Synthetic Report on Case studies

Report Title:	Synthetic Report on the case studies		
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Responsible Project Partner:	BAM	Contributing Project Partners:	TNO

Document data:	File name / Release:	EC4SafeNano_D4.4_Synthetic Report on Case Studies final 2019-11-26.docx		Release No.: 2
	Pages:	24	No. of annexes:	-
	Status:	Final	Dissemination level:	Public
Project title:	**EC4SafeNano**: European Centre for Risk Management and Safe Innovation in Nanomaterials & Nanotechnologies		Grant Agreement No.:	723623
WP title:	The demonstration – Testing the capability to deliver services and benchmark		Deliverable No:	D4.4
Date:	Due date:	April 30, 2019	Submission date:	November 26, 2019
Keywords:	Case studies			
Reviewed by:	Experts at the CS workshops		Review date:	November 20, 2019
	B. Caillard		Review date:	November 26, 2019
Approved by WP leader:	W. Unger		Approval date:	November 26, 2019
Approved by Coordinator:	V. Dulio		Approval date:	November 26, 2019

Berlin, November 2019

 The research leading to these results has received funding from the European Union's Horizon 2020 Research and Innovation Programme, under the Grant Agreement No 723623.

Release History

Release No.	Date	Change
1	11.10.2019	Initial version
2	20.11.2019	Update based on feedback from experts at the CS workshops

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EC4SafeNano Project

The European Centre for Risk Management and Safe Innovation in Nanomaterials and Nanotechnologies, EC4SafeNano, is a 2016-2019 Coordination and Support Action, funded by the European Commission. EC4SafeNano is coordinated by INERIS, and operated together by major European risk institutes with the support of numerous associated partners, gathering all stakeholders involved in Nanomaterials and Nanotechnologies (regulators, industry, society, research, service providers...).

A central challenge to ensure the sustainable production and use of nanotechnologies is to understand and effectively control the risks along the industrial innovation value chain. Knowledge about nanotechnology processes and nanosafety issues (hazards, fate, risk...) is growing rapidly but the effective use of this knowledge for risk management by market actors is lagging behind.

EC4SafeNano will promote a harmonized vision of expertise in risk assessment and management for the public and private sectors to enable the safe development and commercialization of nanotechnology. The main objective of EC4SafeNano is to design harmonized services in risk assessment and management and a sustainable structure to deliver these services. For that, the project will gather stakeholder needs and expertise resources. It will demonstrate the efficiency of the proposed solution on case studies.

Executive Summary

Nanomaterials are a relatively new class of materials possessing various novel properties and for which a regulatory framework still has to be established by regulators. This is accompanied by the development of grouping strategies and structure-activity relationships, which requires datasets of well characterized nanomaterials. Especially the surface chemical characterization of nanoparticles is crucial, since the surface properties largely determine their biological and environmental fate. How the surface chemistry changes under relevant ageing conditions is of particular interest, because exposure of the environment will normally occur not to the pristine material but to a nanoform that underwent some kind of transformation. This is also relevant for their use in workplace atmosphere where their growing production and use can result in an increasing number of workers exposed to transformed nano-objects and their aggregates and agglomerates (NOAA), especially in the scarcity of appropriate risk management measures.

Based on the EC4SafeNano Task 4.1 survey Question 2 (Rank 2 with 353/475 points): "How can the surface chemistry and reactivity of engineered nanoparticles be determined and how can one take into account the behavior and transformation in relevant media (workplace atmosphere, waste, water…) throughout their life cycle? (case study on e.g. TiO2 or SiO2)" it was decided to launch a case study addressing this issue.

In this case study the surface chemical transformations upon ageing of a representative set of titanium dioxide nanoparticles has been investigated. The materials have been analyzed by various analytical techniques in a cooperation among five EC4SafeNano project partners. By using time-of-flight secondary ion mass spectrometry (ToF-SIMS) in combination with principal component analysis (PCA) it was possible to identify even subtle changes in the surface chemistry of the investigated materials.

The results of the Case Study will be streamlined into OECD activities. This organization recommended in the OECD Report on the Assessment of Biodurability of Nanomaterials and their Surface ligands (2017): "The surfaces of NMs may be the principal determinants of their durability under conditions of dissolution and/or biodegradation in biological and environmental fluids. Surfaces of NMs are modified through the use of surfactants, capping agents or attached ligands. it is therefore important to study the (bio)-durability of the surface coatings and ligands of NMs in biological and environmental media as well as their impact on the (bio)-durability of these core NMs."[1]

A parallel second case study was also launched which focused on the risk assessment of transformed NOAA in workplace atmosphere. The potential source of the exposure in such a scenario is the wear and tear of nanocoated working gloves which might release NOAA in the workplace atmosphere. Upon release, the workers who wear these gloves while operating may inhale, touch or ingest NOAA. This may have consequences on the health of these workers.

Based on a tier-0 investigation of the available data in the pertinent scientific literature, we found that a potential release of NOAA from these nanocoated gloves do not pose any adverse health risk to the workers and the workers are safe to use them, provided relevant risk management measures are in place. However, the scope of this investigation remains preliminary and tentative as the exact composition of the nanocoating and its amount on the gloves are presently unknown, and consequently the presence of other potentially hazardous substances cannot be excluded.

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1 Introduction

In work package 4 (WP4) "The demonstration – testing the capability to deliver services and benchmark", it is the aim to demonstrate the functionality of the EC4SafeNano Centre in terms of providing expertise and governance. Specific objectives are to demonstrate that the Centre with the consortium partners and associated partners *collectively* can provide technical expertise and science-based answers to questions raised by stakeholders. To this end, case studies covering multiple aspects in the field of safety of nanotechnology were launched to address and demonstrate the collective process for provision of high-quality answers to questions received from stakeholders from all targeted groups (EC, member states, industry, civil society and non-governmental organizations).

It was the main goal of task 4.1 (T4.1) to identify a list of relevant questions and prioritize these questions for the selection of the case studies which are planned and performed in T4.2 to demonstrate the operational capability of the EC4SafeNano hub and to evaluate its performance later in tasks T4.3 and T4.4. The list of questions originated from two sources: 1) already existing knowledge or available questions among partners of the consortium and the Joint Research Centre (JRC), and 2) the output from WP1, more specific questions raised in the survey on the inventory of needs, as expressed by different stakeholder groups. Thereafter, prioritization of questions was made with the help of different stakeholders. The result was a ranked list of questions from which those with the highest priority were selected for the case studies. The results of these two case studies are briefly presented in following sections.

2 Case Study on Transformations of Nano-TiO_2

2.1 Introduction

It is important to know how nanomaterials will transform due to ageing since it is unlikely that exposure to them will take place in their pristine state. Usually nanomaterials get aged upon their release or are surrounded by a matrix due to their incorporation into a product. It is therefore not sufficient to characterize the pristine materials with regard to their fate, hazard and environmental impact. This holds especially true for the surface chemistry of nanomaterials, since their surface characteristics usually determine their fate and biological interactions. Therefore, the complete physical-chemical surface characterization should be performed thoroughly also for aged nanomaterials following likely and realistic ageing scenarios. A nice overview about possible ageing scenarios of nano-enabled products throughout their life-cycle is given by Nowack *et al*.[24-25] The environmental fate of pristine nano-TiO_2 and aged paint containing nano-TiO_2 (UV exposure and abrasion) has furthermore been studied by Larue *et al*. and Nowack *et al*.[2,3] A general relevant ageing process is the adsorption of inorganic ions or natural organic matter (NOM) on the surface, which has recently been reviewed by Grassian *et al*. and Parak *et al*.[4,5] Stirring TiO_2 nanoparticles in moderately hard reconstituted water (MHRW) leads to a coverage of the surface by adsorbed carbonate as identified with ATR-FTIR.[6] In a similar manner the binding of the protein bovine serum albumin (BSA), citric acid and humic acid has been studied.[4,7] The adsorption of NOM onto aggregates of citrate capped TiO_2 nanoparticles has furthermore been investigated using a combination of SEM and ToF-SIMS by Szakal *et al*.[8]

For the selected materials of this case study, some prior investigations into their behavior upon ageing have to be considered. In a comprehensive study funded by the German Environmental Agency, the stability of the surface coatings of JRCNM62001a (former code: NM103) and JRCNM62002a (NM-104) has been investigated.[9] As described previously, those particles consist of a rutile TiO_2 core, an inorganic Al_2O_3 and different organic coatings. JRCNM62001a is equipped with the hydrophobic PDMS, whereas JRCNM62002a is equipped with the hydrophilic glycerol. The ageing of those materials was performed by stirring and ultrasonication in pure water and additionally under variation of several parameters, such as pH value, ionic strength (Ca^{2+}), and the dissolved organic content (DOC). The transformation of the nanoparticles has been investigated by the quantification of released coating material using LC-MS and ICP-OES and by identifying changes in the NP properties, such as zeta-potential, isoelectric point and size distribution. The aged nanoparticles have furthermore been investigated using SEM-EDX and ToF-SIMS. Based on the performed experiments it is concluded that most of the organic coating is released from the surface, whereas the Al_2O_3 layer stays intact even after severe stress conditions. This is corroborated by the fact that the aged nanoparticles do not lead to radical formation under UV irradiation as has been observed by EPR experiments. Furthermore, an influence of the ionic strength and the DOC content on the zeta potential was clearly demonstrated. It seems that especially DOC is of importance and leads to a (steric) stabilization of the nanomaterials and a negative zeta potential.[9]

A similar ageing study has been performed by Auffan *et al.* and Labille *et al.* for T-Lite SF (BASF) nanoparticles in water, that again consist of a TiO_2 core, an inorganic Al_2O_3 and an organic polydimethylsiloxane PDMS coating.[10,11] The aged particles have been studied using a combination of ATR-FTIR Spectroscopy and NMR spectroscopy (^{13}C, ^{29}Si, ^{27}Al). With those techniques it was observed that the organic coating of the particles is mostly removed upon ageing in water, whereas the Al_2O_3 layer seems to be stable.[10] Those nanoparticles are usually used as UV filters in sunscreens, and therefore also the ageing of those composite materials has been studied by the same group.[12] Furthermore, nanoparticles aged according to their protocol have been

tested with regard to their toxicity for aquatic species (daphnia magna)[13] and plants.[14] Although the inorganic coating, which is responsible to suppress the formation reactive oxygen species, seems to be stable in water, Virkutyte *et al.* report that this coating gets depleted by dispersion of similar particles in chlorine-containing swimming pool water.[15]

Therefore, the ageing in simulated swimming pool water was chosen as one representative medium to investigate the ageing of the selected nanomaterials. Additionally, the ageing upon climatic weathering was chosen, since no studies are known to date how the long-term exposure to UV-light and the variation of temperature and humidity affects the surface chemistry of nanomaterials. This investigation is based on the existing tests for the ageing of paints using climate chambers[3] and BAM has experience in these kind of ageing tests for the weathering of polymeric materials.[16,17]

2.2 Design of the Case Study

2.2.1 Nanoparticulate TiO_2 samples used for testing

To investigate the surface chemical transformations of representative nano-TiO_2 samples after ageing in relevant media, a set of samples had to be selected. Nano-TiO_2 belongs to the 11 nanomaterials selected by the OECD to be tested for 59 endpoints.[18, 19] Representative test materials with different sizes, crystalline phases and surface coatings are provided by the Joint-Research Center (JRC) of the European Commission and thereby assure the comparability among studies.[20] The JRC Repository offers 6 TiO2 materials of which only 5 are true nanomaterials following the EC recommendation for a nanomaterial with at least one dimension below 100 nm. Those materials have been extensively studied with regard to their physical-chemical properties,[21,22] their environmental fate,[23,24] and toxicology.[25] Although most OECD endpoints have already been well investigated, the characterization of the surface chemistry and the identification of degradation products upon ageing have not yet been addressed adequately.[26] They have therefore been chosen as materials for this case study. Additionally the material BAM-P110 was selected, which is a reference material certified for the BET specific surface area provided by BAM.[27] Since the initial list of materials contains three uncoated anatase samples, it was decided that only one of those materials needs to be investigated within this case study, to reduce the number of samples and because similar behavior of those materials can be expected. Due to the availability of higher quantities for BAM-P110 and more gaps in the existing data matrix for this material, it was selected to represent uncoated anatase NPs. An overview about the four selected nano-TiO_2 materials is given in Table 1.

Table 1: List of nano-TiO_2 NPs used in the Case Study

Sample	Name	Producer	Production Process	Crystalline Phase	Primary Particle Size	Surface Coating	Amount of Sample
JRCNM62001a	UV TITAN M262	Sachtleben	Sulfate	rutile	21 nm	Al_2O_3,PDMS	6 g
JRCNM62002a	UV TITAN M212	Sachtleben	Sulfate	rutile	21 nm	Al_2O_3, glycerol	6 g
JRCNM01005a	P25	Evonik	Chloride	anatase/ rutile	15-24 nm	-	6 g
BAM-P110	NO-0058-HP	IOLITEC	n.g.	anatase	22 nm	-	18 g

As can be seen from Table 1, all materials possess a different composition. The materials JRCNM62001a and JRCNM62002a are composed of a TiO_2 (rutile) core and an inorganic Al_2O_3 coating, however, they differ in their additional organic coating. For JRCNM62001a, the polymer polydimethylsiloxane (PDMS) is present on the surface, whereas JRCNM62002a contains a glycerol coating. These materials represent TiO_2 nanoparticles as they are usually applied as UV-filters in cosmetic products. The other two samples represent uncoated TiO_2 samples, which differ with regard to their crystalline phases. Whereas JRCNM01005a is composed of a

mixture of anatase and rutile, the sample BAM-P110 consists of pure anatase. These kinds of materials usually find applications in the fields of catalysis, pigments and coatings

2.2.2 *Ageing of nano-TiO_2: Selection of the Ageing Conditions*

It is important to know how nanomaterials will transform due to ageing since it is unlikely that exposure to them will take place in their pristine state. Usually nanomaterials get aged upon their release or are surrounded by a matrix due to their incorporation into a product. It is therefore not sufficient to characterize the pristine materials with regard to their fate, hazard and environmental impact. This holds especially true for the surface chemistry of nanomaterials, since their surface characteristics usually determine their fate and biological interactions. Therefore, the complete physical-chemical surface characterization should be performed thoroughly also for aged nanomaterials following likely and realistic ageing scenarios. A nice overview about possible ageing scenarios of nano-enabled products throughout their life-cycle is given by Nowack et al.[28-29] The environmental fate of pristine nano-TiO_2 and aged paint containing nano-TiO_2 (UV exposure and abrasion) has furthermore been studied by Larue et al. and Nowack et al.[30,31] A general relevant ageing process is the adsorption of inorganic ions or natural organic matter (NOM) on the surface, which has recently been reviewed by Grassian et al. and Parak et al.[32,33] Stirring TiO_2 nanoparticles in moderately hard reconstituted water (MHRW) leads to a coverage of the surface by adsorbed carbonate as identified with ATR-FTIR.[34] In a similar manner the binding of the protein bovine serum albumin (BSA), citric acid and humic acid has been studied.[32,35] The adsorption of NOM onto aggregates of citrate capped TiO_2 nanoparticles has furthermore been investigated using a combination of SEM and ToF-SIMS by Szakal et al.[36]

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Therefore, the ageing in simulated swimming pool water was chosen as one representative medium to investigate the ageing of the selected nanomaterials. Additionally, the ageing upon climatic weathering was chosen, since no studies are known to date how the long-term exposure to UV-light and the variation of temperature and humidity affects the surface chemistry of nanomaterials. This investigation is based on the

existing tests for the ageing of paints using climate chambers [31] and BAM has experience in these kind of ageing tests for the weathering of polymeric materials.[44,45]

2.2.3 *Climatic Weathering under UV Irradiation (Ageing Procedure 1)*

Artificial UV-irradiation tests were carried out in a fluorescent UV lamp device of the type Global UV Test 200 (Weiss Umwelttechnik GmbH, Reiskirchen, Germany), based on ISO 4892-3.[46] The spectral distribution – characterized by UVA-340nm lamps and spectrally neutral filtering using a PVDF-membrane that separates lamps and samples. As this spectral distribution is limited to UV and near VIS, radiation heating can be neglected ($T_{Surface}$-$T_{Chamber}$ < 2 K). The device allows temperature and humidity to be controlled independently.

While normally samples are irradiated vertically mounted on the rear wall of the sample compartment in this case the powder samples had to be orientated horizontally. The irradiation was directed to them via a 45° mirror, as can be seen in Figure 4. This mirror reduces the UV irradiance hitting the vertical sample plane by about one third due to the geometrical reduction of the irradiating area out of the fluorescent lamps in the door of the device. The samples were placed in glass crystallizing dishes covered with fused quartz plates that are transparent for the applied UV light (GE 124 fused quartz, Plano GmbH).

The used ageing cycle is characterized by the conditions given in Table 2. The cycle was repeated for a total duration of 1000 h (41.6 d). The powders have been stirred with a spatula every week to assure a consistent exposure. The UV radiant exposure received by the samples for this period was about 100 MJ/m2 which compares to an averaged maximal annual outdoor exposed sample in Central Europe of about 180 MJ/m2.

Table 2: Parameters of the UV-ageing scenario.

Duration / h	Temperature / °C	% Relative Humidity
6	60	38
2	8	76
Continuous UV irradiation with an UV-irradiance of 29 W/m^2 in the sample plane.		

2.2.4 *Dispersion of TiO_2 NPs in Swimming Pool Water (Ageing Procedure 2)*

The nanoparticles were dispersed in simulated swimming pool water (SPW) according to a protocol adapted from Virkutyte et al.[43] Therefore, each TiO_2 material is dispersed in swimming pool water (ingredients are listed in Table 3) with 2 parts per million (ppm) of free chlorine, since the chlorine level should stay between 1.0 and 3.0 ppm to maintain a healthy pool. Clorox bleach (The Clorox Company, USA) containing 5.8% as Cl_2 was used as the chlorine source. Sodium hydrogen sulfate ($NaHSO_4$) was used to maintain pH at 6. Despite the actual pH in swimming pools is 7.8, slightly acidic conditions were adopted to maximize the amount of Free Available Chlorine (FAC) available in the SPW to enhance reproducibility. All the experiments were performed in the presence of fluorescent light and were not covered.

The particles were dispersed in 50 ml of SPW at a concentration of 2g/L and let to age at room temperature for 45 min while being rotated continuously using a tube rotator set at 15 rpm speed. The samples were then washed through centrifugation with 7850 rpm at 4 °C for 2 hours. After the removal of the supernatant, 20 ml of DIW was added to the NP pellets and the solutions were vigorously mixed using a vortex to break the pellet and separates the particles. The obtained solution was then centrifuged for 30 min at 7850 rpm and the supernatant was discharged. This was repeated three times and after the third wash and addition of 20 ml of DIW to the pellet, the obtained samples were vortexed, stored in the freezer at -80 °C for 24 hours and finally freeze dried for about three to four days to obtain the aged nanomaterials as a powder.

Table 3: Swimming Pool Water (SPW) Composition (pH 6)

Chemicals	Quantity
DI water (L)	2
$CaSO_4 \bullet 2\ H_2O$ (g)	0.765
$NaHSO_4$ (g)	0.24
$NaHCO_3$ (g)	0.30
5.8 % NaClO (mL)	1110 or (2 ppm)

2.2.5 Assembly of a Task Force and Survey on Analytical Methods Used

The work of a task force consisting of BAM, RISE, UoB, DEMOKRITOS and PLUS was initiated during a telephone conference 12. June 2018 and is coordinated by BAM. In Table 4 an overview about the selected measurement techniques to characterize the nanomaterials, the responsible partners and the corresponding available SOPs, collected in WP2, is given. Within the OECD Guidance Manual for the Testing of Manufactured Nanomaterials, the following techniques are recommended to study the surface chemistry: EELS, XPS, AES, TGA and TG-MS.[18] The pristine materials have already been studied to some extent, however, still rarely with high-end surface analytical techniques.[47] Furthermore, few investigations on the surface chemistry of aged nanoparticles have been performed yet, which therefore has been identified as a general future need.[48-50]

Table 4: Overview on used phys-chem characterization techniques, the responsible partners and available SOPs

Descriptor	Technique	Partner	Potential Backup	SOPs (collected in WP2)
Particle Size	TEM / SEM	DEMOKRITOS	BAM / RISE	NanoReg D.10, nanOxiMet, NanoValid
	DLS	UoB	PLUS / RISE	NanoReg D2.08, nanOxiMet, NanoValid, JRC Report, UBA Report
	NTA	PLUS		
Atomic Composition	ED-XRF	DEMOKRITOS		NanoReg D2.04
	EDX	DEMOKRITOS	BAM / RISE	NanoReg D2.04
Surface Area	BET	PLUS	RISE / UoB / DEMOKRITOS	NanoReg D2.11, nanOxiMet, NanoValid
Surface Charge	Zeta Potential	UoB	PLUS / RISE	NanoReg D2.09, NanoValid, JRC Report, UBA Report
Surface Coating Quantification	Extract. / GC-MS	UoB		NanoReg D2.04
	TGA	UoB	RISE	NanoReg D2.04
Surface Chemistry	XPS	RISE	BAM / UoB	NanoValid
	ATR-FTIR	RISE	DEMOKRITOS	(UBA Report)
	ToF-SIMS	BAM		(ACENano), (UBA Report)

2.3 Results and Conclusions

This case study was aimed at identifying and characterizing transformations occurring in the surface chemistry of nanomaterials due to the ageing under relevant ageing conditions. To that end, a set of four well-characterized TiO_2 nanomaterials with diverse surface chemistry was selected and either exposed to long-term UV irradiation (UV-ageing) or swimming pool water (SPW-ageing). With the help of a multi technique approach it was possible to characterize the nanomaterials with regard to their different surface chemical properties and to confirm the presence of their specified coatings by comparison of the results for their pristine state with reported observations. Consequently, the same techniques have been used to investigate the transformations that result from the different ageing treatments.

The basic characterization of the pristine nanomaterials regarding their size, shape, crystallinity and bulk chemical composition was achieved using transmission electron microscopy (TEM) together with energy dispersive X-ray spectroscopy (EDS) and energy dispersive X-ray fluorescence (ED-XRF). A combination of more surface sensitive methods, such as X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to analyze the presence and chemical nature of the inorganic and organic layers present on the surface. Finally, the aggregation behavior of the nanomaterials has been analyzed using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and their BET surface area.

A general trend that was observed for the UV-aged samples is the decrease of organic material on the nanomaterial surface. Whereas this effect is relatively small for the polymeric PDMS coating of sample JRCNM62001a, it is much stronger for the small molecule glycerol of sample JRCNM62002a and the adventitious carbon species that are present on the formally uncoated materials JRCNM01005a and BAM-P110. Additionally, the partial removal of anionic impurities can be observed, which originate from the respective production processes of the nanomaterials. For JRCNM01005a this is Cl^- originating from the chloride process, and for JRCNM62001a and JRCNM62002a SO_4^{2-} from the sulfate process. They probably get washed out due to the high humidity during the UV-weathering experiment. The inorganic Al_2O_3 coating seems to be completely unaffected by the UV-treatment confirming that the protective function of this coating for TiO_2 UV-filters is stable and effective. No clear effect was observed on the surface area and the aggregation behavior as it was analyzed with BET and DLS. For the samples that were aged in artificial swimming pool water (SPW), a complete removal of the organic coatings PDMS and glycerol was observed. At the same time, an increase of the amount of adventitious carbon species was observed for all nanomaterials. The dispersion in simulated swimming pool water, containing many additives, seems to lead to an increased adsorption of organic matter. Furthermore, it can be observed that the high levels of anionic impurities get washed out of the samples. Instead all SPW-aged samples exhibit similar levels of Cl^- and Ca^{2+} as residues from the swimming pool water. Interestingly, a second effect of the SPW treatment was observed for the sample BAM-P110. A drastically increased aggregation was observed solely for this sample by the techniques DLS, NTA, and BET. Remarkably, all other samples do not show a significant change in aggregate size.

The multi technique approach furthermore allows us to evaluate the capabilities and limitations of the applied methods regarding their suitability to address the endpoint surface chemistry and their sensitivity to identify even small surface chemical transformations. An overview about all methods and their respective findings is given in Table.

Transmission electron microscopy (TEM) is the only technique that can simultaneously confirm particle size, shape, crystallinity, and together with Energy-dispersive X-ray spectroscopy (EDS) the bulk chemical composition. It is therefore able to deliver multiple crucial nanoparticle descriptors (endpoints) in only one measurement. However, its capability to address the endpoint surface chemistry is rather limited. In this work TEM was mainly used to determine the presence of an amorphous inorganic layer. Although thin layers were detected on the surfaces that appeared to be amorphous, no unambiguous conclusion could be made since the observed structures could alternatively result from bending of the crystalline planes or of crystalline structures that are out of focus. Consequently, it was impossible to clearly identify transformations of that inorganic layer upon ageing. Possibly with the investment of more time and resources such uncertainties might be overcome. Furthermore, additional information could be obtained about crystalline defects or the presence of multiple crystalline domains. It even could be tried to visualize organic coatings by optimization

of sample preparation procedures that do not dissolve the organic coatings. Finally, a statistical analysis of a large number of particles could be performed to obtain a quantitative size distribution instead of an estimated primary particle size as it was done for this study.

With energy dispersive X-ray fluorescence spectroscopy (ED-XRF) it is possible to analyze the bulk chemical composition comparable to the information obtained by EDS. This is an attractive option in case no TEM/EDS is available or if a cheaper solution is required. Within this study, the presence of the elements Ti, Al, Si and S has been analyzed in a qualitatively manner. With more resources, also a quantification could be established. However, this technique has the general disadvantage that it is difficult to detect lighter elements such as C, N and O due to the strong dependence of the X-ray yields on the atomic number. Furthermore, ED-XRF only provides a bulk chemical analysis and therefore gives very limited insight into the surface chemical composition. Although it was possible to monitor the depletion of PDMS coating for JRCNM62001a based on the Si signal, it is impossible to know if the polymer is present on the nanoparticle surface or elsewhere in the sample.

The presence of organic material on the surface of the nanomaterials can generally be analyzed using thermogravimetric analysis (TGA). However, due the presence of physiosorbed water and volatile organic residues from the production process on the surface, the quantification of the weight loss might be difficult if an extensive pre-heating is avoided to prevent additional ageing of the materials as in this case study. To distinguish between the endothermic evaporation of solvents and exothermic decomposition of organic molecules, differential thermal analysis (DTA) can be applied. For the investigated materials, no clear trend for the mass loss during TGA was observed for pristine and aged samples. The total weight loss is in the range of 1-4% in all cases and it seems that this method is not sensitive enough to identify small changes in the surface chemistry composition for this set of nanomaterials. The coupling of TGA with mass spectrometry could give additional information about the released molecules, however, was not applied in this study. Therefore, simple TGA seems to be suitable only as a first screening method to get a rough information about the presence of the volatile and combustible matter that is present in the sample.

More information about the molecular structure of the organic matter that is present in the samples can be derived from Fourier-transformed infrared (FT-IR) spectroscopy. This technique allows to identify chemical functional groups based on the excitation of characteristic bond vibrations. It was possible to identify the presence PDMS for the sample JRCNM62001a, and the presence of less specific organic molecules for JRCNM62002a, whereas for the uncoated materials only vibrations from the TiO_2 lattice and adsorbed water molecules have been observed. Furthermore, it was possible to qualitatively observe that the amount of the respective organic molecules decreases upon ageing. Although the relatively surface sensitive attenuated total reflectance (ATR) technique was used in this study, the information depth of the technique is with ca. 1 µm still drastically larger than the particle size of the analyzed nanomaterials. Chemical information with higher surface sensitivity can be obtained using X-ray photoelectron spectroscopy (XPS), which provides an information depth of 2-10 nm. However, for the analyzed set of samples with particle sizes around 20 nm still significant bulk contribution to the XPS spectra have to expected. XPS allows to quantitatively analyze the surface elemental composition and thus also to quantify the previously described transformations. It was possible to show that all pristine nanomaterials have similar amounts of organic matter adsorbed on their surface, even if no coating was specified. By analyzing the C 1s spectra, it was furthermore possible to investigate the chemical nature of the organic molecules and thereby to distinguish and to characterize the different organic surface layers. This was especially useful for the identification of glycerol, which was difficult to detect by all other methods. The only technique in this study that has an information depth which is much lower than the primary particle size of the nanomaterials is time-of-flight secondary ion mass spectrometry (ToF-SIMS). With an information depth of less than 1 nm, this technique allows to really probe the outmost layer of the nanomaterials. Although the obtained data is non-quantitative, even small surface chemical transformations can be identified with the help of principal component analysis. The identification of characteristic fragment ions, furthermore gives the opportunity to gain insights into the molecular structure of the surface layers that are not accessible by any other method.

Table 5: Overview about the capabilities of the applied characterization methods.

Technique	Descriptors	Specific Information for the Pristine NMs	Observed Surface Chemical Transformation	Limitations
TEM	- Primary Particle Size - Crystalline Phase - Identification of crystalline and amorphous domains - Morphology - (Aggregation)	- Primary particles sizes around 20 nm - Irregular shapes - Distinction between rutile and anatase - Presence of amorphous surface layers	increased aggregation for SPW-aged BAM-P110 (qualitative)	- Crystalline domains might appear as amorphous due to bending or bad focusing - Sample preparation requires a dispersion of NMs
EDS	- Bulk chemical composition	Presence of Al for Al_2O_3 coated materials was confirmed	None	- not surface sensitive - ensemble method - not quantitative
ED-XRF	- Bulk chemical composition	Presence of Al and Si for the respectively coated materials was confirmed	Depletion of the Si content for aged JRCNM62001a	- not surface sensitive - not quantitative - low X-ray yields for light elements (Z<11) such as C, O, N
BET	- Surface area - Porosity	- Similar surface areas for all NMs - Match reported values	Drastic decrease of surface area for SPW-aged BAM-P110	- requires prior activation at 100°C and vacuum
DLS	- Hydrodynamic diameter - Particle size distribution - Aggregation	- Mainly small aggregates with hydrodyn. diameters of 80-100 nm	Drastic increase of aggregate size for SPW-aged BAM-P110	- requires a dispersion of NMs - dominated by larger aggregates due to scattering
ζ-potential	- Surface charge - Dispersion stability	- similar ζ-potentials between +20 and +30 mV	Decrease of the ζ-potential for SPW-aged BAM-P110 below 20 mV	- requires a dispersion of NMs
TGA	- Volatile fraction - combustion of organic material	Weight loss for all NMs in the range of 1-4 %	No clear trends	- requires activation
ATR FT-IR	- Identification of functional groups	- Specific absorption bands for PDMS coating - Less specific bands for glycerol coating	Decrease of the absorption bands corresponding to the organic coatings	- not surface sensitive (information depth > 1µm) - no detection of adventitious carbon
XPS	- Quantitative elemental composition at the surface (2-10 nm) - Information about chemical states	- All coating materials can be quantified - All NMs show a similar amount of carbon on their surface - The different organic layers can be distinguished due to different chemical binding environment	- decrease of the carbon content - removal of glycerol as observed by reduction of C-O component in C 1s spectra	- Hydrogen is not detectable - Surface sensitivity (2-10 nm) not sufficient for NMs in the size range of the information depth
ToF-SIMS	- Chemical composition of outmost layers (< 1 nm) - multivariate data analysis	- all samples can be clearly distinguished based on characteristic fragment ions	- removal of organic compounds - removal of impurities such as Cl^- and SO_4^{2-}	Semi-quantitative

Finally, the techniques dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and surface area determination by N_2 sorption experiments (BET) provide information about the aggregation and agglomeration behavior of the nanoparticles. With all methods, a strong aggregation of the sample BAM-P110 after ageing in swimming pool water (SPW) and no significant changes in all other cases were observed. The measurement of the ζ-potential shows that for this strongly aggregating sample, the electrostatic stabilization is lowest explaining the observed behavior. For the given set of samples, BET has the advantage over DLS and NTA that it is measured using the nanomaterial as a powder and therefore does not require an additional dispersion step which might cause additional ageing of the particles. For powder samples, thus BET is advantageous, whereas DLS is the method of choice for already dispersed samples. Unfortunately, these methods do not provide an insight into the transformations of the surface chemistry on a molecular level that might lead to the observed effects. It was furthermore not possible to explain the unique aggregation behavior of SPW-aged BAM-P110 with the help of the other techniques.

By comparing all methods, it becomes obvious that each method addresses different aspects of the complex endpoint surface chemistry. To obtain a comprehensive picture, therefore, it seems insufficient to concentrate on a single analysis technique. Depending on the question of interest, usually a combination of multiple complementary techniques is required to obtain a satisfactory answer. If a completely unknown nanomaterial has to be characterized and the presence and chemical nature of a surface coating has to be investigated for the first time, significantly more effort has to be invested than for the investigation of a new batch of an already well-characterized nanomaterial. It has to be noted that most of the techniques are rather bulk analytical methods when applied to nanomaterials and only ToF-SIMS and XPS can be considered truly surface sensitive with information depths < 10 nm. Only if the transformations of already well-characterized nanomaterials are of interest, methods such as TGA, FT-IR and ED-XRF can be used to investigate potential changes assuming that the principal distribution of layers in a structure does not change. However, it might be impossible to prove that the identified species is indeed present on the surface and not elsewhere in the sample.

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2.5 Cost and Time Analysis

Table 1: Overview about invested time for each part of the analysis

Activity	Institution	Time invested
Case Study Management and Reporting	BAM	7 Person Months
UV Ageing	BAM	5h labor (excluding 1000 h exposure time)
Dispersion Ageing	UoB	32 h
ToF-SIMS Measurement	BAM	36 h (3 h / sample)
ToF-SIMS Analysis with PCA	BAM	24 h (2 h / sample)
DLS and ζ-Potential Analysis	PLUS	8 h
UV/Vis Analysis	UoB	8 h
NTA Analysis	PLUS	8 h
BET Analysis	PLUS	8 h
TGA Analysis	PLUS	8 h
XPS Analysis	RISE	confidential
FT-IR Analysis	RISE	confidential
HR-TEM Analysis	DEMOKRITOS	12 h (1h /sample)
ED-XRF Analysis	DEMOKRITOS	22 h (2 h / sample)

Total allocated time: ~1400 h
Total cost: ~85.000€

2.6 Identified Issues regarding the technical content of the case study

During the case study some technical issues have been encountered, and the following Table 2 provides an overview about the individual problems and the respective solutions that have been found.

Table 2: Overview about encountered technical issues working on the case study

Method	Partner	Issue	Solution
Ageing	UoB	A broken vial during freeze drying lead to a loss and contamination of samples. Therefore, the ageing procedure had to be repeated.	Each sample should be freeze dried individually to avoid cross contamination.
DLS	PLUS, UoB	A harmonization of dispersion protocols was difficult since some SOPs require specific instrumentation that was not available in all labs.	Agreement on a dispersion protocol that can be followed in both laboratories based on the NANOGENOTOX protocol.[69]
XPS	BAM, RISE	The partners follow different approaches for data analysis. RISE prefers to analyze the samples as if nothing was known about them, BAM prefers to use known information from different techniques to interpret the data.	
BET, TGA	PLUS	Both methods usually require a heating step prior to the analysis. This had to be avoided or minimized to prevent additional ageing of the nanomaterials.	For BET, a relatively low degassing temperature of 100 °C was chosen. No pre-treatment was used for the TGA analysis.

2.7 Identified Issues Regarding the Governance of EC4SafeNano

Although the case study was intended to test the operational functioning of the centre, this was difficult to achieve due to several reasons. During the time of the case study selection, neither the business model (WP5) nor the catalogue of services (WP3) was yet developed and thus it was difficult to predict the future activities of the center. Due to the lack of a real-life customer, the case studies were selected based on a priority ranking of questions by the stakeholders of the centre as it is described in D4.1, and an alignment of the results with the fit and gap analysis, which was performed in WP3. Furthermore, no organizational structures and procedures have been in place that could have been tested. However, it is important for future activities of the centre to have clear procedures in place to assign project leaders and task force members for specific customer requests. Additionally, for each contact person there should always be a backup in place to ensure a smooth transition in the case of personnel fluctuation as it was observed to happen in a longer project.

For the sake of this case study, the management task was conducted by BAM as the WP4 leader. The task force was assembled by analyzing the competences of each project partner that were registered in the WP1 survey. Unfortunately, some project partners (NRCWE, VITO, TECNALIA) with adequate competences to contribute to this case study have been unavailable due to budget limitations. A general problem was that no specific budget was foreseen in the DoW and not all project partners had resources available in WP4. Therefore, the contribution of each partner has to be seen as an investment into the future center and the inclusion of associated partners was not possible.

3 Case Study on Occupational risk assessment of nanocoated gloves

3.1 Introduction

Commercial nanocoatings for industrial gloves are claimed by their manufacturers to impart an extremely strong and long-lasting layer on the gloves surface which protects their fabric against oil, moisture, bacteria and eventual abrasion. However, it is unknown whether the on-site workers who wear these nanocoated gloves are safe against the exposure to nano-objects and their aggregates and agglomerates (NOAA) which may get released due to wear and tear of the nanocoating over time. In the present study, a risk assessment was carried out which aimed to assess any potential risk that these nanocoated gloves pose towards the workers.

In occupational settings, three principal routes of exposure are possible: inhalation, dermal and oral. The inhalation route of exposure occurs when the airborne NOAA enters the respiratory tract via breathing. NOAA can then deposit on the various parts of the upper or lower respiratory system and translocate to other organs (e.g. blood, adjacent lymph nodes, brain). Dermal exposure becomes applicable when the NOAA comes in contact with the skin of a worker, penetrate the dermis (skin barrier) and be eventually absorbed systemically. The oral route of exposure results either from contaminated skin (dermal) by hand-mouth contact or from inhaled particles that are cleared by the mucociliary escalator from the lungs that end up in the oropharynx and are subsequently swallowed. All three exposure routes were covered in this evaluation, which was divided in three sections: hazard in which the intrinsic toxic properties of the nanocoating were considered, exposure in which exposure estimates were presented, and risk in which hazard and exposure were combined to assess the possible health risks of occupational exposure.

3.2 Summary of the findings

Based on a tier-0 investigation of the available data in the pertinent scientific literature (details provided in D4.3), we found that the potential release amount of NOAA from the nanocoated gloves was not enough to pose any adverse health risk to the workers and the workers are safe to use them, provided relevant risk management measures are in place. However, the scope of this investigation remains preliminary and tentative as the exact composition of the nanocoating and its amount on the gloves were unknown to the investigators, and consequently the presence of other potentially hazardous substances cannot be excluded.

3.3 Organization of the work

There are no existing standard methods, regulations or guidelines for the risk assessment of the nanocoated gloves in question which could be referred during the case study. Consequently, TNO sought the assistance from EC4SafeNano consortium by presenting the case study to center partners. A discussion session was held amongst the partners and a task force of volunteering partners, who have proven expertise in nanomaterials characterization and risk assessment, was assembled. TNO agreed with the task force members to prepare a protocol detailing the risk assessment methodology and share it with task force members to have their agreement. On the basis of mutually agreed protocol, TNO carried out the risk assessment and provided a first draft of the assessment report to the task force members. Subsequently, the task force members reviewed the report and provided their feedbacks to TNO. TNO incorporated the respective feedbacks in the final risk assessment report and included its full text version in D4.3.

3.4 Identified issues and relevant recommendations

3.4.1 Operational functioning

The present case study provides an example for an expert consultancy service that can be delivered by EC4SafeNano in future. The detailed description and methodology of the case study, as provided in D4.3, can provide the basis for the quality criteria that can be set by the center as harmonized ways of working. Nevertheless, when this case study was carried out, there were no organizational structures and procedures in place which could have been tested for the operational functioning of the center. This is, in particular, relevant to the assembly of the task force for this case study which was done without following any specific procedure but on a volunteering basis. It is, therefore, important for future activities of the center to have clear procedures in place to assign task force members for specific customer requests.

3.4.2 Harmonization

Before starting the risk assessment process, TNO ensured that the assessment protocol was agreeable to all task force members, thus eliminating any eventual harmonization related issues. Thus, this practice of preparing a study protocol which shall be agreeable to all contributing task force members, should be implemented in future for the EC4SafeNano services for which there is no existing standard or harmonized protocol.

3.4.3 Budget

The amount of budget allocated to TNO and other contributing task force members for this case study was found to be sufficient to carry out the assessment and prepare the final report.

3.4.4 Confidentiality

Although not implemented during the execution of this case study, EC4SafeNano should have a standard Non-disclosure agreement (NDA) in place which allows a restricted knowledge share of confidential material, knowledge, or information that is relevant to future case studies. This way, a potential client of the center can remain assured that any information, deemed as sensitive or confidential by the client, shall remain restricted to or by third party (i.e. someone other than client and EC4SafeNano) if the client deems so.

3.5 Added value of EC4SafeNano

The high level of expertise of the contributing task force members in nanomaterials related risk assessment ensures that EC4SafeNano is capable of dealing with higher tier full-fledged nanomaterials risk assessments for future projects. Particularly in the case of the present case study, the capability of the task force members to be able to assess the potential risks without any technical issue with only limited available information on product and its use conditions further supports their expertise. The combined efforts of relevant experts from different EU countries during the execution of the case study also ensures that the delivered results are of high quality, EU-wide accepted and harmonized to a large extent.

Conclusion from TNO case study (on EC4SafeNano performance)

The present case study provides an ideal example of type of service demands which EC4SafeNano can receive from its potential clients, particularly from the industrial sector. Thanks to the collective expertise of its consortium partners, inclusion of EC4SafeNano in the case study added a comprehensive approach to the investigation. During the execution of the case study, we encountered some organization related issues on which we have provided some insights and pertinent recommendations that should be implemented during the development of the center to avoid those issues in the future. These recommendations should assist Ec4SafeNano in the longer term to have the organizational structure in place to meet the demands of the different stakeholders, including regulatory authorities on governance decisions on the safety of the nanomaterials and nanotechnologies.

4 EC4SafeNano workshops on Case Studies

4.1 Overarching issues and conclusions

Both reports of the case studies were presented to external and EC4SafeNano experts in dedicated workshops in order to discuss the performance of the center shown during the case studies. Technical and governance issues were addressed in questionnaires given to those experts and their feedback was carefully considered by the center. This activity was planned as tasks 4.3 and 4.4 in the DoW of the project.

The first case study was set up to test the capability and performance of the EC4SafeNano center to analyze transitions of nanomaterials by methods of phys-chem characterization. The second case study provides an example for an expert consultancy service that can be delivered by EC4SafeNano in future. In addition, the described case study and methodology can provide the basis for the quality criteria that can be set by the center as harmonized ways of working (i.e. following the procedure as used in this case study) for the services for which there are no existing standards,

When these case studies were planned, there were no organizational structures and procedures in place which could have been tested for the operational functioning of the center. This is relevant to the assembly of the task force for this case study which was done without following any specific procedure but on a volunteering basis. It is, therefore, important for future activities of the center to have clear procedures in place, as a part of the business model, to assign task force members for specific customer requests. Moreover, once the organizational structures and procedures are in place, invested budget is also expected to reduce for the services similar to this case study. Furthermore, there was delay in choice of the case studies and building the services (Catalogue of Services, WP3). These delays were foreseen as critical implementation risks (see 1.3.5 in the DoW) and mitigation measures were taken. So a preliminary list of already existing questions and another preliminary list of existing services were used at the beginning of the work on WP4.

The main conclusion of the workshops on topics addressed by the case studies is that there is a high level of expertise of the contributing task force members in nanomaterials related risk assessment ensures that EC4SafeNano is capable of dealing with higher tier full-fledged nanomaterials characterizations and risk assessments for future projects and regular service.

4.2 Experts Feedback on Case Study on Transformations of Nano-TiO_2

The technical content of the case study was considered of high quality by the external experts, but they viewed CS2 to be a research activity and not a service of the center. A case study like CS2 could be also done in response to regulatory needs, i.e. by organizations developing standards and regulations. That is true. However, the comprehensive character of CS2 allows the center now to derive and define specific services offered by the center in the field of LCA of nanomaterials addressing highly relevant physical and chemical endpoints defined by the OECD (OECD ENV/JM/MONO (2010) 46). The content of the full CS2 report will be published by EC4SafeNano partners illustrating (and surely canvassing customers) the comprehensive measurement capabilities of the center. It will underpin the offers of the center in the catalogue of services developed by WP3. The services derived from CS2 will address needs of manufacturers in LCA of their nanomaterial products including effects of exposure to different environments. Standardized UV weathering and an aquatic environment were selected for CS2 because they are highly relevant for performance tests of

NPs on the one hand side and their environmental impact on the other, i.e. addressing two principally different groups of potential customers.

The main conclusion from CS2 is that the center, by forming a task force managing the request, has the capability to follow changes of phys-chem parameters (endpoints specified by the OECD) of nanoparticles exposed to different environments on different levels of information, complexity and costs. Simple approaches as TGA and high-end methods as XPS and ToF-SIMS delivering very detailed chemical information can be offered to costumers. The procedure of building the task force itself is a critical issue (as mentioned by the experts) and in the EC4SafeNano governance document some criteria have been proposed and will be decided in detail as a part of the internal business model of the center.

The experts were somewhat struggling with a clear definition who the customer of this particular case study is or could be. What can be the demand from a client? The answer is that a portfolio of different methods of phys-chem characterization of nanoparticles (different in the level of information delivered and the cost of application) tested in the CS can be now delivered as analytical services by the center to the industry, i.e. to producers and users of nanomaterials. Motivations for them can be quality management for nano products and their LCA in terms of performance. Another motivation for clients from the industry may arise from future regulatory demands, e.g. the preparation of dossiers for new nanomaterials for REACH/ECHA by manufacturers. Other costumers can be parties involved in the OECD standardization process. Of course, the OECD cannot be a costumer because their business model is based on in-kind contributions of member states. However, to develop OECD TGs and GDs practical work is required and that work is often executed as part of funded national or European projects. Those projects may act as costumers of the center which offers specific analytical services fit for purpose as shown by CS2. Here we refer to new project proposals for the development of OECD guidance documents (GD) on (1) the Identification and quantification of the surface chemistry and coatings on nano- and microscale materials, ENV/CHEM/NANO(2018)4/ADD2, and (2) Aquatic (Environmental) Transformation of Nanomaterials, ENV/CHEM/NANO(2018)4/ADD5, which are supported by the funded MALTA II European project. The leaders of the two projects mentioned here already expressed their interest to get access to the results of CS2.

The experts were also struggling with the costs of CS2. CS2 was aiming on the full use of all relevant measurement capabilities at the partners of the center and a full study of the effect of ageing of nanomaterials exposed to different environments – it has the character of a piloting research project and the costs are reasonable. However, based on knowledge developed by CS2 custom tailored offers on different levels of costs and information delivered will be developed by the center as part of its catalogue of services which will be competitive in terms of costs.

Another important comment by the experts was that the CS2 report in their hands cannot be the report sent to the customer. This question had been addressed by the partners involved in CS2 and contributing to the catalogue of services (WP3). The conclusion is that the center definitely has to deliver analytical reports according to the requirements of ISO 17025, including description of measurement protocols, standards, uncertainty of measurement and a conclusion addressing the question raised by the costumer. A respective template for test reports was developed by BAM (D4.2, Annex 4) and was accepted by the center's partners.

The experts pointed to the fact that there are standards that rule the application of the methods used in CS2 for phys-chem analysis of nanoparticles. This is also requested by ISO 17025 and became part of the template of the test report to be used by EC4SafeNano for reporting results. Recently, BAM contributed to a survey on existing standards in a text book (Charles A. Clifford et al., Chapter 5, International standards in nanotechnologies, in: CHARACTERIZATION OF NANOPARTICLES - Measurement Processes for Nanoparticles Edited by VASILE-DAN HODOROABA, WOLFGANG E.S. UNGER and ALEXANDER G. SHARD, ISBN: 9780128141823, Elsevier, 2019). Here international standards on sample preparation, electron microscopies, scanning probes, suspension-based size measurement methods, surface area, surface chemical analysis and material specific standards developed by ISO/TC 229 Nanotechnologies, CEN/TC 352 Nanotechnologies, IEC TC 113 Nanotechnology for electrotechnical products and systems and ASTM International TC E56

Nanotechnology are reviewed. These standards will be considered for services of the center derived from CS2.

4.3 Experts Feedback on Case Study on Occupational Risk Assessment of Nanocoated Gloves

From technical point of view, the experts found that the analysis was very well done and useful. Most of the clients are not expected to master a lot of technical information on methodologies etc. and in a way there is a need also for educating them. The language used in the report was found to be appropriate for this target people to comprehend. Nevertheless, some specific technical aspects were mentioned on which more information was required. These points are now addressed, and more information is now provided in the report.

Contextual information which has been provided by the customer was also considered by the experts to be important to be included in the report. Such information is already known to the customer and the assumptions made on the basis of this contextual information have to be included in the report. The perspective of the client should be clearly documented as well. The risk assessment was performed based on the conditions that the client have provided to the center and it should be ensured that these conditions are well understood, summarizing in a way what we have understood by the client. This would also serve in better clarifying and justifying the underlying assumptions of the analysis.

One of the experts found the final report to be broader than the scope of the case study. According to the expert, most of customers have no or medium knowledge concerning this situation; The scope of their client is more commercial while this study was a bit broader. The report was more extensive, over-technical and advanced compared to the understanding of the client. It can also be beneficial for the customer because it is more than what they are expecting. Other expert opinions considered that although the report can be less extensive, some technical proof and specific advice (whether the gloves are safe or not) have to be included. This would support the decision of the client towards using on not these gloves.

The speed of the response was found to be reasonable by the experts. On the other hand, personnel effort was higher than expected. It was pointed out that this was a first experience within the center and there was not a standard protocol to follow. Some things were not clear for some task members, while there were also some internal issues. For such services, if the center is running and organizational structure and procedures are already in place, decision times could be minimized and personnel effort will be lower as compared to this case study.

The experts also commented on the fact that there are countries which more expensive than other, with different costs and salaries. This is also reflecting in the results; There are differences in the responses regarding the personnel effort and the total cost. The total cost should be adjusted otherwise it could be a deterrent for a customer from e.g. southern Europe.

One of the experts considered that the center could use but not develop harmonized methods (i.e. OECD). It was pointed out that the center could contribute in doing the pre-standardization work i.e. before the method goes to OECD. In the center there are many people of high expertise, including experience on the development of standards. In the governance structure for the future center, we foresee certain activities which will be refined when the center is established. These include that the members of the center would engage in activities to prepare the ground for standardization.

Many experts felt that there is not significant added value regarding the confirmation and certification. However, it was not clear if all the experts have understood properly these general questions.

Finally, it was mentioned that even if the additional benefits of such a center are proposed to a potential client, it is to the client to decide if they want to use the center or move towards an individual service provider. Considering risk governance at European level, such a center is really needed and its role cannot be undertaken by an individual service provider. The center could establish an environment to reach consensus among many people, and deliver services with these additional characteristics, including the time and the resources that are needed.