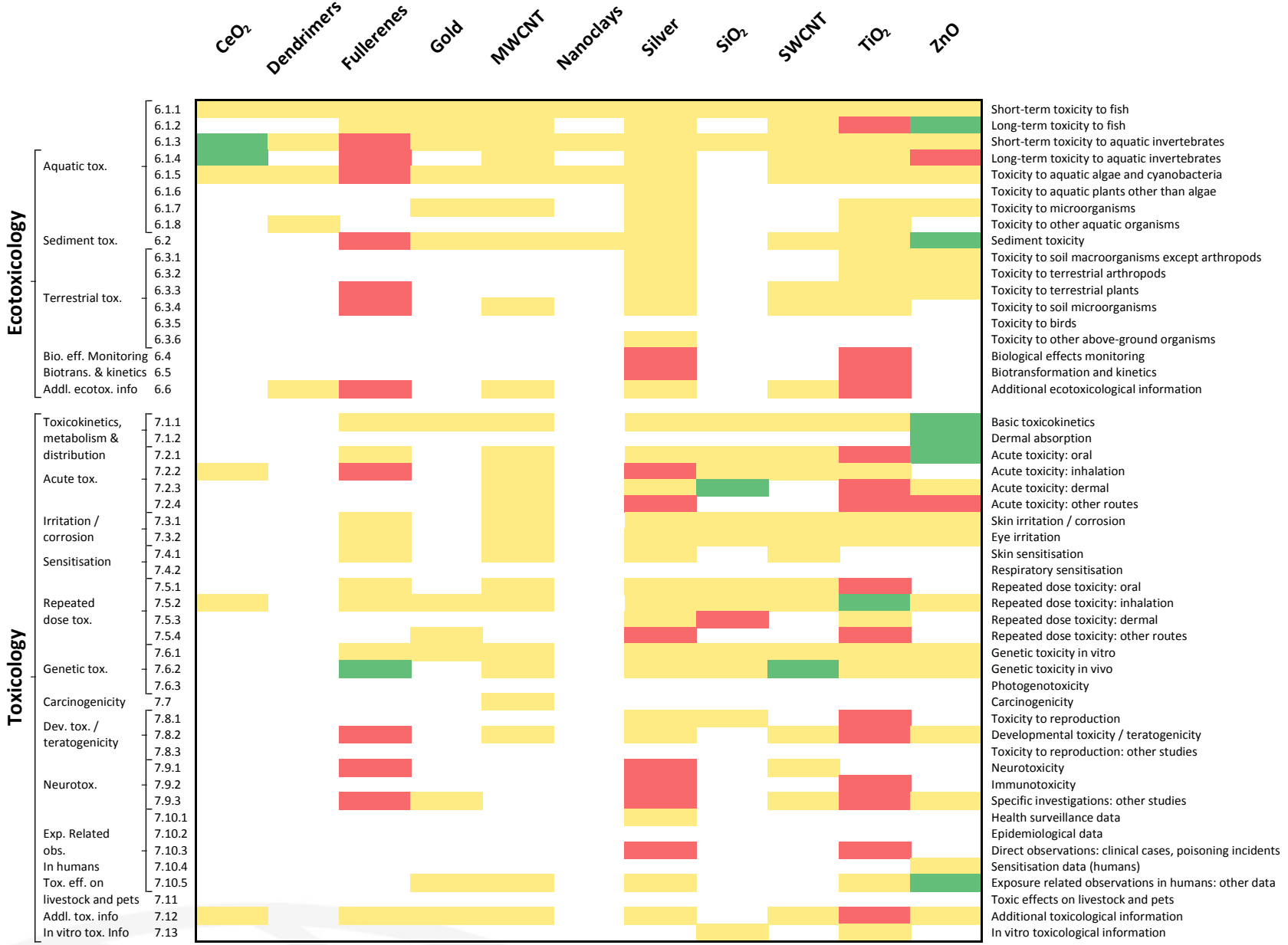


Figure 6. Heat map of comparison of data availability between this study and the OECD Endpoint finder Excel Spreadsheet.



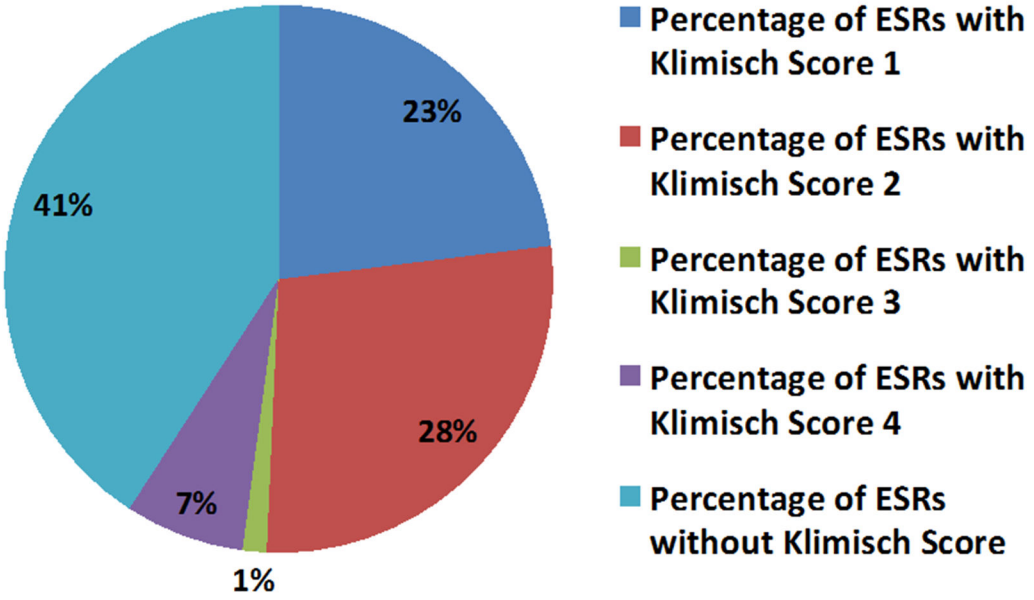
Red = data availability identified only by the OECD Endpoint finder Excel Spreadsheet; Yellow = data availability identified by both this study and the OECD Endpoint Finder Excel Spreadsheet. Green = data availability identified only by this study.

3.2.3 Klimisch score

Figure 8 shows the availability of Klimisch score at third-level endpoint for each type of nanomaterial, for ecotoxicological and toxicological information. For all the ESRs, Klimisch score was available in 462 ESRs (i.e., 59% of all the ESRs).

Figure 7 shows the overall availability of Klimisch score of the 11 types of nanomaterials.

Figure 7. The overall availability of Klimisch score for ecotoxicological and toxicological information

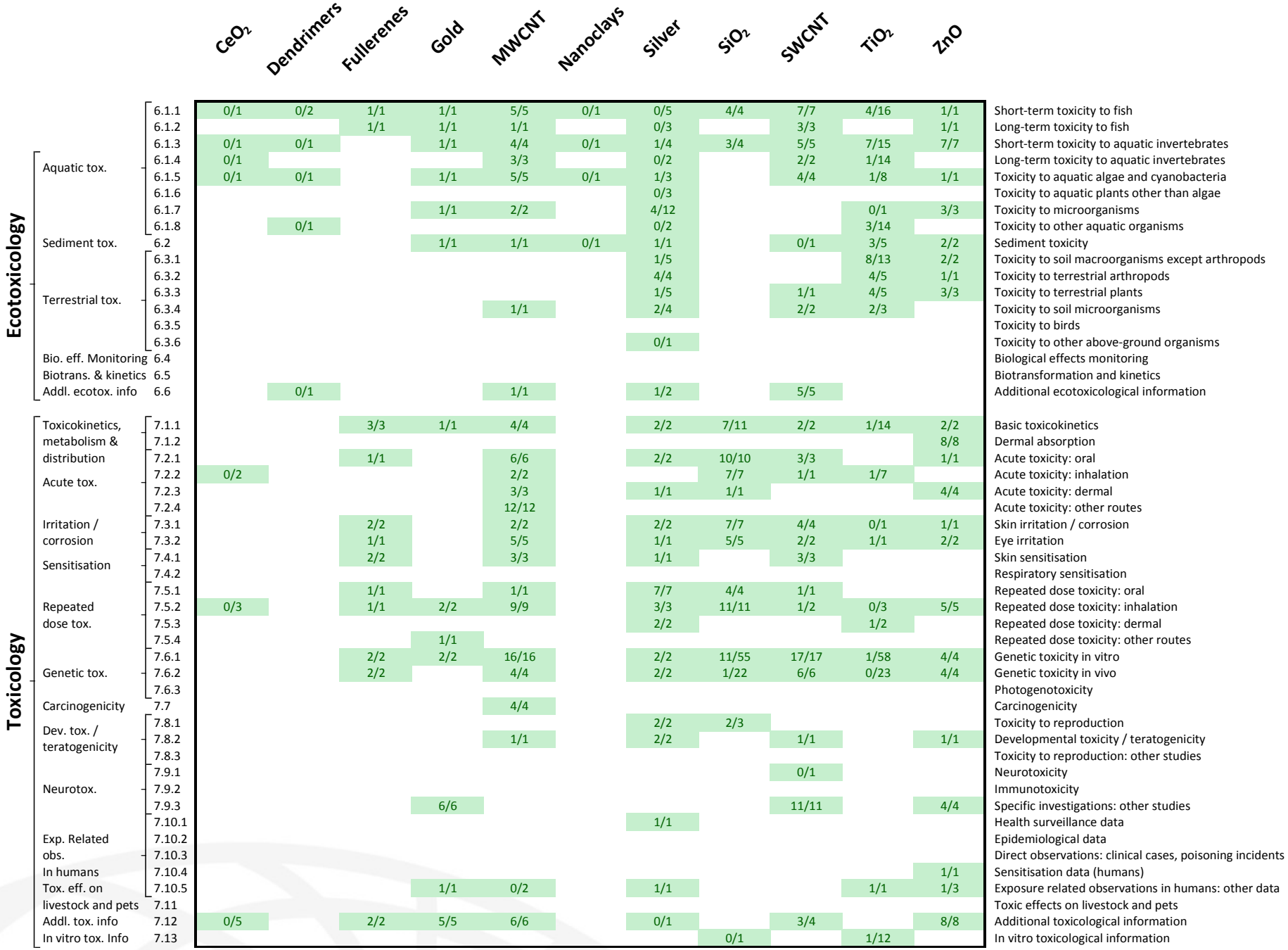


For individual type of nanomaterials, fullerenes, gold nanoparticles and ZnO have the highest availability of Klimisch score (100%) while CeO_2, dendrimers and nanoclays have the lowest (0%). The details of availability were tabulated (*Table 4*).

Table 4. Details of availability of Klimisch score for ecotoxicological and toxicological information of each type of nanomaterials

NM	No. of ESRs	No. (%) of ESRs with Klimisch score 1	No. (%) of ESRs with Klimisch score 2	No. (%) of ESRs with Klimisch score 3	No. (%) of ESRs with Klimisch score 4	No. (%) of ESRs without Klimisch score
CeO_2	14	0	0	0	0	0
Dendrimers	6	0	0	0	0	0
Fullerenes	19	10 (53%)	8 (42%)	1 (5%)	0	0
Gold	24	0	23 (96%)	1 (4%)	0	0
MWCNT	103	49 (48%)	29 (28%)	1 (1%)	22 (21%)	2 (2%)
Nanoclays	4	0	0	0	0	0
Silver	88	10 (11%)	37 (42%)	0	0	41 (47%)
SiO_2	145	39 (27%)	35 (24%)	0	0	71 (49%)
SWCNT	88	36 (41%)	41 (47%)	0	7 (8%)	4 (5%)
TiO_2	221	28 (13%)	15 (7%)	1 (0.5%)	0	177 (8%)
ZnO	69	8 (12%)	28 (41%)	8 (12%)	25 (36%)	0

Figure 8. The availability of Klimisch score at third-level endpoints of each of the 11 types of nanomaterials for ecotoxicological and toxicological information. x/y indicates x ESRs out of y ESRs have Klimisch score.

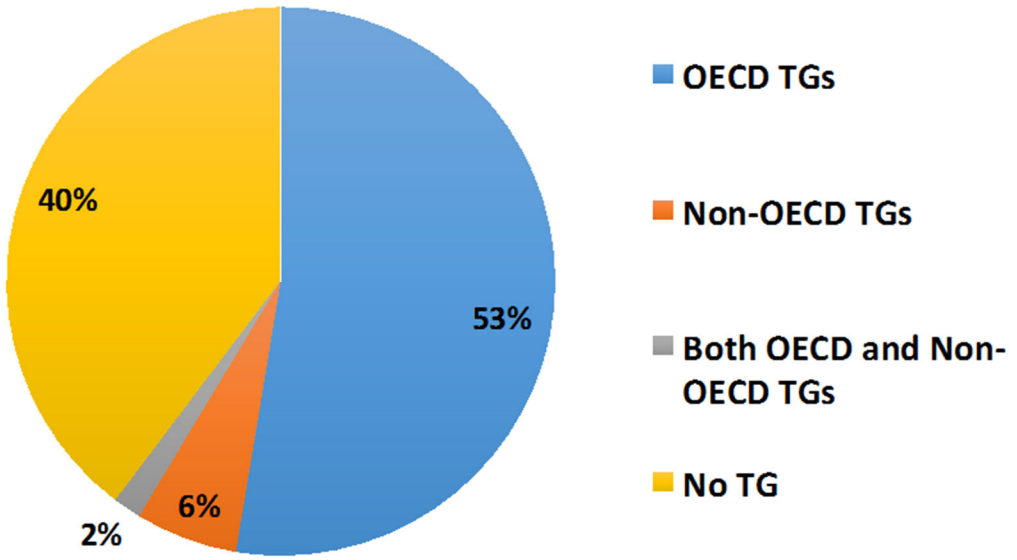


			CeO_2	Dendrimers	Fullerenes	Gold	MWCNT	Nanoclays	Silver	SiO_2	SWCNT	TiO_2	ZnO	
Ecotoxicology	Aquatic tox.	6.1.1	0/1	0/2	1/1	1/1	5/5	0/1	0/5	4/4	7/7	4/16	1/1	Short-term toxicity to fish
		6.1.2			1/1	1/1	1/1		0/3		3/3		1/1	Long-term toxicity to fish
		6.1.3	0/1	0/1		1/1	4/4	0/1	1/4	3/4	5/5	7/15	7/7	Short-term toxicity to aquatic invertebrates
		6.1.4	0/1				3/3		0/2		2/2	1/14		Long-term toxicity to aquatic invertebrates
		6.1.5	0/1	0/1		1/1	5/5	0/1	1/3		4/4	1/8	1/1	Toxicity to aquatic algae and cyanobacteria
		6.1.6							0/3					Toxicity to aquatic plants other than algae
		6.1.7				1/1	2/2		4/12			0/1	3/3	Toxicity to microorganisms
		6.1.8		0/1					0/2			3/14		Toxicity to other aquatic organisms
	Sediment tox.	6.2				1/1	1/1	0/1	1/1		0/1	3/5	2/2	Sediment toxicity
	Terrestrial tox.	6.3.1							1/5			8/13	2/2	Toxicity to soil macroorganisms except arthropods
		6.3.2							4/4			4/5	1/1	Toxicity to terrestrial arthropods
		6.3.3							1/5		1/1	4/5	3/3	Toxicity to terrestrial plants
		6.3.4					1/1		2/4		2/2	2/3		Toxicity to soil microorganisms
		6.3.5												Toxicity to birds
		6.3.6							0/1					Toxicity to other above-ground organisms
	Bio. eff. Monitoring	6.4												Biological effects monitoring
	Biotrans. & kinetics	6.5												Biotransformation and kinetics
	Addl. ecotox. info	6.6		0/1			1/1		1/2		5/5			Additional ecotoxicological information
Toxicology	Toxicokinetics, metabolism & distribution	7.1.1			3/3	1/1	4/4		2/2	7/11	2/2	1/14	2/2	Basic toxicokinetics
		7.1.2											8/8	Dermal absorption
		7.2.1			1/1		6/6		2/2	10/10	3/3		1/1	Acute toxicity: oral
	Acute tox.	7.2.2	0/2				2/2			7/7	1/1	1/7		Acute toxicity: inhalation
		7.2.3					3/3		1/1	1/1			4/4	Acute toxicity: dermal
		7.2.4					12/12							Acute toxicity: other routes
	Irritation / corrosion	7.3.1			2/2		2/2		2/2	7/7	4/4	0/1	1/1	Skin irritation / corrosion
		7.3.2			1/1		5/5		1/1	5/5	2/2	1/1	2/2	Eye irritation
	Sensitisation	7.4.1			2/2		3/3		1/1		3/3			Skin sensitisation
		7.4.2												Respiratory sensitisation
	Repeated dose tox.	7.5.1			1/1		1/1		7/7	4/4	1/1			Repeated dose toxicity: oral
		7.5.2	0/3		1/1	2/2	9/9		3/3	11/11	1/2	0/3	5/5	Repeated dose toxicity: inhalation
		7.5.3							2/2			1/2		Repeated dose toxicity: dermal
		7.5.4				1/1								Repeated dose toxicity: other routes
	Genetic tox.	7.6.1			2/2	2/2	16/16		2/2	11/55	17/17	1/58	4/4	Genetic toxicity in vitro
		7.6.2			2/2		4/4		2/2	1/22	6/6	0/23	4/4	Genetic toxicity in vivo
		7.6.3												Photogenotoxicity
	Carcinogenicity	7.7					4/4							Carcinogenicity
	Dev. tox. / teratogenicity	7.8.1							2/2	2/3				Toxicity to reproduction
		7.8.2					1/1		2/2		1/1		1/1	Developmental toxicity / teratogenicity
		7.8.3												Toxicity to reproduction: other studies
	Neurotox.	7.9.1									0/1			Neurotoxicity
		7.9.2												Immunotoxicity
		7.9.3				6/6					11/11		4/4	Specific investigations: other studies
	Exp. Related obs. In humans	7.10.1							1/1					Health surveillance data
		7.10.2												Epidemiological data
		7.10.3												Direct observations: clinical cases, poisoning incidents
		7.10.4											1/1	Sensitisation data (humans)
	Tox. eff. on livestock and pets	7.10.5				1/1	0/2		1/1			1/1	1/3	Exposure related observations in humans: other data
		7.11												Toxic effects on livestock and pets
	Addl. tox. info	7.12	0/5		2/2	5/5	6/6		0/1		3/4		8/8	Additional toxicological information
	In vitro tox. Info	7.13								0/1		1/12		In vitro toxicological information

3.2.4 Testing Guidelines (TGs)

Figure 9 shows the availability of TGs in ESRs of ecotoxicological and toxicological information. The 782 endpoint studies adopted 94 TGs in total, 54% of which were OECD TGs. Approximately 55% of the endpoint studies adopted OECD TGs while 8% adopted non-OECD TGs. No TGs were adopted in approximately 40% of the endpoint studies.

Figure 9. Availability of TGs in ESRs of ecotoxicological and toxicological information



3.2.5 Use of cell/tissue/organism, species and route of administration in the Testing Programme

In the database, we documented the use of cell/tissue/organism, species, and route of administration in the Testing Programme. This provides a quick access to look up relevant information without going through the lengthy dossier documents.

3.2.6 Availability of exposure concentration or dose and exposure duration/frequency

In the database, we documented exposure concentration or dose for each ESR as well as exposure duration/frequency. This provides a quick access to look up relevant information without going through the lengthy dossier documents.

Since particle (or fibre) number concentration is of great importance for determining the potential risks from exposure to nanomaterials, the ESRs using particle number concentration (in the Testing Programme, most of the ESRs using mass concentration) were tabulated (*Table 5*).

Table 5. ESRs using particle number concentration in the Testing Programme

NM	EP No.	EP	Endpoint study record
CeO_2	7.5.2	Repeated dose toxicity: inhalation	2011-08-04 RIVM Subacute inhalation toxicity study with Ceria Dry CeO_2 in rats
CeO_2	7.5.2	Repeated dose toxicity: inhalation	2012-03-07 RIVM Kinetics of inhaled nano & micro cerium oxide
C60	7.5.2	Repeated dose toxicity: inhalation	Repeated dose toxicity: inhalation.001
Gold	7.5.2	Repeated dose toxicity: inhalation	7440-57-5, Repeated dose toxicity-inhalation, Sung, 2011, RS, K
MWCNT	7.7	Carcinogenicity	Mitsui MWCNT-7: Carcinogenicity.001
MWCNT	7.7	Carcinogenicity	Mitsui MWCNT-7: Carcinogenicity.002
MWCNT	7.10.5	Exposure related observations in humans: other data	Hanwha CM-100 (1) Exposure related observations in humans: other data.001
MWCNT	7.10.5	Exposure related observations in humans: other data	Hanwha CM 100 (2) Exposure related observations in humans: other data.001
Silver	7.5.2	Repeated dose toxicity: inhalation	7440-22-4, Repeated dose toxicity-inhalation, Ji, 2007, RS, K
Silver	7.5.2	Repeated dose toxicity: inhalation	7440-22-4, Repeated dose toxicity-inhalation, Kim, 2009, RS, K
Silver	7.5.2	Repeated dose toxicity: inhalation	7440-22-4, Repeated dose toxicity-inhalation, Sung, 2009, RS, K
Silver	7.5.3	Repeated dose toxicity: dermal	Repeated dose toxicity: inhalation.002
Silver	7.6.2	Genetic toxicity in vivo	7440-22-4, Genetic toxicity in vivo - Micronucleus test, Kim, 2011, RS, K
Silver	7.10.5	Exposure related observations in humans: other data	7440-22-4, Exposure related observations in humans- other data, Lee, 2011, RS, K
SWCNT	7.5.2	Repeated dose toxicity: inhalation	Super Growth Repeated dose toxicity: inhalation.001
SWCNT	7.9.3	Specific investigations: other studies	Super Growth SWCNT Intratracheal Instillation.001

3.2.7 Manufacture, use and exposure of NMs

The manufacture, use and exposure information, which is extremely limited in the dossier documents, was extracted from Section 3 for the 11 types of nanomaterials of the Testing Programme. *Table 6* shows all the information available across the 11 types of nanomaterials in Section 3. The details of data are not a summary of information available in Section 3, but precisely *all* the available information in Section 3.

Table 6. Data available with regard to manufacture, use and exposure of nanomaterials in the Testing Programme. "-" indicates no information available.

NM	**EP No.**	**EP**	**Details of data**
CeO_2	-	-	-
Dendrimers	-	-	-
Fullerenes	3.1	Technological process	Method of manufacture of substance: Combustion method with sublimation purification
Gold	-	-	-
MWCNT	3.1	Technological process	Nikkiso MWCNT: CVD method using floating metal catalyst Arkema Grphistrength C100: CVD using supported metal catalyst for continuous production by fluidized bed NANOCYLTM NC7000: Catalytic Carbon Vapor Deposition using supported metal catalyst for (semi)-continuous production in a moving bed reactor Mitsui- MWNT-7: Floating Chemical Vapor Deposition BaytubesR: Chemical Vapor Deposition using supported metal catalyst for (semi)-continuous production by fluidized bed Hanwha MWCNT: Cheaptubes.com MWCNT: Catalytic Carbon Vapor Deposition using supported metal catalyst for (semi)-continuous production in a moving bed reactor
Nanoclays	-	-	-
Silver	3.2	Esitmated quantities	10000 … 50000 tonnes
SiO_2	-	-	-

NM	EP No.	EP	Details of data
SWCNT	3.1	Technological process	Method of manufacture of substance: Principal SWCNT 1; Nikkiso SWCNT; e-DIPS (enhanced Direct-Injection- Pyrolytic-Synthesis) method is adopted for continuous production of SWCNTs. Principal SWCNTs 2; Super Growth; Water-assisted CVD using Fe catalyst developed by AIST(National Institute of Advanced Industrial Science and Technology) Alternate SWCNT 1; Helix; chemical vapor deposition Alternate SWCNT 2; Carbolex; carbon arc synthesis Alternate SWCNT 3; CNI; high-pressure CO disproportionation process (HiPCO) using Fe(CO)5 as a catalyst and purified by acid treatment
TiO_2	None		None
ZnO	None		None

In the IUCLID-format, Section 3 allows reporting human exposure scenarios. OECD did not have the intention to systematically collect such information. Consequently, as listed in *Table 6*, such information was missing for all of the 11 types of nanomaterials in the dossiers. However, some limited but relevant information, such as exposure related observation in humans, health surveillance data and sensitization data, was identified in Section 7.10 for 5 types of nanomaterials, as shown in *Table 7*. Most of the nanomaterials in *Table 7* were not well characterized. The identities of the nanomaterials used in the tests were not well documented in the dossiers. The full details were documented in an Excel spreadsheet named "Exposure" in the database.

Table 7. Available human exposure data in the dossiers across all the 11 types of nanomaterials. "-" indicates no available information.

NM	EP No.	EP	ESR	TG	NM ID	Klimisch score
Gold	7.10.5	Exposure related observations in humans: other data	7440-57-5, Exposure related observations in humans- other data, Anonymous, Year, RS, K	-	cAuNP	2
MWCNT	7.10.5	Exposure related observations in humans: other data	Hanwha CM-100 (1) Exposure related observations in humans: other data.001	-	Hanwha CM 100	-
MWCNT	7.10.5	Exposure related observations in humans: other data	Hanwha CM 100 (2) Exposure related observations in humans: other data.001	-	Hanwha CM 100	-

NM	EP No.	EP	ESR	TG	NM ID	Klimisch score
Silver	7.10.1	Health surveillance data	7440-22-4, Health surveillance data, Lee, 2012, RS, K	-	Unknown ID	2
Silver	7.10.5	Exposure related observations in humans: other data	7440-22-4, Exposure related observations in humans- other data, Lee, 2011, RS, K	-	Unknown ID	2
TiO_2	7.10.5	Exposure related observations in humans: other data	Exposure related observations in humans: other data.001	-	Unknown ID	1
ZnO	7.10.4	Sensitisation data (humans)	Disregarded.Nano.Cantor (1994), HRIPT	-	Unknown ID	4
ZnO	7.10.5	Exposure related observations in humans: other data	Disregarded.Nano.Kuschner (1995), inhalation	-	Unknown ID	4
ZnO	7.10.5	Exposure related observations in humans: other data	Disregarded.Nano.Fink (1997), photoirritation	-	Unknown ID	4
ZnO	7.10.5	Exposure related observations in humans: other data	Disregarded.Nano.Cantor (1994), photoirritation	-	Unknown ID	4

3.3 REFINED ANALYSIS OF THREE DOSSIERS

The dossier documents of fullerenes, SWCNT and zinc oxide were reviewed in more detail to assess the availability of characteristics in comparison to recommended minimal data requirements recommended in several leading peer-reviewed journals.

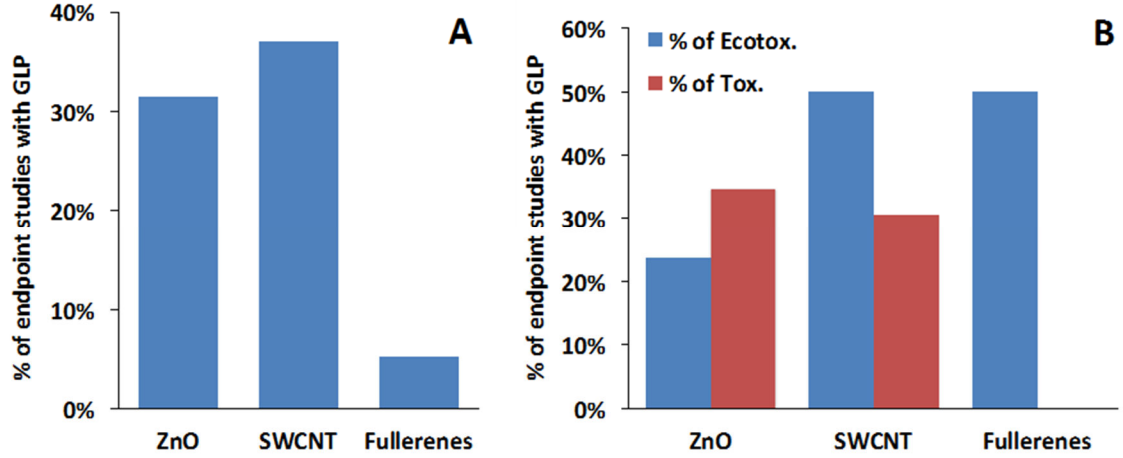
The refined analysis was focused on ecotoxicological and toxicological information. We reviewed 70, 89 and 19 ESRs of zinc oxide, SWCNT and fullerenes, respectively, and documented 178 rows and 26 main columns of data. For all the characteristics of interest, we identified whether there was any presence of relevant information in the dossiers. In addition, for purity, we extracted the degree of purity. Already at this level it was clear that there was very limited information in the dossiers that would inform about the nanoscale, while the situation was better for chemistry descriptors as well as the experimental circumstances (see also chapter 3.3.3 about Data completeness).

3.3.1 GLP compliance

The availability of GLP compliance in the ESRs (i.e. whether labs adhered to a quality system of management controls in the research labs while creating the data presented in the ESR) was examined in the refined analysis. *Figure 10* A shows the overall percentage of endpoint studies performed with GLP

compliance for zinc oxide, SWCNT and fullerenes. In general, less than 40% of the ecotoxicological and toxicological endpoint studies were performed with GLP compliance. Only 1 out of 19 endpoint studies of fullerenes was performed with GLP compliance. Approximately 30% of toxicological endpoint studies of zinc oxide and SWCNT were GLP-compliant, while no toxicological endpoint study of fullerenes was GLP-compliant (Figure 13 B). Half of ecotoxicological endpoint studies of SWCNT followed GLP compliance while the percentage for zinc oxide was less than 30%. The percentage for fullerenes was also 50% because in total only 2 ecotoxicological ESRs were available for fullerenes.

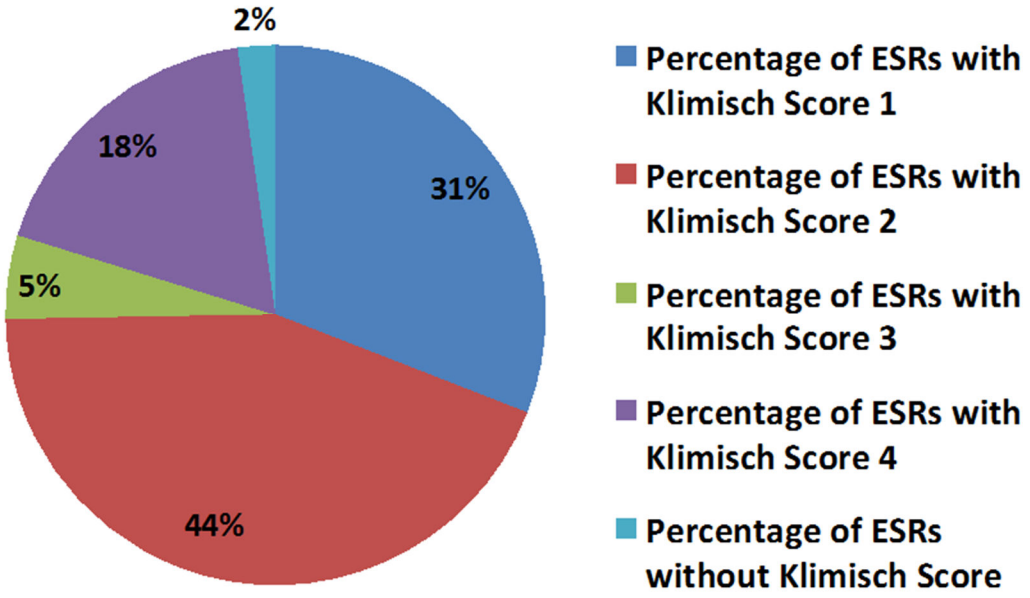
Figure 10. Overall percentage of endpoint studies with GLP compliance (A) and separate percentages of ecotoxicological and toxicological endpoint studies with GLP compliance (B) for zinc oxide, SWCNT and fullerenes in the refined analysis.



3.3.2 Klimisch score

Of all the 178 ESRs we reviewed in the refined analysis, Klimisch score was not available for only 4 ESRs of SWCNT. *Figure 11* shows the overall availability of Klimisch score of the 3 types of nanomaterials of the refined analysis. The availability of Klimisch score in the refined analysis (98%) was significantly higher than that of all the 11 types of nanomaterials in the initial analysis (59%).

Figure 11. The overall availability of Klimisch score of ecotoxicological and toxicological information for zinc oxide, SWCNT and fullerenes.



The separate availability on Klimisch score for zinc oxide, SWCNT and fullerenes is shown in *Figure 12*A. Approximately 90% of the ecotoxicological and toxicological endpoint studies of SWCNT and fullerenes were assigned either the top rank of 1 (reliable without restriction) or the second rank of 2 (reliable with restriction), while only half of the endpoint studies of zinc oxide were assigned with a Klimisch score of 1 or 2. As shown in *Figure 12* B, ecotoxicological and toxicological endpoint studies of zinc oxide had similar Klimisch score distribution. For SWCNT, more studies with Klimisch score of 1 and 2 were found in toxicological studies than ecotoxicological ones. Since toxicological endpoint studies of fullerenes significantly outnumbered ecotoxicological ones, the Klimisch score distribution of toxicological endpoint studies generally represent the overall Klimisch score distribution of ecotoxicological and toxicological endpoint studies.

Figure 12. Separate availability of Klimisch score of ecotoxicological and toxicological endpoint studies of zinc oxide, SWCNT and fullerenes

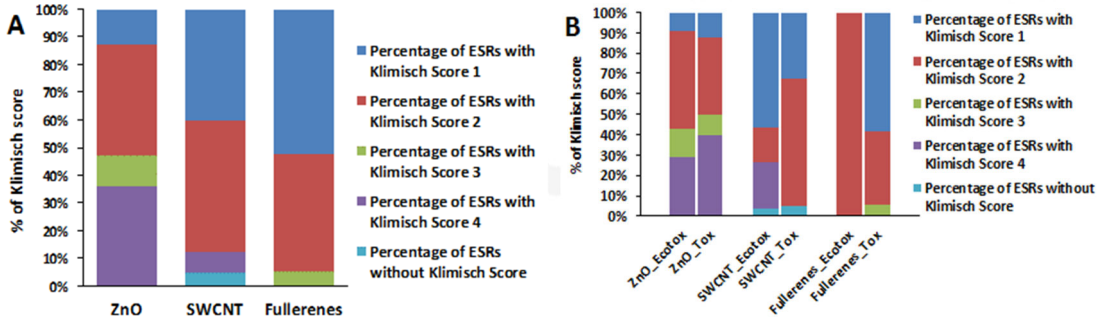
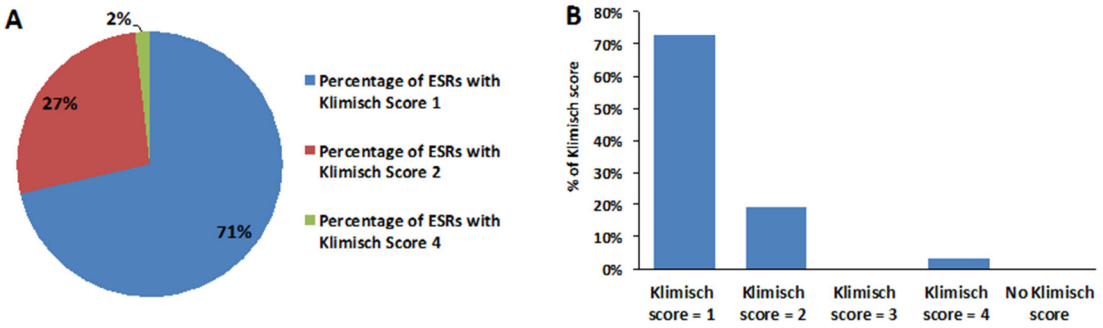


Figure 13 shows the correlation between GLP compliance and Klimisch score. For all 56 endpoint studies performed with GLP compliance in the refined analysis, 55 studies (98%) had a Klimisch score of 1 or 2 and only 1 (2%) had a Klimisch score of 4 (*Figure 13* A). Over 70% of endpoint studies with a Klimisch score of 1 were GLP-compliant while only approximately 20% of endpoint studies with a Klimisch score 2 were GLP-compliant. Almost no endpoint studies with a Klimisch score lower than 2 were performed with GLP compliance (*Figure 13* B).

Figure 13. Correlation between GLP compliance and Klimisch score in the refined analysis: (A) The percentage of different Klimisch scores for ESRs with GLP compliance; (B) The percentage of ESRs with GLP compliance for ESRs with different Klimisch scores



3.3.3 Data completeness

The data completeness of ESRs in the refined analysis was assessed according to the minimal data requirements recommended by articles from leading peer-reviewed journals. The completeness was calculated as percentage of ESRs with available data (only presence and absence) for each characteristic at third-level endpoint. For a number of characteristics that may undergo significant changes in different conditions (e.g., different degrees of agglomeration and size distributions in various testing mediums or after different periods of time following preparation), only those in endpoint studies that clearly showed relevant characterization had been conducted in the testing system were considered to be available. For example, if an endpoint study had only particle size without showing whether the size had been measured in the testing system by the data submitter, this characteristic would be considered not available in the endpoint study. The raw data of completeness assessment can be found in relevant spreadsheets of the database. The results were presented in heat map for zinc oxide, SWCNT, fullerenes and the 3 types of nanomaterials as a whole (Figure 17 in the annex).

The characteristics were grouped into 3 categories as follows.

- Chemistry: characteristics describing intrinsic properties of the material, including chemical composition, purity/impurities, persistence, surface chemistry and surface charge
- Nanoscale descriptor: characteristics describing properties specific to risk assessment for nanomaterials, including degree of aggregation/agglomeration, morphology (including aspect ratio), particle size (average, range, etc.), particle size distribution, particle mass concentration, surface area and particle number concentration
- Circumstance: characteristics describing parameters in relation to exposure scenarios, including details on the matrix/dispersant/solvent surrounding the nanomaterial, physical/chemical form of released/detected nanoparticles, exposure duration and exposure frequency

In general, characteristics that did not require sophisticated characterization had significantly high availability for most endpoints. These characteristics can be further categorized into 2 groups as follows to explain the reasons for high availability.

- Easy to obtain (available from suppliers and generally not dependent on how the nanomaterials are processed): chemical composition, purity
- Easy to measure and/or control (they are decided in the experiment design): particle mass concentration, details on the matrix/dispersant/solvent surrounding the nanomaterial, physical/chemical form of released/detected nanoparticles, exposure duration and exposure frequency

The only exception is impurity, which should have been easily available from material suppliers and included into "easy to obtain". In the refined analysis, 50% (14 out of 28) of the ESRs with degree of purity below 98% did not report impurities. The separate percentages for zinc oxide, SWCNT and fullerenes are 100% (5 out of 5), 39% (9 out of 23) and 0%, respectively. It should be noted that, for degree of purity presented as a range, the lower limit was used as the degree of purity.

From the perspective of characteristic grouping, the availability of characteristics of "circumstance" was significantly high, with all the 4 characteristics available for most of the endpoints. In the "chemistry" group, degree of purity was available for much more endpoints than other characteristics without considering composition, which was available for each ESR as we did not assess whether the data submitter validated the chemical composition of the tested nanomaterials. No ESR described anything about persistence of the test nanomaterials. Some ESRs described surface chemistry of the tested nanomaterials, such as surface functionalization and coating. It should be noted that we checked the presence and absence of relevant information with regard to surface chemistry. Such information was considered not available if nothing was mentioned about surface functionalization and coating in an ESR. However, it was considered available if an ESR mentioned the nanomaterials were without surface functionalization

or coating. Surface charge was available in 1 ERS that clearly mentioned surface charge was measured in the testing system. Among the characteristics of "nanoscale descriptor", particle mass concentration, which is less nano-specific than the other characteristics in this group, was the only one available in almost all the ESRs for the reasons abovementioned. In contrast, in most of the ESRs, data submitter did not well document relevant information or conduct relevant measurements of the other characteristics. The following criteria indicate what information was based on for assessing the presence and absence of the characteristics under "nanoscale descriptor".

- Degree of aggregation/agglomeration: whether ESRs mentioned that the degree (or size) of aggregation/agglomeration of the nanomaterials in the testing system was examined.
- Morphology (including aspect ratio): whether ESRs mentioned that the nanomaterials in/from the testing system were examined with microscopy or other necessary approaches.
- Particle size (average, range, etc.): whether ESRs mentioned that the size of nanomaterials in/from the testing system was measured.
- Particle size distribution: whether ESRs mentioned that size distribution of nanomaterials in/from the testing system was measured. A simple particle size range (e.g., from x nm to y nm) was not considered as size distribution in our assessment.
- Surface area: whether ESRs mentioned that the surface area of nanomaterials in/from the testing system was measured.
- Particle number concentration: whether ESRs mentioned that the number concentration of nanomaterials in/from the testing system was measured.

Following particle mass concentration, particle size had the second highest availability across the 3 types of nanomaterials under "nanoscale descriptor". Morphology was the third one, however, it was unavailable for 18 endpoints in the refined analysis. Only a few ESRs clearly claimed that the data submitter had characterized degree (or size) of aggregation/agglomeration, particle size distribution, surface area and particle number concentration. In particular, particle number concentration was only available for nanomaterial aerosols in studies of repeated dose toxicity via inhalation.

3.4 SUB-SAMPLING

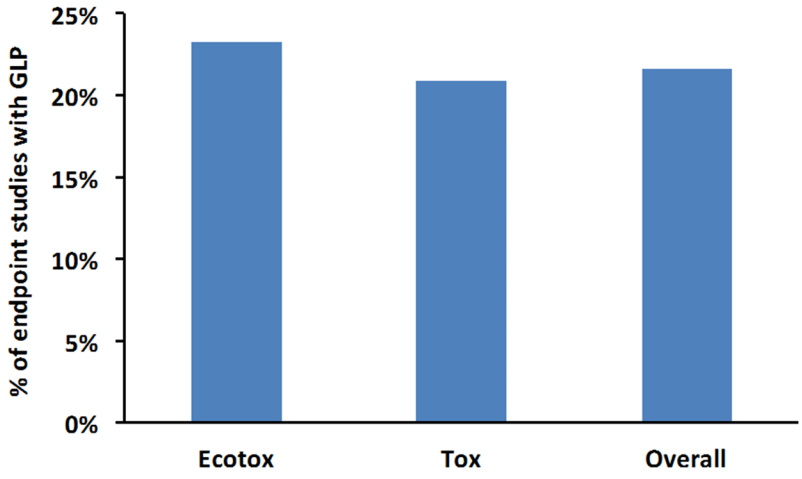
In order to compare the general trend of data availability of ESRs for refined analysis with that of all of the ESRs, approximately 10% of all ecotoxicological and toxicological ESRs from 11 types of nanomaterials was randomly selected, reviewed and assessed with the same method as the refined analysis. Following the random sampling, a stratified strategy was applied to ensure, at each second-level endpoint, at least 10% of ESRs were assessed. After the random sampling and stratified sampling, at least 10% of ESRs for each type of nanomaterials at second-level endpoint were covered. In total, 17% of all the ecotoxicological and toxicological ESRs, including 75 ESRs selected from random sampling and 59 ESRs selected from stratified

sampling, were assessed in the sub-sampling analysis. The number of ecotoxicological ESRs and the number of toxicological ESRs are 43 and 91, respectively. The results were presented in an Excel spreadsheet of 134 rows and 27 columns of data. The subsampling showed a similar picture as the full refined analysis of the three selected nanomaterial in that very limited nanoscale relevant information was available.

3.4.1 GLP compliance

The availability of GLP compliance in the ESRs was examined in the sub-sampling analysis. *Figure 14* shows the percentage of endpoint studies performed with GLP compliance for ecotoxicological ESRs, toxicological ESRs and all ESRs in the sub-sampling analysis. Approximately, 22% of the endpoint studies randomly selected for sub-sampling were GLP-compliant, almost the same as the percentage of both ecotoxicological and toxicological endpoint studies. The percentage is lower than that of zinc oxide and SWCNT, which are 31% and 37%, respectively. It is also lower than the average percentage of endpoint studies with GLP compliance of the refined analysis (i.e., 31%) for zinc oxide, SWCNT and fullerenes. It indicates that, in general, the endpoint studies in the sub-sampling analysis are less likely to be reliable than the ones in the refined analysis.

Figure 14. Percentage of endpoint studies with GLP compliance in sub-sampling analysis.



3.4.2 Klimisch score

Figure 15 shows the overall availability of Klimisch score of in the sub-sampling analysis. Slightly over half of the ESRs had a Klimisch score of 1 or 2 while slightly over one third of the ESRs were not assigned a Klimisch score. From the perspective of Klimisch score, only about half of the endpoint studies in the sub-sampling was reliable for regulatory purposes. The proportions of

different Klimisch scores in the sub-sampling analysis are similar to those in the initial analysis (*Figure 7*), indicating that the ESRs selected for the sub-sampling analysis were a good reflection of overall ecotoxicological and toxicological information.

Figure 15. The overall availability of Klimisch score for ecotoxicological and toxicological information in the sub-sampling analysis

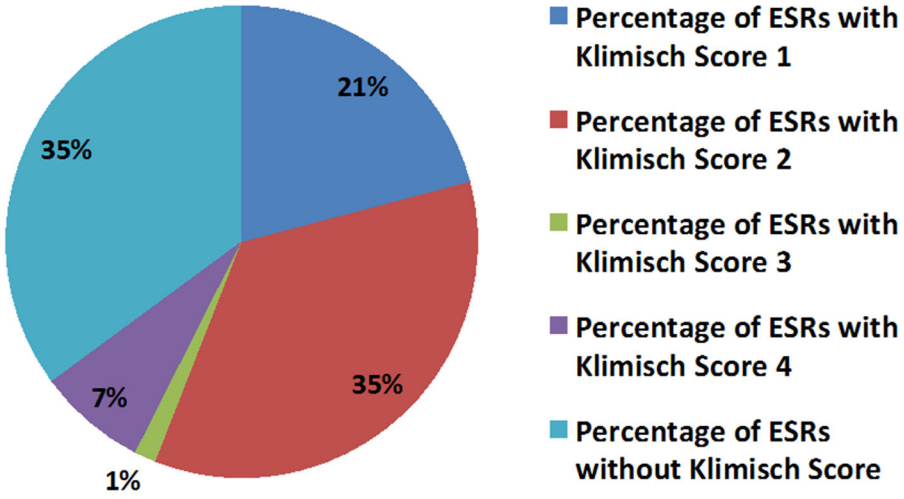
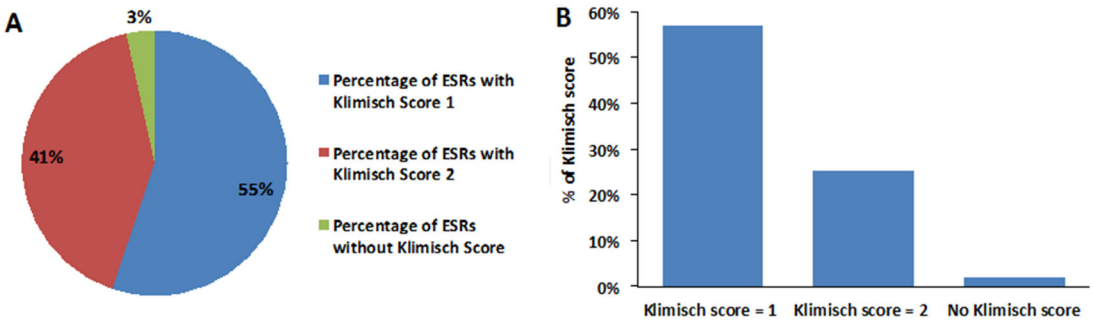


Figure 16 shows the correlation between GLP compliance and Klimisch score in the sub-sampling analysis. For all 29 endpoint studies performed with GLP compliance in the sub-sampling analysis, 28 studies (97%) had a Klimisch score of 1 or 2 and only 1 (3%) was not assigned a Klimisch score (*Figure 16* A). Over 50% of endpoint studies with a Klimisch score of 1 were GLP-compliant while only approximately 26% of endpoint studies with a Klimisch score 2 were GLP-compliant. Only 1 endpoint study with a Klimisch score lower than 2 (including no those without a Klimisch score) was performed with GLP compliance (*Figure 16* B).

Figure 16. Correlation between GLP compliance and Klimisch score in the sub-sampling analysis: A. The percentage of different Klimisch scores for ESRs with GLP compliance; B. The percentage of ESRs with GLP compliance for ESRs with different Klimisch scores



The percentage of ESRs with Klimisch score 1 and 2 in the sub-sampling analysis is almost the same as the one in the refined analysis (*Figure 13*) while the percentage of ESRs with GLP compliance having a Klimisch score 1 and 2 is also almost the same in the sub-sampling analysis and in the refined analysis. The difference is that the percentage of ESRs with Klimisch score 1 is approximately 15% higher in the refined analysis than in the sub-sampling analysis while the percentage of ESRs with Klimisch score 2 is correspondingly 15% lower. It indicates that, in general, the endpoint studies in refined analysis are more reliable than the ones in sub-sampling analysis.

3.4.3 Data completeness

The data completeness of ESRs in the sub-sampling analysis was assessed according to the minimal data requirements recommended by articles from leading peer-reviewed journals with the same approach as in the refined analysis. The raw data of completeness assessment can be found in the spreadsheet named "Sub-sampling" of the database. The results, which were was calculated as percentage of ESRs with available data (only presence and absence except for purity) for each characteristic at third-level endpoint, were presented in a heat map (shown in Figure 18 in the Annex). More endpoints were covered in the sub-sampling analysis than in the refined analysis because more types of nanomaterials were sampled.

When comparing the overall data completeness of all the ESRs for each characteristic in the refined analysis and the sub-sampling analysis (see also Figure 18 in the Annex), one can observe a common trend for both analysis approaches. In general, sub-sampling results had the same trend of data completeness as refined analysis results, indicating that the trend of data completeness of the refined analysis did not deviate from the general trend of data completeness of ecotoxicological and toxicological information. Six characteristics (namely chemical composition, particle mass concentration, details on the matrix/dispersant/solvent surrounding the nanomaterial, physical/chemical form of released/detected nanoparticles, exposure duration and exposure frequency), which were also distinguished from the refined analysis, had significantly higher availability than the other characteristics, because they were either easy to obtain or easy to measure and/or control. The percentages of availability of a few characteristics were also comparable for the two analyses, including surface chemistry, morphology, particle size and surface area. A few characteristics had considerable higher percentages of availability in the sub-sampling than in the refined analysis, although the absolute difference is usually less than 10%. The primary reason is that these characteristics were generally not available for the 3 types of nanomaterials selected for the refined analysis, such as surface charge, degree of aggregation/agglomeration, particle size distribution and particle number concentration. Taking particle number concentration as an example, as shown in *Table 5*, only 3 out of 16 ESRs with particle number concentration were from the 3 types of nanomaterials of the refined analysis.

4 DISCUSSION

The eleven dossiers that were published by OECD document that a considerable amount of endpoint studies were conducted on ENM. In total 113 testing protocols were used, many of these protocols (65) corresponded to OECD testing guidelines. However, only about half of the related endpoint study records were done using unaltered OECD guidelines. Thus, only a small portion of studies would actually meet the rigid criteria according to the mutual acceptance concept by OECD. The reasons for modifications of test guidelines is not clear from the information given in the dossiers. It would be particularly important to understand whether an endpoint study protocol was modified to account for challenges related to nano-specific ENM properties or for other reasons.

At first sight the dossiers seem to document well the toxicity of the materials that were tested. However, the refined analysis reveals that most studies do not provide details about the size or the size distribution of NM test material, characteristics deemed important to understand potential risks of nano-scale materials. It seems that most studies solely relied on the characterisation of the initial raw material as delivered to them, thus without any further assessment of what they used.

Less than 2% of the study records document size distribution to be assessed in the actual test media (aerosol, dispersion, feed) as part of the experiment. Instead, most studies use mass rather than number or size distribution (thus not following scientifically recommended reporting practice), which is more pronounced even in inhalation studies. However, for many of the studies reporting mass concentrations, it is not certain that the presence of the nanomaterial was confirmed qualitatively or quantitatively in the final preparation (aerosol, dispersion, feed) used for testing. This leaves only a minute number of studies that provided a full nano-specific characterisation of nanomaterials in the actually delivered material preparation (aerosol, liquid, feed, etc).

Information about impurities is often missing. Although it would be good to document impurities in all ESRs, it is understandable, to some extent, that information about impurities are not available if the degree of purity is high (e.g., ≥98%). However, in principle, even purity as high as 98% is not a sufficiently "safe" level because a trace amount of toxic impurities can dominate the overall toxicity of the tested nanomaterial and thus mislead the risk assessment. Therefore, the approach to report impurities should be assessed. One approach could define a threshold above which it is not necessary to report impurities as long as one can prove that at the tested level, all of the possible impurities are far below levels able of causing toxic effects to the test organism.

Considering documented challenges in preparing materials for delivery in test systems and organisms, most of the endpoint study records in the dossiers are therefore associated with two important uncertainties:

a) Whether the expected *amount* of nanomaterial was delivered (risk of loss of material during the preparation)
b) In what *form* the material was delivered and thus which if any nano-characteristics may have influenced the outcome of the tests.

In addition, for soluble materials, it is in most cases undocumented in the records whether the material was still in particular form or in ionic form.

These uncertainties lead to several problems for interpreting the dossiers as a whole.

First, assuming that the material was actually delivered to the test cells or organism in the intended quantities and that the test guidelines do work with the nanomaterials, the dossiers document the toxicity of these tested materials delivered in this form. However, due to the lack of nano-specific data, it is not possible to make any statements about the influence of individual nano-specific characteristics to the observed toxicity.

Second, the absence of nano-specific characterisation of the material as it was presented to the biological test system means that it is problematic to understand whether the tested form represents material properties as they can be found in real life, i.e. in industrial production or consumer products.

OECD assigned study records with a Klimisch score. The Klimisch score aims to reflect the degree of reliability for regulatory purposes (from 1 as very reliable to 3 as not reliable and 4 as not assignable). Usually, only studies with scores 1 and 2 are considered suitable for European regulatory purposes such as REACH. The Klimisch Score of the assessed studies was not well correlated to the degree of details provided. Actually, many Klimisch 1 score studies gave very few details about what they did and even less details with regard to nano-specific data. Thus, the Klimisch score, while usually informing about the reliability of a study for regulatory purposes, does not inform whether a study in a dossier can be used to improve our understanding of nano-specific aspects of the material.

The dossiers contain very little human exposure data and environmental fate. This existence of these sections stems from the decision of OECD to adopt the IUCLID format. However, OECD did not have the intention to collect this type of data, which is stated in a footnote of the OECD nano-dossier website, "*For the sake of completeness, the spreadsheet identifies all of the endpoints available in IUCLID. Not all of the IUCLID endpoints were addressed in the Testing Programme, nor were they intended to be addressed.*" Unfortunately, OECD does not further specify which of the data was intended to be systematically collected, which was occasionally available and which of the not available data was not intended to be collected. It would be very useful if OECD could include this information also in the actual dossiers, e.g. by writing under the relevant section headers in each dossier "These endpoints were not

addressed in the Testing Programme, nor were they intended to be addressed."

It is regrettable that OECD had decided to **not** collect exposure and environmental fate data, because one of the biggest challenges in assessing the risks of nanomaterials is the absence of sufficient information to develop scenarios about typical worker, consumer and environmental exposures. These scenarios would also be helpful to understand whether concentration ranges used in toxicological studies were in an exposure-relevant range.

5 CONCLUSIONS

The initial question of the presented study was to assess whether the data in the dossiers would allow for a risk assessment, which may be used also for regulatory purposes. However, the dossiers contain insufficient data informing about exposure to workers, humans and ecosystems, which allows the conclusion that the dossiers alone are insufficient to assess the RISK of nanomaterials in the sense of risk being a function of exposure and hazard. Furthermore, even when exposure information is known by regulators, they still cannot draw conclusions about nano-specific risks because nano-specific information on the hazard side is mostly missing. Thus, our analysis confirms the statement made by OECD when publishing the dossiers that they should not be used for regulatory risk assessment.

The dossiers do not contain sufficient information that would allow a risk assessment.

Knowing about how many people are exposed to approximately what levels, what nanoforms and in which situations is important not only to calculate the risk but also to identify where the biggest needs are for regulatory efforts and to identify the most suitable regulatory tools.

Now, the OECD website on which the dossiers are published suggests that "The outcomes of this Testing Programme provide information on the "intrinsic properties" of nanomaterials, that is, on properties of nanomaterials which are unique to the nanoscale dimension of these materials." It further states that "Understanding the intrinsic properties of nanomaterials is crucial to choose existing, adapt or create appropriate risk evaluation and management strategies for Manufactured Nanomaterials.".

However, because of the deficiency of the dossiers to document the actual delivered form of nanomaterials to the testing organisms, it is very challenging to make any firm statements regarding the nano-specificity of the observed hazard data and the extent to which the endpoint changes were linked to different nano-scale properties.

The lack of characterisation of actual used nanomaterial preparations is a serious challenge.

The lack of characterization of actually used dispersions, aerosols and feed is a serious challenge to risk assessment. It is one that is difficult to overcome *post-hoc* because most studies do not indicate dispersion protocols used. This prevents assessment *post-hoc* whether the final dispersion was likely to contain the intended mass concentration, how much agglomeration may have occurred, and how the preparation protocols may have influenced the size-distribution, functionalisation or other properties of the nanomaterial as it it reached the target.

The dossiers do not allow conclusions on the appropriateness of current Testing Guidelines for assessing ENM hazards.

Can the OECD dossiers at least inform about nano-characteristics of the raw material that influence their toxicology? Unfortunately, there is not enough nano-specific information in the dossiers

to answer this question, though having this information would be highly desirable for regulators as well as companies that are interested in a "safe by design" approach. Also read-across may be very challenging on the basis of the current dossiers.

Could the dossiers be used as a basis for testing the tests, thus to assess if the existing TGs may need adaptations when testing nanomaterials? The dossiers do not contain a systematic testing of the influence of nano-specific characteristics on the outcome, nor do they provide data that would allow assessment of the effect of nanoscale features on the test guidelines. It also would be important to systematically test how and why nano-properties can lead to artefacts, and what can be done to avoid or at least recognise them. Thus, the dossiers in their current format do not allow making statements on the quality of the TGs for nanomaterial testing.

Absence of observed toxicity does not need to equal no toxicity.

One could be tempted to state that for substances that were identified as low toxicity, it is not necessary to do a very detailed additional assessment of the influence of the nanoscale characteristics, because the risk will be low anyway. However, most endpoint studies did not characterize the finally used test-solution or aerosol, thereby leaving the doubt that artefacts in the preparation could have resulted in a partial or total loss of material or toxicologically relevant properties. It is also not clear whether suitable protocols were used for creating the preparations used to expose the test organisms. Thus, in a worst case assumption, hazardous material may not have been recognized as such. A major challenge to many of the endpoint study records is that they do not document any characterisation work done on the used nanomaterial preparations (aerosol, dispersions, feed) and that they do not mention whether the possibility of artefacts was assessed. Together, this implies that no *observed* toxicity does not necessarily equal no toxicity.

6 RECOMMENDATIONS

While the dossiers in their current format have clear deficiencies for risk assessment and for describing which of the nano-specific properties contribute how much to the observed toxicity, they still represent an impressive amount of data. We recommend to complete the dossiers where easily possible and to use the lessons gained in the previous efforts of the WPMN to move on with the goal to create in the next step data, databases and protocols that provide a support for regulatory risk assessment and management.

In particular, we recommend that the following options are considered:

- Expand the currently ongoing programme to systematically test the appropriateness of Testing Guidelines, including how to identify and prevent artefacts
- Develop Testing Guidelines for making nanomaterial preparations such as aerosols, dispersions and feed
- Define a minimum set of characteristics to be reported when testing nanomaterials following OECD TG
- Support the build-up of widely accessible exposure registries and databases
- Fill the gaps of current dossiers

6.1 SYSTEMATICALLY TEST THE EXISTING TESTING GUIDELINES

A large number of research studies have already developed protocols that are suitable within the frame of their study for work with nanomaterials. A series of scientific articles also described how endpoint assessments can be falsified by the introduction of nanomaterials. Large projects in the EU such as QNano, MARINA, NanoValid and Nanoreg have developed and used strategies to improve testing protocols with round robin approaches. This experience should be used for testing the OECD testing guidelines and to improve existing guidelines (if needed) to make them suitable for nanomaterial testing. It is likely that most TG will not require a complete re-work, but that it will suffice to address reporting of nano-specific characteristics and preparation methods (e.g. dispersion protocols), as outlined in the following two sections.

6.2 DEVELOP TG FOR MAKING DISPERSIONS AND OTHER TEST PREPARATIONS

Preparation of dispersions and other test preparations containing nanomaterials is challenging. It is a field where many research groups have already assessed possible strategies. It therefore seems feasible to develop in a reasonable time under OECD guidance, a set of protocols that meet strict requirements made by OECD for its TGs.

6.3 DEFINE A MINIMUM OF NANOMATERIAL CHARACTERISTICS TO BE REPORTED

If a laboratory reports data according to current TG, it will inform about the tested materials but will not be required to inform about nano-specific characteristics of the preparation of nanomaterials in the form it was given to the test organisms. Not having these characteristics in a laboratory report represents a major roadblock for using the reports to assess whether the preparation was free of artefacts and whether nano-specific characteristics that can pose problems were sufficiently assessed.

To address the problem of modification (thus change of the material-type) or ionisation (thus disappearance of the nano-form) of non-persistent materials, it would be also useful to check whether the ENM is stable in the test solution for the duration of the experiment. Ideally, one would also characterize the ENM in the actual test system (cell, animal) during and at the end of the test, though for many materials this may not be possible at reasonably low exposure concentrations.

Finally, considering that it is time consuming and costly to make a full physico-chemical characterization, it would be useful if OECD could support efforts to identify key metrics that allow to assess if (and what) further more detailed assessment of ENM characteristics in the actual preparation seem warranted.

6.4 SUPPORT THE BUILD-UP OF EXPOSURE DATABASES

Having more information about occupational long-term exposure together with a description of health and cause of mortality is critical to confirm that exposures believed to be safe are actually not associated to elevated ill-health, thus that risk management efforts are effective (this is actually valid for chemicals in general). While it may seem difficult to follow workers for decades, following them for a few years will enable identification of most health effects that develop over time. A comparison with air pollution effects could be especially useful because for air pollution, both, public health as well as occupational health dose-response data exists.

There are several governmental bodies collecting such data and bodies such as PEROSH (in regard to workers' exposure) are trying to build up databases. It would be useful if OECD at least endorsed such efforts, if not support them actively by helping define international guidelines for exposure data collection and sharing. This would allow regulators and risk managers world-wide to profit from knowing about observed levels and situations where unhealthy exposure concentrations can be reached. It will also help knowing if large parts of the population can be exposed (even at low levels) to a situation or if it concerns only a few individuals.

6.5 FILL THE GAPS OF THE CURRENT DOSSIERS

The dossiers lack information about nano-specific characterisation, preparation protocols and exposure data.

Characterisation: It is possible that some of the labs actually characterised the dispersion and aerosol before or while exposing the test organisms, but that this information did not make it into the dossiers. We recommend to assess if this is likely to be the case for many of the tests. If so, this information should be retrieved and added to the dossiers.

Preparation protocols: Protocols on how to create nanomaterial preparations (aerosol, dispersion, feed) for these experiments should be collected and made available. This will allow an assessment of the potential for artefact generation during the preparation steps, and thus will allow differentiating between endpoint study records that are more or less reliable with regards to actual exposure concentration and the presentation of nanomaterial characteristics.

Exposure data: Human and ecosystem data is not well represented in the dossiers. However, it would be useful if the OECD WPMN could build upon the testing Programme by including exposure as an important area where information should be collected. Such information will be very useful to develop scenarios of exposure and to define priorities. (see also *6.4 Support the build-up of exposure databases*).

Annex

Table 8. TGs adopted in the Testing Programme: TGs with deviations and their associated nanomaterials were highlighted in ***yellow and bold italic***.

EP No.	Endpoint	TG code	TG title	NM tested
4. Physical and chemical properties				
4.1	Appearance/physical state/colour		WPMN guidance manual	MWCNT, SWCNT
4.4	Density	ISO 787:11	General methods of test for pigments and extenders -- Part 11: Determination of tamped volume and apparent density after tamping	SiO_2
4.5	Particle size distribution (Granulometry)	EN 481	Workplace atmospheres. Size fraction definitions for measurement of airborne particles	SiO_2
		ISO 9276-2	Representation of results of particle size analysis -- Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions	SiO_2
		NIST 960-1	Particle Size Characterization (Special publication 960-1)	SiO_2, TiO_2
4.7	Partition coefficient	OECD 107	Partition Coefficient (n-octanol/water): Shake Flask Method	Dendrimers
		OECD 117	Partition Coefficient (n-octanol/water), HPLC Method	Dendrimers
4.8	Water solubility	OECD 105	Water Solubility	Dendrimers, Gold, SiO_2
		ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Silver
4.13	Flammability	EN 14034	Determination of explosion characteristics of dust clouds	SiO_2, TiO_2
		VDI 2263, Part 1	Dust fires and dust explosions; hazards, assessment, protective measures; test methods for the determination of the safety characteristic of dusts	TiO_2

EP No.	Endpoint	TG code	TG title	NM tested
4.14	Explosiveness	VDI 2263, Part 1	Dust fires and dust explosions; hazards, assessment, protective measures; test methods for the determination of the safety characteristic of dusts	TiO_2
4.19	Stability: thermal, sunlight, metals	OECD 113	Screening Test for Thermal Stability and Stability in Air	ZnO
4.23	Additional physico-chemical information	ISO 10801	Nanotechnologies -- Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method	Silver
		ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Sliver
4.24	Agglomeration/aggregation	ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Gold, Silver
			WPMN guidance manual	MWCNT
4.25	Crystalline phase	ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Silver
4.26	Crystallite and grain size	OECD 110	Particle Size Distribution/ Fibre Length and Diameter Distributions	Silver
		ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Silver
4.28	Specific surface area	ISO 9277:1995	Determination of the specific surface area of solids by gas adsorption using the BET method	TiO_2
		ISO 9277:2010	Determination of the specific surface area of solids by gas adsorption - BET method	Fullerenes, MWCNT, SiO_2, SWCNT, ZnO
4.29	Zeta potential	ENV/JM/MONO(2009)20/REV	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision	Gold, Silver

EP No.	Endpoint	TG code	TG title	NM tested
4.30	Surface chemistry	ISO 15472	Surface chemical analysis -- X-ray photoelectron spectrometers -- Calibration of energy scales	ZnO
4.31	Dustiness	EN 15051:2006	Workplace atmospheres - Measurement of the dustiness of bulk materials - Requirements and reference test methods	MWCNT, TiO2, ZnO
		DIN 55992-2	Determination of a parameter for the dust formation of pigments and extenders - Part 2: Drop method	MWCNT
4.32	Porosity	ISO 15901-1:2005	Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption - Part 1: Mercury porosimetry	Fullerenes, MWCNT, SWCNT
		JIS K 1150-1994	Test method for silica gel	MWCNT
4.33	Pour density	ASTM D 1513-05	Standard Test Method for Carbon Black, Pelleted—Pour Density	MWCNT, SWCNT
		DIN/ISO 9136	Abrasive grains -- Determination of bulk density	MWCNT
		JIS K 6219-2 (2006)	Carbon black for rubber industry - Characteristics of pelletized carbon black - Part 2: Determination of pour density	Fullerenes
4.34	Photocatalytic activity	ISO 22197-1	Fine ceramics (advanced ceramics, advanced technical ceramics) -- Test method for air-purification performance of semiconducting photocatalytic materials -- Part 1: Removal of nitric oxide	Fullerenes, MWCNT, SWCNT
		JIS R 1701	Fine ceramics (advanced ceramics, advanced technical ceramics)-Test method for air purification performance of photocatalytic materials	Fullerenes, MWCNT, SWCNT
5. Environmental fate and pathways				
5.1.3	Phototransformation in water	OECD guideline draft	Phototransformation of Chemicals in Water - Direct and Indirect Photolysis	Fullerenes

EP No.	Endpoint	TG code	TG title	NM tested
		OECD 316	Phototransformation of Chemicals in Water – Direct Photolysis	Fullerenes
5.2.1	Biodegradation in water: screening tests	OECD 106	Adsorption -- Desorption Using a Batch Equilibrium Method	Silver
		OECD 301 F	Ready Biodegradability: Manometric Respirometry Test	MWCNT, SWCNT
		OECD 302 C	Inherent Biodegradability: Modified MITI Test (II)	MWCNT, SWCNT
5.2.2	Biodegradation in water and sediment: simulation tests	OECD 106	Adsorption -- Desorption Using a Batch Equilibrium Method	TiO_2
		OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	CeO_2
		OECD 303	Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms	TiO_2
		OECD 303A	Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms	CeO_2, TiO_2
5.4.1	Adsorption / desorption	OECD 106	Adsorption -- Desorption Using a Batch Equilibrium Method	CeO_2, Silver, TiO_2
5.4.4	Other distribution data	DIN 19528	Leaching of solid materials	Silver
		OECD 312	Leaching in Soil Columns	TiO_2
5.6	Additional information on environmental fate and behaviour	OECD 106	Adsorption -- Desorption Using a Batch Equilibrium Method	Silver, TiO_2
		OECD 303	Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms	Silver
6. Ecotoxicological information				
6.1.1	Short-term toxicity to fish	ASTM E729-96	Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians	TiO_2

EP No.	Endpoint	TG code	TG title	NM tested
		DIN 38415 T6	German standard methods for the examination of water, waste water and sludge - Subanimal testing (group T) - Part 6: Toxicity to fish; Determination of the non-acute-poisonous effect of waste water to fish eggs by dilution limits (T 6)	Silver
		Fish Embryo Toxicity[7]		***TiO_2***
		ISO 15088:2007	Water quality -- Determination of the acute toxicity of waste water to zebrafish eggs (Danio rerio)	Dendrimers
		OECD 203	Fish, Acute Toxicity Test	Fullerenes, ***Gold***, MWCNT, Nanoclays, Silver, SiO_2, SWCNT, TiO_2
		OECD 204	Fish, Prolonged Toxicity Test: 14-Day Study	MWCNT
		OECD 210	Fish, Early-life Stage Toxicity Test	Silver
		OECD 212	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	CeO_2, MWCNT, Silver, TiO_2
		OECD 236	Fish Embryo Acute Toxicity (FET) Test	SWCNT, ***TiO_2***
		OECD draft guideline	Fish, test with fish embryos	Silver
		OECD Fish Embryo Toxicity Test (May 2006)		***SWCNT***
6.1.2	Long-term toxicity to fish	OECD 204	Fish, Prolonged Toxicity Test: 14-Day Study	MWCNT, SWCNT
		OECD 210	Fish, Early-life Stage Toxicity Test	Silver, ZnO
		OECD 212	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	Silver
		OECD draft guideline	Fish, test with fish embryos	Gold

[7] Declared as a deviation of a TG but original TG not mentioned in ESR.

EP No.	Endpoint	TG code	TG title	NM tested
		Fish embryo toxicity test: the OECD draft proposal for a new guideline (2006)		Silver
6.1.3	Short-term toxicity to aquatic invertebrates	ASTM E1706-05	Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates	SWCNT
		ASTM E729-96	Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians	SWCNT, TiO_2
		ASTM E-2317-04	Standard Guide for Conducting Renewal Microplate-Based Life-Cycle Toxicity Tests with a Marine Meiobenthic Copepod	SWCNT
		British Standard (BS 1996)		Dendrimers
		EPA-821-R-02-012	Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms	TiO_2
		ISO 6341:2012	Water quality -- Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) -- Acute toxicity test	TiO_2
		OECD 202	Daphnia sp. Acute Immobilisation Test	CeO_2, ***Gold***, MWCNT, Nanoclays, Silver, ***SiO_2***, SWCNT, TiO_2, ***ZnO***
		OECD 203	Fish, Acute Toxicity Test	Silver
		OECD 204	Fish, Prolonged Toxicity Test: 14-Day Study	***Silver***
		SOP of Daphtoxkit FTM magna		ZnO
		SOP of Protoxkit F		ZnO
		SOP of Thamnoxkit FTM		ZnO
6.1.4	Long-term toxicity to aquatic invertebrates	EPA Method 1002	Cladoceran,Ceriodaphniadubia, SurvivalandReproductionTest	***TiO_2***

EP No.	Endpoint	TG code	TG title	NM tested
		EPS/11RM/33		TiO_2
		OECD 202	Daphnia sp. Acute Immobilisation Test	TiO_2
		OECD 211	Daphnia magna Reproduction Test	CeO_2, MWCNT, Silver, SWCNT, TiO_2
		OECD 212	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	TiO_2
6.1.5	Toxicity to aquatic algae and cyanobacteria	EPS1/RM/25	Biological Test Method: Growth Inhibition Test Using a Freshwater Alga	TiO_2
		ISO 8692	Water Quality - Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum	Silver, TiO_2
		OECD 201	Freshwater Alga and Cyanobacteria, Growth Inhibition Test	Dendrimers, ***Gold***, MWCNT, Nanoclays, Silver, SWCNT, TiO_2
		OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	MWCNT
6.1.6	Toxicity to aquatic plants other than algae	***ISO 20079***	Water quality -- Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) -- Duckweed growth inhibition test	***Silver***
		ISO 5667-16	Water quality -- Sampling -- Part 16: Guidance on biotesting of samples	Silver
		OECD 221	Lemna sp. Growth Inhibition Test	Silver
6.1.7	Toxicity to microorganisms	***DIN 38412-48***	German standard methods for the examination of water, waste water and sludge - Bio-assays (group L) - Part 48: Toxicity test with Arthrobacter globiformis for contaminated solids (L 48)	***Silver***
		EPA (2007) Nanotechnology White Paper, EPA, U.S.A. and OECD (2008) Current Developments in Manufactured Nanomaterials in Korea		Silver

EP No.	Endpoint	TG code	TG title	NM tested
		ISO 11348-2:2007	Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 2: Method using liquid-dried bacteria	Gold
		ISO 11348-3	Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria	Silver
		OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	MWCNT, ZnO
		OECD (2008) Current Developments in Manufactured Nanomaterials in Korea		Silver
6.1.8	Toxicity to other aquatic organisms	OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	Dendrimers, Silver, TiO_2
6.2	Sediment toxicity	ASTM E1367-99	Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods	ZnO
		ISO 10872:2010	Water quality -- Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)	***TiO_2***
		OECD 203	Fish, Acute Toxicity Test	***TiO_2***
		OECD 219	Sediment-Water Chironomid Toxicity Using Spiked Water	***Gold***, ***MWCNT***, Nanoclays, Silver, TiO_2
		OECD 225	Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment	***TiO_2***
		OSPARCOM 1995		***ZnO***
6.3.1	Toxicity to soil macroorganisms except arthropods	ASTM E2172 - 01(2014)	Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans	Silver
		ISO 15685	Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation	Silver

EP No.	Endpoint	TG code	TG title	NM tested
		ISO 17512-1	Soil quality -- Avoidance test for determining the quality of soils and effects of chemicals on behaviour -- Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei)	TiO_2
		OECD 207	Earthworm, Acute Toxicity Tests	TiO_2
		OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	Silver
		OECD 216	Soil Microorganisms: Nitrogen Transformation Test	Silver
		OECD 217	Soil Microorganisms: Carbon Transformation Test	Silver
		OECD 219	Sediment-Water Chironomid Toxicity Using Spiked Water	Silver
		OECD 222	Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)	Silver, TiO_2
		OECD 303A	Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms	Silver
6.3.2	Toxicity to terrestrial arthropods	ASTM E2172 - 01(2008)	Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans	TiO_2
		ASTM E2172 - 01(2014)	Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans	Silver
		OECD 207	Earthworm, Acute Toxicity Tests	TiO_2
		OECD 226	Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil	TiO_2
6.3.3	Toxicity to terrestrial plants	OECD 208	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	Silver, TiO_2
		USEPA Ecological Effects Test Guidelines Terrestrial Plant Toxicity, Tier I (Seedling Emergence) 1996		TiO_2
6.3.4	Toxicity to soil microorganisms	OECD 216	Soil Microorganisms: Nitrogen Transformation Test	MWCNT, Silver, SWCNT, TiO_2

EP No.	Endpoint	TG code	TG title	NM tested
		OECD 217	Soil Microorganisms: Carbon Transformation Test	Silver, TiO_2
6.6	Additional ecotoxicological information	OECD 209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	Silver, SWCNT
		ISO 21427-1:2006	Water quality -- Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 1: Evaluation of genotoxicity using amphibian larvae	MWCNT
7. Toxicological information				
7.1.1	Basic toxicokinetics	***OECD 412***	Subacute Inhalation Toxicity: 28-Day Study	***TiO_2***
		OECD 413	Subchronic Inhalation Toxicity: 90-day Study	***SiO_2***
		OECD 417	Toxicokinetics	***Silver***, ZnO
7.1.2	Dermal absorption	OECD 427	Skin Absorption: In Vivo Method	ZnO
		OECD 428	Skin Absorption: In Vitro Method	ZnO
		SCCNFP/0750/03 (Oct. 2003)	Basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients	ZnO
7.2.1	Acute toxicity: oral	OECD 401	Acute Oral Toxicity	SiO_2, ZnO
		OECD 420	Acute Oral Toxicity - Fixed Dose Procedure	***MWCNT***
		OECD 423	Acute Oral toxicity - Acute Toxic Class Method	MWCNT, ***Silver***, SWCNT
		OECD 474	Mammalian Erythrocyte Micronucleus Test	Fullerenes, MWCNT, ***SWCNT***
7.2.2	Acute toxicity: inhalation	***OECD 403***	Acute Inhalation Toxicity	MWCNT, ***SiO_2***
7.2.3	Acute toxicity: dermal	***OECD 402***	Acute Dermal Toxicity	MWCNT, ***Silver***, ZnO
		OECD 407	Repeated Dose 28-day Oral Toxicity Study in Rodents	***ZnO***
7.2.4	Acute toxicity: other routes	OECD 404	Acute Dermal Irritation/Corrosion	MWCNT

EP No.	Endpoint	TG code	TG title	NM tested
		OECD 431	In Vitro Skin Corrosion: Human Skin Model Test	MWCNT
7.3.1	Skin irritation / corrosion	EU method B.40 BIS (In vitro Skin Corrosion) 2008		ZnO
		OECD 404	Acute Dermal Irritation/Corrosion	Fullerenes, MWCNT, ***Silver***, ***SiO_2***, SWCNT, TiO_2
		OECD 431	In Vitro Skin Corrosion: Human Skin Model Test	MWCNT, ZnO
		National standard protocol (No. IPC/05-92) corresponding to US EPA		SiO_2
		Patch-Test; Hazardous Substances, Part 191, Section 11, FDA, Washington, 1965		***SiO_2***
		SOP of the ZEBET 2006		ZnO
7.3.2	Eye irritation	Draize-Test; Hazardous Substances, Part 191, Section 12, Federal Register, Vol. 37, No. 83, FDA, Washington		SiO_2
		Harbell J.W. et al. (2009): COLIPA Program on Optimization of Existing In Vitro Eye Irritation Assays for Entry into Formal Validation: Technology Transfer and Intra/Inter		ZnO
		MatTek Corporation, Ashland, MA 01721, USA: EpiOcularTM human cell construct: Procedure details, Version 3.1a of February 10, 2010		ZnO
		OECD 405	Acute Eye Irritation/Corrosion	Fullerenes, MWCNT, ***Silver***, SiO_2, SWCNT, TiO_2
		OECD 437	Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants	MWCNT, ZnO
7.4.1	Skin sensitisation	***OECD 406***	Skin Sensitisation	Fullerenes, MWCNT, ***Silver***, SWCNT
		OECD 429	Skin Sensitisation - Local Lymph Node Assay	MWCNT
7.5.1	Repeated dose toxicity: oral	**OECD 407**	Repeated Dose 28-day Oral Toxicity Study in Rodents	Fullerenes, ***Silver***, ***SiO_2***, SWCNT
		OECD 408	Repeated Dose 90-Day Oral Toxicity Study in Rodents	***Silver***, SiO_2

EP No.	Endpoint	TG code	TG title	NM tested
		OECD 420	Acute Oral Toxicity - Fixed Dose Procedure	MWCNT
		OECD 422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	***Silver***
7.5.2	Repeated dose toxicity: inhalation	OECD 407	Subacute Inhalation Toxicity: 28-Day Study	MWCNT
		OECD 412	Subacute Inhalation Toxicity: 28-Day Study	***CeO_2***, ***Gold***, ***Silver***, ***SiO_2***, SWCNT, ***TiO_2***, ***ZnO***
		OECD 413	Subchronic Inhalation Toxicity: 90-day Study	***Gold***, MWCNT, ***Silver***, ***SiO_2***, ***ZnO***
		OECD 453	Combined Chronic Toxicity/Carcinogenicity Studies	TiO_2
7.5.3	Repeated dose toxicity: dermal	OECD 408	Repeated Dose 90-Day Oral Toxicity Study in Rodents	Silver
		OECD 412	Subacute Inhalation Toxicity: 28-Day Study	Silver, ***TiO_2***
7.5.4	Repeated dose toxicity: other routes	***OECD 407***	Subacute Inhalation Toxicity: 28-Day Study	***Gold***
7.6.1	Genetic toxicity in vitro	(Japan) Guidelines for Screening Mutagenicity Testing Of Chemicals		Fullerenes, SWCNT, TiO_2
		EU Method B.17 (Mutagenicity - In Vitro Mammalian Cell Gene Mutation Test)		SiO_2
		OECD 471	Bacterial Reverse Mutation Test	Fullerenes, ***Gold***, MWCNT, ***Silver***, SiO_2, SWCNT, ZnO
		OECD 473	In vitro Mammalian Chromosome Aberration Test	Fullerenes, ***Gold***, MWCNT, ***Silver***, ***SiO_2***, SWCNT, ZnO
		OECD 476	In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes	MWCNT, SiO_2, TiO_2, ZnO
		OECD 482	Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells in vitro	SiO_2
		OECD 487	In Vitro Mammalian Cell Micronucleus Test	***SiO_2***, ***TiO_2***

EP No.	Endpoint	TG code	TG title	NM tested
7.6.2	Genetic toxicity in vivo	OECD 412	Subacute Inhalation Toxicity: 28-Day Study	MWCNT
		OECD 474	Mammalian Erythrocyte Micronucleus Test	Fullerenes, MWCNT, ***Silver***, SiO_2, SWCNT, TiO_2, ZnO
		OECD 488	Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays	TiO_2
7.8.1	Toxicity to reproduction	***OECD 415***	One-Generation Reproduction Toxicity Study	***SiO_2***
		OECD 416	Two-Generation Reproduction Toxicity	SiO_2
		OECD 422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	***Silver***
7.8.2	Developmental toxicity / teratogenicity	***OECD 414***	Prenatal Development Toxicity Study	***MWCNT***, ZnO
		OECD 422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	***Silver***
7.9.3	Specific investigations: other studies	EU Directive 67/548/EEC, Annex V, B41		ZnO
7.12	Additional toxicological information	National Committee for Clinical Laboratory Standards (NCCLS)		ZnO

Figure 17. Heat maps showing data completeness for ecotoxicological and toxicological information: (A) zinc oxide, (B) SWCNT, (C) fullerenes, (D) zinc oxide, SWCNT and fullerenes combined as a whole. The blank space indicates that no endpoint study was conducted.

A

			Chemistry						Nanoscale descriptor							Circumstance			
			Chemical composition	Purity	Impurities	Persistence	Surface chemistry	Surface charge	Degree of aggregation / agglomeration	Morphology (including aspect ratio)	Particle size (average, range, etc.)	Particle size distribution	Particle mass concentration	Surface area	Particle number concentration	Details on the matrix /dispersant /solvent surrounding the NM	Physical/chemical form of released/detected NPs	Exposure duration	Exposure frequency
Ecotoxicology	Aquatic tox.	6.1.1	100%	100%	0%	0%	0%	0%	0%	100%	100%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.3	100%	14%	0%	0%	29%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.4																	
		6.1.5	100%	0%	0%	0%	0%	0%	0%	100%	100%	100%	100%	0%	0%	100%	100%	100%	100%
		6.1.6																	
		6.1.7	100%	33%	0%	0%	0%	0%	0%	67%	67%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.8																	
	Sediment tox.	6.2	100%	0%	0%	0%	50%	0%	0%	50%	100%	0%	100%	0%	0%	100%	100%	100%	100%
	Terrestrial tox.	6.3.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.3.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	0%	0%
		6.3.3	100%	100%	0%	0%	0%	0%	0%	67%	33%	0%	100%	33%	0%	100%	100%	100%	100%
		6.3.4																	
		6.3.5																	
		6.3.6																	
	Bio. eff. Monitoring	6.4																	
	Biotrans. & kinetics	6.5																	
	Addl. ecotox. info	6.6																	
Toxicology	Toxicokinetics, metabolism & distribution	7.1.1	100%	100%	0%	0%	100%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	100%	100%
		7.1.2	100%	50%	0%	0%	25%	0%	0%	13%	13%	0%	100%	0%	0%	100%	100%	100%	100%
		7.2.1	100%	100%	100%	0%	0%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	0%	0%
	Acute tox.	7.2.2																	
		7.2.3	100%	50%	0%	0%	25%	25%	0%	0%	25%	25%	100%	0%	0%	75%	100%	75%	100%
		7.2.4																	
	Irritation / corrosion	7.3.1	100%	100%	0%	0%	100%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.3.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Sensitisation	7.4.1																	
		7.4.2																	
	Repeated dose tox.	7.5.1																	
		7.5.2	100%	80%	20%	0%	80%	0%	0%	0%	80%	0%	100%	0%	0%	80%	100%	60%	60%
		7.5.3																	
		7.5.4																	
	Genetic tox.	7.6.1	100%	100%	0%	0%	75%	0%	0%	0%	0%	0%	100%	0%	0%	50%	100%	100%	100%
		7.6.2	100%	100%	0%	0%	100%	0%	0%	0%	75%	0%	100%	0%	0%	100%	100%	100%	100%
		7.6.3																	
	Carcinogenicity	7.7																	
	Dev. tox. / teratogenicity	7.8.1																	
		7.8.2	100%	0%	0%	0%	100%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	100%	100%
		7.8.3																	
	Neurotox.	7.9.1																	
		7.9.2																	
		7.9.3	100%	40%	0%	0%	20%	0%	0%	0%	0%	0%	100%	0%	0%	40%	100%	60%	60%
	Exp. Related obs. In humans	7.10.1																	
		7.10.2																	
		7.10.3																	
		7.10.4	100%	0%	0%	0%	100%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Tox. eff. on livestock and pets	7.10.5	100%	0%	0%	0%	33%	0%	0%	0%	0%	0%	100%	0%	0%	67%	100%	100%	100%
		7.11																	
	Addl. tox. info	7.12	100%	13%	0%	0%	0%	0%	13%	38%	63%	13%	63%	0%	0%	25%	75%	38%	38%
	In vitro tox. Info	7.13																	

B

			Chemistry						Nanoscale descriptor							Circumstance			
			Chemical composition	Purity	Impurities	Persistence	Surface chemistry	Surface charge	Degree of aggregation / agglomeration	Morphology (including aspect ratio)	Particle size (average, range, etc.)	Particle size distribution	Particle mass concentration	Surface area	Particle number concentration	Details on the matrix /dispersant /solvent surrounding the NM	Physical/chemical form of released/detected NPs	Exposure duration	Exposure frequency
Ecotoxicology		6.1.1	100%	71%	57%	0%	0%	0%	0%	14%	14%	0%	100%	0%	0%	100%	100%	86%	86%
		6.1.2	100%	33%	33%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.3	100%	40%	0%	0%	20%	0%	0%	0%	0%	0%	100%	0%	0%	80%	100%	100%	100%
	Aquatic tox.	6.1.4	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.5	100%	0%	25%	0%	0%	0%	0%	25%	25%	0%	75%	0%	0%	100%	100%	75%	75%
		6.1.6																	
		6.1.7																	
		6.1.8																	
	Sediment tox.	6.2	100%	100%	100%	0%	0%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	0%	0%
		6.3.1																	
		6.3.2																	
	Terrestrial tox.	6.3.3	100%	100%	100%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.3.4	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	0%	0%	100%	100%
		6.3.5																	
		6.3.6																	
	Bio. eff. Monitoring	6.4																	
	Biotrans. & kinetics	6.5																	
	Addl. ecotox. info	6.6	100%	0%	0%	0%	20%	0%	0%	0%	0%	0%	80%	0%	0%	40%	80%	80%	80%
Toxicology	Toxicokinetics, metabolism & distribution	7.1.1	100%	50%	0%	0%	0%	0%	0%	50%	50%	50%	100%	0%	0%	100%	100%	100%	100%
		7.1.2																	
	Acute tox.	7.2.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.2.2	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.2.3																	
		7.2.4																	
	Irritation / corrosion	7.3.1	100%	0%	25%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.3.2	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Sensitisation	7.4.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	67%	67%
		7.4.2																	
	Repeated dose tox.	7.5.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.5.2	100%	0%	0%	0%	0%	0%	50%	50%	50%	0%	100%	0%	50%	100%	100%	100%	100%
		7.5.3																	
		7.5.4																	
	Genetic tox.	7.6.1	100%	71%	47%	0%	12%	0%	12%	6%	6%	0%	100%	0%	0%	47%	100%	71%	71%
		7.6.2	100%	17%	17%	0%	0%	0%	0%	50%	50%	0%	100%	17%	0%	83%	100%	100%	100%
		7.6.3																	
	Carcinogenicity	7.7																	
	Dev. tox. / teratogenicity	7.8.1																	
		7.8.2	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.8.3																	
	Neurotox.	7.9.1	100%	0%	0%	0%	100%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	100%	100%
		7.9.2																	
		7.9.3	100%	50%	67%	0%	0%	0%	0%	17%	17%	8%	100%	0%	0%	92%	100%	100%	92%
	Exp. Related obs. In humans Tox. eff. on livestock and pets	7.10.1																	
		7.10.2																	
		7.10.3																	
		7.10.4																	
		7.10.5																	
		7.11																	
	Addl. tox. info	7.12	100%	25%	0%	0%	25%	0%	0%	0%	0%	0%	0%	0%	0%	25%	100%	25%	25%
	In vitro tox. Info	7.13																	

C

			Chemistry						Nanoscale descriptor							Circumstance			
			Chemical composition	Purity	Impurities	Persistence	Surface chemistry	Surface charge	Degree of aggregation / agglomeration	Morphology (including aspect ratio)	Particle size (average, range, etc.)	Particle size distribution	Particle mass concentration	Surface area	Particle number concentration	Details on the matrix /dispersant /solvent surrounding the NM	Physical/chemical form of released/detected NPs	Exposure duration	Exposure frequency
Ecotoxicology	Aquatic tox.	6.1.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	0%	100%	100%	100%
		6.1.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.3																	
		6.1.4																	
		6.1.5																	
		6.1.6																	
		6.1.7																	
		6.1.8																	
	Sediment tox.	6.2																	
	Terrestrial tox.	6.3.1																	
		6.3.2																	
		6.3.3																	
		6.3.4																	
		6.3.5																	
		6.3.6																	
	Bio. eff. Monitoring	6.4																	
	Biotrans. & kinetics	6.5																	
	Addl. ecotox. info	6.6																	
Toxicology	Toxicokinetics, metabolism & distribution	7.1.1	100%	67%	0%	0%	0%	0%	0%	0%	33%	0%	100%	0%	0%	100%	100%	100%	100%
		7.1.2																	
		7.2.1	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Acute tox.	7.2.2																	
		7.2.3																	
		7.2.4																	
	Irritation / corrosion	7.3.1	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.3.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Sensitisation	7.4.1	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.4.2																	
	Repeated dose tox.	7.5.1	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.5.2	100%	100%	0%	0%	0%	0%	0%	0%	100%	100%	100%	0%	100%	100%	100%	100%	100%
		7.5.3																	
		7.5.4																	
	Genetic tox.	7.6.1	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.6.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.6.3																	
	Carcinogenicity	7.7																	
	Dev. tox. / teratogenicity	7.8.1																	
		7.8.2																	
		7.8.3																	
	Neurotox.	7.9.1																	
		7.9.2																	
		7.9.3																	
	Exp. Related obs. In humans	7.10.1																	
		7.10.2																	
		7.10.3																	
		7.10.4																	
	Tox. eff. on livestock and pets	7.10.5																	
		7.11																	
	Addl. tox. info	7.12	100%	0%	0%	0%	0%	0%	50%	50%	100%	0%	50%	0%	0%	100%	100%	100%	100%
	In vitro tox. Info	7.13																	

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			Chemistry						Nanoscale descriptor							Circumstance			
			Chemical composition	Purity	Impurities	Persistence	Surface chemistry	Surface charge	Degree of aggregation / agglomeration	Morphology (including aspect ratio)	Particle size (average, range, etc.)	Particle size distribution	Particle mass concentration	Surface area	Particle number concentration	Details on the matrix /dispersant /solvent surrounding the NM	Physical/chemical form of released/detected NPs	Exposure duration	Exposure frequency
Ecotoxicology	Aquatic tox.	6.1.1	100%	67%	44%	0%	0%	0%	0%	22%	22%	0%	100%	0%	0%	89%	100%	89%	89%
		6.1.2	100%	60%	20%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.3	100%	25%	0%	0%	25%	0%	0%	0%	0%	0%	100%	0%	0%	92%	100%	100%	100%
		6.1.4	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.5	100%	0%	20%	0%	0%	0%	0%	40%	40%	20%	80%	0%	0%	100%	100%	80%	80%
		6.1.6																	
		6.1.7	100%	33%	0%	0%	0%	0%	0%	67%	67%	0%	100%	0%	0%	100%	100%	100%	100%
		6.1.8																	
	Sediment tox.	6.2	100%	33%	33%	0%	33%	0%	0%	33%	100%	0%	100%	0%	0%	100%	100%	67%	67%
	Terrestrial tox.	6.3.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		6.3.2	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	0%	0%
		6.3.3	100%	100%	25%	0%	0%	0%	0%	50%	25%	0%	100%	25%	0%	100%	100%	100%	100%
		6.3.4	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	0%	0%	100%	100%
		6.3.5																	
		6.3.6																	
	Bio. eff. Monitoring	6.4																	
	Biotrans. & kinetics	6.5																	
	Addl. ecotox. info	6.6	100%	0%	0%	0%	20%	0%	0%	0%	0%	0%	80%	0%	0%	40%	80%	80%	80%
Toxicology	Toxicokinetics, metabolism & distribution	7.1.1	100%	71%	0%	0%	29%	0%	0%	14%	57%	14%	100%	0%	0%	100%	100%	100%	100%
		7.1.2	100%	50%	0%	0%	25%	0%	0%	13%	13%	0%	100%	0%	0%	100%	100%	100%	100%
	Acute tox.	7.2.1	100%	40%	20%	0%	0%	0%	0%	0%	20%	0%	100%	0%	0%	100%	100%	80%	80%
		7.2.2	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.2.3	100%	50%	0%	0%	25%	25%	0%	0%	25%	25%	100%	0%	0%	75%	100%	75%	100%
		7.2.4																	
	Irritation / corrosion	7.3.1	100%	43%	14%	0%	14%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.3.2	100%	60%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Sensitisation	7.4.1	100%	40%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	80%	80%
		7.4.2																	
	Repeated dose tox.	7.5.1	100%	50%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
		7.5.2	100%	63%	13%	0%	50%	0%	13%	13%	75%	13%	100%	0%	25%	88%	100%	75%	75%
		7.5.3																	
		7.5.4																	
	Genetic tox.	7.6.1	100%	78%	35%	0%	22%	0%	9%	4%	4%	0%	100%	0%	0%	52%	100%	78%	78%
		7.6.2	100%	58%	8%	0%	33%	0%	0%	25%	50%	0%	100%	8%	0%	92%	100%	100%	100%
		7.6.3																	
	Carcinogenicity	7.7																	
	Dev. tox. / teratogenicity	7.8.1																	
		7.8.2	100%	0%	0%	0%	50%	0%	0%	0%	50%	0%	100%	0%	0%	100%	100%	100%	100%
		7.8.3																	
	Neurotox.	7.9.1	100%	0%	0%	0%	100%	0%	0%	0%	100%	0%	100%	0%	0%	100%	100%	100%	100%
		7.9.2																	
		7.9.3	100%	47%	47%	0%	6%	0%	0%	12%	12%	6%	100%	0%	0%	76%	100%	88%	82%
	Exp. Related obs.	7.10.1																	
		7.10.2																	
		7.10.3																	
	In humans	7.10.4	100%	0%	0%	0%	100%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	100%	100%
	Tox. eff. on	7.10.5	100%	0%	0%	0%	33%	0%	0%	0%	0%	0%	100%	0%	0%	67%	100%	100%	100%
	livestock and pets	7.11																	
	Addl. tox. info	7.12	100%	14%	0%	0%	7%	0%	14%	29%	50%	7%	43%	0%	0%	36%	86%	43%	43%
	In vitro tox. Info	7.13																	

Figure 18. Heat maps showing data completeness for ecotoxicological and toxicological information in the sub-sampling analysis. The blank space indicates that no endpoint study was conducted. The light brown colour indicates that the endpoints were not sampled but ESRs were available in the dossiers.

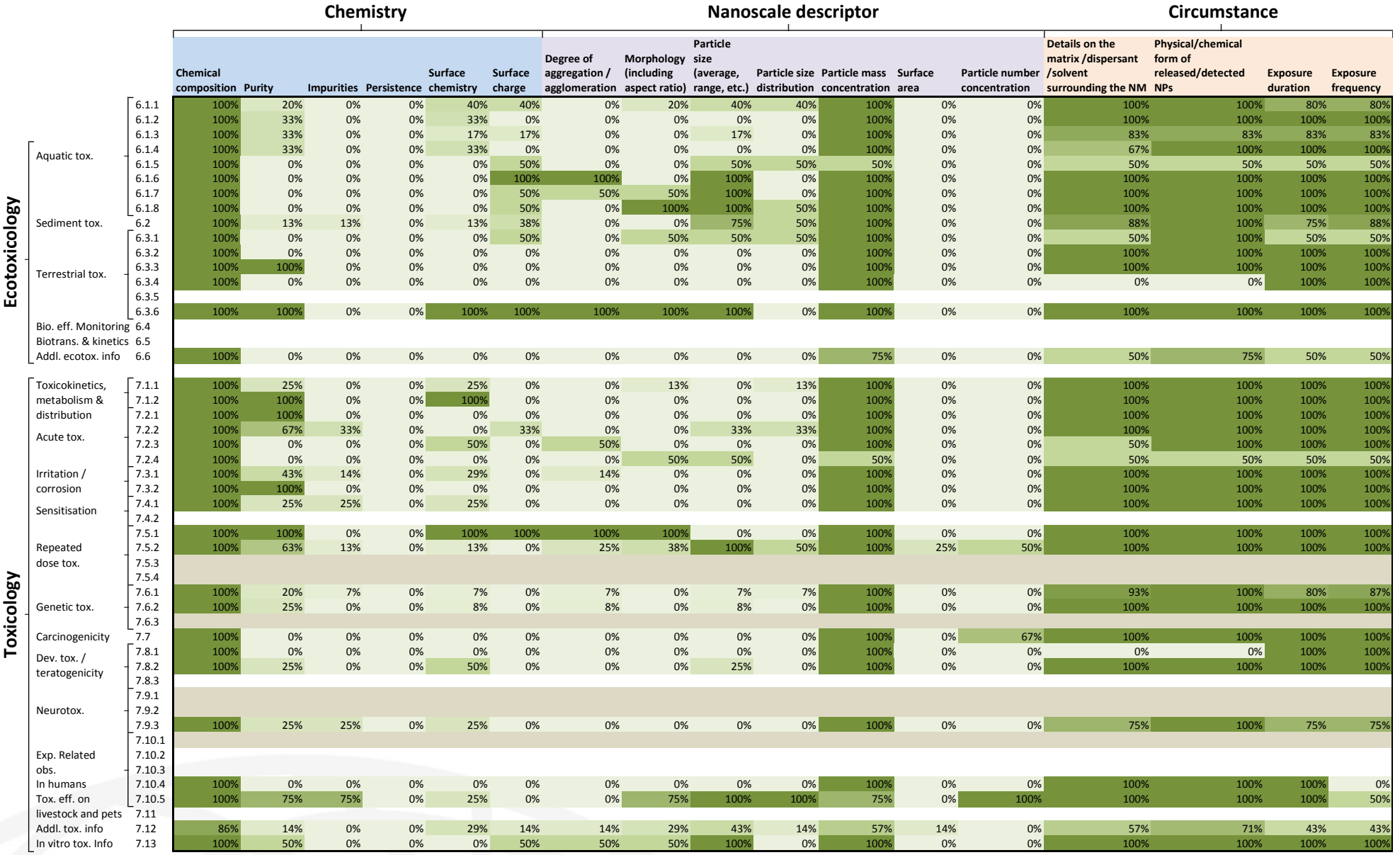

Figure 19. The overall data completeness of all the ESRs for each characteristic in the refined analysis and the sub-sampling analysis.