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Literature study on the uses and risks of nanomaterials as pigments in the European Union

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Disclaimer

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Abstract

This report provides a review on the safe use of nano-sized pigments when used by professionals, workers or consumers. Extensive literature searches were carried out in combination with analyses of data from national nano-inventories to capture the current state of play. The report provides a list of nano-sized pigments currently identified on the EU market and discusses potential risks arising from their use in paints, coatings, printer toner cartridges, personal care products, tattooes as well as other potential uses of nanopigments.

Data for hazard and risk assessment are not available for the vast majority of identified nanopigments. Evidence-based conclusions on the safety of most nanopigments uses cannot be drawn, however, some general observations and conclusions can be derived. Dry pigments present the highest concern because they can be easily inhaled and ingested. Exposures to nano-sized pigments that are integrated into polymer, paint or coating matrices are not thought to be significant, and the risks to consumers from such uses are low.

The report also discusses uncertainties associated with the identification of nanopigments (and nanomaterials in general), measurements of exposure to nano-sized pigments and methods of toxicity testing.

Executive Summary

This project was initiated by EUON to investigate the safe use of the pigments at nano scale (nanosized or nanopigments) in consumer products, as well as their safety when used by professionals and workers, by means of a literature search. The potential risks arising from these uses was to be assessed by reference to toxicological data on identified nano-sized pigments, as well as any available data on exposure potential.

In order to achieve the aim of the project, a key task was to prepare an inventory of nano-sized pigments ("nanopigments") currently known to be on the EU market, and to map these to identified consumer and professional uses, where possible. It was agreed early in the project that the scope of inventorisation should focus on pigments that are declared to be marketed as nanoforms.

Inventorisation

Official sources consulted for this project to derive a current list of known nano-size pigments on the EU market were the ECHA REACH registration database, which has a search facility allowing discrimination of the disseminated registrations covering nanoforms, as well as publications by the Belgian and French national inventories, and the current EU catalogue of nanomaterials used in cosmetic products. A publication of the Danish Environmental Protection Agency, using data from the Danish Product Register, was also used (Danish EPA 2015a).

The inventorisation began by searching the ECHA REACH database. A preliminary list of 348 pigments was identified, which are registered as bulk-form substances. This list was obtained by searching for "pigment" in the disseminated REACH database search function. Using the facility that allows filtering by declared nanoforms it was observed that, as of April 2018, only 23 substances in total have been identified as nanomaterials in the REACH database. Of these, 8 substances are identified as having a pigment use in the lifecycle descriptions of the public REACH dossiers. 4 additional substances were identified that are fillers/ extenders that are commonly used in conjunction with pigments, and thus may present a co-exposure potential.

To supplement this list, data were extracted from the Danish, French and Belgian nano inventories, as well as the current EU catalogue of nanomaterials used in cosmetic products. The Danish list (175 pigments) does not specifically distinguish nano-sized pigments – it assumes that all pigments declared in the Product Registry fall under the EU definition. This list was however used to supplement the REACH list (23 nanomaterials), as some pigments were not REACH registered when the Danish EPA report was published in 2015.

A pigment was definitively confirmed as a nanopigment existing on the EU market when it appeared on either the REACH database and/or in the Danish list and it appeared in the French or Belgian nano inventories, and/or was identified from an industry source, or is listed in the EU catalogue of nanomaterials used in cosmetic products.

Additional efforts were made to collect information from industry sources. A small number of respondents provided only general information. In follow-up, an extensive internet search was conducted to collect product brochures and technical data sheets for pigments products supplied by the same companies. Of 398 retrieved publications, 49 provided evidence of nano-sized pigments, however these were principally related to Titanium Dioxide (TiO_2) and Carbon Black. In general, public-domain product information does not provide sufficient characterisation data, such as particle size distributions, to determine whether a pigment is marketed specifically in nano-form.

Within the scope of this project, only clearly declared nanopigments were used to define a current inventory of 81 substances (Appendix 1) that are definitively identified as nanopigments (including the 4 extender/filler pigments) and can thus be definitively confirmed

to be on the EU market as of April 2018.

On April 26th 2018 Member states voted to amend the REACH annexes to explicitly include new requirements for the reporting of nanoforms of substances[1]. This will provide a framework for the description of nanomaterial characteristics and to address risk assessment and risk management of nanomaterials. In this light, a more authoritative inventory of nanomaterials – and consequently nano-sized pigments, is likely to become reality over the coming years.

Hazard Assessment

Nanomaterials exhibit numerous unique properties that render them beneficial in applications in practically all fields of human activity. These unique physical and chemical properties may, at the same time, potentially lead to toxicity.

Adverse effects of a nano-size material cannot always be derived from the known toxicity of the macro-sized material of the same chemical composition, as novel phenomena, such as quantum effects, size-dependent properties, and unique toxicokinetics become apparent as their dimensions approach nanoscale. Moreover, from a toxicological point of view, nanomaterials represent an extremely diverse group of substances with various toxic potentials. While the chemical composition basically determines biological behaviour and potential toxicity of conventional chemicals, nanomaterial toxicity depends on numerous physical and chemical properties, in particular, the size, shape, crystalline structure, surface electric charge, chemical compositions of the core and shell (surface coating), and purity. The combination of these properties governs the ability of nanomaterials to enter biological systems, distribute in the bloodstream and lymphatic system, and penetrate into cells, tissues, and organs, as well as their interactions with cells and intracellular structures and macromolecules. Nanomaterials particularly interact with immune-competent cells, as these are responsible for elimination of the potentially dangerous objects from the body.

For the present report, an extensive literature search was performed to identify relevant toxicological data applicable in the risk assessment of nanopigments. It has been found that the scientific literature on the toxicity of nanopigments is mostly restricted to a small number of inorganic nanomaterials with a wide range of applications (beyond pigment-related uses), such as nano-TiO_2, nano-ZnO, carbon black, nano-Ag, nano-Au and to a lesser extent nano-iron oxides (e.g. Fe_2O_3), nano-aluminium oxides, nano-silica (SiO_2), and Barium sulphate ($BaSO_4$). Reports of nanotoxicological studies on organic nanopigments are rare.

Reliable nano-specific toxicological data for risk assessment are currently not available for the vast majority nanopigments identified in the inventory. Moreover, due to a large number of factors affecting toxicity of nanomaterials (such as their variability and dynamic behavior in the environment), and in the absence of standardised testing methodologies for nanomaterials, available toxicological data are generally inconsistent, and reported results are often contradictory. However, for well-tested substances general conclusions can still be drawn.

In this report, a summary of toxicological data is provided in selected nanomaterials used as pigments for which these data were available. Focus has been placed on endpoints representing most concerns in relation to human exposure, and on toxicokinetics as a crucial factor affecting toxicity upon different exposure routes. Furthermore, the role of nanomaterial characteristics in the observed toxicity was taken into account. To compensate for the lack of information on most of the less commonly used nanopigments, general toxicological

[1] http://ec.europa.eu/transparency/regcomitology/index.cfm?do=search.documentdetail&Dos_ID=15915&DS_ID=56122&Version=2

observations that are valid for insoluble nanoparticles are provided.

Exposure Assessment

Exposure scenarios (ES) for identified nanopigment uses were developed within this study based on the literature review. ES include a set of information on materials, operation conditions and applied risk management measures. These can represent a valuable tool for exposure assessment and subsequently for the risk assessment of nanomaterials (NMs). Currently, ES for nanomaterials cannot be understood the same way as for conventional chemicals. Whereas for standard chemicals (not in nanoform), ES describe conditions under which the risks are controlled, for many nanomaterials it is not yet possible to ensure their safe use due to the lack of exposure limits for most of them. Nevertheless, the ES can be used to benchmark different process operations and control measures, and to provide general guidance on how to reduce human exposures.

In total, three industrial ES (including 10 contributing exposure scenarios – CES), two professional ES (with 2 CES) and 4 consumer ES have been elaborated. Two scenarios were adopted from NANEX[2] and MARINA[3]/GUIDEnano libraries on exposure scenarios. The scenarios were focused on the following domains: (i) production of TiO_2 and Fe_2O_3; (ii) production and use of printing inks; (iii) use of paints; (iv) personal care products with TiO_2. Hypothetical generic scenarios were also outlined to support future thoughts on potential exposure to nanopigments and products containing nanopigments.

The NANEX template, which is based on the REACH exposure scenario format, was used to describe the ES. The completeness of most of the ES is very low due to lack of contextual information available in the literature. Most of the scenarios on industrial and professional uses are supported by the measurement data. However, different monitoring and sampling strategies, procedures and measuring tools have been used in particular studies. The absence of harmonised standardised operating procedures hindered comparison of the studies and therefore the use of the experimental data for decision-making within the risk management process.

Risk

Risk assessment involves a determination of quantitative or qualitative estimate of risk related to a well-defined exposure situation and a recognized hazard. Preliminary risk assessment of nanopigment uses was carried out based on two different approaches: (i) comparison of experimental measured data on exposure available in studied literature with proposed exposure limits for nanomaterial of concern; and (ii) use of control banding (CB) tools considering the worst case scenario (where the data were missing). Due to gaps in hazard identification and usable dose response relationships, preliminary risk assessment is often based more on the exposure considerations and actual exposure limits or exposure reference doses.

The Stoffenmanager Nano Module[4] was used to qualitatively assess occupational health risks from inhalation exposure to Manufactured Nano Objects (MNOs) for risk banding. This approach was applied to industrial and professional uses of nanopigments. Health risks from consumer exposure were described qualitatively due to the inapplicability of existing Control Banding (CB) tools and the unavailibilty of exposure limits. Due to lack of data, the default inputs for worst case scenarios were chosen. Where a control banding tool was not applicable

[2] http://nanex-project.eu/mainpages/exposure-scenarios-db.html
[3] http://www.marina-fp7.eu
[4] https://nano.stoffenmanager.nl

(for example, in consumer scenarios) a qualitative description of activity and possible risk is provided. The industrial and professional exposure scenarios, dealing with dry and free nanopigments which could become airborne, present the highest concern since there is evidence from animal studies for potential adverse health effects of inhaled (nano) particles.

Due to the absence of good methodologies specific to consumer and professional exposure scenarios, exposure to nanosized TiO_2 was assessed within manufacturing environment. One exposure scenario for occupational exposure to TiO_2 – Packing into bags - is of high concern based on the comparison of recommended OEL for nano-TiO_2 and OEL for ultrafine particles. The recommended OEL for nano-TiO_2 is exceeded due to the significant generation of dusts. Considering the particle number concentration, the measured mass concentrations are, in the worst case scenario, more than 1,200 times higher than the proposed OEL. The risk level obtained from the StoffenmanagerNano for the six exposure scenarios, all reach category I, i.e. high priority. It should be noted that the risk level is highly influenced by the ES data incompleteness. Evidence-based conclusions on the safety of production of nano-TiO_2 cannot be drawn based on the available literature. However, with respect to the precautionary principle, the high potential for inhalation exposure indicates a potential risk for human health.

The production of nano-Fe_2O_3 was also assessed. A scenario related to feeding of material into a semi-open container for washing was identified as the highest concern. The proposed OEL for ultra-fine particle number concentration is exceeded up to 3.3 times.The risk level for the three Exposure Scenarios (ES) (production, material feeding and packaging) reach category I and II, i.e. high and middle priority. The risk level is highly influenced by the ES data incompleteness. The CB tool automatically accounts the worst case scenarios when the input parameter is unknown. Evidence-based conclusions on the safety of production of nano- Fe_2O_3 cannot be drawn based on the available literature. However, the high potential for inhalation exposure indicates a potential risk for human health.

One ES for the production of printing inks containing TiO_2 was developed based on the available literature. This ES is not of high concern based on the comparison between the measured particles concentration and the recommended reference value. The measured concentrations are 2.5 times and 4 times lower than the proposed OEL. On the basis of this exposure the risk level for this ES reached category II, i.e. medium priority.

One ES for professional use of photocopiers was developed based on the available literature. The completeness of this ES is relatively high compared to other ES described for industrial uses. However, comprehensive risk assessment is still not possible due to lack of knowledge on product characteristics, and the absence of contextual information on exposure and risk management measures. There are significant differences between 'during-activity' and 'non-activity' particle number concentrations (12 times higher particle number concentrations during the printing in comparison with the background). The evidence found in the studies on consumer exposure to nanoparticles emitted from the use of printer toner cartridges shows that exposures can vary from small amounts of particles up to more than million of particles/cm^3. Based on the available measurements, the proposed OELs won't be exceeded considering the geometric mean (GM) values. However, the transient peaks are of high concern, and indicate a potential risk for human health.

Clear evidence-based conclusions on the safety of using paints containing nanomaterials cannot be drawn based on the presented data. The risk assessment is impeded by lack of information on almost all necessary inputs, including lack of information on chemical composition of the paints, characterization of nanomaterials contained in the paint, release factors, daily intake, etc. The potential for exposure is considered medium as the nanomaterial is suspended in a liquid matrix. Neither the release of nanomaterials nor the inhalation exposure during the drying period was demonstrated. However, spray applications may generate aerosols containing nanoparticles which could theoretically result in exposure of the lungs.

The presence of nano-sized pigments in tattoo inks represents a special case, as these inks are injected directly into the skin. However, based on review of the available data, clear evidence-based conclusions on the safety of nanomaterials in tattoo inks cannot yet be drawn. The risk assessment is impeded by lack of information on almost all the necessary input data (no information on nanomaterials in tattooes, characterisation of nanomaterials contained in the tattoos, release factors of nanomaterials, etc.).

In summary, it is clear that more information on the use of nano-sized pigments and their potential for release and exposure in occupational, consumer and environmental contexts is needed in order to derive comprehensive and realistic risk assessment. Dry pigments present the highest concern because they could be easily inhaled and ingested. Exposure to nano-sized pigments that are integrated into polymer, paint or coating matrices are not thought to be significant, unless these are further processed by abrasion, for example by sanding. The likely risk from standard painting techniques could be in accidental ingestion of pigments due to eating, drinking or smoking via inadvertent hand to mouth contact, or through direct inhalation of fine liquid aerosols containing nano-sized pigments.

Significant data gaps are observed regarding contextual knowledge on exposures as well as available experimental data on release and exposure. Only a very general conclusion could be drawn based on the precautionary principle: where there is a potential for exposure there is a potential risk for human health, principally from inhalation exposure to airborne nanopigments, which is identified to be of highest concern.

Uncertainties

Several uncertainties have been indentified in this project. From the outset, defining what exactly constitutes a nanopigment presents a challenge, in that data on particle-sizes and nano-fractions are generally not publically available. General assumptions must therefore be made when conducting risk assessments.

In light of the current EU definition of nanomaterials, some industry associations have stated that it could be presumed that many pigments should be considered nanopigments according to the EU definition of nanomaterial, especially since particles incorporated in agglomerates or aggregates are included in the definition.However, currently most of pigments in the REACH database have not been declared in nanoforms.

Currently, REACH includes only a small number of substances declared as nanoforms, so this cannot be viewed as a comprehensive source of information. National registries dealing with nanomaterials are currently established in France, Belgium and Denmark, and their databases are confidential. Only data released in public reports in 2015 and 2016 were available for use in this project. The current EU catalogue of nanomaterials used in cosmetic products was also consulted.

Several problems were encountered with hazard and exposure assessment specifically for nano-sized pigments. First of all, mass-based dose metric is normally reported in toxicological studies, while number-based particle size distribution is often not discussed. Without reporting a standard dose metric (particle number-based or mass-based concentration, surface area, etc.) it is difficult to assess the risks in a real-world exposure. The "standard" metric in exposure measurement for standard chemicals is the mass concentration, whilst for nanomaterials a more appropriate approach can be the use of surface area or of the particle number-based concentration. As there is not yet an agreement on the most appropriate exposure metric to be used in exposure assessment of nanomaterials, the recommendation of using multiple metrics has been proposed.

Although national and international projects have been initiated to define standard testing regimes and exposure scenarios, there are no conclusive outcomes on how to assess risk posed by nanomaterials. This is compounded by the difficulty in measuring real-word exposure under various conditions. Several particle size-measurement methods exist, however there are currently no officially agreed methods available. Most published data lack adequate dose metrics to allow comparison with exposure estimations. Therefore, estimations of consumer / professional exposure must be based on highly conservative assumptions, often based on mass metrics, which are unsuitable for the satisfactory assessment of nanomaterial / nanoparticle exposures.

In general, there are few public-domain data on the use of nanopigments in consumer products, unless these are voluntarily declared, and therefore it is difficult to accurately gauge overall exposure/risks to nanopigments. For the most part, the chemical identity and/or characterisation of nanomaterials applied in consumer products are not well described, if at all.

Even the presence of nanomaterials in most products is uncertain, as mandatory reporting is country-specific, often voluntary and/or with exemptions for nanopigments. A case in point – currently the ECHA REACH database lists only 23 registrations in total where a nanoform is identified. With such a paucity of publicly-available information it is extremely difficult to conduct a comprehensive assessment of risks posed by nanopigments without making significant assumptions on nearly all assessment inputs.

1. Background and Introduction

1.1 Terms of reference

The project was initiated by EUON to investigate the safe use of the pigments at nano scale (nanosized or nanopigments) in consumer products, as well as their safety when used by professionals and workers by means of a literature search. The risks from these uses were to be assessed by reference to toxicological data on identified nano-sized pigments, as well as any available data on exposure potential.

In order to achieve the aim of the project, a major integrant task was to prepare an inventory of nano-sized pigments ("nanopigments") in consumer and professional products currently known to be on the EU market.

In parallel to the literature search, other sources of information were consulted in order to build an inventory of pigments currently on the EU market and, where possible, map these to specific professional and/or consumer uses.

1.2 Defining nano-sized pigments

Pigments can be defined as substances consisting of particles that are practically insoluble in the vehicle or substrate in which they are incorporated, and which impart colour through selective absorption and scattering of light. Pigments are used for colouring paints, inks, plastics, fabrics, cosmetics, food, and other materials. Most pigments are dry colourants, solid at room temperature and usually ground into a fine powder. Powdered pigment is added to neutral, colorless material binder (vehicles, fillers, extenders) that suspends the pigments and provides adhesion. The distinction between a pigment and a dye is that, a pigment is insoluble in its vehicle, resulting in a suspension. A dye is usually itself a liquid or is dissolved in a liquid vehicle.

Coloured pigments are materials that change the color of reflected or transmitted light as the result of wavelength-selective absorption. This physical process differs from fluorescence, phosphorescence, and other forms of luminescence, in which a material emits light.

Pigment opacity (hiding power) is governed by two principles - the ability of particles to absorb and scatter of visible light. The ability of a pigment particle to absorb visible light depends on its chemical composition. For example, Carbon black absorbs all wavelengths of visible light ($\lambda \approx 400 - 800$ nm) and therefore full opacity can be obtained using only small amounts in dispersion. On the other hand, white pigments such as Titanium Dioxide (TiO_2) and fillers such as barium sulphate ($BaSO_4$), do not absorb visible light at all. Coloured pigments absorb different wavelengths of visible light.

Scattering and absorption of light by pigments is also a function of particles size. For a specific wavelength of light, the optimal particle diameter for scattering is about half the wavelength of the light (Beetsma 2017).

Particles of TiO_2 pigments used to provide white colour to coatings and inks have a particle size closer to 200-230 nm, which is optimal for the scattering of white light (Beetsma 2017).Smaller particles of TiO_2 (less than 25 nm) do not scatter light but rather have the ability to absorb UV radiation. A coating containing nano-TiO_2 is transparent because the particles hardly scatter visible light. Nano-sized titanium dioxide pigments, with a particle diameter far below 100 nm, are therefore effective as UV-absorber. They can also be used as "transparent pigments" for applications where the substrate must be visible, for example in wood finishes, but also in specialised automotive coatings, plastic applications, artists' colours, cosmetics and inks.
Modern pigment applications require consideration of specific properties such as dispersibility,

colour strength, light and weather fastness, migration resistance, colour shade and hiding power. These properties depend both on the chemical composition of pigments but also on the size and morphology of the constituent particles.

Pigments are broadly classified as either organic or inorganic. Inorganic Pigments are usually metallic salts precipitated from solutions or metallic oxides. Earth pigments are naturally-occurring coloured substances (principally iron oxide) found in rocks and soils. The preparation process of inorganic pigments is usually simple and consists of mineral washing, drying, pulverizing and mixing into a formulation. Examples of inorganic pigments include TiO_2 (Anatase, rutile), Zinc Oxide, iron oxides, and aluminium oxides. Inorganic pigments have lower cost, simple production process, and higher yield than organic pigments. They are usually stable in the presence of organic solvents and they also provide excellent light and heat resistance and weatherability. Organic Pigments are carbon-based but may also contain inorganic metallic elements that aid stabilisation. They are chemically synthesized from materials such as coal tar and petroleum distillates that are transformed into insoluble precipitates. Traditionally, organic pigments are used as mass colourants. They are popular in plastics, synthetic fibers and as surface coatings-paints and inks.

Organic pigments are generally classified into azo pigments and non-azo pigments. Azo pigments includes monoazo yellow, orange, diazo compounds, naphthol, naphthol AS, azo lake, benzimidazolone, Bisazo condensation, and metal complexes. Non-azo pigments can be further subdivided into pigments such as heterocyclic and fused ring including phthalocyanine, quinacridone, perylene and perinone, thioindigo, anthraquinone, dioxazine, isoindolinone and isoindoline, diketo-Pyrrole-pyrrole (DPP), triarylcarbonium, and quinophthalone. (Kent 2007)

Due to their larger surface area, organic pigments provide higher color strength, however for the same reason their dispersibility is usually poorer (Buxbaum and Pfaff 2005).

Extenders / Filler pigments are solid components that are usually mixed with pigments, and their contribution to colouring is secondary. They are finely ground natural materials, most commonly rare-earth minerals, and they have little effect on the colour of the product, but they do provide enhancement of mechanical, thermal, and barrier properties as well as reducing cost. Filler pigments are differentiated from other pigments in that they usually have little or no effect on the coatings' optical properties other than gloss.

Common extenders are calcium carbonate, silica, micas, clays such as kaolin, and barytes (the natural form of barium sulfate). Although they are not 'true' pigments, they support the functionalistation of pigments. These substances are also discussed in this report in the context of safety because of the possibility of co-exposure, and because some have been declared as nanoforms in REACH, and are used in significant volumes.

Using nano-sized pigments and fillers confers a number of funtional advantages on materials such as better surface appearance, improved resistance to fading, UV and chemical resistance, thermal and electrical conductivity, and improved corrosion resistance.

1.2.1 Pigments in the context of current EU Definition of nanomaterials

In 2011 the European Commission (EC) published its current recommendation on the definition of a nanomaterial (2011/698/EU) [5]:

"Nanomaterial means *a natural, incidental or manufactured material containing particles, in an*

[5] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32011H0696

unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm".

" *'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components*"

" *'aggregate' means a particle comprising of strongly bound or fused particles*"

The purpose of the definition is to allow determination of when a material shall be considered as nanomaterial for regulatory purposes.

However, challenges are being faced by industries in determining whether a material can be considered as a 'nanomaterial' according to this definition, as it uses a threshold related to the number-based size distribution of particles. However, size of pigments is often measured by using techniques (e.g. Dynamic Light Scattering, DLS) that provide a size distribution based on mass, volume, or scattered light intensity, which must to be converted into a number based distribution for regulatory purposes. Such conversion may introduce major errors. Moreover, techniques such as DLS are unable of determining constituent particles whithin aggregates / agglomerates, as requested under the EC definition[6].

Currently, the use of transmission electron microscopy (TEM), done by directly imaging and counting particles, is often necessary for providing particle size distribution information as required by the EC definition. However, sample preparation and counting protocols needs to be standardised in order to make results achieved by different industries comparable.

While it is possible to determine the size distribution of primary particles in pure nanomaterials using electron microscopy, measurement of nanomaterials in mixtures and articles is more complex.

For polydisperse materials (i.e.those with a wide particle size distribution) such as pigments, the requirements of the EC Definition to determine whether the relative number of particles with a size between 1 nm and 100 nm is above or below 50%, can be challenging using existing methods. However, many materials and expecially most of pigments are manufactured under strictly controlled conditions, leading to the manufacture of pigments with small polydispersibility. As a consequence, their size distribution can often be measured with a single method (Nanodefine 2017)

In order to support the implementation of the 2011 definition of nanomaterial, in 2013 the Commission established the NanoDefine project[7] to provide recommendations on suitable methodologies aimed at determining whether an unknown material can be considered as a 'nanomaterial' according to the EC definition.

A number of recommendations were made in order to deal with the major technical challenges that were identified, resulting in calls for development of clarifying guidance on the conditions under which these methods can be used to identify a nanomaterial by employing appropriate quantity or metrics conversion. Among the technical issues, aspects of the EC definition that require clarification have also been analysed and discussed, such as for example the exact

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6 http://publications.jrc.ec.europa.eu/repository/bitstream/JRC92531/jrc_eurocolour%20report_final.pdf

7 http://www.nanodefine.eu/

meaning of the term 'particle', and the identification of constituent particles within agglomerates and aggregates (NanoDefine 2017).

1.3 Scope and uncertainties relating to the project

The scope of this study was limited to those types of products/pigments that have been specifically declared to be marketed in nanoform. Therefore, it was clear from the outset that the biggest challenge of this project will involve the compilation of a current and comprehensive inventory of declared nanopigments that are confirmed to be currently on the EU market, and to map these to the specified known uses. This was determined through inspection of existing national nano inventories, the EU catalogue of nanomaterials used in cosmetic products, the ECHA REACH database of disseminated registered substances and sector-specific sources. It was noted that it is possible to specify "nanoforms" when searching the publicly disseminated database of REACH registrations. Only public domain data were used in this project.

Although the project is described as a literature review based on primary data sources only, it was clear from the outset that the work required would be more extensive and must draw on a wide range of data sources, including industry data.

It can be assumed that most pigment samples will contain a nanosized fraction which may not be measureable – and therefore remain undeclared – in many cases. Only general assumptions can be made in these cases and therefore the review will aim to look to describe generic use and exposure situations where pigments may be used, but without detailed characterisation of their particle size distributions.

With regards to the requirement to assess exposures during the entire lifecycle of products containing nanopigments, consideration was given to complexity of service life, and in particular waste. For the purpose of this review it was proposed to assess risks for product use by professionals and consumers, and in service life up to the point that a product becomes waste.

The assessment of human exposures from waste streams would necessitate a greatly detailed modelling of environmental releases, for example from incineration units, or through water waste streams. This would require a significant additional inputs, including measured environmental data, to form part of an exposure assessment of Man via the Environment. This is nascent area of research that is beyond the scope of this review[8].

The project specification points to assessment of the risks to professionals and consumers and this will be the main focus of the work. However any beneficial information retrieved from other sources, for example industrial use and emissions, was included and discussed where relevant.

2. Inventory of Nano-sized pigments in the EU market

There is currently no central EU register of nanomaterials, however, steps have been taken towards ways of monitoring the presence of nanomaterials used in products in the EU. Nanomaterial registers are in place, or currently being established, in France, Belgium, Denmark, Norway and Sweden. The Swedish registry is anticipated to be fully operational by 2019. Each country has a different approach to the types of nanomaterial that must be reported, and what data should be submitted. For example, the Danish Product Register

[8] http://www.oecd.org/chemicalsafety/nanomaterials-in-waste-streams-9789264249752-en.htm

requires reporting of nanoforms, but nanopigment uses in paint, wood preservative, glue and fillers, as well as use in rubber articles, textiles and printing inks are specifically exempted from this requirement (Danish EPA, 2015a).

In 2017 the EU commission founded an "EU Observatory for Nanomaterials" (EUON) under the auspices of the European Chemical Agency (ECHA). EUON depends on other sources for the provision of information about nanomaterials, for example declarations of nanoforms in REACH registrations, as well as data from existing nanomaterial registers in EU member states.

In April 2018, the EU REACH committee voted to amend REACH annexes so that registrants will have to provide more information on the chartacteristics, uses and risk of substances marketed as nanoforms. These amendments are subject to approval by the Parliamnet and Council before adoption by the Commission[9].

2.1 Data Sources Used

Official sources consulted for this project to derive a current list of known nano-size pigments on the EU market were the ECHA REACH registration database, which has a search facility allowing discrimination of nanoform registrations, as well as publications by the Belgian and French nanomaterial inventories, and the current EU catalogue of nanomaterials used in cosmetic products. A publication of the Danish Environmental Protection Agency, using data from the Danish Product Register, was also referenced (Danish EPA 2015a).

Published data from REACH, French (2016), and Belgian (2016) authorities were the primary sources used to build the inventory, along with the most recent catalogue of nanomaterials used in cosmetic products placed on the EU market.

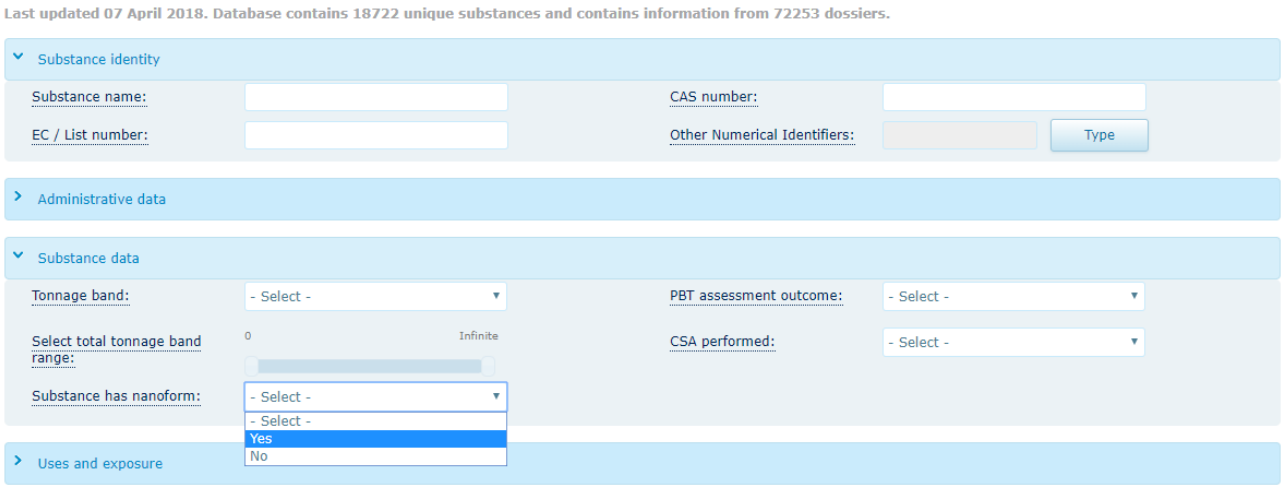
These data were to be supplemented by information derived from the literature review in this project, as well as any information that could be derived from industry or online sources (Annex 1).

2.1.1 ECHA REACH Database

The REACH public database on registered substances allows for searching of registrations based on whether use of a nanoform has been declared (Figure 1). Upon executing this search (6th April 2018) with no other parameters, a list of 23 substances is returned (Table 1).

[9]https://echa.europa.eu/-/echa-welcomes-improved-clarity-on-nanomaterials-in-the-eu-member-states-vote-to-amend-reach-annexes

Figure 1: Search function of REACH database for substances declared as nanoforms



When cross-checked against the life cycle descriptions in the REACH dossiers (i.e. checked against the public REACH dossiers for indication of uses as pigment), 8 substances declared as nanoforms can be identified as having a primary pigment use for product colouration. These are highlighted in blue rows in Table 1.

The status of 4 substances - highlighted in yellow rows – is less clear. These substances are commonly used as extender or functional pigment additives to improve paint and ink properties, therefore their primary purpose may not be to provide colour. For example, silicon dioxide is often added to products as a corrosion inhibitor, filler/reinforcing filler, flow modifier, glossing agent matting (glossing of flatting) agent, process regulator or aid, stabiliser, and viscosity control agent.

These substances are included in the inventory as they have a historical or potential use as true pigments. Furthermore, they may present nano-specific risks from co-exposure to pigments with which they are co-formulated.

Although pigmentary and extender uses can be determined, it is not possible to differentiate the volumes used as nanoforms from bulk forms using data from the REACH database.

It must therefore be assumed that the list of declared nanoforms derived from the REACH database is neither comprehensive nor representative. From comparison with other sources – detailed in the next sections - the REACH database currently does not identify the majority of nanopigments that are currently declared on the EU market as in most cases they are not reported as nanomaterials by registrants.

Therefore, in order to supplement this list, and to begin building a wider inventory, further searches of the REACH database were perfomed so as to extract a master list of pigments to be used as the basis for comparison with other sources. Several generic searches were performed and the data collated.

Firstly, the general search term ‘pigment’ was entered into the substance name field. This returned a list of 253 results, which were exported to Microsoft Excel format.

Next, the database was searched by Product Category. The Product Category PC0 was selected and the search term ‘pigment’ was entered into the ‘other uses’ search box (figure 2). This returned 112 results.

In order to delineate Consumer and Professional uses of these pigments, the above two

searches were repeated while selecting the appropriate checkboxes in the lifecycle section (figure 3).

Consumer uses were identified for 148 substances using the search term 'pigment' in the substance name field whilst selecting the 'Consumer Uses' checkbox. 84 results were returned for consumer uses where the term 'pigment' was used instead in the PC0 'other uses' box.

Professional uses were identified for 171 substances using the search term 'pigment' in the substance name field whilst selecting the 'Widespread uses by professional workers' checkbox. 12 results were returned for professional uses where the term 'pigment' was used instead in the PC0 'other uses' box.

The resulting lists were merged in Microsoft Excel to form a master list of 342 registered substances identified as pigments, though not specifically as nano-sized pigments. 4 additional substances representing significant high-tonnage extender pigments / additives / fillers were added to bring the total list to 346 substances. However, none of these substances could at this stage be identified specifically as pigments marketed in nanoform, therefore it was necessary to cross-check this list against other data sources before they could be included in the inventory in the scope of this project.

This list is provided in Appendix 4.

Figure 2: Search of REACH database for substances declared 'pigment' in product category (PC) 0.

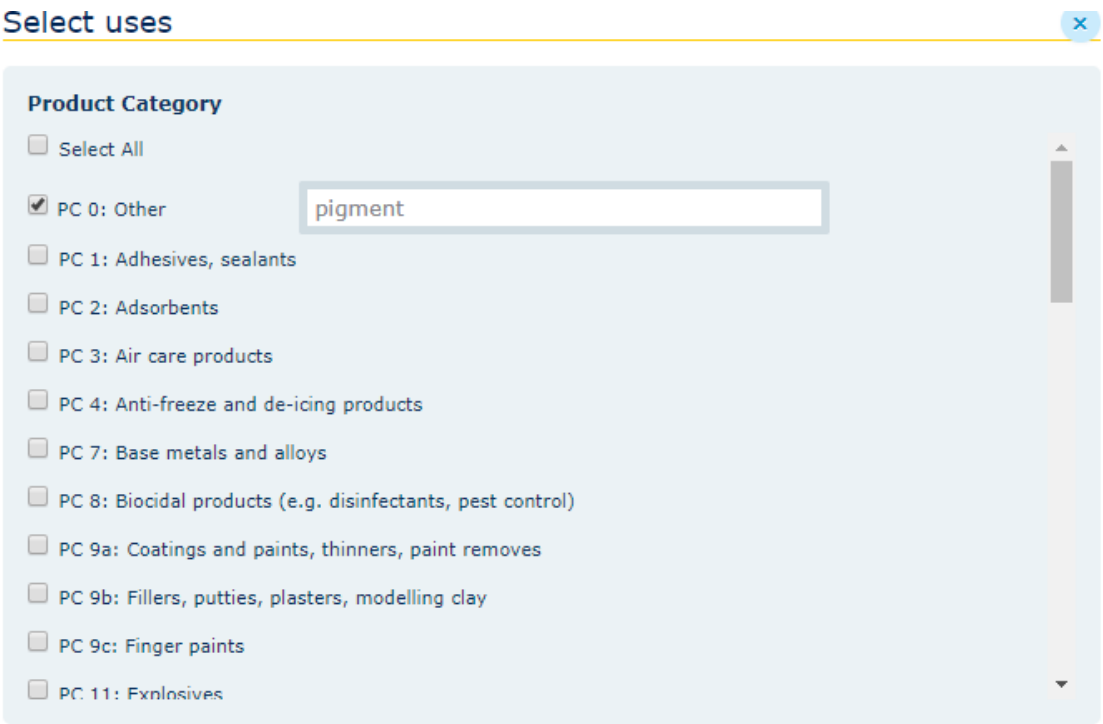


Figure 3: Search of REACH database for substances declared 'pigment' in product category (PC) 0, filtered by consumer uses.

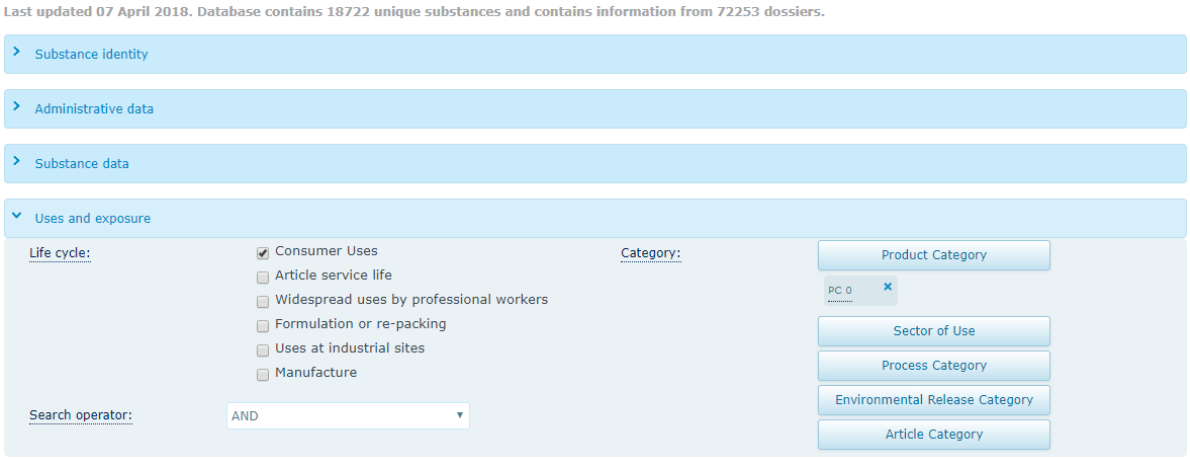


Table 1: Current (April 2018) listing of substances declared as nanoforms in the ECHA REACH database of registered substances
Rows in blue are confirmed pigments, rows in yellow are commonly used extender/filler pigments, white rows are non-pigments

Name	EC / List no.	CAS no.	Registration type	Submission type	Total tonnage band	Pigment Use declared?	Product Categories relevant to pigment use*
(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.0⁴,⁹]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	939-379-0	-	Full	Joint	100 – 1,000	Yes	PC9a, PC9b, PC18, PC32
Aluminium oxide	215-691-6	1344-28-1	Full	Joint	10,000,000+	Yes	PC9a, PC9b, PC9c, PC0: phosphorescent and fluorescent
Calcium carbonate	207-439-9	471-34-1, 7440-70-2	Full	Joint	1,000,000 – 10,000,00	Yes (filler)	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39
Calcium titanium trioxide	234-988-1	12049-50-2	Full	Joint	0 - 10	Yes	PC18
Cerium And Iron Oxide Isostearate.	442-240-2	-	NONS	Individual	Confidential	No	-

Name	EC / List no.	CAS no.	Registration type	Submission type	Total tonnage band	Pigment Use declared?	Product Categories relevant to pigment use*
Cerium dioxide	215-150-4	1306-38-3	Full	Joint	1,000+	No (Wood treatment)	PC15
Cesium tungsten oxide	466-380-9	52350-17-1	Full	Joint	0 - 10	No	-
Diiron trioxide	215-168-2	1309-37-1, 7439-89-6	Full	Joint	100,000 – 1,000,000	Yes	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39
Fumes, silica Amorphous silicon dioxide particles from the volatilization and vaporization of furnace feed materials in the manufacture of ferrosilicon and silicon.	273-761-1	69012-64-2	Full	Joint	100,000 – 1,000,000	Yes (extender)	PC1, PC9a, PC9b, P32
Graphite	231-955-3	7782-42-5	Full	Joint	100,000 – 1,000,000	No (Carbon additive)	-
Iron hydroxide oxide yellow This substance is identified in the Colour Index by Colour Index Constitution Number, C.I. 77492.	257-098-5	51274-00-1	Full	Joint	100,000 – 1,000,000	Yes	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39

Name	EC / List no.	CAS no.	Registration type	Submission type	Total tonnage band	Pigment Use declared?	Product Categories relevant to pigment use*
Iron manganese trioxide	235-049-9	12062-81-6	Full	Joint	10,000 – 100,000	Yes	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39
Manganese ferrite black spinel This substance is identified in the Colour Index by Colour Index Constitution Number, C.I. 77494.	269-056-3	68186-94-7	Full	Joint	10,000+	Yes	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39
Multi-Walled Carbon Nanotubes (MWCNT), synthetic graphite in tubular shape	936-414-1	-	Full	Joint	10 - 100	No	-
Reaction mass of (E)-3,7-dimethylocta-1,3,6-triene and (R)-p-mentha-1,8-diene and (Z)-3,7-dimethylocta-1,3,6,-triene and 7-methyl-3-methyleneocta-1,6-diene and DL-borneol and bornan-2-one and cineole and linalool and linalyl acetate and p-menth-1-en-4-ol	946-855-1	-	Full	Joint	0 - 10	No	-

Name	EC / List no.	CAS no.	Registration type	Submission type	Total tonnage band	Pigment Use declared?	Product Categories relevant to pigment use*
Silicate(2-), hexafluoro-, disodium, reaction products with lithium magnesium sodium silicate	285-349-9	85085-18-3	Full	Joint	10 - 100	Yes Extender / additive	PC1, PC9a, PC 18, PC26, PC18, PC39
Silicon dioxide	231-545-4	7631-86-9, 112926-00-8	Full	Joint	1,000,000+	Extender / additive	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39
Silver*	231-131-3	7440-22-4	Full	Joint	100,000 – 1,000,000	No	PC9a
Single Wall Carbon Nanotubes (SWCNT)	943-098-9	-	Full	Joint	0 - 10	No	-
Titanium dioxide	236-675-5	13463-67-7	Full	Joint	1,000,000 – 10,000,000	Yes	All PCs
tris(4-phenylphenyl)-1,3,5-triazine	479-950-7	31274-51-8	Full	Joint	10 - 100	No UV Filter in cosmetics	-
tris(λ²-iron(2+) ion) 15-methylhexadecanoic acid trioxidandiide	476-890-3	-	Full	Individual	100 – 1,000	No Fuel Additive	-

Name	EC / List no.	CAS no.	Registration type	Submission type	Total tonnage band	Pigment Use declared?	Product Categories relevant to pigment use*
Zinc oxide	215-222-5	1314-13-2, 7440-66-6	Full	Joint	100,000 – 1,000,000	Yes	PC1, PC9a, PC 9b, PC 9c, PC18, PC32, PC34, PC39

*Product categories (PC) derived from REACH lifecycles.

2.1.2 Danish Product Register

The Danish nanodatabase[10] comprises solely mixtures and articles which are intended for sale to consumers and which contain nanomaterials that can be released or whose nanomaterials release carcinogenic, mutagenic and /or reprotoxic substances (CMRs) or environmentally hazardous substances.

Products exempted from the nanoproduct register include those regulated under other legislation, such as food contact materials, cosmetics and medical devices. Other exemptions generally apply to products that may contain nano-sized pigments, for example:

- mixtures and articles containing unintentionally-produced nanomaterials;
- articles containing "fixed" nanomaterials, unless the substances might be released during use;
- printed articles, such as newspapers or labels, containing nanomaterials used in the ink;
- textiles containing nanomaterials in the colours or dyes;
- other products, such as paints, wood preservatives, glues and fillers, that contain nanoscale pigments used solely as colourants; and
- rubber articles that contain nano carbon black or silicon dioxide.

In order to assess the extent and impact of nano-sized pigments in consumer products, the Danish Environmental Protection Agency published a survey of products with nanosized pigments focusing on products exempt from the Danish Nanoproduct Register Denmark EPA (Danish EPA 2015a).

In order to account for the exempted materials, the general approach was to consider that all pigments are potentially nanomaterials based on the existing EU definition of nanomaterial. On this basis, the report extracted a list of 175 pigments from the Danish Product Register for cross-checking with the master REACH list.

A number of deviations from REACH data were identified from the Danish list. In particular, a small number of substances did not appear in the REACH database. In these cases the declared Colour Index (CI) was used to identify the correct EC/CAS numbers, with the corrected entries highlighted in the table in Appendix 1.

The corrected Danish list was therefore added to the inventory of this project for comparative purposes, however as the scope of this work was to identify nano-sized pigments that are currently declared as used on the EU market, it was necessary to further cross-check this list against other nanoproduct inventories and industry data, as detailed below.

Another Danish initiative - The Nanodatabase[11] is an inventory of consumer products containing nanomaterials developed by the DTU Environment, the Danish Ecological Council and Danish Consumer Council. DTU Environment is responsible for the development of the database, the data collection, the scientific assessments of the nanomaterials used in the various consumer products and the NanoRiskCat categorization. This database contains listings for 3,037 products as of April 2018. However, searched for product containing pigments returned few results (Pigment - 7 products, Carbon Black 17 products) which tended towards cosmetic products. This source was deemed to be of limited use for this project and therefore was not used.

[10] https://indberet.virk.dk/myndigheder/stat/MST/Nanoproduktregister

[11] http://nanodb.dk

2.1.3 French Nano Inventory

France was the first EU country to introduce a nano-product register[12]. As of 1 Jan 2013, the declaration of nanomaterials in products containing nanoparticles with volume >100g per calendar year is mandatory under Regulation Articles L. 523-1 to L. 523-5 of the French Environmental Code.

The inventory uses the 2011 definition of the European Commission, excluding naturally-occurring nanomaterials. Manufacturers, importers and distributors must report and update their declarations before 1 May each year through the portal website.

The most recent data available for this project was from 2016[13]. This list was scanned for declared pigment uses and the results were added and cross-checked against the master list prepared from the REACH data.

60 declared nano-sized pigments were extracted from the French inventory.

2.1.4 Belgian Nano Inventory

Similar to the French inventory, the inventory of Belgium has a notification threshold of >100g per calendar year[14]. The most recent public list was provided by the Belgian Nano inventory adminstrator. The list was scanned for any substances identified as having a pigment use. 42 substance in nanoforms identified as pigments by NACE[15] (Nomenclature des Activités Économiques dans la Communauté Européenne) code were extracted from the Belgian inventory.

Of note was that the two major known nanopigments – Titanium dioxide and Carbon black – were not identified as pigments by the Belgian inventory list.

2.1.5 Catalogue of nanomaterials used in cosmetic products placed on the EU market

According to the EU cosmetics regulation[16], the European Commission is required to publish a catalogue of nanomaterials used in cosmetic products on the European market. Notifications are currently made via the Cosmetics Product Notification Portal (CPNP)[17],which is not publicly accessible. However data was available through the most recent (2017) published catalogue[18], which identifies 13 substances as colorants, of which 12 can be identified as pigments (with one azo dye additionally listed).

2.1.6 Other sources

Norway has a register of dangerous substances where 'nano' is included as additional specification[19].

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[12] https://www.r-nano.fr/

[13] Ministère de l'Environnement, de l'Énergie et de la Mer (2016) Éléments issus des déclarations des substances à l'état nanoparticulaire RAPPORT D'ETUDE 2016

[14]https://www.health.belgium.be/en/environment/chemical-substances/nanomaterials/register

[15] http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

[16] http://eur-lex.europa.eu/eli/reg/2009/1223/2016-08-12

[17] http://ec.europa.eu/growth/sectors/cosmetics/cpnp

[18] http://ec.europa.eu/DocsRoom/documents/24521

[19]http://www.miljodirektoratet.no/en/Areas-of-activity1/Chemicals/The-Product-Register/Guide-to-Completing-the-Form

The Swedish Chemicals Agency, Kemi, will require companies to provide information on nanomaterials in chemical products and articles to the Swedish products register by 2019.

Registers also exist in Canada, Australia or Switzerland, and in the United States, where the Project on Emerging Nanotechnologies hosts an inventory of nanotechnology-based consumer products currently on the market[20]. However the contents of the above sources fall out of scope for the identification of nano-sized pigments currently on the EU market.

A number of online inventories containing information about nanomaterials and nanoproducts exist, such as the Consumer Product Inventory (CPI) of the Project of Emerging Nanotechnologies (PEN)[21], the Nanoproduktdatenbank maintained by the Bund für Umwelt und Naturschutz Deutschland (BUND)[22] and the inventory established by the European Association for the Coordination of Consumer Representation in Standardisation (ANEC)[23]. However these databases suffer from a number of limitations limiting their use on this project, in particular because they are are lacking in the description of pigments or are limited to claims by manufacturers.

2.1.6.1 Voluntary Industry Survey

Early in the project it was realised that data derived from primary literature, nano inventories and reports from international bodies would not be sufficient to allow mapping of specified nanopigment types to commercial applications, particularly consumer and professional applications currently on the EU market. There are simply not enough data in the primary literature to establish such links.

It was therefore decided to initiate direct contacts with industry (manufacturers/importers/ distributors), industry associations, and recognised experts in order to collect data for an inventory on nano-sized pigments that would be as comprehensive and current as possible.

To this end, a list of 127 industry contacts was drawn-up from various sources, including known sector organisations, known EU pigment manufacturers , and from lists of distributors and likely importers derived from searches of internet databases. Only five responses had been received, of which only two provided specific data (titanium dioxide used in sunscreens, links to information on carbon black). A sector organisation indicated a willingness to provide data, but could not meet the timeline. This trade association had surveyed its members. It was explained that members would not be volunteering any information as they expressed confidentiality concerns, time pressure, and worries about the purpose of the survey, and whether it was a forerunner to specific regulatory action.

Therefore the voluntary industry survey was unsuccessful in achieving its purpose of gathering product-specific data directly from pigment manufacturers, importers, and distributors.

2.1.6.2 Industry Online Information sources

In the absence of specific literature data on branded products containing nano-sized pigments, the websites of companies targeted with the industry survey were searched for commercial or technical information related to their pigment products. These included product brochures, Technical Data Sheets (TDS), Safety Data Sheets (SDS) and general information.

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[20] http://www.nanotechproject.org/inventories/consumer/

[21] http://www.nanotechproject.org/cpi/

[22] https://www.bund.net/chemie/nanotechnologie/nanoprodukte-im-alltag/nanoproduktdatenbank/

[23] http://www.beuc.eu/safety/nanotechnology

In total, 398 publications were retrieved. These were scanned for information on particle sizes, in search of descriptions including "nano", "nm" and "ultrafine".

Of these publications, 49 made specific reference to nanosized pigments, with the majority describing ultrafine Titanium dioxide and nanosized carbon black. However, most of these publications make minimal or only general reference to nano-sized pigments and their uses, and most do not provide a particle size distribution. Only a small number of marketed nanopigments have been identified from this literature. This made an inventory mapping of nano-sized pigments a major challenge that could not be satisfactorily addressed in this project.

In a bid to address this deficiency, a wider internet search was initiated, to pull-in any relevant data on nanopigments uses, including from non-EU sources. A number of branded products were identified and, where possible, mapped to specific applications in the inventory. Because of commercial sensitivities the list of branded products is kept confidential and not published in this report.

Therefore the mapping of consumer and professional uses largely depended on reported uses in the REACH registrations, on the assumption that nanopigment uses accurately reflect the uses declared in the 'bulk form' registrations.

2.2 Preparation of the Inventory listing

Cross-checking of the master list generated from the REACH database against data from other sources, specifically the National nano inventories of France and Belgium, and the list of pigments prepared by the Danish EPA in 2015, as well as the EU catalogue of nanomaterials used in cosmetic products placed on the EU market, and finally industry sources, showed that, with a small number of exceptions, all nano-sized pigments are already captured in the REACH database, where the majority are only registered in bulk form (see Appendix 1).

Whenever a pigment in the master list was identified as appearing on either the French or Belgian inventory, or identified from an industry source or via the list of 23 REACH-declared nanoforms, or appears on current EU catalogue of nanomaterials used in cosmetic products placed on the EU market, then that pigment was identified as definitely appearing on the EU market, and therefore included in the inventory in the scope of this project.

Two substances are worthy of special consideration: Nano-sized silver (Ag) is declared in the REACH database for its use in speciality paints due to its antimicrobial qualities, not necessarily for pigment use. However, nano-silver is also declared as a colourant in the EU catalogue of nanomaterials used in cosmetic products so it is included.

Rutile – a form of titanium dioxide distinct from anatase – is not specifically listed in REACH or the national inventories by CAS number, however it is included as it a common pigment which is not always distinguished from the commonly-used CAS number for titanium dioxide (13463-67-7) which specifically refers to the anatase form.

The resulting list of 81 substances currently known to be nanopigments, including common fillers, on the EU market is presented in Appendix 1.

2.3 Inventory mapping the uses of identified nano-sized pigments

The next step in inventorisation was to map each identified nanopigment to its known uses. A definitive branded product could be linked to a specific nanopigment use in only a small number of cases. These were included in the master inventory list. However, since pigments – because of their specific function - have a narrow set of uses, it was feasible to map them to the declared lifecycle uses of their bulk forms as declared in the REACH registrations.

Therefore, for each of the 81 identified nanopigments (including the 4 extender pigments), the public REACH dossiers were scanned for lifecycle uses and Product Categories (PCs) so as to be mapped as shown in Appendix 2 (consumer uses) and Appendix 3 (Professional uses). Not all identified nanopigments are as yet REACH registered, therefore these lists contain fewer substances than the main inventory list. Further, not all pigments have both Professional and Consumer uses.

The results are largely as expected. For most of the identified pigments uses are mainly and consistently declared in Paints and Coatings, Ink and Toners, and polymers / rubbers. Other uses identified are in textiles, personal care products/cosmetics, with minor uses in leatherware, adhesives and cleaning products.

Notable was that some registrants will declare all Product Categories as a rule, whether realistic or not, and this presents some difficulties when assigning correct uses. Nevertheless, the standard uses of pigments as colourants emerges as a clear pattern as evidenced by the Product Categories declared.

2.4 Uses of Nano-sized pigments in Consumer and Professional products

Pigments provide a wide range of colouring applications in products such coatings, decorative and protective paints, plastics, printing inks, ceramics, candles, paper products, pharmaceuticals, rubber materials, cements, abrasives, soaps, textile fibres, foodstuffs, decorative cosmetics, and sunscreens.

As discussed in section 1, many pigments used for various applications can incidentally contain a fraction with a median size below 100 nm. Practically, for most applications, pigments will agglomerate and aggregate to form larger particles in dispersion so that the paints, for example, will mostly have particles with a median size well above the 100 nm.

However, for some applications, it is desirable for the pigments to be present in smaller particle sizes and the dispersed particles have a particle size distribution closer to the particle size distribution of the primary particles. These 'nano-enabled' products would specifically use pigments with a median particle size below 100 nm.

It is generally not possible to distinguish between incidental and nano-specific uses, unless these are declared by the product manufacturer. Moreover, under the REACH descriptor system there is, as yet, no possibility to define nano-specific uses.

Referring to the inventory mapping of Professional and Consumer uses presented in Appendices 2 and 3, it can be seen that, when publically available, most declared uses of pigments fall under defined REACH Product Categories (PC), with nearly all pigments being declared for use in coatings and paints (PC9a), inks and Toners (PC18) , and in polymers (PC32).

Other commonly declared product categories are Fillers and putties (PC9b), Finger Paints (PC9c), Leather Treatment (PC23), Paper/Board treatment (PC26), Polishes and Waxes (PC31), cleaning products (PC35) Textiles (PC34), and cosmetics and personal care products (PC39). Food and food contact applications, though outside of REACH scope, are also indicated.

Service life activities are also declared for most pigments, in that they may be subject to grinding and abrasion processes, for example through sanding of painted surfaces, or through milling of plastics, where exposure to dry particles is possible. In such cases, individual nano-sized paricles are likely to remain matrix-bound in larger particles. These are further

elaborated in the Professional uses under PROC 21 and PROC 24 (Low/High energy manipulation of substances bound in articles).

Professionals users can come in contact with dry pigment powders and fillers during the formulation of custom batches. Consumers will generally not come in contact with dry pigment powders except in special cases, in particular artistic uses where custom colour combinations may be desirable. In such cases there is a concern for potential inhalation exposure of airborne particles, some of which may be in the nano range.

In the majority of cases, however, pigments will be supplied in pre-prepared liquid dispersions, or in a ready-to use product such as a paint, polymer or cosmetic product. In these cases the pigments are already dispersed in a liquid or solid matrix, dramatically lowering the potential for inhalation exposure to particles.

Paints and Coatings
One scenario where liquid-bound particles may still be inhaled is through the use of spray paints and coatings, where aerosols have the potential to reach the lower lung, carrying particles in the droplets. This situation may arise commonly in professional workshops, for example in automotive repair centres where coatings may be re-touched. In such cases the use of adequate occupational control measures is required, including the use of spray extraction hoods, respiratory and skin protection. However consumer use of spray paints is also common, leading to wide potential for skin and inhalation exposure to aerosols.
Further service life of paints and coatings can also lead to the possibility of inhalation exposure from dusts generated through abrasion / sanding.

Transparent coatings represent a case where nano-sized pigments are intentionally used in a product, where the underlying material should still be visible through the coating. For example, the use of nano-sized TiO_2 for metallic effect paints allows for greater light-scattering when used in combination with metallic pigments.

Nano-sized primary pigments and extender pigments also confer additional desirable properties, for example anti-scratch and anti-corrosion coatings as commonly used in automotive paints.

Finger, face paints and Toys (including modelling wax)
Uses of pigments in these applications outwardly presents a concern in that children are likely to be exposed, principally by skin contact, but also by unintended oral exposure by mouthing and ingestion.

Ink and Toners
Pigments used in inks are required to have a small particle size diameter in order to offer the highest image resolution. Many manufacturers therefore market pigment grades specifically indicated to be in the nano-range, particularly for ink-jet applications where transparency and high colour strength is desirable.

Use of nano-sized pigments in printing toners presents potential for professional and consumer exposure, as particles are emitted during use. Evidence shows that exposures can vary from small amounts of particles up to more than a million of particles/cm^3. This presents a concern for professional office workers and consumers who may inhale the resulting emissions.

Nanographic printing is a specialised application that uses a heated blanket onto which 'nanoink' particles are ejected, forming a thin layer (500 nm thick) as the solvent (water) dries. This is then transferred to the substrate (paper, plastic, etc.) by an impression cyclinder. The potential for release of nanoparticles during this process is very low.

Nanomaterials such as TiO_2 and colloidal and fumed silica may be used for coating of ink jet papers, allowing better adhesion of ink jet inks to the surfaces of glossy paper for example.

Polymers and Rubbers
Pigments are widely used to colour plastic and rubbers. Carbon black is widely used in rubbers to provide colour, but its main function is to provide rubber reinforcement, where the main use is in tyres. During the use life of a tyre, abrasion will cause significant loss, but evidence suggest that this is in the form of non-respirable rubber particles that remain bound in the matrix.

Textiles
Pigments are used in textile printing, primarily using digital inkjet printing technology which is similar to standard inkjet printing.

Food and Food Contact Uses
Uses in food leads to clear possibility of oral exposure to nanoparticles where these are used – the most common case being titanium dioxide - an approved food colourant (E171) which is used to give whiteness and opacity to foods, but can also provide abrasive effects, for example in chewing gums marketed for teeth-whitening effects. However, nanoforms are currently not specifically approved for use as food colourants, although their presence is known to be incidental, as in the case of TiO_2 in chewing gums.

Cosmetic Uses
Cosmetic uses of pigments lead to principally skin exposure to products containing nano-sized pigments, for example in lipsticks and skin creams.
Nano-sized titanium dioxide and zinc oxide pigments are commonly used in cosmetics sunscreens to absorb UV radiation for the purposes of skin protection. Nano-sized titanium dioxide in particular is efficient at absorbing and scattering both UVA and UVB rays, providing protection against all aspects of UV light that can affect skin, and it is also transparent. Nano silver and nano gold are also declared as colorants in the EU catlogues of nanomaterials used in cosmetic products.

Tattoo Inks – a special case
Tattooing introduce pigments and dyes in the dermis by puncturing the skin. Only seven Member States already have national legislation on tattoos based on a Council of Europe Resolutions ResAP (2008)1 and ResAP (2003)1) on requirements and criteria for the safety of tattoos and permanent make-up (ECHA, 2017a). In many EU countries, no legislative requirements are set for tattoo dyes at all (reviewed in a JRC report - Piccinini et al., 2015a). Pigments used in tattoo and permanent make-up (PMU) products are not specifically produced for such purposes. They are primarily manufactured for textile, car or plastic applications, and generally lack high purity standards (Piccinini et al., 2015b). Carbon black is the most used pigment in tattoo and PMU inks (Piccinini et al., 2015b). Thus tattoo pigments may contain levels of impurities that are not appropriate to be injected into humans (ECHA, 2017a). More than 100 colourants and additives are in use in tattoo inks according to a JRC report with numerous impurities (Piccini et al., 2016). The absence of appropriate data for ink composition, information on the intrinsic properties of some components/impurities hamper the risk assessment (Laux, et al., 2016).

Niederer et al. (2018) performed a market survey in Switzerland to identify pigments used in tattoo. Samples were collected from tattooing and permanent make-up studios and from different importers in the course of 2009-2017. Out of 396 tattoo inks and 55 PMU inks, four prohibited pigments (Pigment Green 7, Pigment Red 122, Pigment Violet 19 and 23) were found. It was shown that banned pigments are rarely declared, but rather masked by listing non-present legal pigments and label forging. Results of this survey highlights the urgency of widespread market controls (Niederer et al., 2018).

Other source of health risk of tattoos is related to presence of toxic contaminants. These can originate from degradation of the tattoo ink (e.g. aromatic amines formed by reductive

cleavage), or be present as impurities derived from the processing of the pigments. Screening of about 300 ink samples in Italy showed that about 40% of the monitored inks are not regular according to European Resolution ResAP(2008) (Forte et al., 2009). In general, purity of tattoo inks is not very high (between 70 and maximum 90%, depending on the source) (Piccinini et al., 2015b).

The contaminants of main concern in tattoo inks are substances such as identified carcinogens and skin sensitisers:

Polycyclic aromatic hydrocarbons (PAHs)
PAHs may be generated during production of carbon black. The PAHs concentration in black tattoo inks ranges from < 1 to more than 200 µg/g ink as shown in analyses of various commercial inks performed by Regensburger et al. (2010). PAHs have been linked to carcinogenesis, skin sensitization, skin photosensitivity, and immunotoxicity. PAHs can also act as photosensitizers, generating singlet oxygen under light exposure. However, adverse health effects of PAH contamination of tattoo inks have not been studies are bioavailability of particle-adhered PAHs in inks may be limited (Jacobsen et Clausen, 2015). Lehner et al. (2014) identified PAHs from black tattoos in regional lymph nodes.

Heavy metals
Nickel was detected in every ink out of 58 inks studied (mostly in small amounts and exceptionally also in higher concentrations) (Jacobsen et al. 2012). Nickel is a contact allergen with the highest sensitisation rate. People with a nickel allergy can therefore develop a serious skin disease following tattooing with nickel contaminated inks.

Aromatic amines
Primary aromatic amines are used in the synthesis of azo colorants. Azo colorants can be converted back to their initial reagents by reductive cleavage. O-anisidine was detecte(d in black ink (Lehner et al., 2011a). Aromatic amines can have carcinogenic, mutagenic, toxic for reproduction and sensitizing effects.

Other toxic substances detected in tattoo inks include: Trichlorobenzene, phenol (up to 385 µg/g; Regensburger et al., 2010), phthalates (0.12 to 691.2 µg/g in black inks; Lehner et al., 2011a) that are toxic for reproduction. Lehner et al. (2011a) detected in black inks: hexachloro-1,3-butadiene (0.08–4.52 µg/g), methenamine (0.08–21.64 µg/g), dibenzofuran (0.02–1.62 µg/g), benzophenone (0.26–556.66 µg/g), and 9-fluorenone (0.04–3.04 µg/g). These impurities may exhibit various toxic effects, e.g. carcinogenic, sensitizing, irritating. Some of the contaminants are known carcinogens and allergens.
In addition to impurities, tattoo inks contain a wide range of **additives**, such as surfactants (to promote dispersion and stabilization), thickening agents, binding agents, thixotropic agents (to inhibit the sedimentation of pigment dispersions during long term storage), preservatives, solvents and fillers (Piccinini et al., 2015b).

The presence of nanoparticles in the tattoo inks was investigated by Høgsberg et al. (2011) using laser diffraction, electron microscopy. Analyses of 58 typical tattoo inks with samples of red, blue, green, yellow, white and black from 13 different manufacturers showed that the vast majority of the tested tattoo inks contained significant amounts of NPs except for the white pigments, in which the smallest primary particle size was approximately 100 nm. The black pigments were almost pure NPs. After tattooing, carbon black nanoparticles form µm-size aggregates. Using atomic force microscopy, aggregated ink nanoparticles were observed in close proximity to collagen fibrils in cryo-sections of tattooed skin (Grant et al., 2015).

According to a study by Engel et al. (2008), the concentration of pigments in freshly tattooed skin ranged from about 0.60 to 9.42 mg/cm2 (mean value 3.2 mg/cm2; 75th percentile chosen to reflect a worst case situation = 3.59 mg/cm^2) for ink containing 25% Pigment Red 22. Injected amount of ink was estimated to be 14.36 mg/cm2 (75% percentile, 25% pigment in the ink) (Engel et al., 2008; ECHA, 2017a). The typical maximum area of a full colour tattoo

that can be made in one session (in one day) is estimated to be 300 cm^2 (ECHA, 2017a). Thus the quantity of pigment soon after tattooing a maximum size tattoo at a single tattoo session would correspond to approximately 960 mg pigment and 4 308 mg ink.

After injection of tattoo ink in the skin, pigment is embedded in the dermis among collagen fibres. Quantity of pigment in the skin decreases over time with only 1-13% remaining in the skin after several years (Lehner et al., 2011a). A part of the pigment may leave the skin with the bleeding during or directly after tattooing. Shortly after tattooing, pigments are also distributed to the lymphatic system and then stored permanently in the regional nodes (Bäumler, 2015). Schreiver et al. (2017) provided analytical evidence of tattoo particles being distributed inside the human body with smaller (nano)particles transported to the lymph nodes. The exact size limit preventing this translocation is unknown yet. In general, processes that decrease particle size promote reduction of the pigment concentration in the skin as larger pigment particles do not pass the lymph nodes. Disintegration due to light-induced decomposition of pigment, and possibly also enzymatic activities or recurring activities of the macrophages contribute to the transport of pigment particles (Bäumler, 2015). As mentioned above, modern-day tattoo inks contain nanoparticles (Høgsberg, et al., 2011; Grant et al., 2015). The extent to which pigment containing nanoparticles may reach internal organs and lead to clinical symptoms has not been studied and is unknown (CHDP, 2015) Pigment nanoparticles may reach the blood circulation and become distributed to several organs, in particular the liver. Sepehri et al. (2017) investigate the systemic distribution of tattoo pigments in extensively tattooed mice. Using TEM, they identified intracellular tattoo pigments in the skin, in lymph nodes and also in the Kupffer cells (liver macrophages) 1 year after tattooing, indicating systemic distribution of tattoo nanopigments (carbon black and red azo pigments). Due to the migration of the chemicals and particles through the body, systemic adverse health effects should be taken into account. Over the months or years after tattooing pigments may be degraded (in particular upon sun exposure) (Baumler, 2015; Serup, 2017). The major part of tattoo colorants (up to 99% in red pigments) disappears from skin months or years after tattooing due to decomposition and transport (Lehner et al., 2011b).

Pigment degradation may be a long-term source of low amount of potentially hazardous compounds. The degradation process is intensified by light exposure. Moreover, light exposure has been shown to increase production of reactive oxygen species (ROS) from inks components and impurities (e.g. PAHs might generate singlet oxygen under UV irradiation). Allergens causing tattoo reactions can probably be formed inside the dermis through a process of haptenization, which can be slow and develop after weeks, months, or years (Serup et Carlsen, 2014).

Accelerated degradation and decomposition of the ink in the skin is used to remove unwanted tattoos. The removal is performed by laser irradiation causing pigment crystals to superheat and break into fragments which are washed away by lymphatic transport (Ferguson et al., 1997). Laser treatment-induced break down of carbon black particles generated nanoparticles with the mean diameter of 6 to 50 nm (Piccinini et al., 2015b). Laser irradiation of red pigment causes release of substantial amounts of carcinogenic aromatic amines into the body (Kent et Graber, 2012).

The real incidence of adverse tattoo reactions is currently unknown. However, surveys have shown high incidence of tattoo-related complications. In a survey carried out in German-speaking countries (Klugl, et al., 2010), about 68% of tattooed people reported skin problems and 6.6% reported systemic reactions after tattooing. Health problems persisted in 9% of tattooed people several weeks after tattooing and 6% reported persistent health problems. Reactions can appear months or years after the tattooing, which is a long period of sensitisation induction. Although the exact mechanism has not been elucidated yet, the delayed complication indicate that intradermal deposit of tattoo pigments results in lifelong exposure and can potentially have a negative effect on human health (Laux et al., 2016). In a EU report (Piccinini et al., 2016), adverse effects related to tattooing were reviewed. In summary, the majority of complications are inflammatory, allergic reactions and poorly

understood coincidental diseases implying autoimmunity.

Impurities may account for some of the observed effects, e.g. ROS production from PAHs upon sun exposure is a possible mechanism of photosensitivity (Bickers and Athar, 2005).

Nanoparticle can be the cause of various adverse effects of tattooing. Carbon black nanoparticles aggregates and agglomerates and clusters of several microns have been observed in the dermis. Such large agglomerates are recognized as foreign bodies by immune cells. Immune response may result in granuloma formation in the tattooed site and even trigger sarcoidosis in other organs, supposedly by an autoimmune mechanism (Kluger, 2013). Pigment particles accumulated and agglomerated in lymph nodes may also cause swelling and inflammation of the node (Serup, 2017).

Photosensitivity is a common side-effect of tattooing. Photochemical reactions to pigment or pigment-breakdown products in situ in the skin with can lead to generation of ROS. Nanoparticles in tattoo inks may cause the formation of highly reactive molecules via a number of mechanisms. Activation of immune cell during (nano)particle phagocytosis trigger ROS production. Surface defects on particles may be involved in the catalysis of ROS, with smaller particles producing more ROS due to high surface area. Nanoparticles adsorb organic chemicals on their surface which in the presence of biological reductants may be involved in redox cycles and generation of radicals (Jacobsen et Clausen, 2015).
ROS production can lead to oxidative stress which can cause damage to biomolecules including DNA. Based on a literature review, Kluger and Koljonen (2012) stated that no direct cause/effect relationship could be established between tattoos and reported skin tumours, and the occurence seems to be coincidental. However, the large-scale studies investigating the association between tattoos and (skin) tumours are not available (Kluger and Koljonen, 2012) and direct causal relationship between tattooing and (skin) cancer has been so far neither proved nor excluded (Piccinini et al., 2016).

3. Primary Literature Review

3.1 Methodology

The major task of this project was to perform a review of data in the primary literature relating to hazards and exposure to nano-sized pigments. All performed literature searches have been documented transparently to allow full reproducibility. The literature search methodology is described in detail in Appendix 6 of this report.

The following chemical, biomedical and multidisciplinary citation databases and indexing services were used for the literature searches:

- Chemical Abstracts (SciFinder) (http://www.cas.org/products/scifinder)
- Web of Science (http://webofknowledge.com/WOS)
- Scopus (http://www.scopus.com/)
- PubMed (http://www.ncbi.nlm.nih.gov/pubmed)

All retrieved references with abstracts were extracted to a specially-developed reference management system.

As a starting point in collecting published literature, a broad general search for chemicals used as pigments in nanoforms was conducted (SEARCH 1), using the combination of keywords *nanopigm* OR (nano* AND pigm*)*. After removal of duplicates, the searches in the databases listed above yielded **5,866 articles.**

Titles and abstracts of the retrieved references were screened to determine eligibility for acceptance. When a reference was found to satisfy the inclusion criteria, it was marked as 'Accepted' and then categorised according to the subject matter.

After the initial scan of the abstracts, the appointed project experts in Toxicology, Exposure/Risk assessment and characterisation conducted a second review of accepted abstracts to cross-check and verify their acceptance and categorisation.

To complement this general search, a modified search (SEARCH 2) was performed for the most common nanopigment types (the most commonly studied in scientific literature) identified in SEARCH 1. During the screening of titles and abstracts in SEARCH 1, the reviewers recorded nano-sized substances occurring in the title and/or abstract as the basis for subsequent SEARCH 2. For these searches, names or CAS numbers of individual chemicals or groups of chemicals were used in combination with keywords indicating adverse effects or keywords typical in toxicological or epidemiological studies. In particular, the keywords terms listed in Table 2 were used:

Table 2: Material-specific search terms used in Search No. 2.

Substance(s)-related keywords	Keywords for toxicity searches	Keywords for epidemiology searches	General limiting keywords
TiO_2, titanum dioxide, 13463-67-7	(tox* OR advers* OR poison* OR damag* OR acut* OR chronic* OR repeated*) AND (vivo OR vitro)	epidemi* OR cohort* OR case-control* OR "correlation study" OR "correlation studies" OR "intervention study" OR "intervention studies" OR "randomized trial" OR "randomised trial" OR "case report" OR "case reports" OR "case series" OR "cross-sectional"	nano*
ZnO, zinc oxide, 1314-13-2			
Carbon black, 1333-86-4			
BaS, barium sulfate, barium sulphate, 7727-43-7			
Fe_2O_3, ferric oxide, 1309-37-1			
$CaCO_3$, calcium carbonate, 471-34-1			
spinel*			

carboxamide*			
dione*			

Note: The "*" symbol stands for a wildcard character used in the searches to capture groups of substances which were commonly occurring in nanomaterial inventories (French, Belgian).

This yielded a total of **3,903 references** after duplicates were removed.

For SEARCH 2, other substances (not listed in Table 2) encountered in the papers screened within SEARCH 1 were considered. In particular, the following substances encountered in the SEARCH 1 were considered for further investigation in SEARCH 2:

- Co(2)+-doped [alumina/titania nanoceramic pigments.]
- Graphene oxide
- Chromium(III) oxide
- Sericite
- $CoAl_2O_4$ (cyan), Au (magenta), $(Ti,Cr,Sb)O_2$ (yellow) and $CoFe_2O_4$ (key)
- Al-doped $BiFeO_3$ coated mica-titania
- Cerium oxide
- Co_2-xMxTiO_4 composite oxide nanoparticles
- calcium iron oxide [$(CaFe_2O_4)$
- Attapulgite/bismuth yellow hybrid pigments
- Prussian blue-polyaniline:polystyrene sulfonate (PB-PANI:PSS)
- metal-free phthalocyanine pigment (Pc)
- $CoCr_2O_4$
- CaO-doped and SiO2-coated CeO_2
- Oxotitanium phthalocyanine (TiOPc)

After piloting the search strategy for SEARCH 2, the above listed substances were not included in SEARCH 2 at the end for one of the following reasons:

a) pilot search in Web of Science produced no relevant hits
b) there was no indication that the substance was used as a pigment in its nanoform marketed in the EU

It was noted that SEARCH 2 collected a large number of references to toxicological studies on nanomaterials, and that many of these would be irrelevant to the project. Other irrelevant studies collected in SEARCH 2 in large numbers were related to photosynthetic pigments.

Particular emphasis was placed on extracting studies indicating exposure or epidemiological data, which are more directly relevant.

Selection of toxicological data proved more challenging. After discussion with the ECHA project team, it was agreed to prioritise those studies that would be fit for regulatory submission, i.e. studies aligning with the Standard information requirements for REACH, for example acute, subacute and subchronic toxicity studies in vertebrates (via oral, inhalation or dermal routes), as well genotoxicity, sensitisation and other studies deemed relevant. It was noted that, in some cases human toxicokinetic data were available, specifically in relation to use of pigments in tattoo inks.

As an extension of WP2, two further systematic searches (SEARCH 3 and SEARCH 4) were performed to capture any residual literature on additional pigments identified in the nano inventory.

Similarly to Searches 1 and 2, scientific literature dealing with the substances listed in the following Table 3 was searched for in Web of Science, SCOPUS and SciFinder (SEARCH 3) with nano* as a limiting keyword (see Appendix 6 for details on the search methodology). These substances were identified to be used as pigments in their nanoform and marketed in the EU based on the data from the Danish, French and Belgian nano-registries. To make sure all relevant literature was captured, substances used in SEARCH 3 were compiled from the Danish, French and Belgian nano-registries combined, i.e. if the substance appeared in at least one of these registries, it was included in SEARCH 3 (opposed to substances listed in Appendix 1, where the substances were considered only if appearing on at least two of the inventories).

SEARCH 4 then captured all references indexed in SciFinder with Chemical Abstracts concept heading "Pigments" and nano* used as a keyword in the search.

SEARCH 3 returned **1,372 citations** while SEARCH 4 returned **471 results**.

In total, 11,602 abstracts were scanned across all searches 1-4.

Table 3: List of substances used in additional literature searches for newly identified pigments (SEARCH 3)

CAS	Name
1345-16-0	Cobalt aluminate blue spinel
14059-33-7	Bismuth vanadium tetraoxide
101357-19-1	Benzenamine, N,N-dimethyl-, oxidized, molybdatetungstatephosphates
1047-16-1	5,12-dihydroquino[2,3-b]acridine-7,14-dione
1325-87-7	Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, molybdatetungstatephosphate
1328-53-6	Polychloro copper phthalocyanine
2512-29-0	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide
147-14-8	29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper
15793-73-4	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]

2512-29-0	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide
2786-76-7	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide
2814-77-9	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol
2465-29-4	3,6-bis(methylamino)xanthylium chloride
3468-63-1	1-[(2,4-dinitrophenyl)azo]-2-naphthol
3520-72-7	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]
35636-63-6	Dimethyl 2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]terephthalate
36888-99-0	5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid
6471-50-7	4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide
4424-06-0	Bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione
5468-75-7	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]
67892-50-6	barium bis[2-chloro-6-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]
5280-66-0	Manganese, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex
5280-68-2	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide
82199-12-0	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(2-methoxyphenyl)azo]-3-oxobutyramide
5567-15-7	Pigment Yellow 83
6041-94-7	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide
61847-48-1	Methyl 4-[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[(2-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]benzoate
75627-12-2	Xanthylium, 3, 6-is(ethylamino) 9-[2-(methoxycarbonyl) phenyl] - 2, 7- dimethyl- , molybdatesilicate
6358-30-1	8,18-dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine
6358-31-2	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide
6410-32-8	3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide

6041-94-7	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide
5280-68-2	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide
6486-23-3	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide
6535-46-2	3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide
67989-22-4	benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-, N-Me derivatives, molybdatephosphates
8007-18-9	Antimony nickel titanium oxide yellow
10101-66-3	Ammonium manganese(3+) diphosphate
12656-85-8	Lead chromate molybdate sulfate red
12737-27-8	Chromium iron oxide
68186-85-6	Cobalt titanite green spinel
68186-87-8	Cobalt zinc aluminate blue spinel
68186-90-3	Chrome antimony titanium buff rutile
68186-91-4	Copper chromite black spinel
68187-11-1	C.I. Pigment Blue 36
58339-34-7	Cadmium sulfoselenide red
68187-40-6	Olivine, cobalt silicate blue
68187-49-5	cobalt chromite green spinel
68187-51-9	Zinc ferrite brown spinel
68187-54-2	tin antimony grey cassiterite
68412-74-8	cobalt zinc silicate blue phenacite
101357-30-6	Silicic acid, aluminum sodium salt, sulfurized
57455-37-5	C.I. Pigment Blue 29
102184-95-2	Silicic acid, zirconium salt, cadmium pigment-encapsulated
1103-38-4	Barium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate]
1324-76-1	[[4-[[4-(anilino)phenyl][4-(phenylimino)-2,5-cyclohexadien-1-ylidene]methyl]phenyl]amino]benzenesulphonic acid
1325-75-3	Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, molybdatetungstatephosphate

1326-03-0	Xanthylium, 9-(2-carboxyphenyl)-3,6-bis(diethylamino)-, molybdatetungstatephosphate
1326-04-1	Xanthylium, 3,6-bis(diethylamino)-9-[2-(ethoxycarbonyl)phenyl]-, molybdatetungstatephosphate
1503-48-6	Quino[2,3-b]acridine-6,7,13,14(5H,12H)-tetrone
2379-74-0	6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one
2387-03-3	1-Naphthalenecarboxaldehyde, 2-hydroxy-, 2-[(2-hydroxy-1-naphthalenyl) methylene]hydrazone
2425-85-6	1-(4-methyl-2-nitrophenylazo)-2-naphthol
3049-71-6	2,9-bis[4-(phenylazo)phenyl]anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone
3089-17-6	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione
3564-22-5	3-hydroxy-4-[(4-methyl-2-nitrophenyl)azo]-N-(3-nitrophenyl)naphthalene-2-carboxamide
3905-19-9	N,N'-phenylene-1,4-bis[4-[(2,5-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]
4051-63-2	4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone
4216-01-7	7H-Benzo[e]perimidine-4-carboxamide, N-(9,10-dihydro-9,10-dioxo-1-anthracenyl)-7-oxo-
4216-02-8	Bisbenzimidazo[2,1-b:1',2'-j]benzo[lmn][3,8]phenanthroline-6,9-dione
4378-61-4	4,10-dibromodibenzo[def,mno]chrysene-6,12-dione
4531-49-1	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methoxyphenyl)-3-oxobutyramide]
4948-15-6	2,9-bis(3,5-dimethylphenyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone
5045-40-9	3,3'-[(2-methyl-1,3-phenylene)diimino]bis[4,5,6,7-tetrachloro-1H-isoindol-1-one]
5160-02-1	C.I. Pigment Red 53, barium salt
5280-80-8	3,3'-[(2,5-dimethyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(5-chloro-o-tolyl)benzamide]
5281-04-9	C.I. Pigment Red 57, calcium salt
5521-31-3	2,9-dimethylanthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone
5580-57-4	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]

5590-18-1	3,3'-(1,4-phenylenediimino)bis[4,5,6,7-tetrachloro-1H-isoindol-1-one]
5979-28-2	N,N'-(3,3'-dimethyl[1,1'-biphenyl]-4,4'-diyl)bis[2-[(2,4-dichlorophenyl)azo]-3-oxobutyramide]
6358-30-1	8,18-dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine
6358-37-8	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-methylphenyl)-3-oxobutyramide]
6358-85-6	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutyramide]
6358-87-8	Diethyl 4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxylate]
6372-81-2	Barium bis[2-[(2-hydroxy-1-naphthyl)azo]benzoate]
6407-75-6	4-[(2,5-dichlorophenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one
6410-26-0	4-[(2-chlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide
6448-95-9	3-hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-phenylnaphthalene-2-carboxamide
6471-49-4	3-hydroxy-4-[(2-methoxy-5-nitrophenyl)azo]-N-(3-nitrophenyl)naphthalene-2-carboxamide
6486-23-3	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide
6528-34-3	2-[(4-methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide
6985-92-8	methyl 2-[[3-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-hydroxy-1-naphthyl]azo]benzoate
10142-77-5	Benzenesulfonic acid, 5-chloro-2-[2-(2-hydroxy-1-naphthalenyl)diazenyl]-4-(1-methylethyl)-, barium salt (2:1)
12224-98-5	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, molybdatetungstatephosphate
12225-08-0	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2,5-dimethoxy-4-[(methylamino)sulphonyl]phenyl]azo]naphthalene-2-carboxamide
12225-18-2	N-(4-chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl]phenyl]azo]-3-oxobutyramide
12236-62-3	2-[(4-chloro-2-nitrophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide
12237-62-6	Ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts

12238-31-2	Manganese, 4-[(4-chloro-5-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex
12239-87-1	Copper chlorophthalocyanine
12286-65-6	Calcium bis[3-nitro-4-[[2-oxo-1-[(phenylamino)carbonyl]propyl]azo]benzenesulphonate]
12768-99-9	C.I. Pigment Orange 42
14154-42-8	Chloro[29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]aluminium
14295-43-3	4,7-dichloro-2-(4,7-dichloro-3-oxobenzo[b]thien-2(3H)-ylidene)benzo[b]thiophene-3(2H)-one
14302-13-7	[1,3,8,16,18,24-hexabromo-2,4,9,10,11,15,17,22,23,25-decachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper
14569-54-1	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-chlorophenyl)-3-oxobutyramide]
15680-42-9	[1-[[(2-hydroxyphenyl)imino]methyl]-2-naphtholato(2-)-N,O,O']copper
15782-05-5	C.I. Pigment Red 48, strontium salt
15790-07-5	C.I. Pigment Yellow 104
15993-42-7	N-(5-chloro-2-methoxyphenyl)-2-[(2-methoxy-4-nitrophenyl)azo]-3-oxobutyramide
16043-40-6	Quino[2,3-b]acridine-7,14-dione, 5,12-dihydro-3,10-dimethyl-
16521-38-3	Aluminium, 2-(1,3-dihydro-3-oxo-5-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid complex
17832-28-9	4-(vinyloxy)butan-1-ol
22094-93-5	2,2'-[(2,2',5,5'-tetrachloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]
27614-71-7	[tetrachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper
29204-84-0	bis[2,3-bis(hydroxyimino)-N-phenylbutyramidato-N2,N3]nickel
29920-31-8	Dimethyl 5-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azoterephthalate
30125-47-4	3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide
31775-16-3	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-methoxyphenyl)-3-oxobutyramide]
31778-10-6	C.I. Pigment Red 208
31837-42-0	2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzoic acid

35355-77-2	Manganese, 3-hydroxy-4-[(1-sulfo-2-naphthalenyl)azo]-2-naphthalenecarboxylic acid complex
37300-23-5	C.I. Pigment Yellow 36
42844-93-9	[1,3-dihydro-5,6-bis[[(2-hydroxy-1-naphthyl)methylene]amino]-2H-benzimidazol-2-onato(2-)-N5,N6,O5,O6]nickel
54660-00-3	Pyrrolo[3,4-c]pyrrole-1,4-dione, 2,5-dihydro-3,6-diphenyl-
59487-23-9	4-[[5-[[[4-(aminocarbonyl)phenyl]amino]carbonyl]-2-methoxyphenyl]azo]-N-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide
61512-61-6	C.I. Pigment Orange 51
61951-98-2	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[5-methoxy-2-methyl-4-[(methylamino)sulphonyl]phenyl]azo]naphthalene-2-carboxamide
65212-77-3	Calcium 4,5-dichloro-2-[[4,5-dihydro-3-methyl-5-oxo-1-(3-sulphonatophenyl)-1H-pyrazol-4-yl]azo]benzenesulphonate
67989-22-4	benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-, N-Me derivatives, molybdatephosphates
68227-78-1	N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide
68511-62-6	Nickel, 5,5'-azobis-2,4,6(1H,3H,5H)-pyrimidinetrione complexes
68512-13-0	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, brominated chlorinated
68610-86-6	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(o-anisyl and 2,4-xylyl) derivs.
68987-63-3	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, chlorinated
71832-85-4	Calcium bis[4-[[1-[[(2-chlorophenyl)amino]carbonyl]-2-oxopropyl]azo]-3-nitrobenzenesulphonate]
71872-63-4	2-Naphthalenecarboxamide, N-[4-(benzoylamino)phenyl]-3-hydroxy-4-[2-[2-methoxy-5-[[[3-(trifluoromethyl)phenyl]amino]carbonyl]phenyl]diazenyl]-
72102-84- 2	C.I. Pigment Orange 64
72639-39-5	nitrophenyl 3-[[2-hydroxy-3-[(2-methylphenyl)carbamoyl]-1-naphthyl]azo]-4-methoxybenzenesulphonate
74336-59-7	3-[(4-chloro-2-nitrophenyl)azo]-2-methylpyrazolo[5,1-b]quinazolin-9(1H)-one
74336-60-0	1-[(5,7-dichloro-1,9-dihydro-2-methyl-9-oxopyrazolo[5,1-b]quinazolin-3-yl)azo]anthraquinone

74441-05-7	C.I. Pigment Yellow 181
78521-39-8	6-[[(4-methylphenyl)sulphonyl]amino]hexanoic acid
78952-72-4	2-[[3,3'-dichloro-4'-[[1-[[(2,4-dimethylphenyl)amino]carbonyl]-2-oxopropyl]azo][1,1'-biphenyl]-4-yl]azo]-3-oxo-N-(o-tolyl)butyramide
79953-85-8	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-[2-(4-chlorophenoxy)-5-(trifluoromethyl)phenyl]benzamide]
82199-12-0	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(2-methoxyphenyl)azo]-3-oxobutyramide
83524-75-8	2,9-bis(p-methoxybenzyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone
84632-50-8	Benzonitrile, 3,3'-(2,3,5,6-tetrahydro-3,6-dioxopyrrolo[3,4-c]pyrrole-1,4-diyl)bis-
84632-59-7	Pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis[4-(1,1-dimethylethyl)phenyl]-2,5-dihydro-
84632-65-5	Pyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis(4-chlorophenyl)-2,5-dihydro-
84632-66-6	Pyrrolo[3,4-c]pyrrole-1,4-dione, 2,5-dihydro-3,6-bis(4-methylphenyl)-
85776-13-2	C.I. Pigment Red 253
85776-14-3	2-Naphthalenecarboxamide, N-(4-chlorophenyl)-4-[2-[2,5-dichloro-4-[(dimethylamino)sulfonyl]phenyl]diazenyl]-3-hydroxy-
85958-80-1	[[3-[1-cyano-2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1H-isoindol-1-ylidene](salicylic)hydrazidato(2-)]nickel
85959-60-0	N-(p-chlorophenyl)-2-cyano-2-[2,3-dihydro-3-[tetrahydro-2,4,6-trioxo-1-p-tolylpyrimidin-5(2H)-ylidene]-1H-isoindol-1-ylidene]acetamide
90268-23-8	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(p-anisyl and Ph) derivs.
106276-80-6	Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with p-phenylenediamine and sodium methoxide
215247-95-3	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene
12240-15-2	C.I. Pigment Blue 27

Additional searches were also subsequently carried out based on expert judgment to collect further information relevant for this report (e.g. reviews of national and international authorities, published general reviews on toxicity of nanomaterials, etc.).

3.1.1 Study selection

For the screening of titles and abstracts and subsequent study selection, clear inclusion and exclusion criteria were set a priori (based on pilot screening of retrieved literature) in order to harmonise approaches of individual reviewers participating in the study selection phase. The inclusion and exclusion criteria for study selection are listed in the following Table 4.

Table 4: Inclusion and exclusion criteria for selection of relevant studies.

Inclusion criteria	Exclusion criteria
Study Type	
Studies referring to nanoscale chemicals which are known to be used as pigments (see the description how chemicals known to be used as pigments will be identified in WP1.	- Studies referring to other chemicals (not known to be used as pigments) - Studies referring to chemicals known to be used as pigments, but dealing with their ”standard“ form, not their nano-form
Exposure	
Studies referring to exposure to nanoscale chemicals which are known to be used as pigments.	- Studies referring to exposure to other chemicals (not known to be used as pigments) - Studies referring to exposure to chemicals known to be used as pigments, but dealing with their "standard“ form, not their nano-form
Outcome	
Any effects caused by nanopigments (or chemicals used as pigments in their nano-form) or linked to exposure to nanpigments are included.	No limitations (exclusion criteria) related to outcome are proposed.
Study design	
Any study design with nanopigments (chemicals used in their nano-form as pigments) as tested material or material to which the target was exposed (animal study, human observation study, in vivo study, in vitro study)	*In silico* studies
Geography	
Studies from all countries are eligible	
Recency	

All studies are eligible regardless of the publication date. Quality and reliability to be taken into account when old studies are involved, especially when they report conflicting results.	
Language	
The search strategy was tailored to searching in English language, however, no languages are excluded	
Publication type	
Primary peer-reviewed studies (articles) - Unpublished research reports of studies carried out for REACH in accordance with OECD TG standard methods and under Good Laboratory Practice	- Secondary and tertiary studies (reviews, factbooks, etc.) - Non-peer-reviewed sources (e.g. patents, communication letters, magazine articles,, etc.)

To minimise the risk of exluding potentially relevant publications, a conservative approach was taken in application of the inclusion/exclusion criteria by the reviewers – i.e. in case of uncertainty that a reference can be excluded it was by default included (accepted) in the stage of screening of titles and abstracts to be further investigated in subsequent steps. As a result, out of the 11,602 publications systematically screened for relevance based on their titles and abstracts, 1,328 were identified as potentially relevant. Out of these, 151 were used in preparation of this report. The others were excluded upon further investigation, due to the the fact that the studies did not meet the inclusion criteria listed in Table 4.

The results of the literature review are presented in Appendix 6.

3.2 Data Quality Review

After scanning and tagging of abstract, expert reviews were conducted. This involved a critical analyses of each paper to assess its quality for inclusion. The methods applied for assessing the quality of literature on toxicology and exposure data are presented in Appendix 8. The results of reviewed literature are presented in the chapters 5, 6 and 7. Studies of insufficient quality (lack of transparency or completeness in the description of the study design, methodology and results were excluded. Each study selected as the basis for risk assessment was detailed in a separate data matrix file.

4. Hazards of Nano-sized pigments

4.1 General Nanoparticle Biokinetics and Toxicity

Most available toxicological data on nanomaterials identified as nanopigments deals with toxicity of one of the following three substances: nano-TiO_2, nano-ZnO and carbon black, with nano-TiO_2 being the most often tested nanoform. Regarding the high number of publications on toxicity of nano-TiO_2, nano-ZnO and carbon black, it was necessary to narrow the selection of the papers that are included in the literature review to those that are most relevant for risk assessment.

Information on most relevant endpoints for risk assessment (repeated dose toxicity, acute toxicity, mutagenicity, genotoxicity, carcinogenicity, skin irritation/corrosion, skin sensitization, eye irritation/corrosion, phototoxicity, reproductive & developmental toxicity, immunotoxicity) is lacking for nanopigments, except for nano-TiO_2, nano-ZnO, nano-silica, and carbon black. However, some general observations are valid for insoluble nanoparticles such as pigments.

Nanomaterials exhibit considerable toxicokinetic differences from molecules in the bulk form. The kinetics of the tissue distribution of nanomaterials is largely determined by the nanoparticle uptake in macrophages and other tissue cells, less by the transport by blood flow or by diffusion into tissues.

Once nanomaterials enter a biological environment, biomolecules immediately adsorb to their surface (formation of so called biocorona) which influences their tissue distribution (Monopoli et al., 2012). Different nanomaterial-protein complexes may have different biokinetics, including the potential translocation across biological membranes (Nel et al., 2006). Adsorption of biomolecules depends on characteristics of both the nanomaterials (mainly on surface properties – surface charge, hydrophobilicity/hydrophilicity; and also on the size and shape of nanomaterials) and the biological environment (exposure route determines the biomolecules present at the site of entry) (McClements et al., 2015).

Based on the exposure route and deposition site, nanomaterials can be cleared by various mechanisms. After inhalation, (nano)particles deposited in the conducting airways are removed by mucociliary transport by means of the cilia and the lung lining layer. Consequently, they can be eliminated from the body by coughting and sneezing or swallowed. From the alveolar region (where mucocilliary transport is not present), nanomaterials are removed by alveolar macrophages which then migrate to the tracheobronchial region and got cleared by mucociliary clearance or to mediastinal lymph nodes. Nanomaterials that were not phagocytosed by macrophages, can migrate to the epithelium and translocate into either the blood circulation or the lymphatic system (Kreyling et al., 2013; Geiser et Kreyling, 2010). After oral exposure, most nanomaterials are trapped in mucous linig in intestine and removed into feces by the continuous renewal of the mucus (des Rieux et al., 2006).

Nanoparticles absorbed into the body (i.e. reached the blood circulation after oral, inhalation, less probably dermal exposure) are cleared through three main pathways: hepatobiliary, renal, and mononuclear phagocyte systems. Clearance through the kidney is mostly restricted to very small nanoparticles (less than 6 nm in size) and their elimination is generally fast, within hours to days after administration. Larger-sized nanoparticles are more likely to be taken up and retained by the tissue macrophages or monocytes in blood (mononuclear phagocyte system). Then the internalized nanomaterials may be degraded intracellularly or they will remain within the cells, accumulate in tissues and organs such as the spleen, lymph nodes, bone marrow, and particularly in the liver. Eventually they may be sequestered in the spleen and liver. In the hepatobiliary clearance pathway nanomaterials are excreted through liver into the bile and feces. This process is usually slow, ranging from hours to months or longer (Zhang et al., 2016).

It is known that nanoparticle toxicity heavily depends on their physical and chemical properties, such as the shape, size, aggregation status in the relevant media, crystalline structure, surface electric charge, chemical compositions of the core and shell (surface coating), and purity/contaminants. These properties govern the ability of nanoparticles to enter biological systems, distribute in the bloodstream and lymphatic system, and penetrate into cells, tissues, and organs. Therefore, as a minimum, a standardized assessment of the physicochemical properties above should be investigated prior to the toxicity testing of nanomaterials (Warheit et al. (2008).

Crystalline structure: Crystalline structure may influence other properties of the material (e.g. reactivity, zeta potential) in a way that affects human and environmental toxicity.

Size: Nanomaterial size affects other physicochemical parameters, such as zeta potential, specific surface area, reactivity. It may determine whether the nanoparticle can be internalised into an organism and how it is distributed within the body, as well as inside a cell. Increased specific surface area, reactivity and transport with decreasing size of nanomaterials are considered as factors explaining higher toxicity of smaller particles.

Phagocytic clearance of nanoparticles is less efficient than clearance of fine particles of the same material. This lower efficiency is likely to lead to volumetric overload of macrophages (Pauluhn, 2009). In the context of lung overload, the size was discussed linked to surface area, which is an important dosimetric for particle-induced pulmonary inflammatory effects showing good correlations with responses. Direct cytotoxic effects resulting from the greater surface area and therefore higher reactivity cannot be ruled out (Borm et Kreyling, 2004). However, experimental data are not consistent and decreasing particle size does not necessary determine increased toxic effects. The situation is further complicated by a higher tendency of smaller particles to aggregate/agglomerate and numerous factors affecting this process (such as pH and composition of media for their dispersion, presence of proteins and other biomolecules, nanomaterial surface properties, concentration, etc.). Particle size distribution is a dynamic property that changes based on actual conditions in which the nanomaterial is present. Prevailing experimental data indicate higher toxic potential of nanoparticles as compared to their microsized counterparts. Dose metric may play an important role in the interpretation of the results. It has been reported that in low soluble particles (i.e. TiO_2), surface area is more appropriate metric for characterization of the dose-response relationship (Oberdorster et al., 2005).

Shape: Particle shape may affect the internalisation of nanomaterials, deposition and persistence within the lungs, translocation into the other organs. Shape may also influence mode-of-action, especially in high aspect ratio materials. It is hypothetized that biopersistent fibres may exhibit asbestos-like toxicity (Poland et al., 2008).

Surface modification (coating, functionalization): nanomaterials can be coated to change, enhance or reduce certain characteristics. Surface modification(s) may determine which biomolecules adhere to the nanomaterials, their distribution and cellular uptake, and their toxic effects.

Impurities and contaminants: Nanomaterials can contain impurities derived from the production process, adsorbed chemicals on the particle surface or biological contaminants, such as lipopolysaccharide.

Lipopolysaccharide is a heat-stable molecules from the outer membrane of Gram-negative bacteria that act as a stronger activator of innate immune cells. It can be a misleading factor especially in immunotoxicological studies, as its adsorption can be overlooked and it can trigger strong innate immune response (Bianchi et al., 2015).

There are numerous sources of impurities of nanomaterials originating in their production, the most common arise from use of starting materials for nanomaterial preparation: catalyst,

reactants, byproducts, surface coating agents, surface reaction-generated species, and transformed byproducts of the nanomaterial in biological and environmental media and variations of the intrinsic nanoparticle structures. Contaminants can be introduced even during nanomaterial purification (for which additional chemicals are often used, e.g. acid treatment). Even nanomaterials without any additives may undergo transformations during storage caused mainly by oxygen and light exposure and generate degradation products (Deng et al., 2018). The key characteristics of nanomaterials are their high surface to volume ratio and reactivity. Once nanomaterials enter the environment, they immediately interact with co-existing contaminants, such as organic substances, metal/metalloid ions, organic matter, inorganic ligands, and other nanoparticles. The formation of nanomaterial-contaminant complexes influences their environmental distributions and interactions with organisms, as the bioavailability and toxicity of both nanomaterials and contaminants can be altered (Deng et al., 2017).

Deng and al. (2017), in their compherensive review summarized interactions of nanomaterials and co-contaminants provided evidence that nanomaterials can increase the toxicity of other contaminants by a "Trojan horse mechanism" (introducing adsorbed molecules into the cells,when unabsorbed would not be able to enter the cells), or by altering metabolism of the exposed organism, and disruption of the cell membrane enabling enhanced uptake.

On the other hand, nanomaterials can mitigate the toxic effects of the co-contaminants. The adsorption on nanoparticles can decrease the bioavailable concentration of the contaminant, or they can compete with the contaminant for the binding sites of a receptor, etc. (Deng et al., 2017).

Adsorbed molecules can also mitigate toxicity of the nanomaterial. They can reduce the toxic effect e.g. by decreasing the surface activity or solubility (a known functions of some coatings), or enhance toxicity of the nanomaterial by promoting ROS production. Adsorbed molecules can also change hydrophobicity/hydrophilicity, aggregation/agglomeration tendency that affect nanomaterial internalization into the cells (Deng et al., 2017)

Nanotoxicity is therefore a complex effect of the nanomaterial core, molecules absorbed onto the nanomaterial surface, surface layer of modified (e.g. oxidized) core molecules, degradation products and released ions (Deng et al., 2018).

All these above listed properties (the size, shape, surface modiication, crystalline structure, impurities) govern the ability of nanoparticles to enter biological systems, distribute in the bloodstream and lymphatic system, and penetrate into cells, tissues, and organs (Aillon et al., 2009). Once there, nanoparticles can interact with organelles and macromolecules, altering their structure. In this way, nanoparticles can interfere with intracellular processes and the functioning of whole organs, causing adverse toxicological effects (Sukhanova et al., 2018).

The main mechanisms by which nanomaterials exert their toxic effects is via the generation of reactive oxygen species (ROS) leading to oxidative stress and inflammation. On the molecular level, nanomaterials can induce oxidative damage to proteins and other biomolecules, including DNA, lipid peroxidation affecting also cell membrane integrity. Furthermore, nanoparticles have been shown to cause mitochondrial perturbation, disruption of the cytoskeleton, lysosomes, cell proliferation defects and eventually cell death (Nel et al., 2006; Frolich 2013). Nanomaterials adsorb biomolecules on their surface in a biological environment which may change structures of proteins affecting their functions (causing, e.g., loss of a function, protein fibrillation, exposition of cryptic epitopes that can elicit immune reaction) (Monopoli et al., 2012).

On the tissue level, these perturbations can manifest as organ dysfunctions, cancer development, atherogenesis, thrombosis, and possibly neurodegenerative diseases (Nel et al., 2006; Buzea et al., 2007). Nanomaterials particularly affect immune-competent cells, as these are responsible for elimination of potentially dangerous objects from the body. Inadequate,

excessive or prolonged activation of immune cells results in inflammation and ROS production that can progress to fibrosis and granuloma formation limiting the tissue functionality and making it prone to tumorous malformations. Immunosuppressive effects can result in increased susceptibility to infections or development of a cancerous diseases. On the other hand, inappropriate immunostimulation can lead to autoimmune diseases and hypersensitivity reactions including allergies (Borashi et al., 2017).

For consumer uses, the skin, gastrointestinal and respiratory tract are seen as the most relevant routes of entry of nanomaterials into the body.

4.1.1 Oral exposure

Biokinetics of nanomaterials following the oral exposure depends on their physicochemical properties (the chemical composition, surface properties, size, and shape). That is why a sufficient characterization of the physico-chemical properties is essential to understand the toxicological data and compare the results from different studies. Moreover, interactions with various components within complex food matrices and the gastrointestinal tract (GIT) are also crucial, as the nanomaterial properties may be changed considerably when they are dispersed in food products. This aspect is, however, usually ignored in animal studies, hampering the interpretation and extrapolation of the results for human health. Another important factor affecting biokinetics of ingested nanomaterials is their potential transformation during the passsage through variable environments, especially due to the varying pH and content of surface-active components, enzymes and other active molecules in the GIT. As a result, the composition, dimensions, surface properties, physical state, and aggregation state of nanomaterials can change in the GIT.

In general, gastrointestinal studies show that many nanomaterials are almost entirely removed in the faeces, however gastrointestinal uptake has also been observed (reviewed in Martirosyan et Schneider, 2014; McClements et Xiao, 2017). Nanomaterial absorption in the GIT decreases with increasing material size (Kermanizadeh et al., 2015).

Following oral exposure, nanomaterials can potentionally adversely affect cells and tissues of the GIT, microbial cells that reside in the human GIT which could indirectly alter human health (Bouwmeester et al., 2011). Ingestion of nanomaterials can also affect other organs in the body in case of absorption into the systemic circulation through the GIT (McClements et Xiao, 2017). Uptake of nanomaterials from the gastrointestinal tract will vary depending on nanomaterial properties. However, particles absorbed from the gastrointestinal tract may enter the lymphatics and the blood capillaries and reach the systemic circulation leading to transport and accumulation in remote tissues or organs (Martirosyan et Schneider, 2014).

Many contradictory results are seen with respect to nanoparticle toxicity from food sources. In a recent review of studies on the use of silver, iron oxide, titanium dioxide, silicon dioxide, and zinc oxide nanoparticles in food, it was noted that nanoparticles with different physicochemical properties produce different results depending on the particle dimensions, shape, aggregation state, internal composition, surface composition, crystal form and dose. In many studies, the properties of the nanoparticles were not adequately characterized or reported (McClements et Xiao, 2017). Variable effects of dietary patterns, food matrix, and passage through the gastrointestinal tract were frequently not reported, but these are critical factors for nanoparticle behaviour and toxicity, as mentioned above. It was recommended that standard methods should be developed to adequately test toxicity of oral exposure to nanomaterials under reproducible and human-relevant conditions.

4.1.2 Inhalation exposure

Deposition efficiencies of inhaled particles along the respiratory tract (i.e. in the nasopharyngeal, tracheobronchial and alveolar regions) depend mainly on the particle size and shape (Oberdorster et al., 2005; Bierkandt et al., 2018). Inhaled nanoparticles deposit preferentially in the alveolar region of the lungs with a maximum at 20 nm (Kreyling et al 2013). The main mechanism of deposition of inhaled nanoparticles in the respiratory tract is diffusion. In aggregates/agglomerates or high aspect ratio nanomaterials, other mechanisms, such as inertial impaction, gravitational settling, and interception, are employed (Oberdorster et al., 2005).

The most prevalent clearance mechanism for nanoparticles in the alveolar region for rodents is phagocytosis by alveolar macrophages, whith the gradual movement of the macrophages with internalized particles toward the mucociliary escalator. The retention half-time of solid particles in the alveolar region based on this clearance mechanism is about 70 days in rats and up to 700 days in humans (Oberdorster et al., 2005). Particle clearance from the human peripheral lungs and from those of dogs and monkeys differ from that in rodents: in the three large species even micron-sized particles enter the epithelium and penetrate into interstitial spaces and macrophage-mediated clearance occurs at a rate which is one order of magnitude lower than that of rodents (Kreyling et al 2013).

Translocation of particles may occur to other tissues and organs via the systemic circulation and lymphatic system. Nakane et al (2012) in their systematic review analyzed 61 studies on particle translocation from the respiratory system (covering 29 nanomaterials of different chemical composition) and found that the main factors influencing the translocation were the size, material and route of exposure (inhalation, instillation, etc.). Animal species was considered a less important factor based on the categorical regression analysis. As for the sites where particles were detected, there were 9 reports for the brain, 7 reports for the kidney, 3 reports for the heart, 2 reports for the thyroid, 20 reports for the liver, 4 reports for the spleen, 14 reports for blood, and 4 reports for lung capillary lumens (Nakane et al., 2012). Based on quantitative biokinetic analysis after nanoparticle (iridium, carbon, gold, or titanium dioxide) application to the lungs of a rat model, small fractions of nanoparticles were found in all secondary organs studied including the brain, heart and even in the foetus (Kreyling et al 2013). Fractions in each of the secondary target organs were low (0 .5 % of the administered dose) but depended strongly on particle size in an inverse fashion. However, the translocation was increased (5–10% of the administered dose) in the soft tissue and skeleton (without blood content) and also it was found that the negatively ionic surface charged nanoparticles translocated more rapidly than positively charged nanoparticles of the same size (Kreyling et al 2013). In addition, strong differences of the totally translocated fractions depended on the nanoparticle material, morphological and/or surface properties (Kreyling et al 2013). The portion of translocated nanoparticle mass fraction in animal studies differs significantly. However, reliable human studies have reported less than 1% of the dose delivered to the lungs to be translocated to the blood (reviewed in Geiser and Kreyling, 2010).

Current evidence suggests that the adverse effects of inhaled inorganic nanoparticles – particularly cancer – primarily occur upon their high accumulation in organs. This can cause chronic toxicity due to the perpetual stimulation of the immune system that induces an immune-inflammatory response. Inflammatory mediators can travel in the circulation causing systemic inflammation distant from the point of exposure and indirect toxic effects (De Matteis, 2017).

The rate of clearance from the lung is a major determining factor for the development of lung cancer seen in animal studies. The rat in particular is known to be susceptible to a 'lung overload' effect whereby failure to efficiently clear insoluble nanoparticles from the lung, which may involve direct toxicity to clearing macrophages and their overload - leads to an exaggerated and chronic inflammation response which – evidence suggests – is the primary

mechanism of genotoxicity leading to lung neoplasms (ECETOC 2014). The crucial question from the regulatory toxicology point of view is the relevancy for humans of both non-neoplastic and neoplastic effects observed specifically in rats chronically exposed to extremely high concentrations of poorly soluble particles of low acute toxicity (Borm et al., 2015). Although the defence mechanisms and particle retention metrics in rats are entirely different than those in humans (or even other rodents), based on the precautionary principle, the rat is considered the most sensitive species for assessing lung cancer risk for poorly soluble low toxic particles, as it appears to be the only laboratory animal species that develops lung tumours upon exposure to these particles (Borm et al., 2015).

4.1.3 Dermal exposure

Skin acts as a barrier protecting body from external substances. However, some substances are able to penetrate and permeate this barrier. Many studies on dermal exposure route have shown negligible absorption of nanoparticles through the intact skin, however the general conclusion is that penetration is determined by nanomaterial characteristics (size, chemical composition, surface properties (charge, hydrophobilicity/hydrophilicity), and shape) as well as skin conditions, with damaged, depilated, or irratiated skin being more permeable. Smaller, positively charged nanoparticles have been shown to have enhanced penetration capability. In case of soluble nanomaterials (e.g. ZnO), ions can be absorbed even without nanoparticle penetration and reach the systemic circulation. A vehicle may also play an important role as it affects aggretation/agglomeration and potentially also surface properties of the carried nanomaterial (Filon et al., 2015).

Experimental findings on skin absorption and skin toxicity of nanoparticles do not allow for specific conclusions. Results of existing studied tend to be contradictory in the absence of standardised testing methods (Crosera et al., 2009).

The NANODERM project concluded that adverse health effects for the topical application of sunscreens containing TiO_2 nanoparticles (especially when coated) are not expected for healthy skin. However, several other studies on carbon-based nanoparticles confirm an interaction between human dermal cells and nanosized particles (NANODERM, 2007). The EU's Scientific Committee on Consumer Safety (SCCS), in its assessments of nanosized Carbon Black (SCCS, 2015), TiO_2 (SCCS, 2013; SCCS, 2018) and ZnO (SCCS, 2012a,b), concluded that these particles are at most mild skin irritants and are likely not to cause skin sensitisation due to lack of dermal penetration. In the case of ZnO, nanosized particles were shown not to penetrate the skin unless solubilised Zn ions are released from ZnO nanoparticles (SCCS, 2012a,b). This is in accordance with findings of Kermanizadeh et al. (2015), that based on their extensive literature review on toxicokinetics of nanomaterials, concluded that the risk of nanomaterial-induced damage to secondary organs following dermal exposure is fairly low.

4.1.4 Other routes

Other, less commonly recognized routes of entry – through the eye and olfactory bulb of the nose (Oberdorster et al., 2004) cannot be ruled out, as they have been experimentally proven in various mammals (including rodents and primates) using various nanomaterials and a polio virus (of dimensions corresponding to nanoscale materials). Similarly, there is a possibility of nanomaterial uptake by ophthalmic and maxillary neurons of the trigeminus nerve (reviewed in Oberdorster et al., 2005). Although probability and health impacts of these observations for human health are not clear yet, these pose concerns about potential neurotoxicological effects of nanoparticle exposure (Oberdorster et al., 2009). Nanoparticle accumulation in the rat brain after inhalation was observed via the olfactory bulb (Pujalte et al., 2017; Oberdorster et al. 2009; Kao et al., 2012) as well as both via olphactory bulb and blood circulation (Kreyling, 2013).

Another route of entry related to the dermal one is the puncturing of the skin during tattoo

making. Schreiver et al. 2017 provided analytical evidence of tattoo particles being distributed inside the human body with smaller (nano)particles transported to the lymph nodes. The exact size limit preventing this translocation is unknown yet. Sepehri et al. (2017) identified intracellular tattoo pigments in the skin, in lymph nodes and also in the Kupffer cells (liver macrophages) 1 year after tattooing (in mice), indicating systemic distribution of tattoo nanopigments (carbon black and red azo pigments).

4.1.5 Genotoxicity and Cancer

Numerous studies have postulated three main mechanisms whereby nanoparticles may exert genotoxic effects. Direct primary genotoxicity involves direct interaction of the nanomaterial with the DNA upon entry into cells (and cell nucleus). Indirect primary genotoxicity is the result of e.g. nanomaterial-induced reactive oxygen species (generated by nanomaterials themselves or by mitochondria interacting with nanomaterials in a cell), or of the release of toxic ions from the nanomaterial. Secondary genotoxicity is caused by nanomaterial-induced inflammation resulting in release of reactive oxygen species from activated phagocytes (Magdolenova et al., 2014). This secondary genotoxicity is considered to involve a threshold set by the exposure concentration that will trigger inflammation and overwhelm antioxidant and DNA damage repair capacities.

In the following paragraphs, a summary of available relevant toxicity data on selected nanopigments is provided. These common inorganic pigments encompass those where extensive toxicological data have been derived for the nano–forms that have been found in the open literature. For organic pigments there are few toxicological data on nano-forms as yet.

4.2 Nano-sized Titanium Dioxide (TiO2)

TiO2 (CAS Number 13463-67-7) is a chemically inert substance and it is considered to be non-toxic in the bulk form. The main application of TiO_2 is as a white pigment due to its very high refractive index. It is insoluble in water, organic solvents, as well as in aqueous systems, and under physiological conditions. However, in the nanosize, the behaviour and characteristic of the substance may change significantly and new or enhanced properties occur. Nano-TiO_2 exhibits increased catalytic activity and photocatalytic properties (Shia et al., 2013).

TiO_2 is one of the most produced nanomaterials based on the annual tonnage and also one of the most often investigated nanomaterials in toxicological studies.

Pigmentary TiO_2 is used in a wide range of consumer products, such as cosmetics, paints, dyes and varnishes, textiles, paper, plastics, food, drugs, and even paving stones (Weir et al., 2012). Nanoscale titanium dioxide is transparent, and therefore is used in sunscreen for its UV resistance. Nano-TiO_2 is applied in antimicrobial applications, in air water purification, medical applications, and energy storage (Weir et al., 2012). However, it has been shown that pigmentary-grade TiO_2 always contain an unintentional fraction of particles with dimensions below 100 nm (i.e. within the nanoscale) (Weir et al., 2012; Peters et al., 2014).

Nano-TiO_2 can exist in diverse forms, differing in characteristics such as e.g. the size, shape and surface modifications, with potentially different properties and toxicological effects. The main characteristics that affect biological effects and potential toxicity of nano-TiO2 are:

Crystalline structure: There are two main crystalline forms of (nano)TiO_2, rutile (CAS Number 1317-80-2) and anatase (CAS Number 1317-70-0). The other crystalline form, brookite, is of less importance with regard to both applications (as it is not present in commercially available products) and toxicity assessment.

Anatase is more reactive and thus considered to be more toxic than rutile. Interestingly, even higher reactivity and toxicity has been detected in a mixture of anatase and rutile, a

photocatalytical standard P25 consisting of approx. 20% rutile and 80% anatase particles of average size of 20-25 nm (Sanders et al., 2012). Nanoparticles of comparable sizes (diameter of 52 ± 7 nm and 51 ± 6 nm measured by transmission electron microscopy) and different crystal structures (rutile and anatase, respectively) were used in an *in vitro* study evaluating effect in two cancerous cell lines: A549 cells (human lung carcinoma cells) and MCF-7 cells (human breast cancer cells) (deMatteis et al., 2016). Higher toxicity of the anatase crystalline form (cytotoxic effects, reactive oxygen species production, actin filament reorganization) was due to its higher uptake, at the same size, surface charge and dose compared with rutile nanoparticles. Authors also demonstrated that higher toxicity in anatase was caused by its easier ionization under sunlight at low pH making it more prone to degradation and titanium ion release compared to rutile.

Size: Nanomaterial size is a critical parameter determining the absorption, translocation and interaction with cells, as describe above.

Shape: Comparison of TiO_2 fibres, TiO_2 nanoparticles and crocidolite (a form of asbestos) has shown similar effects for TiO_2 fibres and crocidolite in an *in vitro* study on macrophages (Allegri et al., 2016). Fibre-shaped TiO2 (> 15 µm), but not spheres, induced inflammasome activation and release of inflammatory cytokines through a cathepsin B-mediated mechanism *in vitro* in macrophages (Hamilton et al., 2009).

Surface modification: The most common coatings on nano-TiO_2 are composed of oxyhydrates and oxides of aluminium and silicon. Coating can e.g. provide hydrophobic/hydrophilic surface or can limit photocatalytic properties of TiO_2 under UV exposure (such as aluminium-coated TiO_2 nanoparticles in sunscreens). As photocatalytic activity is related to reactivvity and reactive oxygen species production, such coating can significantly decrease toxic effects of the particles (TSA 2013). However, sometimes coating may enhance the toxicity. In an whole-body inhalation study SiO2-coated rutile TiO_2 nanoparticles were the only sample tested that elicited pulmonary neutrophilia in exposed mice mediated through increased expression of proinflammatory cytokine TNF-α and neutrophil chemoattractant CXCL1 by macrophages (Rossi et al., 2009). Neither uncoated nano-TiO_2 nor silica elicited toxic effects.

Impurities and contaminants: Common impurities from nano-TiO_2 production comprise metals (including toxic heavy metals), depending on the methods of nano-TiO_2 production, e.g. chromium, manganese, vanadium, magnesium, aluminum, calcium, silicon, iron. The presence of impurities can enhance reactivity of nano-TiO_2 and toxicity, especially in case of toxic substances, such as heavy metals. Moreover, nanoparticles can act as vectors introducing toxic contaminants into the cells when the adsorption of contaminants on nanoparticle surface is followed by their uptake. For example, nano-TiO_2 was shown to facilitate the accumulation of phenanthrene in the ark shell clams probably by prolongation of the retention time of phenanthrene on the nanoparticle aggregates in the digestive tract (Tian et al., 2014). In a study of Yang et al. (2012), nano-TiO_2 promoted the accumulation of Cd2+ in the single-cell green alga *Chlamydomonas reinhardtii* by forming a complex with the cell membrane and subsequently increased its permeability.

All the forms of TiO_2 are expected to be biopersistent and of poor solubility. The above mentioned physico-chemical parameters might, however, lead to more potent toxicity or to other specific lesions via specific mode of actions.

Unfortunately, detailed characterization of the tested samples is often missing in reported studies, which limits the interpretation of the results. Similarly, a direct comparison of the effects observed in nano-TiO_2 differing in more than one characteristics can be misleading. Comparison of the effects of different nano-TiO_2 observed in different studies is further complicated by different experimental conditions and protocols.

Oral exposure
There are several studies in experimental animals using different forms of nano-TiO_2 with inconsistent conclusions.

Warheit et al. (2015) evaluated three types of TiO_2 (aluminium-coated rutile 145 nm particles; not coated rutile 173 nm particles and anatase/rutile aluminium/silica coated 73 nm nanoparticles) in oral 90-day, 28-repeated dose and acute oral toxicity studies, respectively, following appropriate OECD guidelines. TiO_2 particles did not produce any adverse effects, namely: no adverse histopathological findings, no effects on body or organ weights, food consumption or food efficiency, concomitant with an absence of any significant clinical/pathological chemical endpoints that might have been an indicator of a toxic effect.

In contrast with the conclusion of Warheit et al. (2015), adverse effects of orally administered nano-TiO_2 were reported e.g. in Hu et al. (2010) and Chen et al. (2015). In these studies very small particles (< 10 nm) were tested in mice. The observed neurotoxic and cardiotoxic effects might be related to the extreme small size of nanoparticles. However, the methods of these studies that do not follow any standardized guidelines (e.g. OECD) which impede a direct comparison of the results with the negative outcomes of the OECD studies described above.

Ammendolia et al. (2017) using realistic doses of 1 and 2 mg/kg/day for 5 consecutive days (which is comparable to doses that are expected from real-life oral exposure of the general population taking into account the proportion of particles that are actually nano-sized in consumed food) observed increased length of intestinal villi in only male rats. The goblet cells appeared increased in number, suggesting a hyperplasia likely related to the increased villi size. Authors hypothetize that repeated effects of ingested nano-TiO_2 on intestinal mucosa could lead to an increased risk of tumor development or to progression of existing tumoral processes that can lead to carcinogenesis in the gut. However, the hypothesis was not further investigated. Results of *in vitro* experiments indicate that nano-TiO_2 may act by increasing testosterone levels that can affect Insulin-like Growth Factor 1, a hormone-regulated growth factor. Increased testosterone levels were detected by the same group under the same experimental conditions (Tassinari et al., 2014) with sex-related histological alterations in endocrine-active tissues such as thyroid (both sexes), adrenal cortex (females only), adrenal medulla (both sexes) and ovarian granulosa (in females). The interpretation of these results is complicated by missing toxicokinetic information and proper histopathological investigation of other possibly affected organs.

Zhao et al. (2013) detected dose-dependent fertility reduction, ovarian inflammation and follicular atresia (degeneration) in mice after 90-day intragastric exposure to 2.5-10 mg/kg nano-TiO_2.

Potential genotoxic effects of 3 pigment-grade and 3 nanoscale TiO_2 nanoparticles were compared by analyzing induction of micronuclei and toxicity in bone marrow and peripheral blood reticulocytes from rats orally exposed to 500, 1000 or 2000 mg/kg nano-TiO_2. The study was performed in accordance with OECD guidelines. None of the tested samples exhibited positive results in the applied assays. At the same time, no significant increases in TiO_2 exposed animls over controls were measured in the blood (48 or 72 h) or liver (72 h). Authors suggested that lack of genotoxicity can be attributed to inability of the test material to migrate from the gastrointestinal tract into the blood and then into target tissues (Donner et al., 2016).

Dietary TiO_2 nanoparticles are reported to be mostly excreted in the faeces in experimental animals (Warheit et al., 2015; MacNicoll et al., 2015; Cho et al., 2013). Similar results (little uptake occurred through the intestine) were obtained in a human study with volunteers after a single oral dose of 5 mg/kg of 15 nm, 100 nm, and < 5000 nm TiO_2 particles dispersed in water (Jones et al., 2015). The authors emphashize the role of aggregation/agglomeration *in vivo* (e.g. in the gastric fluid), especially for particles such as TiO_2 which are prone to

agglomeration. As a result, the actual numbers of nanoparticles available for absorption in the gut after ingestion may be less than predicted from a standard particle characterisation in saline/water.

Oral exposure to nano-TiO_2 promoted preneoplastic lesions in the rat colon after pretreatment with carcinogen 1,2-dimethylhydrazine (Pence et Buddingh, 1987). The potential genotoxicity is probably indirect, i.e. it is mediated through inflammation and oxidative stress (Sycheva et al., 2011), thus a threshold for these effects may be derived. Patients with a compromised gastrointestinal system may be more sensitive towards nano-TiO_2-mediated genotoxicity (Kurzawa-Zegota et al., 2017).

Inhalation exposure
The inhalation route is of special interest for low soluble, low toxicity materials, as local tumours have been found only after respiratory exposure and no carcinogenic concern was identified by oral and dermal routes. The main proposed mechanism of carcinogenicity by inhalation is based on the low solubility and biopersistency of the particles leading to pulmonary inflammation and oxidative stress. Secondary genotoxicity and cell proliferation may result in carcinogenicity.

Mild reversible immunomodulatory effects (Bettini et al., 2017), and transient inflammation (Warheit et al., 2006) were detected in rats. Inhalation exposure leads to decrease in the Th2 response (Scarino et al., 2012) and augmented airway hyperresponsiveness (Mishra et al., 2016), caused by ovalbumin. However, no inflammation was detected in healthy (non-sensizited) animals (Scarino et al., 2012; Mishra et al., 2016). In a murine model of diisocyanate-induced occupational asthma, but not in healthy mice, increased inflammation was observed after exposure to nano-TiO_2 (Hussain et al., 2011).

Wallin et al. (2016) investigated the inflammatory response, the acute phase response, and the genotoxic effect of two different TiO_2 nanoparticles following a single intratracheal instillation in mice. The tested materials were an unmodified rutile TiO_2 with endogenous negative surface charge, and a positively charged form. Cellular composition and protein concentration were determined in bronchoalveolar lavage (BAL) fluid as markers for an inflammatory response. Exposure to both TiO_2 nanoparticles induced increased levels of DNA strand breaks in lung tissue at all doses 1 and 28 days post-exposure. Pulmonary and systemic genotoxicity was analysed by the alkaline comet assay as DNA strand breaks in BAL cells, lung and liver tissue. Surface modification of reactive negatively charged rutile TiO_2 to positively charged did not consistently influence pulmonary toxicity of the studied TiO_2 nanoparticles.

Pujalte et al. (2017) investigated disposition kinetics of anatase nanoparticles (20 nm) in rats after 6h inhalation exposure (15 mg/m^3). The highest tissue levels of Ti were found in lungs, reaching peak values 48h after exposure and progressively decreasing over the 14-day post-exposure observation period. Ti levels in blood, lymph nodes and other internal organs (including liver, kidney, spleen) were approximately one order of magnitude lower than those in the lungs. Nanoparticles were eliminated predominantly by mucociliary clearance, subsequently ingested and excreted in feces. Of note, transfer of TiO_2 to the olfactory bulbs and brain was also detected. Oxidative damage was significantly increased in lungs and blood at 24 h but not at later time points.

Gate et al. (2017) compared biopersistence and translocation to extrapulmonary organs of TiO_2 in healthy young adult (12–13-week-old) and elderly rats (19-month-old) after subacute inhalation exposure (4 weeks, 6 h/day, 5 days/week) to TiO_2 nanoparticles (P25 – rutile/anatase, 21.5 nm; 10 mg/m^3) during a 180-day post-exposure period. Large amounts of Ti were initially found in the lungs which were slowly cleared during the post-exposure period. From the day 28, small increase of Ti was found in the spleen and liver of the exposed young adult rats. In the elderly group, translocation to extra-pulmonary organs was significant at the day 90. Ti levels recovered from the spleen and liver of the exposed elderly rats were higher than in those from exposed young adults.

A detail mechanistic study on possible effects of rutile TiO_2 inhalation was performed in rats using a gene expression analysis of lung tissues after exposure to doses equivalent to 1.5, 5, and 15 working days at the Danish occupational exposure level for TiO_2 (6.0 – 9.75 mg TiO_2/m^3). The study found alterations in the expression of several genes associated with ion homeostasis and muscle functions which may potentially interfere with calcium, ion, and lipid homeostasis, and affect pulmonary smooth muscle contraction. Prolonged disturbances in ion homeostasis and airway smooth muscle functioning can potentially contribute to the development of lung diseases such as pulmonary fibrosis, asthma, and even lung cancers (Husain et al., 2013). The proposed mechanisms of action, however, require a series of further experiments to be confirmed.

Dermal exposure
Small laboratory animals' skin represents a skin barrier that is more permeable than that of larger animal species including humans (Magnusson et al., 2001), thus negative results in dermal subchronic and chronic studies performed with accordance with standard (e.g. OECD) protocols should be considered as a reliable source of information on tested nanoparticle safety.

Nano-TiO_2 may have adjuvant effects, that is, it may increase the dermal sensitization potency of dermal sensitizers (such as dinitrochlorobenzene) by augmenting a Th2 immune response. The Th2 response is involved in the defense against extracellular parasites and it is also responsible for triggering various allergic inflammatory responses. However, the dermal sensitization potential of nano-TiO_2 without the previous or concurrent application of a sensitizer was not observed (Hussain et al., 2012; Auttachoat et al., 2014; Park et al., 2011; and reviewed in Schilling et al., 2010).

The route of exposure may affect the results, e.g. parenteral exposure increased auricular lymph node cell proliferation, while oral and dermal exposure (healthy skin) had no effects (Auttachoat et al., 2014).

Adachi et al. (2013) investigated penetration of nano-TiO^2 in hairless rats and the toxicity following subchronic exposure. Titanium was detected in the superficial part of the upper stratum corneum (stratum disjunctum), in keratinized layers of the follicular infundibulum. Moreover, focal parakeratosis and spongiosis indicating development of contact dermatitis was observed in the exposed experimental animals. Authors explained that dermatitis conditions may be caused by mechanical irritation of the skin, or by reactive oxygen species that could be generated by the uncoated TiO2 nanoparticles on the skin surface and cause development of skin inflammation showing spongiotic dermatitis. However, the study did not find any obvious evidences of nano-TiO^2 skin penetration and the influence of subchronic exposure of TiO^2 was not considered significant.

Wide application of nano-TiO_2 in sunscreens requires evaluation of nano-TiO_2 skin penetration and potential toxic effects under UV light exposure. *In vitro* studies have shown that the natural and artificial light promote degradation of nano-TiO_2, particularly its anatase forms, and subsequent dermal penetration (deMatteis et al., 2016). *In vivo* studies investigating further the phenomenon of photoinduced disaggregation of nano-TiO_2, the role of pH and nanoparticle characteristics (size, crystal structure), and the potential for enhanced penetration and toxicity are not currently available.

***In vitro* studies**
Møller et al. (2017) conducted a review of numerous studies on the genotoxicity of TiO2. It was shown that the standard comet assay can discriminate between the genotoxicity of different types of TiO_2, and that anatase TiO_2 appears to be the form with strongest genotoxic potential.

In vitro genotoxicity of TiO_2 nanoparticles at 25, 75 and 125 µM was shown in short term

cultures of human peripheral blood using Chromosomal aberration assay and Comet assay specifically adapted for nanoparticles as a test compound, along with the mechanistic study. The mode of genotoxicity was found to be due to a direct effect, based on an in vitro DNA binding study that showed strong binding affinity of TiO_2 nanoparticles with human genomic DNA, in addition to negative free energy value, which indicated spontaneous binding (Patel et al., 2017).

Proquin et al. (2016) showed that food-grade TiO_2 (E171 colour additive) - a mixture of micro-sized and nano-sized particles - has the capability to induce reactive oxygen species formation in acellular (cell-free medium) conditions, as well as induce DNA damage as determined by the *in vitro* comet and micronucleus assays.

Using representative nanomaterials from European Commission Joint Research Centre Nanomaterials Repository, rutile aluminium-coated hydrophobic and hydrophilic 25nm TiO_2 nanoparticles (NM103, NM104), Jalili et al. (2018) did not detect genotoxic effects: no decrease in cell viability, oxidative stress (glutathion levels not decreased), no significant change in γH2AX (a marker of DNA double strand breaks), negative results in alkaline comet assay (DNA strand breaks) and alkaline comet assay with FPG (DNA oxidative damage) and micronucleus assay (potential underestimation of micronucleus assay was reported due to the interference of nanoparticles with the scoring method). Cellular uptake was confirmed (localization of nanoparticles was observed in cell vesicles, no particles were visible in the cell nucleus).

Summary

In summary, inhalation exposure may be considered the most hazardous route of exposure to nano-TiO_2, since pathological changes were detected in lungs and other organs, and translocation of inhaled particles to other organs was observed in animal studies. Carcinogenic effects have been mostly related to overload conditions in rats and thus may be of limited relevance for realistic human exposure (see below).

TiO_2 has been listed as a possible human carcinogen by some agencies and regulators (e.g. IARC, 2006). Thompson et al. (2016) conducted a systematic review of the literature to characterize the available data and identify candidate datasets upon which environmental toxicity no significant risk level (NSRL) could be derived. They identified 473 human studies, out of which only 7 were epidemiological studies that met inclusion criteria to quantitatively characterize carcinogenic endpoints in humans, however, the authors found out that none of these human studies supported derivation of toxicity criteria. Therefore, animal data were used to derive safety values for TiO_2. NSRL of 300 mg/day was derived. Low-dose linear extrapolation from tumor incidence in the rat lungs resulted in an NSRL value of 44 mg/day. Finally, they compared the obtained limit values with current environmental exposures to TiO_2 and concluded that environmental exposure to respirable TiO_2 (in California) is not likely to pose a health hazard (the mean particulate matter PM 2.5 TiO_2 values in southern California ranged from 0.0059 to 0.0096 mg/m^3, corresponding to ~0.12 mg/day).

Mechanisms of mutagenic/carcinogenic effects of nano-TiO_2 as low soluble biopersisitent particles are inflammation and oxidative stress (secondary toxicity). Direct genotoxicity cannot be excluded, however, has not been directly confirmed *in vivo*.

Cell culture studies also show increased levels of oxidatively damaged DNA after exposure to TiO_2. Exposure to nanosized TiO_2 is associated with genotoxicity in cells, whereas there are still too few reliable studies to assess the genotoxic potential in animal models. Moreover, observed toxic effects may be restricted to UV exposure. UV exposure results in generation electron-hole pairs in nano-TiO_2 that can lead to biological injury through oxygen radical production or electron capture (Xia et al., 2008).

Human data do not support an association between occupational exposure to TiO_2 and risk for cancer (reviewed in TSA 2013).

Oral studies performed according to the standard guidelines with proper toxicokinetic and histopathological evaluation are mostly negative or with less serious local impact (especially limited to the gut tissue). Carcinogenic effects were not observed upon oral exposure. However, based on the reviewed studies, an indirect role in promoting carcinogenicity cannot be excluded. The absence of adverse effects even at very high orally administered doses is most probably linked to limited nano-TiO_2 absorption reported in other studies. The hypothesized mode of action for genotoxic effects of low soluble low toxic (nano)particles requiring a sufficient accumulation of particles to induce inflammation and proliferative lesions, observed in inhalation studies under overload conditions, is not seen to occur from oral exposures.

The ECHA Risk Assessment Committee (RAC) in their recent opinion concluded that the experimental and human evidence does not support TiO_2 to be classified as Carc. 1A or 1B (ECHA, 2017b) based on a weight-of-evidence approach (taking into account that TiO_2 was not shown to be a multisite carcinogen, lung tumour developped especially in female rats, no robust carcinogenicity studies in species other than rats are available, and rat lung tumours only developed under inhalation exposure conditions associated with marked particle loading of macrophages. ECHA (2017c) assumes that practical threshold for lung tumour development can be derived as the mechanism of mutagenicity in lung cells is considered to depend on chronic inflammation and oxidative stress.

4.3 Nano-sized Carbon Black (CB)

Carbon black (CAS Number 1333-86-4) is an almost pure form of elemental carbon. It is a black, finely divided pellet or powder composed of colloidal particles that is chemically and physically distinct from soot and black carbon (Long et al., 20013). Black Carbon refers to fine particles containing elementar carbon. They are found in ambient air as a result of emissions from incomplete combustion of fossil fuels and biomass. Soot is also generated by combustion and is highly heterogeneous (poses significantly higher ash and extractable organic matter contents than carbon black). Chemical and physical properties of soot are highly variable depending on its source. Contrarily carbon black is a manufactured product of almost pure carbon (Long et al., 2013).

Carbon black is predominantly used as a reinforcement of rubber and tyres. As a black color pigment it is present in paints, inks, coatings, laquers, cements, ceramics, paper, and also in cosmetics (IARC, 2010). The particular applications of carbon black are related to its properties, such as the specific surface area, particle size and structure, conductivity and color. Carbon black is in the top 50 industrial chemicals manufactured worldwide, based on annual tonnage (Long et al., 20013).

Most carbon black is produced by the oil furnace process, which is most often referred to as furnace black. The standard form most commonly used to evaluate the toxicity of nano-sized carbn blacks is Printex ® 90, a High Color Furnace (HCF) black, with an average particle size of 14 nm and a surface area of 350 m2/g. Apart from tinting and coloring, nano-sized carbon black can have enhanced properties related to UV protection, conductivity and special effects (thermal insulation or rheology control, respectively, when using as a reducing agent or an antioxidant).

Carbon black is insoluble in water and other solvents, therefore, together with TiO_2 and other materials, it is a representative of poorly soluble low toxicity particles (SCCS, 2015).

Organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), nitro-derivatives of PAHs and sulphur-containing PAHs can be adsorbed onto the carbon black particles surface as they are generated during production of carbon black (Lindner et al., 2017; SCCS, 2015). However, commercial carbon blacks such as Printex 90 consists of carbon with less than 1% organic and inorganic impurities, and are thererfore a relatively 'clean' particles which are

suitable for testing intrinsic toxicological properties (Jackson et al., 2011).

Oral exposure

The acute oral LD50 of carbon black (Printex-140 and Spezialschwarz 4) in rats was found to be greater than the maximum technically feasible dose (10,000 mg/kg bw) in two unpublished acute oral toxicity studies (ECHA, 2018). No mortality and no signs of toxicity were evident. Carbon black is therefore considered to have a low acute toxicity by the oral route (SCCS, 2015).

In another unpublished study, repeated oral administration of carbon black (20-30 nm) to rats for 90 days at doses of 100, 300 or 1000 mg/kg/day produced no adverse effects, leading to a No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg/day (SCCS, 2015).

Oral administration of carbon black (diameter 20 to 30 nm, surface area range 200 - 260 m2/g) to pregnant rats at 100, 300, or 1000 mg/kg bw/day during organogenesis showed no adverse maternal changes or any effects on embryo-fetal development. The No Observed Adverse Effect Levels (NOAEL) for both maternal toxicity and developmental toxicity was 1000 mg/kg bw/d (Ramesh et al., 2012, cited in SCCS, 2015).

2-year oral carcinogenicity studies on carbon black have been conducted in mice and rats, in both cases showing no observed increase in tumour incidence. The dermal application of various carbon blacks to mice showed no carcinogenic effect (IARC, 2010, SCCS, 2015).

Inhalation exposure

Male Sprague-Dawley rats exposed to Printex 90 aerosols in a nose-only exposure chamber for 6h/day, 5 days per week for 13 weeks at a concentration of approximately 9 mg/m^3 displayed only mild to moderate respiratory effects (Lim et al., 2012). Printex 90 (14 nm) was selected as a representative nano-sized carbon black. Three types of aerosolised agglomerates were used, presenting particle mass median aerodynamic diameters of 1.52 µm (group 1), 1.30 µm (group 2), and 0.97 µm (group 3). Average concentrations during the exposure period were 8.8±3.7 mg/m^3 for group1, 8.6±4.5 mg/m^3 for group 2 and 9.0±3.1 mg/m^3 for group 3. Inflammation was evident in the increased cell numbers in the bronchoalveolar lavage (BAL) fluid. Macrophages, and polymorphonuclear leukocytes were increased and carbon black masses were visible in BAL. However, few differences were found between the three differently agglomerated aerosols. There were no significant differences in body weight, lung functions or cytokine levels in BAL fluid. There were no statistically significant differences in pulmonary function between the control group and the three agglomerated carbon black exposed groups. No histopathological symptoms were found to be associated with carbon black exposure in any group. It was concluded that agglomeration did not affect the toxicity of nano-sized carbon particles (Lim et al., 2012).

Particle retention, lung inflammation, and pathology was investigated in female rats, mice and hamsters after inhalation exposure to 0, 1, 7, 50 mg/m^3 (nominal concentrations) of high surface area carbon black (Printex 90) (14 nm aggregate aerosols with aerodynamic diameters ranging from 1.2 to 2.4 µm) for 13 weeks. Group of animals were sacrificed after 5 weeks of exposure to measure retained carbon content in the lungs. Further groups of were sacrificed immediately at 13 weeks and after recovery periods of 3 and 11 months post-exposure to allow evaluation of particle retention and clearance through bronchoalveolar lavage (BAL) analysis (Carter et al., 2006).

Prolonged retention of carbon black particles in the lung was found in rats and mice exposed for 13 weeks to 7 and 50 mg/m^3, and in hamsters exposed for 13 weeks to 50 mg/m^3. One group of rats was also exposed to 50 mg/m^3 of low surface area carbon black, which was more efficiently cleared from the lungs than 50 mg/m^3 high-surface carbon black. This shows that particle surface area is an important determinant of lung clearance, and therefore observed inflammatory effects. Moreover, a greater and more persistent inflammatory and related oxidative stress response was observed in rat lungs. The authors suggested that it is a

likely mechanism by which high lung burdens of particles can induce secondary genotoxicity resulting in cell mutations and subsequent lung tumor formation (Elder et al., 2005). BAL fluid was analysed for markers of inflammation (cell type, reactive oxygen and nitrogen species, and cytokine levels) and lung tissues were evaluated for gene expression of anti-inflammatory mediators. A dose- and time-related effect was seen with all measured parameters, with rats showing the highest levels of ROS, and hamsters showing the lowest levels (Carter et al., 2006). Lung tissue from rats taken from the same study was analysed to show 8-oxo-dG adduct formation, demonstrating that oxidative DNA damage is a primary mechanism of tumour formation (Gallagher et al., 2003), while 32P post-labeling analysis of lung samples showed no induction of DNA-PAH adducts, which would indicate a direct DNA-binding effect (Borm et al., 2005).

Modrzynska et al. (2018) evaluated induction of pulmonary inflammation, pulmonary and hepatic acute-phase response and genotoxicity following exposure to TiO_2, cerium oxide (CeO2) or Carbon Black (Printex 90 14nm) nanoparticles in female C57BL/6 mice. Animals were exposed to a single dose of 162 µg NPs/mouse by either intratracheal instillation, intravenous injection or oral gavage and terminated 1, 28 or 180 days post-exposure. Liver DNA damage was observed using the Comet Assay after intravenous injection and intratracheal instillation of carbon black nanoparticles but not after exposure to TiO_2 or CeO2. Hepatic DNA damage seen with carbon black was caused by translocated particles detected in the liver (Modrzynska et al., 2018).

Increased inflammation and intracellular calcium was reported after single instillation in rats with ultrafine carbon black (14 nm) but not with larger carbon black particles (320 nm) (Brown et al., 2000). The existing literature supports that the inflammation causes serious damage to the lung functions by many mechanisms, most of which are not properly understood (Niranjan and Thakur, 2017).

De Haar et al. (2005) demonstrated that ultrafine carbon black particles can act as adjuvants, that is they may enhance the body's immune response to an antigen, promoting respiratory sensitisation. Mice exposed intranasally with a combination of ovalobumin (OVA) and 200 µg of carbon black (particle size not reported) showed an increased expression of ovalobumin-specific Th2 cytokines and corresponding lymph node enlargement. This was further supported by observed allergic airway inflammation after challenges with OVA.

Furthermore, it has been suggested that Printex-90 decreased the expression profile of CYP gene that may interfere with the detoxification potential of inhaled toxic compounds (Elder et al., 2009). It was also reported that carbon black nanoparticles were associated with accelerated cardiovascular changes, which may compromise "healthy aging" and may trigger cardiovascular diseases (Buchner et al., 2013).

A 24-hour inhalation study with ultrafine carbon black (median mass diameter of 46 nm) at levels which do not induce detectable pulmonary neutrophilic inflammation (172 µg/m^3), showed that it may cause cardiovascular and pulmonary impairment, in the absence of detectable pulmonary inflammation, in individuals suffering from pre-existing cardiovascular diseases (Upadhyay et al., 2008).

A study on the cardiac autonomous nervous system in mice indicated that carbon black (particle diameter 19 nm) can cause cardiovascular dysfucntions independent of apparent myocardial and pulmonary injury (Jia et al., 2012). These results suggest that some of the adverse effects, such as cardiotoxic ones, caused by exposure to carbon black may not be detected in standard studies. Furthermore, some effects may occur even at very low doses in compromised individuals.

Carbon blacks with diameters of 14 nm and 37 nm were tested by inhalation in two studies using female rats and another study in male and female rats. The incidence of benign and malignant lung tumours was significantly increased in female rats in studies, however no

tumour increase was found among the male rats (IARC, 2010).

Three studies by intratracheal administration in rats using carbon blacks of 14 nm and 95 nm diameters showed increased incidence of benign and malignant lung tumours. The increase in tumour frequency was dependent on the size of the particles, with smaller particles showing the highest potency (IARC, 2010).

The developmental toxicity of carbon black (Printex 90, 14 nm) nanoparticles was evaluated in a mouse model using intratracheal instillation. Time-mated mice exposed to carbon black (11, 54 or 268 µg/animal; the dose corresponds to inhalation exposure of 42 mg/m^3 for 10 hours) demonstrated liver DNA damage in mothers and in offspring exposed *in utero*. There were no effects on gestation and lactation. However, exposure produced persistent lung inflammation, and induced small changes in locomotor activity in female offspring in the Open field test. Behaviour of exposed male offspring was changed compared to the controls. The observed change in neurobehaviour was thought to be more likely an indirect effect caused by the maternal inflammation rather than a direct particle effect on the foetus (Jackson et al., 2011).

Human maternal exposure to particulate matter in the air – of which carbon-based nanoparticles can make up a significant fraction - is associated with negative birth outcomes. The mechanisms underpinning these effects have been suggested to be based on oxidative stress through pulmonary inflammation (Shah et Balkhair, 2010).

Dermal exposure
SCCS (2015) concluded that the available unpublished data show that carbon black particles with a diameter of greater than 20 nm are not likely to be absorbed through the intact skin. No information is available in relation to particles smaller than 20 nm (SCCS, 2015).

Carbon black applied to intact and abraded rabbit skin under occlusive conditions for up to 24 hours showed no signs of oedema or erythema. Carbon black is not considered to be irritating to the rabbit skin (SCCS, 2015).

Skin sensitisation of carbon black has been tested using the Guinea Pig Buehler test and the Local Lymph Node Assay (LLNA), in both cases showing no evidence of skin reactions. However, since carbon black particles are unlikely to penetrate the skin, sensitisation is not anticipated, however, it cannot be ruled out in cases where particles could enter via damaged skin, as carbon black particles have been shown to act as immune adjuvants, leading to sensitisation (de Haar et al., 2005; ECHA, 2018; SCCS, 2015). This may be particularly important with regards to the use of carbon black in tattoing in which skin penetration is ensured.

Carbon black is the most used pigment in tattoo and PMU inks (Piccinini et al., 2015b). Sepehri et al. (2017) investigate the systemic distribution of tattoo pigments in extensively tattooed mice. Using TEM, they identified intracellular tattoo pigments in the skin, in lymph nodes and also in the Kupffer cells (liver macrophages) 1 year after tattooing, indicating systemic distribution of tattoo nanopigments (carbon black and red azo pigments). Due to the migration of the chemicals and particles through the body, systemic adverse health effects following tattooing cannot be excluded.

Summary
Carbon black is categorised by the International Agency for Research on Cancer as possibly carcinogenic to humans - category 2B - based on sufficient evidence for the carcinogenicity of carbon black in experimental animals and inadequate evidence in humans (IARC, 2010), however most *in vitro* mutagenicity studies of carbon black have proved negative (IARC, 2010).

The available evidence shows that carbon black does not directly interact with DNA. Carbon black causes inflammation and oxidative stress in the lung leading to mutations, which is a

secondary genotoxic mechanism. Negative results in DNA adduct studies demonstrate the inability of carbon black to produce DNA adducts in the lungs of rats and in human lung epithelial cells.

Lung tumours seen in rats exposed by inhalation to high concentrations of carbon black are likely related to the pro-inflammatory response, providing further evidence that carbon black causes genotoxicity through a secondary mechanism related to observed rat-specific lung overload (Gallagher et al 2003, Borm et al. 2004, Elder et al 2005, Carter et al 2003, ECETOC 2013).

The overall weight of evidence indicates that carbon black nanoparticles should not be considered a direct reproductive toxicant, however SCCS (2015) recommend against applications that may lead to inhalation exposure as it considers animal carcinogenicity studies in carbon black are relevant to human inhalation exposures.

4.4 Nano-sized Zinc Oxide (ZnO)

ZnO (CAS Number 1314-13-2) is a white powder. It is considered non-toxic (except for ZnO fumes, see bellow), and it is widely used as an additive in numerous applications. Zinc is an essential trace element required for numerous basic functions in all living organisms as it plays a unique and extensive role in biochemistry of numerous enzymes and other biomolecules (ADSTR 2005).

ZnO is mainly used in the rubber industry as a vulcanizing activator, in ceramics, and in concrete. Applications where ZnO is added as a white pigment have been decreasing. In the past ZnO was a common white pigment in paintings. Today, it is used in cosmetics, where also UV absorber properties are utilized. The scattering power of ZnO depends on the particle size, with certain sizes of ZnO nanoparticles being transparent.

Overall, nano-ZnO represent one of the most widely used and studied nanomaterials. Its main applications are as polymer fillers and UV absorbers. It can be found in cosmetics (mainly in sunscreens), electronic sensor, solar voltaics, transducer applications, plastics, ceramics, glass, cement, rubber, lubricants, paints, food, batteries, fire retardants, etc. (Ma et al., 2013). Due to wide size variability, the presence of nanoparticles in the pigment-grade ZnO cannot be excluded.

Together with nano-TiO_2, nano-ZnO belong to the category of metal oxide nanomaterials. However, in terms of the interactions with biological systems and potential toxicity, there are substantial differences between the toxic effects and the mechanisms of toxic actions observed within TiO_2 and ZnO nanomaterials. While TiO_2 is a typical low solubility low toxicity nanomaterial, contrarily, the solubility is considered as the main mechanism of nano-ZnO toxicity.

Reducing the particle size increases the reactive surface area, reactivity and zinc ion dissolution rate. In addition to size, other characteristics can change the toxic potential of ZnO particles. Especially surface coating is able to reduce the dissolution rate and toxic effects mediated by zinc ions (Baek et al., 2012).

Nano-ZnO dissolves in biological fluids including artificial gastrointestinal fluid and lung fluid to form Zn2+ that seems to be distributed systematically to organs. Zinc absorption is slightly higher for the small particles compared to the larger ones, which could be due to a higher dissolution rate (The Danish Environmental Protection Agency, 2015).

In order to describe the potential toxicity of nano-ZnO, both data on nano-ZnO and conventional forms (metal, salts, colloids) should be reported.

Oral exposure
Systemic toxicity was investigated in a 90-day oral study and revealed histopatological changes in the pancreas and stomach of rats exposed to 125, 250 and 500 mg/kg/day of nano-ZnO (20 nm). However, sensory responses, motor activity, weight, and urinalysis were not changed in the exposed animals and no fatalities were reported (Park et al., 2014).

Another sub-chronic study with nano-ZnO (100 nm) with rats exposed to 500 mg/kg, 125 mg/kg and 31.25 mg/kg found significant changes in hematological and blood biochemical analysis, which could correlate with anemia-related parameters, in the 500 mg/kg groups of both sexes and significant adverse histopathological effects in the stomach, pancreas, eye, and prostate gland tissues. At 31.25 mg/kg, no adverse effects were detected (Kim et al., 2014). In this study two forms, negatively and positively charged ZnO, were tested and the particle charge did not affect the degree of the lesions.

Oral exposure of rats to 100 and 400 mg/ml nano-ZnO (<50 nm) for 12 weeks decreased sperm cell counts, sperm motility, live and normal sperms, as well as serum testosterone levels. Severe histopathological damage with a significant increase in lipid peroxidation and a decrease in antioxidant enzyme activity in testis was observed (Hussein et al., 2016).

In a prenatal developmental toxicity study (according to the OECD test guideline 414) of ZnO nanoparticles in rats (Hong et al., 2014), ZnO nanopartciles (20 nm, positively charged) were administered to pregnant rats by gavage at 100, 200, and 400 mg/kg/day over the period of gestational days 5–19. Effects on the intrauterine growth and fetal visceral morphology were observed in the group administered with 400 mg/kg/day. Interestingly, no significant difference was found in the Zn content of fetal tissue between the control and high-dose groups. Dose of 200 mg/kg/day did not cause adverse heath effects in this study.

Srivastav et al., (2017) evaluated the genotoxic potential of ZnO nanoparticles after oral administration in mice at dose levels of 300 and 2000 mg/kg body weight. Chromosomal aberration and micronucleus tests were conducted following OECD test guidelines 475 and 474, respectively. DNA damage was evaluated at 0, 24, 48, and 72 h post-treatment using a randomly amplified polymorphic DNA (RAPD) assay; additionally, semen analyses were also performed at 34.5 days post oral exposure. The reactive oxygen species (ROS), as measured by 8-oxo-20-deoxyguanosine and catalase were increased at the highest dosage (2000 mg/kg) of nano-ZnO compared to controls. Aberrant sperm morphology with reduced sperm count and motility were also present in the high-dose group. The results suggested that ZnO NPs are mildly genotoxic in a dose-related manner and this toxicity were induced by generation of ROS.

Inhalation exposure
Inhalation of ZnO (nano)particles has been reported to lead to the development of metal fume fever in occupational conditions. It is an acute disease induced by inhalation of metal oxides which does not usually progress to chronic lung disease. The exact mechanism behind the development of the metal fume fever is not known, but it is believed to involve an immune response (inflammation of the respiratory tract) to the inhaled ZnO with subsequent release of histamine or histamine-like substances (ATSDR, 2005).

Evaluation of pulmonary toxicity of 35nm ZnO nanoparticles following inhalation (4 weeks, 2 and 10 mg/m^3) and intratracheal instillation (0.2 or 1 mg/kg) detected transient inflammation characterized by increased inflammatory markers in BAL fluid of exposed rats. However after a recovery period no persistent inflammation in the rat lungs was observed. It was suggested that well-dispersed ZnO nanoparticles have low toxicity (Morimoto et al. 2016).

Following nasal exposure of rats to ZnO nanoparticles (38 nm, the presence of ZnO nanoparticles in brains and olfactory bulbs was confirmed using transmission electron microscopy (TEM), demonstrating an olphactory bulb-brain tranlocation pathway for ZnO

nanoparticles (Kao et al. 2102).

In a study investigating nano-ZnO neurotoxicty in mice after intraperitoneal exposure (Tian et al. 2015), ZnO nanoparticles (20-80 nm in TEM) caused abnormal cognitive functions and neuronal pathological changes in the hippocampus, with old (18-month) mice exhibiting greater susceptibility to ZnO nanoparticle-induced damage compared to young (6-month) mice. Synergistic influence of aging and ZnO nanoparticles exposure on systemic inflammation was shown as a probable mechanism.

Dermal exposure
With respect to the application of nano-ZnO in cosmetics, mainly sunscreens, the key question is whether or not nano-ZnO might penetrate the skin, affect the living layers of the epidermis and dermis, and be absorbed into the blood stream leading to potential systemic health hazard. This issue was addressed in numerous studies, and prevailing experimental data did not report absorption of nano-ZnO through the skin, as summarized in the reports by SCCS (2012; 2014).

However, zinc from ZnO particles in sunscreens applied outdoors was detected to be absorbed through human skin when exposed to the sun in a real-life environment. It is not clear whether trace amount of zinc detected in blood and urine has been absorbed as ZnO particles or soluble Zn or both (Gulson et al., 2010). Considering the dissolution of ZnO, it is most likely that the zinc was absorbed in the ionic form. Moreover, only a small proportion of Zn ions released from the ZnO nanoparticles may be available for systemic exposure when applied dermally. Interestingly, slightly increased blood and urine Zn levels were detectable some 11 days after application, suggesting that dermally applied soluble nanomaterials may represent a reservoir for prolonged ion release (Gulson et al., 2010). Morever, Filon et al. (2015) pointed that metal nanoparticles can release a greater amount of ions compared to bulk material, due to their high surface/mass ratio.

Ryu et al. (2014) performed a repeated dose 90-day study according to the OECD guideline 411 (with modifications for dosage, biochemical parameters, and histopathologic evaluation) and derived the NOAEL for dermal exposure route at 1000 mg/kg body weight.

Low or absent dermal toxicity, no acute dermal toxicity, no skin irritation and no skin sensitization potential were also observed in other studies as reviewed in Schilling et al. (2010). Schilling et al. (2010) concluded that there is no evidence that ZnO micro- or nano-structured particles pose a phototoxic or photogenotoxic risk to humans.

However, Jang et al. (2012) reported potential photoxic effects of ZnO nanoparticles of 20 and 100 nm, both positively and negatively charged, in EpiDerm (a 3D human skin equivalent model) starting at 10 µg/ml using 3T3 Neutral Red Uptake assay.

As the behavior of nano-ZnO might be different under various conditions, for risk assessment purposes, it is important to evaluate the effects in commercially-relevant formulations and detailed characterization of the particles *in situ* should be performed (i.e. within the test formulation).

***In vitro* studies**
Using *in vitro* multiparametric toxicity screening approach George et al. (2009) describe a mechanisms of toxicity of ZnO nanoparticles based on dissolution and Zn ion release. Dissolution of ZnO nanoparticles and release of Zn2+ were capable of ROS generation and activation of an integrated cytotoxic pathway that includes intracellular calcium flux, mitochondrial depolarization, and plasma membrane leakage. Moreover, the authors showed that iron doping were capable to reduce of ZnO cytotoxicity by slowing the Zn2+ release.

Khan et al. (2015) evaluated the toxic effects of ZnO and TiO_2 nanoparticles at different concentrations (50, 100, 250 and 500 ppm) and compared them with their respective salts

using a battery of cytotoxicity and genotoxicity assays. Both nanoparticles were found to dose dependently generate reactive oxygen species (ROS) in parallel with depletion of glutathione and glutathione-S-transferase levels, and increased superoxide dismutase, catalases and lipid peroxidation. ZnO and TiO_2 nanoparticles produced roughly equal levels of oxidative stress. The genotoxic potential of both nanomaterials was investigated in the *in vitro* alkaline comet assay. DNA damage induced by the nanoparticles was concentration dependent and was significantly greater than their ionic forms at 250 and 500 ppm concentrations. Nanoparticles of ZnO were significantly more genotoxic than TiO_2 at higher concentrations. The toxicity of these nanoparticles was found to be due to the generation of ROS causing oxidative stress.

Summary
Nano-ZnO is a soluble metal oxide nanomaterial. Metal ions released from nano-ZnO are well-known inducers of ROS production, oxidative stress and pulmonary inflammation. Particle dissolution is considered as a key ZnO property involved in oxidative injury (George et al., 2009).

In addition to other nanomaterial properties (surface modification, shape), the solubility rate and Zn2+ release depend also on characteristics of the environment (pH, presence of biomolecules and salts). Importantly, the ion release-madiated toxicity of nano-ZnO can be effectively controlled and by surface coating and dopping (incorporation other atoms, e.g iron, into the ZnO lattice) that result in lower solubility (George et al., 2009).
Potential genotoxic effects of nano-ZnO were reviewed by Schilling et al. (2010). The authors concluded that clastogenic activity was observed in mammalian cells *in vitro* but there was clearly no indication for a clastogenic potential or an aneugenic activity in *in vivo* studies (Schilling et al., 2010).

4.5 Nanosized Barium Sulphate (BaSO4)

BaSO4 (CAS Number 7727-43-7) is an inert and high density material. $BaSO_4$ is a water-insoluble salt of barium. It belongs to low-toxicity low-solubility materials. Due to its inertnes, insolubility amd biocompatibility, $BaSO_4$ is used for radiographic contrast media.

Nano-$BaSO_4$ is mainly used as a filler or additive, and there are also various promising applications of nano-$BaSO_4$ in nanomedicine (e.g. in ortopedic medicine, diagnostic imaging), however it is also listed as a colourant in the EU catalogue of nanomaterials used in cosmetic products.

In the paint and varnish industry, barium sulfate is used as filler mainly due to its inertness and high density. It improves the volume and consistency, viscosity and workability of e.g., fillers, surfacers, and primers. In covering coats and enamels, it is used as "spacer" to improve titanium dioxide pigment scattering or to avoid flocculation of organic or inorganic colored pigments. Compounds that consist of zinc sulfide and barium sulfate as a white pigment (lithopone) have become less important.

There is a limited amount of toxicological studies evaluating potential toxic effects of nano-$BaSO_4$. Most of the available data are from inhalation or *in vitro* experiments. In some studies, nano-$BaSO_4$ is used as a negative control (inert) nanomaterial (e.g. Schwotzer et al., 2017; Schwotzer et al., 2018; Westphal et a., 2015, see bellow).

Inhalation exposure
In a extensive short-term (5-day) inhalation study evaluating 13 different types of nanomaterials and one type of microparticles, $BaSO_4$ (NM-220, representative nanomaterial from the European Commision Joint Research Centre Nanomaterials repository), a no-observed adverse effect concentration (NOAEC) higher than 50 mg/m^3 was derived (corresponding to the highest tested concentration) for $BaSO_4$. Barium was not detectable in the lymph nodes of the animals in the exposure groups and it was only 1.4 µg in the high concentration recovery

group exposed to 50 mg/m^3 $BaSO_4$. (Landsiedel et al., 2014).

Another inhalation study with NM-220 (50.0 mg/m^3 for 1, 28, 90 days; 6h/day, 5 days/week) did not reveal any significant changes in body weights or food and water consumption, no clinical signs due to particle exposure. Nano-$BaSO_4$ exposure caused only a slight and reversible increase of inflammatory cell numbers and enhanced Ki67 levels (a maker for cell proliferation, non persistent effect seen). Barium elimination was extremely rapid in this study and no signs of overload were detected even upon the very high dose used (50 mg/m^3). The only persistent effects were detected in the nasal cavity: mucous cell hyperplasia (non-persistent) and eosinophilic globules (persistent) in the olfactory and respiratory epithelia (from exposure day 28) (Schwotzer et al., 2017). Gene expression analysis performed under the same experimental conditions, revealed changes in pathways related to inflammation, and lung cancer. However the relevance of these finding for risk assessment has not been confirmed in any further experiments (e.g. confirmation of the gene expression findings on a higher then molecular level) (Schwotzer et al., 2018). A limitation of this study is related to application of only one, moreover high and unrealistic, concentration of $BaSO_4$ (50mg/m^3). Mass median aerodynamic diameter was 2.95 ± 2.43 µm indicating presence of big aggregates/agglomerates. Pronounced aggregation/agglomeration may be related to high nanoparticle concentrations.

Cordelli et al (2016) investigated genotoxicity in the blood cells of female rats exposed to aerosol concentrations of 0.1 up to 3 mg/m^3 CeO_2 or 50 mg/m^3 $BaSO_4$ nanomaterials (6h/day; 5 days/week; whole-body exposure) for 3- or 6-months. DNA effects were analysed in leukocytes using the alkaline Comet assay, gene mutation and chromosome aberration assay. No significant changes were observed in leukocytes of the exposed animals.

A biokinetics study comparing tissue distribution of 131Ba over 28 days after inhalation, intratracheal instillation, gavage and intravenous injection of radiolabelled $^{131}BaSO_4$ nanoparticles was performed by Konduru et al. (2014). While the instilled and inhaled $BaSO_4$ nanoparticles were cleared quickly, administration via these routes resulted in higher tissue retention than with ingested nanoparticles. Injected $BaSO_4$ nanoparticles were localized in the reticuloendothelial organs and redistributed to the bone over time. Fecal excretion was the dominant elimination pathway for all three routes of exposure. Interestingly, $BaSO_4$ nanoparticles exhibited lower toxicity and biopersistence in the lungs compared to other poorly soluble nanoparticles such as CeO_2 and TiO_2. The authors suggest that particle dissolution is a likely mechanism, although, in physiological simulant fluids, the same $BaSO_4$ samples dissolved only slightly: 0.1% in phosphate buffered saline and phagolysosomal simulant fluid (pH4.5) and 1% at pH 1 (during 28 days). Further studies are needed to elucidate in vivo behaviour of these nanoparticles.

***In vitro* studies**

Negative results were obtained also in *in vitro* studies in which $BaSO_4$ did not elicit any cellular effects *in vitro*: no cytotoxicity, no reactive oxygen species production, no TNF-α release, no H_2O_2 synthesis by rat NR8383 alveolar macrophages (Wiemann et al., 2016). Simiarly Westphal et al. (2015) did not observed toxic effects in NR8383 cells and the derived LC50 value, a very high concentration of 455.9 µg/cm2, suggests low toxic potential of nano-$BaSO_4$.

Summary

Most studies used the same nanomaterials for their experiments (NM-220, a representative nanomaterial from EU JRC Nanomaterials repository). Thus there is limited information on the effects that may be induced by different nano-$BaSO_4$ characteristics (such as shape, size, surface modifications) on toxicity. However, based on the known properties and categorization of nano-BaSO4 among low solubility low toxicity nanomaterials, it can be presumed, that general rules, such as fibre-toxicity paradigm, increase reactivity with relatively increasing surface area in smaller particles, impurity-mediated toxicity, will be applicable also for nano-$BaSO_4$.

In a comparative inhalation studies, $BaSO_4$ nanoparticles exhibited even lower toxic potential than other poorly soluble nanoparticles (Landsiedel et al., 2014; Konduru et al., 2014).

4.6 Nano-sized Iron Oxides

Iron oxide pigments comprise various substances, among which iron(II) oxide wustite (FeO), iron(III) oxide hematite (α-Fe_2O_3) and iron(II,III) oxide magnetite (Fe_3O_4) are the most known, used and most often studied. The predominant industrial use of iron oxides is in construction, paints/coatings, masonry and tiles (Pease et al., 2016).

The colour of iron oxides range from black (magnetite) and grey (wustite), to yellow (goethite), red (hameatite) and orange (lepidocrocite). Iron Oxide Nanoparticles can also be transparent (at sizes bellow 10 nm) (Mohapatra and Anand, 2010). Steel constructions, cars and concrete products are the main pigment-related uses of iron oxide (nano)particles where they can, among other functions, e.g. reduce corrosion (Mohapatra and Anand, 2010).

Iron oxide nanoparticles are found naturally in the environment (originating mostly from volcanic eruptions). Moreover, they are unintentionally produced by industry and traffic, contributing to air pollution particulates, and also intentionally chemically synthesized for a wide variety of applications, especially in electronics and biomedicine (magnetic resonance imaging, targeted drug delivery, tissue engineering, hyperthermia tumour destruction, chelation therapy, etc.) (Singh et al., 2010). Iron oxide nanoparticles have superparamagnetic properties and in general exhibit good biocompatibility. For biomedical applications, various surface modifications that prevent the nanoparticles from aggregation and increase their lipophilicity and stability have been explored to ehnace biocompatibility of iron oxide nanoparticles and to avoid their clearance from bloodstream by cells of the reticuloendothelial system (Mahdavi et al., 2013).

Iron oxide nanoparticles belong among the low soluble low toxicity particles (Pauluhn, 2012).

Oral exposure
In an acute oral study performed according to the OECD protocol (Test guidelines 420 for acute oral toxicity—fixed dose procedure), female Wistar rats were exposed by oral gavage to 500, 1000 and 2000 mg/kg of bulk and nano iron(III) oxide (Fe_2O_3). No signs of clastogenicity, aneuploidy or polyploidy in the comet assay in peripheral blood cells, peripheral blood micronucleus test, bone marrow micronucleus assay and chromosomal aberrations assay were detected for either iron oxide samples (Singh et al., 2013). However, biochemical changes, negative for bulk iron oxide, were significantly changed in nano iron oxide (Kumari et al., 2013).

Results of a 28-day oral study in Female Wistar rats comparing nano Fe_2O_3 particles (30 nm) and bulk Fe_2O_3 (<5µm) indicated higher toxic potential of nano-forms. Doses 30 and 300 mg/kg/day did not exert any adverse effects in any form, however, the highest tested dose of 1000 mg/kg/day of nano-iron oxide caused changes in biochemical parameters in red blood cells and organs as well as histopathology (necrotic) changes in liver, kidney and spleen but not in the brain and heart (Kumari et al., 2014).

Inhalation exposure
In vivo animal studies on inhalation exposure to iron oxide are limited to micro-sized particles. Overall, their results show overload toxicity typical for low soluble low toxic particles at high exposure doses (reviewed in Pease et al., 2016).

Other routes
Studies performed via other routes than the more likely ones in terms of human exposure, offer however important mechanistic insight.

In a recent study of Dhakshinamoorthy et al. (2017), adult male mice were intraperitoneally administered with 45 nm Fe_2O_3-NPs (25 and 50 mg/kg body weight) once a week for 4 weeks. A significant change in locomotor behaviour and spatial memory was observed in treated animals. Moreover, damage to blood–brain barrier permeability, nanoparticle accumulation, oxidative stress, including lipid peroxidation and DNA damage in brain regions were observed.

***In vitro* studies**
Feng et al. (2018) investigated the influence of particle size and surface coating on the *in vitro* and *in vivo* biological behaviors of IONs (Iron Oxide Nanoparticles). Commercially available IONs with different core size (10 nm or 30 nm) and surface coating, polyethylenimine (PEI) and polyethylene glycol (PEG), were used in this study. While PEG coated IONs did not exhibit toxic effects except for high concentrations, PEI coated IONs toxicity included the disruption of cell membrane integrity, ROS generation, apoptosis, and G2-phase cell cycle arrest in both RAW264.7 macrophages and non-phagocytic SKOV-3 ovarian cancer cells. *In vivo* (intravenous administration) PEI coated IONs were lethal for the exposed SKOV-3 tumor bearing nude mice and BALB/c mice. Among PEGylated IONs, 10 nm ones exhibited relatively higher cellular uptake and tumor accumulation than 30 nm ones. Cytotoxicity of PEI-coated IONPs may originate from the PEI coating material. The study emphasizes the possibility to manipulate toxic effects of IONs by modification of their physicochemical characteristics. Size and coating have been proven as crucial factors to be considered for various IONs applications, enabling significantly decrease/increase toxicity. This further complements the hypothesis, that higher bioavailability and larger surface areas may lead to higher levels of intracellular free iron with decreasing size of iron oxide, exacerbating toxic effects in nano-forms.

Summary
From the weight of evidence available for iron oxides, the "bulk" iron oxides are not human lung carcinogens. Current evidence remains conflicting for "nano" form iron oxides as to their genotoxic potential; in vivo via the oral route, no genotoxicity is seen but genotoxicity/carcinogenicity via the lung appears not to have been investigated. Further studies are needed to draw conclusions for "nano" form iron oxides (Pease et al. 2016).

The potential mechanism of toxicity can be the release of leachable iron (III) from Fe_2O_3. Iron is a transition metal and readily reacts with hydrogen peroxide and oxygen (produced in cells e.g. by the mitochondria) generating highly reactive hydroxyl radicals and ferric ions via the Fenton's reaction. In iron oxide nanoparticles, leachable iron levels were significantly higher than in the micro-forms and the surface area is probable determining the iron leachability (Bhattacharya et al. 2012). Iron oxide nanoparticle-induced iron disruption of homeostasis, as proposed by Kornberg et al. (2017), arises from degradation of IONs in the acidic environment of (phago)lysosomes after their cellular uptake. Released free iron ions cause an excess of reactive oxygen species generation via participation in the Fenton's reaction (see above).

4.7 Nano-sized Aluminium Oxides (Al2O3)

Aluminium oxide, Al_2O_3 (CAS Number 1344-28-1) is an insoluble aluminium compound. At neutral pH, the Al oxides and oxyhydroxides are chemically stable (Krewski et al., 2007). Industrial applications of Al_2O_3 comprise its use in adsorbents, abrasives, lubricants and water-proofing agent (Krewski et al., 2007).

Aluminium oxide flakes coated with highly refractive metal oxides such as titanium dioxide or iron oxide exhibit pearlescent effects and are used in paints for reflective decorative effects (automotive or cosmetic industries). The pearlescent effect is due to light interference rather than to light absorption (Sharrock al 2000, Cramer 2002).

Nano-$Al2O_3$ is mainly used as a filler and additive in numerous applications. Al_2O_3 nanoparticles are stable in biological systems. In addition to insolubility in water, Al_2O_3 nanoparticles are not easily ionized, even in acidic conditions, such as gastric juice (pH 1.5–3.5) and the lysosomal

lumen (pH 4.5–5.0). It results in low reactivity with cellular components (Park et al., 2017b).

Nanosized aluminium oxide particles are also generated during aluminium welding and corundum grinding. Primary aluminium nanoparticles are present in aluminium welding fume and corundum dust (10–35 nm and crystalline 10–75 nm, respectively), however they tend to agglomerate and form larger particles (Schneider et al. 2013). Chronic occupational inhalation of nanoparticle-containing bauxite and other non-reactive Al dusts was associated with pneumoconiosis in exposed workers (reviewed in Willhite et al., 2014). No significant associations have been found between occupational Al exposures and cancer risk (Willhite et al., 2014).

Oral exposure
Park et al. (2017a) compared toxicokinetics of three types of commercially available aluminium-based nanoparticles: two rod-type aluminum oxide nanoparticles with different aspect ratios (short rods with aspect ratio of 2.1 ± 0.4, lenght <20 nm, and long rods with aspect ratio of 6.2 ± 0.6, length > 20 nm, the precise dimensions of the rods were not reported) and spherical Al_2O_3-coated CeO_2 nanoparticles (approx. 15 nm), in a repeated dose oral study (2 and 6 mg/kg, 6 days/week for 28 days). All three types of the tested materials were slightly ionized in the gastric fluid and rapidly particulized in the intestinal fluid. All samples had elevated Al levels in the heart, spleen, kidney and blood at 24 hours after the final dose.

Prabhakar et al. (2011) compared the acute oral toxicity of bulk Al_2O_3 (99%) and 30 nm or 40 nm Al_2O_3 in rats (500, 1000 or 2000 mg/kg). Oxidative stress markers were changed in the nanoparticle-treated group. The histopathological changes were investigated in the brain, liver, kidneys and heart on the day 14 after the treatment. Changes were detected only in the liver (dilated central veins and distended portal tracts) in animals treated with the highest dose of 2000 mg/kg of the nano Al_2O_3.

Inhalation exposure
After 4-week inhalation exposure (6h/day, 5 days/week) to 0.4, and 3 mg/m^3 of 10 and 40 nm Al_2O_3 nanoparticles, no adverse effects were observed. At the highest tested concentration of 28 mg/m^3, particles accumulated in enlarged, foamy alveolar macrophages. Slight to minimal focal septal thickening, increased numbers of epithelial cells and increased inflammatory infiltrate were observed (Pauluhn 2009).

Using the same nanoparticles (short and long Al_2O_3 rods and aluminium coated cerium nanoparticles; Park et al., 2017a) and AlOOH nanoparticles (200 nm), Park et al. (2017b) investigated pulmonary biopersistence and subsequent toxicity 13 weeks after single intratracheal instillation. The uptake of all nanoparticles by pulmonary immune cells was observed, but the total number of pulmonary immune cells only increased significantly in mice treated with AlOOH nanoparticless. Long rods and AlOOH nanoparticles increased the proportion of eosinophils in the lung, whereas AlCeO2 nanoparticles increased that of basophils.

The highest toxicity was observed in the mice treated with AlOOH nanoparticles, which showed lower biostability (increasd degradability). Aspect ratio and biostability may be important factors in the determination of the biopersistence of nanoparticles and the subsequent biological response. Modification of nanoparticles by the addition of a hydroxyl group aggravated their biological activity by increasing generation of ROS. Short Al_2O_3 rods exhibited lowest toxicity out of the tested samples.

Using whole-body dynamic inhalation model, Li et al. (2017) observed emphysema and small airway remodeling in murine lungs, accompanied by enhanced inflammation and apoptosis after seven-day exposure to 40 nm Al_2O_3 nanoparticles (0.4 mg/m^3; 8h/day). As a mechanism, the authors determined down-regulation of PTPN6 leading to inflammation and apoptosis, and ultimately resulting in the development of the observed induction of chronic obstructive

pulmonary disease-like lesions.

Adamcakova-Dodd et al. (2012) did not find severe pulmonary damage or inflammation (based on LDH, IL-6, IFN-γ, MIP-1α, TNF-α and MIP-2 in BAL fluid) in male C57Bl/6 mice exposed to 2–4×2800 nm Al_2O_3 nanowhiskers in a whole body exposure chamber (3.3 mg/m^3, 4 h/day, 5 days/week for two or four weeks). Methacholine challenge revealed no evidence of airway hyperreactivity. Histology did not find any signs of airway remodeling, inflammation, lymphoid aggregates or fibrosis. The length of the nanowhiskers observed in transmission electron microscopy was 50 to 300 nm (not the claimed 2800 nm). After 4 weeks, 58% of the Al_2O_3 nanowhiskers were dissolved in artificial lysosomal fluid (at pH 4.5) and about 15% in Gamble's solution (pH 7.4) which represents extracellular fluid. Authors suggest that high dissolution rate in lysosomal fluid is caused by the presence of citrate.

Other routes
After intraperitoneal injections of 10 nm Al_2O_3 nanoparticles (single dose of 3.9-8.5 g/kg; or repeated dose of 1.3 mg/kg every 48h for 28 days) Al bioaccumulation was observed in the brain, liver, kidneys, intestine, and spleen. Increase in DNA damage in the brain cells was detected in the comet assay, suggesting possible genotoxic effects of Al_2O_3 nanoparticles . LD50 24 hours post-exposure was calculated at 15.10 mg/kg. However, neither histopathology evaluation, nor analyses of nanoparticle presence in the tissues were performed within the study (Morsy et al., 2016).

After intravenous injection (1.25 or 5mg/kg), Park et al. (2017b) detected higher accumulation of longer rods (aspect ratio of 6.2 ± 0.6; length > 20 nm) as compared to short Al_2O_3 rods (aspect ratio of 2.1 ± 0.4; length < 20 nm) in mice (the precise dimensions of the rods were not reported).). Authors concluded that accumulation following a single intravenous injection with rodtypes of Al_2O_3 nanoparticles is strengthened by a high aspect ratio and, subsequently, this accumulation has the potential to influence immune functions (antigen-presentation /chemotaxis).

Summary
There is only a limited number of published studies reporting in vivo toxicity o Al203 nanomaterials. Reactive oxygen species production, oxidative stress and inflammatory responses are reported to be involved in toxicity of low soluble low toxic nanomaterials. Surprisingly, Adamcakova-Doff et al. (2012) observed relative high level of dissolved Al in artificial lysosomal fluid after 24h incubation, concluding that this topic requires further investigation, considering that Al_2O_3 is categorised among the low soluble particles.

4.8 Nano-sized Silica (SiO2)

Silica nanoparticles have a wide range of applications, expecially as additives to cosmetics, drugs, printer toners, varnishes, and food. Moreover biomedical and biotechnological are intensively developed (Napierska et al., 2010). Nano-silicate pigments can be used in paper coatings and large volumes are found in pigment-related applications as a common extender supporting the functionalistation of pigments (Yousseff et al., 1998; Abu-Ayana et al., 2005; Palraj et al., 2015).

Silica is a common filler and extender for paint due to its inertness and low cost. The hardness and inertness of silica improves resistance to wear, burnish, abrasion and stains. The narrow size distribution and bright white color, minimizes binder demand and yields an excellent color. It is also often used to replace as much as 50% of titanium dioxide in paint formulations (Palraj et al., 2015; Ralston et Eppler, 1995).

Silica (silicon dioxide, SiO_2) is a compound that appears in two major forms, crystalline and amorphous, according to the arrangement of the atoms.

Crystalline silica has structures with repeating patterns of silicon and oxygen. Chemical structures of amorhous silica are more randomly linked. The crystalline structure significantly affect properties and behaviour of silica, including toxic effects. Quartz (CAS Number 14808-60-7) is the most common form of crystalline silica and is commonly found in the environment. Occupational exposure to crystalline silica (especially at high doses and long-term durations) is associated with increased risk of development of several pulmonary diseases, including silicosis, chronic obstructive pulmonary disease, and lung cancer. Silicosis is a irreversible deadly disease, characterized by progressive lung fibrotic changes and is specifically caused by crystalline silica (Borm et al., 2011; ADSTR, 2017).

Amorphous silica (CAS Number 7631-86-9) is mainly a synthetized material and generally is considered less harmful than crystalline forms (Murugadoss et al,. 2017). However, data are insufficient to determine whether or not amorphous silica causes lung diseases in humans. The major problem of epidemiological studies assessing exposure to biogenic amorphous silica is its contamination with crystalline silica (Merget et al., 2002). Overall, amorphous silica has been far less studied in humans than the crystalline form (Napierska et al., 2010). No human data specifically evaluating exposure to nano-silica are available yet.

As described for other nanomaterials, numerous physico-chemical properties (in addition to the crystal structure, also particularly size, shape, surface propersties, impurities) affect toxicity of nano-silica. Kim et al. (2009) observed higher toxicity of silica nanoparticles compared to microsized particle both *in vivo* and *in vitro*.

Catranova et al. (1997) reported that quartz of high iron content produced more radicals and more inflammation *in vitro* than samples with low iron content (probably by generation of OH radicals via the Fenton reaction). The iron content of quartz as an important factor affecting the potential to generate free radicals was confirmed in other studies (e.g. Fenoglio et al., 2001). On the other side, neutralization of nano-silica surface may lead to mitigation of toxicicity (Duffin et al., 2001).

Moreover, the synthesis route of amorphous silica have been reported to have significant impact on toxicity of amorphous silica nanoparticles (ADSTR, 2017). Pyrogenic or fumed silica is prepared at a vapour phase and typically has a very high specific surface area and in comparison with silica synthesized by the wet route exhibited noticeably different biological effects (generally a higher toxic potential). The production process determines surface moieties (such as silanols, silanolates, and siloxanes) in silica nanoparticles which critically affect their toxicity (ADSTR, 2017).

Oral and Dermal exposure
In their extensive review on toxicilty of silica nanoparticles, Murugadoss et al. (2017) summarized that adverse effects of amorphous silica were mainly observed in acutely exposed animals, while no signs of toxicity were noted in chronically dosed animals in dermal and oral studies.

Inhalation exposure
Most *in vivo* studies on silica toxicity was performed by exposure via the respiratory tract (Napierska et al., 2010).

As summarized by Napierska et al. (2010) and a follow up review by Murugadoss et al. (2017), inhalation exposure to synthetic amorphous silica (colloidal silica, fumed silica and precipitated silica) of various sizes and under various exposure conditions elicits in exposed animals (reversible) inflammation, granuloma formation and emphysema, but no progressive fibrosis of the lungs was reported.

Clearance of micro-sized amorphous silica from the lungs during the post-exposure period has been reported to be rapid in both acute and subchronic studies in rats (Art et al., 2007; Johnston et al., 2000). Arts et al. (2007) showed that after nose-only exposure to various amorphous silica samples at concentrations of 1, 5 or 25 mg/m^3 for five consecutive days

(6h/day) the clearance of amorphous silica from the lungs during the post-exposure period was rapid as amounts of silicon were mostly below the detection limit 1 month after the exposure. Contrarily in quartz-exposed animals, silicon was found in the lungs at comparable levels 0-, 1- and 3-months post-exposure accompanied with progressive inflammation.

Summary
With regards to the toxic effects of nano-sized silica, two forms, crystalline and amorphous silica, are considered separately. Crystalline silica (mostly represented by quartz in toxicological studies) exhibits more severe adverse health effects, especially after inhalation exposure. As summarized by Borm et al. (2011). the most likely mechanism for quartz pulmonary toxicity *in vivo* is inflammation (i.e. a secondary, thresholds mechanism).

Amorphous silica is classified as partly soluble (ECETOC, 2014). Solubility has been defined as the key clearance mechanism involved in amorphous silica removal from the lung. Biopersistency of quartz is probably the main factor of its pulmonary toxicity (Arts et al., 2007).

4.9 Nano-sized Silver (nano-Ag)

Nano-sized silver may be used in paints as an antimicrobial agent, but it is also listed as a colourant in the EU catalogue of nanomaterials used in cosmetic products placed on the EU market. In the past, antimicrobial properties of silver were used in a form of e.g. nitrate. However, in the form of nanoparticles, the surface area available for microbes increases substantially.

The main mechanism by which silver nanoparticles exhibit their toxic effects is release of silver ions. Therefore, all factors, that influence the rate, extent, location and/or timing of silver ion release may afftect nano-Ag toxicity (Xiu et al., 2012), namely physico-chemical properties of nanomaterials (size, shape, surface coating) and properties of the environment (e.g. pH, salt content, temperature, etc.). Kittler et al. (2010) observed a significant increase in toxicity of nano-Ag during storage which, as authors stated, was due to the release of Ag ions.

Cronholm et al. (2013) compared uptake, cytotoxic and genotoxic effects of Ag nanoparticles and Ag salts ($AgNO_3$). High uptake of nanoparticles compared to salts was detected. Ag nanopaticles did not induce toxicity, and after 24h a decrease in aggregate/agglomerate size was observed due to release of toxic ions. The authors concluded that te results are consistent with the Trojan horse type mechanism in which the NM taken-up in cells through active mechanisms of internalization can release intracellularly the toxic ions .However, intracellular dissolution of silver was slower than that of CuO, the other tested metal nanoparticles, that were completely dissolved after 4h.

Oral exposure

Lubich (2012) tracked polymer coated and uncoated Ag nanoparticles or a silver nitrate solution to measure silver levels in tissues in male rats after 28-day oral exposure. Silver nitrate consumption lead to 10 times higher Ag concentration in the livers and spleens than Ag NPs. Interestingly, Ag nanoparticles were also observed in tissues of ion-treated rats, indicating precipitation of silver as insoluble salts that form nanoparticles. These findings shows that *in vivo* silver may change form between ions and nanoparticles.

Oral administration of 60 nm silver nanoparticles in carboxymethyl cellulose at doses 30-1000 mg/kg/day for 28 days (according to a OECD protocol) resulted in a dose dependent accumulation of silver content in blood, stomach, brain, liver, kidneys, lungs and testes indicating systemic distribution of exposed mice. Gender differences were found, with females accumulating twice more Ag in their kidneys compared to males at all doses (Kim et al., 2008). Cholesterol values were significantly increased in the blood of the groups treated with 300 or 1000 mg/kg suggesting possible liver damage. Kim et al. (2008) evaluated also the DNA damage in rats after oral exposure using OECD protocol for bone marrow micronucleus test. No significant effect on micronucleated polychromatic erythrocytes or bone marrow cells were found, however the presence of the nanoparticles in bone marrow was not reported.
Ag tissue concentrations were higher in females than in males also in a 90-day oral study (Kim

et al. 2010). Moreover, in the sub-chronic study a higher incidence of bile duct hyperplasia and liver necrosis (with no dose-response) was observed in all groups.

Inhalation

Stebounova et al. (2011) found minimal inflammatory or toxicological effects on the lung as judged by traditional measurements in bronchoalveolar lavage fluid or by histopathology in mice exposed for 2 weeks by whole-body inhalation to Ag nanoparticles of primary size 5 ± 2 nm (3.3 mg/m^3, 4 hours/day). Based on the mass of Ag delivered to the murine lungs (803 µg Ag/g lung (dw)), the authors derived that it corresponds to a hypothetical lung burden accumulated by a 70 kg person exposed to 1.0 mg/m^3 for 16.6 hours. According to the authors, Ag nanoparticles induce significant inflammation in vivo at much higher exposure doses than other metals (e.g. Cu NPs, TiO_2 NPs).

90-day inhalation study using aerosolized silver nanoparticles (18-19 nm) at concentration 49-515 µg/ m^3 showed systemic distribution with significant dose-dependent increases in silver concentrations in the blood, liver, olfactory bulb, brain and kidneys in exposed rats. Concentrations were similar in males and females except for kidneys where the female accumulated two or three times more silver (Sung et al., 2009). These results are in agreement with the observations made after oral exposure in the study of Kim et al. (2008). Dose-dependent decrease in tidal volume, minute volume and peak inspiration flows accompanied by histopathological changes (mixed cell infiltrate and chronic alveolar inflammation) were seen in the lungs. Highest dose caused a higher incidence of bile-duct hyperplasia and perivascular infiltrate in the liver.

Based on a 12-week inhalation toxicity study in rats exposed to 14-15 nm Ag nanoparticles, Song et al. (2012) calculated human NOAECs of 47 µg/m^3 (with heavy exercise) and 23 µg/m^3 (with light exercise) using the Multiple Path Particle Dosimetry Model (MPPD).

Kim et al. (2008,) evaluated DNA damage in rats laso after inhalation exposure using OECD protocol for bone marrow micronucleus test. As obtained also via the oral route, no significant effect on micronucleated polychromatic erythrocytes or bone marrow cells were found, however the presence of the nanoparticles in bone marrow was not reported.

Dermal exposure

Vlachou et al. (2007) found increased serum silver levels in patients with burns who received treatment with dressings containing nanocrystalline silver (15 nm) for 28 days or less (depending on clinical requirement). 3 months after cessation of the treatment, the levels returned to the initial values. No haematological or biochemical indicators of toxicity associated with silver absorption from the dressings were reported.
Samberg et al. (2010) applied silver nanoparticles of two different size (20 and 50 nm) to skin of pigs (0.34-34.0 µg/mL for 14 days) and investigated their penetration. 50 nm particles were detected within the superficial layers of the stratum corneum and 20 nm particles in the top layer of the stratum corneum (2010).

In a skin sensitization study performed according to the OECD protocol (guinea-pig maximization test), Kim et al. (2012) found discrete and patchy erythema in one out of 20 guinea-pig exposed to 10 nm Ag nanoparticles (average size 10 nm). The test material was reported as a weak skin sensitiser.

Genotoxicity in vitro

Comet assay was used to assess genotoxic effects of 5 - 45 nm silver nanoparticles in human peripheral blood cells and in mice. In vitro Comet assay showed DNA damage at all doses (10, 25 and 50 µg/mL) in the initial hour of exposure and only at the two high doses after the first hour: DNA damage further decreased over time. Results of the in vivo comet assay were negative (Tavares et al., 2012). An in vivo micronucleus test in rats after oral and inhalation

exposure was also negative (Kim et al., 2008).

Other routes
In a recent review, Ema et al. (2017) collected significant effects reported in the literature on the reproductive and developmental toxicity of silver nanoparticles in laboratory animals. A wide range of effects were reported, including testicular/sperm toxicity in males and ovarian and embryonic toxicity in females. Maternal injection of Ag NPs delayed physical development and impaired cognitive behavior in offspring. Ag was observed in testes, visceral yolk sac (administration during early gestation in mice), placenta, breast milk, and pre- and postnatal offspring after injection during late gestation in rats. Authors concluded that further studies employing the state-of-the-art methodologies, and administration route and doses relevant to human exposure are needed to fill the data gap and to confirm the reproducibility of study results.

4.10 Nano-sized gold (nano-Au)

Gold nanoparticles (AuNPs) have been extensively evaluated for their potential biomedical applications, for example as drug carriers, contrast agents, or therapeutics (Fratoddi et al 2014). Nanogold may also be used in consumer applications such as food packaging, beverages, toothpaste, automobiles, and lubricants (Sung et al., 2011) and is also declared as a colourant in the EU catalogue of nanomaterials used in cosmetic products.

Gold in its bulk form, and particles of micron size range or larger, are generally seen as inert and of low toxicity. Most *in vivo* studies on the toxicology of gold nanoparticles have focussed on biomedial applications and therefore have tended to use the intravenous route of administration, using very small particles (<10nm) that are capable of translocation. It is generally observed that the absorption, uptake, tissue distribution and toxicity of gold nanoparticles (AuN) is principally size-dependent (Zhang et al 2011).

Oral Exposure

The limited *in vivo* data on orally-administered AuNP suggests that the toxicity of orally ingested AuNP at therapeutic or biologically relevant doses is low, however tissue distribution and toxicity is strongly size-dependent.

In the study of Hillyer and Albrecht (2001), AuNPs (4, 10, 28, and 58 nm) were administered to mice in drinking water at concentrations of 200 ug/ml over 7 days. Tissue distribution was evaluated through visualising particles by TEM and quantified by ICP-MS. Distribution was found to be inversely related to size: the smallest particles (4 nm) were largely retained while larger particles (58 nm) were not detectable in any evaluated tissues.

The smallest particles (4 nm) were found in highest amounts in the kidneys, whereas 10 nm particles were mostly found in the stomach. 28 nm particles were retained in the stomach and small intestine, suggesting that the smallest particles were better able to cross the intestinal walls while larger particles became trapped in the mucus layer or intestinal wall.

Zhang et al (2010) administered AuNP (13.5 nm) solutions orally to mice at doses ranging from 0.138–2.2 mg/kg for 14 to 28 days. Low concentrations of gold nanoparticles did not cause any obvious decrease in body weight or appreciable toxicity. At higher doses, observed adverse effects included decreased body weight and enlarged spleens with decreased peripheral red blood cells beginning at 1.1 mg/kg. Particles were visualised by TEM in the red blood cells of the high dose groups (other tissues not evaluated).

No significant differences in other organ weight indices were noted and no deaths were reported. In this particular study, the finding of AuNP in red blood cells coupled with decreased peripheral red cells suggests AuNP-related hemolysis (Zhang et al, 2010).

In a 28 day study using gum-stabilised AuNP(average size 14nm), oral administration in rats at doses up to 300 mg/kg showed no adverse effects. Organ and body weights, histology, hematology, clinical chemistry all appeared normal (Dhar et al. 2010).

Schleh et al (2011) showed that negatively charged AuNPs were accumulated more than positively charged particles. Rats were administered radiolabelled gold nanoparticles of variable sizes and either negative (1.4-200 nm) or positive (2.8 nm) surface charge nanoparticles with opposite surface charges by intra-oesophageal instillation. The highest accumulation in secondary organs was mostly found for 1.4 nm particles; the negatively charged particles were accumulated mostly more than positively charged particles. 18 nm AuNPs showed a higher accumulation in brain and heart compared to other sized particles, however the reason for this specificity was not understood.

Inhalation Exposure

Sung et al (2011) investigated the toxicity of gold nanoparticles by inhalation in Sprague Dawley rats. 4 groups of 10 animals were exposed to gold nanoparticles (average diameter 4-5 nm) at dose of 0, 0.04 μg/m^3 , 0.38 μg/m^3, and 20 μg/m^3 for 6 hours/day, 5 days/week, for 90-days in a whole-body inhalation chamber.

Body weight, food consumption, and lung function were recorded weekly, and blood samples were collected for hematology and clinical chemistry tests. Cellular differential counts and cytotoxicity measurements, such as albumin, lactate dehydrogenase (LDH), and total protein were also monitored in a cellular bronchoalveolar lavage (BAL) fluid. No statistically significant differences were found in cellular differential counts. Lung function test measurements, including tidal volume and minute volume, were measured and showed a tendency to decrease comparing control and dose groups during the 90-days of exposure.

At necropsy, dose-related changes were only observed in the lung. Histopathologic examination showedfocal inflammation with an inflammatory infiltrate, and increased macrophages in the high-dose rats. Tissue distribution of gold nanoparticles showed a dose-dependent accumulation of gold in only lungs and kidneys. There was no significant increase in gold concentration in the olfactory bulb.

Based on changes observed in the lung histopathology and lung function in high-dose animals, the median dose concentration (0.38 μg/m^3) was selected as the No Observed Adverse Effect Level (NOAEL) (Sung et al. 2011).

Schulz et al (2012) investigated the genotoxic effects of AuNPs (2, 20 and 200 nm) administered by single intratracheal instillation (18 μg) to male adult Wistar rats. After 72 hours, comet assays of lung tissue and micronucleus tests in bone marrow. No DNA damage was detected.

Measurements of clinical pathology parameters in bronchoalveolar lavage fluid (BALF) and blood indicated no local reactions in the lungs, or any adverse systemic effects. The conclusion was that different-sized AuNPs tested were non-genotoxic and showed no systemic and local adverse effects at the given doses.

Yu et al (2007) demonstrated the potential for gold nanoparticles to translocate from the lung to other organs over time, with the potential to cause significant effects in exposed tissues. Rats were exposed to aerosolized AuNPs in an exposure chamber at airborne particle concentrations of 2×106/cm^3, with more than 75% of the particle diameters between 30 and 110 nm. Tissue concentrations were measured by ICP-MS after 5 and 15 days.

After 5 days, significant amounts of Au were detected in the lung and olfactory bulb. After 15 days, gold accumulation was detected in the lung, esophagus, tongue, kidney, aorta, spleen, septum, heart and blood (Yu et al 2007).

Dermal Exposure

Skin absorption potential of AuNPs has been suggested to have therapeutic potential in transdermal delivery systems, yet there is little available data on their skin absorption, and dermal toxicity has not been described.

Sonavane et al (2008) studied the *in vitro* permeation of AuNPs of three different sizes through rat skin and showed that the smaller AuNPs penetrated in the deeper layer of the skin. AuNPs showed size-dependent permeation through rat skin: 15 nm AuNPs showed higher permeation compared to 102 nm and 198 nm AuNPs. TEM study of rat skin revealed accumulation of smaller size nanoparticles in deeper skin regions whereas larger particles were observed mainly in epidermis and dermis.

Larese et al (2010) similarly demonstrated that AuNPs are able to penetrate the human skin, also by using a Franz diffusion cell method. The total amount of gold permeating through intact and damaged human skin during a 24-h period was measured. AuNPs (as small as 12.6 ± 0.9 nm) were shown to permeate the skin in greater amount than other NPs, such as silver nanoparticles. As AuNPs do not release Au ions in physiological condition, penetration happens only for particles.

In vitro studies

In vitro studies using gold nanoparticles have demonstrated that both size and surface chemistry play a crucial role in determining toxicity of AuNPs.

Xia et al (2017) investigated the effects of particle size on the genotoxicity of gold nanoparticles. AuNPs (5, 20, and 50 nm, with same composition) were assessed in a battery of in vitro assays (Comet assay in HepG2 cells, chromosomal aberration in Chinese hamster Lung (CHL) cells) and in the in vivo mouse micronucleus test. Mechanistic data were also derived from cell cycle analysis and reactive oxygen species (ROS) measurements.

In the comet assay, 5 nm AuNPs producted a dose-dependent increase in DNA damage after 24-h exposure. 5 nm AuNPs were shown to promote the production of reactive oxygen species (ROS) and induce cell cycle arrest in G1 phase at tested plate concentrations of 1.67, 5, and 12.5 mg/mL). In contrast, 20 and 50 nm AuNPs did not induce DNA damage at the same tested concentrations.

AuNPs did not increase the frequency of chromosomal aberrations in Chinese hamster lung (CHL) cells in the either the presence or absence of metabolic activation (S9). There was no cytoxicity observed up to the maximum concentration (12.5 mg/mL) of all three particle sizes.

The micronucleus test used an extended exposure time. Mice received intravenous injections of AuNPs at doses of 0.17 or 0.5 mg/kg per day for up to 14 days (cumulative dose of 2.38 and 7 mg/kg). Examination of the bone marrow showed a significant, dose-dependent increase in micronuclei frequencies for 5 nm AuNPs, but not for the 20 and 50 nm – sized particles).

The authors suggest that the smaller particles elicit a stronger genotoxic effect due to their higher cellular uptake and increased ROS production (Xia et al. 2017).

Pan et al (2009) showed that 1.4 nm gold nanospheres resulted in necrosis, damage to mitochondria and induction of oxidative stress, while 15 nm gold nanospheres did not harm the cells.

AuNPs of diameter 1.4 nm capped with triphenylphosphine monosulfonate (TPPMS) were much more cytotoxic than 15-nm nanoparticles (Au15MS) of the same chemical composition. It was shown that cyctotoxicity was caused by oxidative stress via ROS. Pretreatment of the nanoparticles with reducing antioxidants (N-acetylcysteine, glutathione, and TPPMS) reduced the toxicity of the 1.4 nm particles.

Studies on human leukaemia cell lines treated with gold nanospheres of different sizes (4, 12, 18 nm in diameter) and covered by various capping agents have shown that they are non-cytotoxic (Connor et al., 2005). Similar results were obtained with AuN of 3.5 nm diameter, which were shown to penetrate the cells by endocytosis without inducing toxicity (Shukla et al 2005).

However, other studies have shown that AuNPs are cytotoxic at high concentrations. Goodman et al. (2004) demonstrated that cationic gold nanospheres (2 nm) are cytotoxic when measured in the colorimetric microtiter (MTT) assay, whereas anionic nanoparticles were not toxic to the same cell lines (Goodman et al 2004).

Other routes:

Chen et al. (2013b) showed sex-differences in the effects of polyethylene glycol (PEG) coated AuNPs on mice livers and kidneys when administered by an intraperitoneal injection. It appeared that AuNPs (4.4 and 22.5 nm) caused more serious liver toxicity and infection in male mice than female mice, while 22.5, 29.3, and 36.1 nm AuNPs caused more pronounced kidney damage in female mice than male mice. However, no significant effects were seen in the reproductive organs of either sex.

Semmler-Behnke et al.(2014) showed that radiolabelled AuNPs (1.4nm, 18nm, and 80nm) administered intravenously to pregnant and non-pregnant rats showed marked biokinetic differences. All sizes of AuNP were found in the placentas and amniotic fluids of pregnant animals, however 1.4 nm AuNPs accumulated at the uterine wall at concentrations two levels of magnitude higher than for 18 nm or 80 nm AuNPs. Translocation of AuNPs from maternal blood into the foetuses was observed, but only tiny fractions of 1.4nm and 18nm particles were found. 80 nm AuNPs did not cross the placenta.

4.11 Other pigments

Only sporadic reports of toxicity testing on other nano-sized pigments were captured in this literature review.

Prussian blue nanoparticles (PBNPs) (CAS Number 14038-43-8), have a history of use for cancer magnetic resonance imaging and photothermal therapy. Chen et al. (2016) investigated changes in the biochemical and immunity indicators after daily injection of these nanoparticles through tail vein of mice. Scanning electron microscopy images indicated that the average particle size of the PBNPs was around 100 nm, showing a uniform cubic structure. Histological examination showed that the nanoparticles accumulated primarily in the liver and spleen. The frequency of T-cells in the spleen decreases after the first injection day, then recovered to the normal level after 60 days of injection. The frequency of T-cells in the blood was also initially decreased after the first injection, but subsequently increased, returning to normal levels after 7 days of injection. Acute liver damage was suspected by significantly raised serum indexes of liver functions (alanine transaminase, aspartate transaminase, total bilirubin, and alkaline phosphatase) after 1 h of injection, but levels gradually decreased to normal after 60 days of injection. The results indicate that PBNPs show in vivo acute toxicity, but animals recover after 60 days of repeated dosing, indicating that longer-term toxicity is

low.

Hofmann et al. (2016) studied the short-term inhalation toxicity of five organic diketopyrrolopyrrole (DPP) pigments and two inorganic iron-oxide-based pigments in GLP-compliant studies on Wistar rats. Groups of animals were exposed head-nose to concentrations of 30 mg/m^3 to each of the materials for 6 h/day on 5 consecutive days. Particle characterization studies were performed to compare the effects of five DPP-based pigments and two inorganic iron-oxide-based (Pigment Red 101):

- mixed chlorinated DPP isomers (CAS Number 84632-67-7, 88949-44-4, 84632-65-5) (39 nm diamter, 62 m2/g surface area)
- fine meta-chloro DPP (CAS Number 84632-67-7) (10-50 nm, 64 m2/g)
- coarse meta-chloro DPP (CAS Number 84632-67-7) (70–200 nm, 42 m2/g)
- fine Pigment Red 254 (CAS Number 84632-65-5) (43nm, 94 m2/g)
- coarse Pigment Red 254 (CAS Number 84632-65-5) (233nm, 16 m2/g)
- fine Pigment Red 101 (CAS Number 1309-37-1) (4-21nm, 107 m2/g)
- coarse Pigment Red 101 (CAS Number 1309-37-1)(48-90 nm, 12 m2/g)

Animals were bronchially lavaged and sacrificed after 3 weeks of recovery. Histopathology of the respiratory tract showed that mixed chlorinated DPP isomers, Pigment Red 254, and meta-chloro DPP caused pigment deposits. Alveolar macrophage phagocytosis was evident, along with slight hypertrophy/hyperplasia of the bronchioles and alveolar ducts. However, there was no evidence of inflammation. Only pigment deposition and pigment phagocytosis were observed after exposure to Pigment Red 101.
All tested pigments were well tolerated well and caused only marginal effects in BAL fluid. For meta-chloro DPP isomer it was observed that the coarse material was slightly more potent than the nano-form. It was hypothesized that this was becasue of the needle-like crystal form of the coarse material. The NOAEL was 10 mg/m³ for coarse meta-chloro DPP, and 30 mg/m³ air for fine meta-chloro DPP, as well as fine and coarse Pigment Red 101. While the reasons for a NOAEL of 30 mg/m^3, a concentration well above the doses generally reported for lung overload in rat, were not discussed in the study, it was noted that the particles tended to concentrate in the lumen of bronchio-alveolar junction, indicating that not all particles reached the alveoli. Furthermore, after 3-weeks of exposure-free period, the hypertrophy initially seen after 4 days of exposure on 30mg/m^3 animals had regressed completelely.

Pang et al. (2017) studied the toxicity of nanoscale copper phthalocyanine (n-CuPc) released from automotive coatings in a an *in vitro* macrophage model (J774 A1). Fragments from automobile coatings with n-CuPc as well as from a reference coating were obtained by sanding. Size distribution and agglomeration of both the pristine n-CuPc and sanding fragments were measured to provide mean particle sizes of 333 nm and 6141 nm, respectively. J774 A1 cells were exposed to either pristine n-CuPc, fragments containing n-CuPc, fragments of the reference materials, or $CuSO_4$ representing the ion concentration in n-CuPc or controls for 24 h. There was no significant difference in the toxicity induced by fragments of n-CuPc and the referecne materials after 24 h, with the calculated EC50 values of 242.9 and 246.6 µg/ml, respectively. The results suggest that when the n-CuPc pigments was embedded in a polymer, their toxicity was reduced. The observed no difference in toxicity when compared with the reference material was surmised to be because the matrix dominated the toxicity of released fragments, preventing the toxicity of the embedded pigment.

4.12 Summary of hazard data on nanomaterials used as pigments

In the toxicological literature there is a bias towards investigating a small number of nanomaterials that are also used as nanopigments, in particular TiO_2, Carbon black and ZnO.

Adverse effects of a nano-size materials cannot always be determined from the known toxicity of the macro-sized material of the same chemical composition. The toxicity of soluble nanomaterials is generally mediated by soluble ions, whereas poorly soluble nanomaterials may exhibit nano-specific effects

Quantum effects, size-dependent properties, and unique toxicokinetics become apparent as particle dimensions approach nanoscale. Nanomaterial-specific toxicity has been shown to depend on physical and chemical properties including shape, size, crystalline structure, surface electric charge, chemical compositions of the core and shell, and on the presence of impurities and contaminants.

Although *in vivo* and *in vitro* studies have yet to be standardised for study of the unique characteristics of nanomaterials, it is often observed that they may elicit oxidative stress and chronic inflammation when clearance mechanisms are overloaded, particularly in the lung and liver, leading to secondary genotoxicity and potentially neoplastic and reproductive/developmental effects from the systemic circulation of inflammatory mediators.

With the absence of standard testing guidelines, tests have been performed under a variety of conditions, sometimes leading to conflicting results. For example, aggregation of particles in the testing media can lead to variations in exposure regimes.

Toxicity of nanoparticles after dermal and oral exposure has been demonstrated to be low under conditions relevant to expected low-level human exposures.

Oral studies with nanomaterials have shown that observed systemic effects are most likely related to the absorption of particularly small nanoparticles (<10nm) from the gastrointestinal tract, and their subsequent translocation and accumulation in other organ systems. However, studies using realistic human doses show that dietary nanoparticles are generally not absorbed in the gut, and are excreted in the faeces.

Experimental findings on skin absorption and dermal toxicity of nanoparticles are conflicting in the absence of standardised testing methods. However, most studies conclude that topical application of nanoparticles on healthy skin - for example in the case of sunscreens containing TiO_2 - poses little risk of absorption.

Animal inhalation studies have typically used high doses under conditions that are not relevant for human exposure. For example, the rat is particularly sensitive to lung overload-mediated effects due to inefficiency in clearing insoluble nanoparticles from the lung. This has been shown to lead to an exaggerated chronic inflammation response which is believed to be the principal mechanism of genotoxicity leading to lung neoplasms observed in rat studies.

5. Epidemiology of nano-sized pigments

Epidemiology of TiO2
Several studies have been conducted, mainly in production workers or in other occupations exposed to TiO_2 (Warheit 2013). For all of these studies, no causative link has been demonstrated between TiO_2 exposures and cancer risk.

Examined mortality and cancer risk was assessed in a cohort of 1576 male workers exposed for more than a year in two TiO_2 production plants in the United States. It was reported that

mortality due to cancer was significantly lower than expected. Nested case-control assessments demonstrated no significant associations with risks of lung cancer, chronic respiratory diseases or radiological abnormalities (Fryzek et al. 2003, Warheit 2013).

A retrospective cohort mortality study of 4,241 workers at 4 production facilities in the United States concluded that for workers exposed to TiO_2 particles, there were no correlations between occupational exposures and increased risks of lung cancer or other adverse health effects (Fryzek et al., 2003).

The largest cohort study was conducted on 15,017 TiO_2 workers employed for >1 year in 11 plants in six European countries (Boffetta et al., 2004). The investigators concluded that there was no evidence of an exposure–response relationship with respect to lung carcinogenic effects. In recent publications, a cohort study of 3,607 DuPont TiO_2 production workers, followed in the years 1935–2006 reported a lack of positive association between occupational exposures to dust and mortality, lung cancer, non-malignant respiratory disease or heart diseases (Ellis et al., 2010, 2013). The Epidemiological Working Group at the International Agency for Research on Cancer (IARC 2006) concluded based upon the available data, that the study results do not suggest an association between occupational exposures to titanium dioxide particles and risk for lung cancer (Warheit 2013, IARC, 2006; Baan et al., 2006). In support of these conclusions, the findings from additional epidemiological studies in occupational workers exposed to TiO_2 particles have demonstrated negative correlations between exposures and lung cancer (Siemiatycki, 1991; Boffetta et al., 2001; Ramanakumar et al., 2006, 2008). Becker 2011 provides a critical review of the available data on TiO_2. The epidemiological studies did not provide data on primary particle size or size distribution of the TiO_2 particles, i.e. these studies did not contain any details about the actual exposure to nano-TiO_2 (Warheit 2013).

On the basis of available epidemiological studies it is not possible to draw conclusions about the risk of cancer that is associated with exposure to nano-TiO_2. Thus far, epidemiological studies of TiO_2 are inconclusive, and there are no studies looking specifically at nano-TiO_2. At present no definitive conclusions can be drawn about the carcinogenic effect of nano-TiO_2 on humans.

On the basis of the animal data, it can only be said that nano-TiO_2 is suspected of being potentially carcinogenic to humans, since the available studies are not adequate to make a robust evaluation of its carcinogenicity. On June 9, 2017, the European Chemicals Agency (ECHA) announced that the Committee for Risk Assessment (RAC) "*concluded that the available scientific evidence on TiO_2 meets the criteria in the [Classification, Labeling and Packaging (CLP)] Regulation to classify titanium dioxide as a substance suspected of causing cancer through the inhalation route*." (ECHA 2017b)

A systematic review of the literature was conducted to characterize the available data and identify candidate datasets upon which toxicity values could be derived. A survey of mechanistic data relevant for lung cancer was used to support quantitative inhalation risk assessment approaches. A total of 473 human studies were identified, 7 of which were epidemiological studies that met inclusion criteria to quantitatively characterize carcinogenic endpoints in humans. None of these studies supported derivation of toxicity criteria (Thompson et al., 2016).

Pelclova et al. 2015 carried out a study (in the years 2012 and 2013) with 36 workers exposed for 9 years (mean length of exposure) to (nano)TiO_2 pigment and 45 controls. Condensate of the exhaled breath condensate (EBC) titanium and markers of oxidation of nucleic acids (including 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), 5-hydroxymethyl uracil (5-OHMeU)) and proteins (such as o-tyrosine (o-Tyr), 3-chlorotyrosine (3-ClTyr) and 3-nitrotyrosine (3-NOTyr)) were analysed from samples of their exhaled breath. Titanium in the EBC was significantly higher in production workers ($p<0.001$) than in research workers and unexposed controls. Accordingly, most EBC oxidative stress markers, including in

the preshift samples, were higher in production workers than in the two other groups. Multiple regression analysis confirmed an association between the production of TiO_2 and the levels of studied biomarkers. The concentration of titanium in EBC may serve as a direct exposure marker in workers producing TiO_2 pigment; the markers of oxidative stress reflect the local biological effect of (nano)TiO_2 in the respiratory tract of the exposed workers.

In a follow-up study (Pelclova et al. 2017) investigated the impact of short-term exposures on the markers of health effects in office workers relative to production workers from the same factory. Twenty-two office employees were examined. These workers were occupationally exposed to (nano)TiO_2 aerosol during their daily visits of the production area for an average of 14±9 min/day. Median particle number concentration in office workers was not available, while in the production area concentrations were reported as $2.32\times104/cm^3$. About 80% of the particles were <100 nm in diameter. A panel of biomarkers of lipid oxidation, specifically malondialdehyde (MDA), 4-hydroxy-trans-hexenal (HHE), 4-hydroxy-trans-nonenal (HNE), 8-isoprostaglandin F2α (8-isoprostane), and aldehydes C6-C12, were studied in the EBC and urine of office workers and 14 unexposed controls. Nine markers of lipid oxidation were elevated in the EBC of office employees relative to controls ($p<0.05$); only 8-isoprostane and C11 were not increased. These results suggest that even short exposure to (nano)TiO_2 aerosol would induce oxidative stress in the respiratory tract.

Significant association was found in the multivariate analysis between the employment in the TiO_2 production plant and EBC markers of lipid oxidation. The EBC markers in office employees reached about 50% of the levels measured in production workers, and the difference between production workers and office employees was highly significant ($p<0.001$). None of these biomarkers were elevated in urine. In a previous study of Pelclova et al., 2015, titanium was under the limit of detection (LOD=1.2µg/L) in the urine samples of the workers exposed to TiO_2 for longer time. The presence of TiO_2 was found only in EBC. One major challenge with sensitive biomonitoring techniques, however, is their non-specificity and difficulty in interpreting the meaning of their physiological values in the context of chronic disease development and damage-repair kinetics.

Liou et al. 2015 reviewed the epidemiological studies of workers' exposure to nanomaterials (TiO_2, multiwalled carbon nanotubes, iron oxides, CNT, <100 nm) and characterized their study designs, findings, and limitations. In total 15 (11 cross-sectional, 4 longitudinal, 1 pilot decriptive study). All 11 cross-sectional studies showed a positive relationship between various biomarkers and nanomaterial exposures. Three of the four longitudinal studies showed a negative relationship; the fourth showed positive findings after a 1-year follow-up. Each study considered exposure to nanomaterials as the independent variable. Exposure was assessed by mass concentration in 10 studies and by particle number concentration in six studies. Six of them assessed both mass and particle concentrations. Some of the studies had limited exposure data because of inadequate exposure assessment. Generally, exposure levels were not very high in comparison to studies performed in human inhalation chamber, where usually a high exposure level is used, but there were some exceptions. Most studies involved a small sample size, from 2 to 258 exposed workers. These studies represent the first wave of epidemiological studies of nanomaterial workers. They are limited by small numbers of participants, inconsistent (and in some cases inadequate) exposure assessments, generally low exposures, and short intervals between exposure and effect. Still, these studies are a foundation for future work; they provide insight into where nanomaterial workers are experiencing potentially adverse effects that might be related to nanomaterial exposures.

On the basis of available epidemiological studies it is not possible to draw conclusions about the risk of cancer that is associated with exposure to nano-TiO_2. Thus far, epidemiological studies of TiO_2 are inconclusive, and there are no studies looking specifically at nano-TiO_2. At present no definitive conclusions can be drawn about the carcinogenic effect of nano-TiO_2 on humans. Some studies are showing the effect of nano-TiO_2 on the oxidative stress markers. Multiple regression analysis confirmed an association between the production of TiO_2 and the

levels of studied biomarkers. The concentration of titanium in EBC may serve as a direct exposure marker in workers producing TiO_2 pigment; the markers of oxidative stress reflect the local biological effect of (nano)TiO_2 in the respiratory tract of the exposed workers.

Epidemiological studies of Carbon black

The meta-Analysis carried out by Morfeld et al. (2016) examined cardiac mortality in three cohorts of carbon black production The mortality from all causes, heart disease (HD), ischemic heart disease (IHD) and acute myocardial infarction (AMI) were analysed. Fixed and random effects (RE) meta-regression models were fit for employment duration, and for overall cumulative and lugged quantitative carbon black exposure estimates. Full cohort meta-SMRs (RE) were 1.01 (95% confidence interval (CI) 0.79–1.29) for HD; 1.02 (95% CI 0.80–1.30) for IHD, and 1.08 (95% CI 0.74–1.59) for AMI mortality. For all three outcomes, meta-SMRs were heterogeneous, increased with time since the first and the last time of exposure,and peaked after 25–29 or 10–14 years, respectively. Meta-Cox coefficients showed no association with lugged duration of exposure (because the effects of exposure to some substances or particles are more likely associated with recent than more distant past exposure, exposure estimates are lugged by some specific time-period; for example, in analyses lugged by 10 years, only cumulative exposure occurring in the 10 years prior to each age-specific risk set is considered). A small but imprecise increased AMI mortality risk was suggested for cumulative exposure (RE-hazards ratio (HR) = 1.10 per 100 mg/m^3-years; 95% CI 0.92–1.31), but not for lugged exposures. The results do not demonstrate that airborne CB exposure increases all-cause or cardiac disease mortality.

The aim of another cross-sectional study was to establish and identify the health effect markers of workers with potential exposure to nanoparticles (20–100 nm) during manufacturing and/or application of nanomaterials (Liou et al. 2013). The types of nanomaterials handled by the 227 exposed individuals were carbon nanotubes, titanium dioxide, silica dioxide, and other nanomaterials including nanoresins, nanosilver, nanogold, nanoclay, nanoalumina, and metal oxides. Depression of antioxidant enzymes and increased expression of cardiovascular markers were found among workers handling nanomaterials. There are several limitations to this cross-sectional epidemiologic study. Firstly, the cross-sectional study results may not be interpreted as causal association due to a lack of temporality (that is, a cross-sectional study is a type of observational study that analyzes data from a population, or a representative subset, at a specific point in time and not over long-term observations as with cohort studies). Secondly, the small size of the study population limits its conclusions to general ones only. Third, a lack of exposure assessment meant it was not possible to define the dose–response relationship. Fourth, the heterogeneity of nanomaterials made it difficult to find a sufficiently large group of workers exposed to the same particles and to present potential health effects of any one nanomaterial. Fifth, misclassification of exposure was possible.

In the cohort study published by Dell et al., 2015 the carbon black exposure and risk of malignant and non-malignant respiratory disease mortality was assessed. The study evaluated lung cancer and respiratory disease mortality associations with cumulative inhalable carbon black exposure among 6634 US carbon black workers. No consistent associations were observed between cumulative inhalable carbon black exposure and respiratory disease mortality. Quantitative carbon black exposure estimates were not related to lung cancer or non-malignant respiratory disease mortality.

Epidemiology of Iron oxides

From the weight of evidence available for iron oxides, the “bulk” iron oxides are not human lung carcinogens. Current evidence remains conflicting for “nano” form iron oxides as to their genotoxic potential; in vivo via the oral route, no genotoxicity is seen but genotoxicity/carcinogenicity via the lung appears not to have been investigated. Further studies are needed to draw conclusions for “nano” form iron oxides (Pease et al. 2016). Epidemiological studies reporting positive findings in cancer risk of iron oxide (nano)particles

have numerous confounding factors (mainly lacking separation of iron oxide exposure from exposure to other known or suspected carcinogens). Interpretation of such data is therefore problematic. In selected epidemiological studies that eliminate the most common confounding factors, no excesses of lung cancer occurences were reported in iron oxide exposed groups (Pease et al., 2016).

Pelclova et al. (2016) evaluated markers of oxidative stress and inflammation in exhaled breath condensate (EBC) and urine samples of 14 workers exposed to iron oxide aerosol (primarily α-Fe_2O_3) for an average of 10 ± 4 years. Median mass exposure concentrations during analyses were 0.083 mg/m^3 (66,800 particles/cm^3). While markers in urine did not significantly differ among exposed and control group, almost all markers of lipid, nucleic acid and protein oxidation were elevated in the EBC of exposed workers. Negative results in urine samples indicates that workers exhibit rather local than systemic oxidative stress.

Conclusion

Of the identified epidemiological data found, most were identified for TiO_2. However, even for TiO_2 the epidemiological studies are inconclusive, and there are no studies looking specifically at nano-TiO_2. At the present no definitive conclusions can be drawn about the carcinogenic effect of nano-TiO_2 on humans. Nevertheless, on the basis of the animal data TiOi was classified as a substance suspected of causing cancer through the inhalation route.

There are several limitations in the available epidemiologic studies. Most of them are cross-sectional studies and the studies results are not interpreted as causal association due to a lack of temporality. Furthermore, the small size of the study population is a limitation for drawing general conclusions. The lack of exposure assessment made impossible to analyze the dose–response relationship. In addition, the heterogeneity of nanomaterials made it difficult to find a sufficiently large group of workers exposed to the same particles and to present potential health effects of concrete and specific nanopigment. Finally, misclassification of exposure was possible.

Curently, there are no sufficient epidemiological data to confirm causal relationship between the exposure to nanomaterials in general and/or nano-pigments in particular, and adverse health effects.

6. Potential exposure to nano-sized pigments from consumer and professional products

The following sub-chapters present summaries of published data on exposure, risk assessment and life cycle of the nano-pigments found in the literature.

6.1 Summary of published data on exposure

From the literature search, fourteen studies related to exposure to nanopigments (7 industrial, 2 professional and 10 consumer) were critically assessed based on the qualitative methodology described in Appendix 8. The studies are presented in details in Chapter 7.

Published exposure data were related to the following nano-pigments or incidental particles (incidental nanoparticles originated from products containing nano-pigments): TiO_2, $CaCO_3$; ZnO; Cr6 +; Al_2O_3; Fe_3O_4; MWCNT; AlOOH; nickel (Ni), chromium (Cr), manganese (Mn), and cobalt (Co); iron oxides; silicon dioxide (SiO_2); nano-Fe_2O_3; and Unspecified (nanopigments in general). The vast majority of data was related to TiO_2. The clear identification of the substance(e.g. CAS number) occurred just for TiO_2, namely CAS 13463-67-7. The crystalline structure was mentioned only for TiO_2 (rutile and anatase). Particle size/diameter was reported in a limited number of studies, and it was in the range of tens to hundreds of nm, exceptionally up to micrometres (not "nano"). Other characteristics of nano-pigment (surface area, crystal structure, shape surface modifications, impurities etc.) were described exceptionally.

Occupational exposure data was related to exposures occurring within the manufacture of nano-pigments. Professional exposure data was published only for the use of printers. Within the exposure studies, only 3 performed a characterisation of the material measured. In these studies there was a general lack of exposure scenarios description; often the individual activities associated with the production and use of nano-containing products are missing. The exposure route assessed in the studies is mostly inhalation.

A quantitative exposure assessment was carried out in the identified literature (van Broekhuizen 2012; Pauluhn 2011; Huang et al. 2010; van Ravenzwaay 2009) by workplace /exposure measurements, comparison of measured data with background levels and/or proposed exposure limits. Broekhuizen 2012 aimed to set exposure limits. The studies do not

use uniform methodology on workplace/exposure measurements. The experimental data on exposure are heterogenous, depending on measurement equipment.

Zou (2015) tried to compare the different metrics (the number concentration (NC), surface concentration area (SAC), and mass concentration (MC)) of nanoparticles in workplaces measured by different monitoring tools used for exposure measurement. The study investigated the relationship between particle size distribution, mass concentration and surface area concentration. Different measuring tools measured different particle size ranges and were based on different detection principles. The conclusion of the study was that NC and SAC metrics are significantly distinct from the MC in characterizing exposure to airborne nanoparticles. Number concentration and surface area concentration were found to be more relevant for assessing the exposure to nanomaterials compared to traditional approach of mass concentration. Simultaneous measurements of the NC, SAC, and MC should be conducted as part of nanoparticle exposure assessment strategies and epidemiological studies.

Simultaneous measurements of the NC, SAC, and MC should be conducted as part of nanoparticle exposure assessment strategies and epidemiological studies.

Related to consumer exposure, most data were found for exposure to nano-pigments via food, paints and printer toners. The most commonly occurring uses of pigments in products are paints (waterborne paints), food (candies, sweets, chewing gums), food supplements, cosmetics (sunscreens), and toners. Product name and the concentration of nanomaterial in the product were rarely mentioned, and are lacking in most of the cases. No study focused on exposure via environmental media (i.e. air, water and sediment, soil and dust) was identified. Only studies on release of nano-pigments from different uses were reported.. The release studies (described in the Life cycle assessment chapter 6.2) were used as a basis for environmental exposure assessment.

Consumer exposure estimation is often difficult as it requires knowledge of the nature of the products used, the circumstances of their intended and reasonably foreseeable use, as well as the amount of substances in consumer products or articles used per event and the frequency and duration of the event. Furthermore, release and subsequent exposure also takes place from articles or reacted/dried mixtures, which can be influenced by water or saliva contact, skin contact, elevated temperature, mechanical abrasion or by slow emission from the article matrix over service life. Very often, assumptions must be made and estimation has to be based on default values or reasoned judgement (Mackevica and Hansen, 2016).

Overall, it can be said that the quantity and quality of data available on nano-sized pigments related to consumer and professional exposure in the open literature is still extremely low. The ranking of studies as low or medium quality was mainly due to the incompleteness of information presented. Nano-pigment release was asessed for the environment, but without any further link to human exposure and the potential health impact. The possible releases of nano-pigments from different products are presented without exposure scenario data (i.e. information on how often exposure occurs, how long the exposure takes, under what microclimate and another conditions). Release experiments are performed at concentrations exceeding the real values measured in the workplace and the environment. Measurement tools used do not allow to measure the exposure to particles only in the range up to 100 nm but up to 1000 nm.

Studies focused on nanoparticle release from printing activities (Pirela et al 2015, Martin et al. 2015, Pirela et al., 2017) identified the following elements within the subsequent chemical analysis :e.g. Fe, Mg, Si, Cr, Ni, Cu, Sn and Ti. Nevertheless, these findings cannot be used for the nanopigment risk assessment, because it is not clear whether they originate from ink nano fraction or are part of incidental particles arising from toner printing activity. Sometimes a nanomaterial is described in the study based on the EU definition (2011/698/EU), however release/exposure assessment is carried out for larger particles (not nano-size only).

Based on evaluated studies, TiO_2 is the most studied nano-pigment within its production and application in different products (paints, food, cosmetics, etc.). Some studies prove the relationship between oxidative stress following exposures to these nanoparticles (Pelclova et al. 2015, Pelclova et al., 2017). Attention should therefore focus on further research in this area related to potential risk to humans and the environment.

6.2 Summary of published data on Risk Assessment and Management

Twelve studies were found and marked as relevant for risk assessment. Most of the studies were assessed as low quality according to the evaluation criteria (see Appendix 8), because they contain few concrete details relevant to risk assessment of nano-pigments for human health.

In the studies of Hogsberg 2011 and Schreiver 2017, the authors were focused on the identification and characterisation of pigments in skin tattoos and the possible particles sizes without direct link to potential health risk. Some studies were oriented on exposure assessment (Dudefoi 2017, Jorgensen 2017, Kaegi 2008, Kaegi 2017), e.g. the oral exposure from chewing gum, the measurement of nano-pigment release from paints within weathering etc., without combining exposure and hazard data. Other studies provided rather general guidance on risk assessment or present hazard information with a short summary of the epidemiological studies.

Other studies provided mainly general guidance, for example only discuss risk asessment or presenting more hazard information with a short summary of the epidemiological studies. Som et al (2011) reviewed nanomaterial effects on the environment and human health on the basis of selected environmental and nanotoxicological studies and on their own environmental exposure modeling studies. They argued that the behavior of nano-TiO_2 is mainly governed by agglomeration processes that lead to its elimination from the water column and sedimentation. Depending on the composition of the water, the form of nano-TiO_2 or the presence of biofilms, rather rapid and complete removal from water is observed; ZnO is easily soluble in water and is expected to be rapidly dissolved.

Methods for prediction of biological effects and hazard potential of metal and metal oxide nanomaterials in human tissues using expert knowledge are mentioned in some studies (Warheit 2013, Landsiedel 2017). These studies could be partially useful to develop the exposure scenarios and/or following risk assessment.

Only one study - published by Heringa et al. (2016) - was assessed as high quality regarding human health risk assessment of nanopigments. This study was focused on the risk assessment of titanium dioxide nanoparticles via oral exposure, including toxicokinetic considerations. TiO_2 intake was based on ingestion via food, supplements and toothpaste, and on the average measured total Ti or TiO_2 particle levels in such products, according to the Dutch National Food Consumption Survey (DNFCS) (Rompelberg et al. 2016).

Human health risks were assessed using two different approaches: based on intake, i.e. external doses (Approach 1), and based on internal organ concentrations using a kinetic model (Approach 2). Results showed that with Approach 1, a human health risk is not expected for effects in liver and spleen, but a human health risk cannot be excluded for effects on the ovaries (effects on ovary found in Tassinari et al., 2014; the uncertainty of this conclusion stated by authors (Heringa et al. 2016) is based on extrapolation from a very short term study – 5 days – to chronic exposure). The short exposure time in the Tassinari et al. (2014) study results in a rather large uncertainty in the extrapolation to chronic exposure. It is unknown whether the effects seen after this short duration will also occur after longer duration, when the animals have had the chance to adapt to the exposure. When based on organ concentrations of TiO_2 NPs (Approach 2), a potential risk for liver, ovaries and testes was found. The authors argues that currently estimated risk can be influenced by factors such as absorption potential, form of TiO_2, particle fraction, particle size and physico-chemical

properties in relation to toxicity, among others.

A toxicokinetic modelling study (Geraets et al., 2014) investigated the tissue distribution and blood kinetics of various titanium dioxide nanoparticles in rats up to 90 days post-exposure after oral and intravenous (i.v.) administration of a single or five repeated doses. Suspensions of titanum dioxide containing agglomerates /aggregates in the range 80–150 nm were administered to rats orally or i.v. . Several tissues such as liver, spleen, kidney, lung, heart, brain, thymus, reproductive organs were evaluated for titanium (Ti) content. After single i.v. administration, titanium was still detected in all tissues 24 hours post dosing with liver being the target tissue, followed by spleen and lung. Repeated dosing showed up to 5 times higher tissue titanium levels (µg/tissue) compared to single dose, indicating that no significant elimination occurred in the first 24 hours, and that the accumulation is dose proportional. Redistribution from liver to the spleen was observed over the 90 day post-exposure period, with slow tissue elimination and apparent accumulation in the spleen. By comparison, in the oral studies, repeated oral exposure at an overall dose of 11.5 mg TiO_2 resulted in titanium levels that were near or below the detection limit in liver and spleen, indicating a very low absorption from the gastrointestinal tract. However, there was some evidence to support absorption via the gastrointestinal tract, as increased levels of titanium could be detected in some liver and lymph nodes of the orally-exposed animals. The authors conclude that, although oral bioavailability is very low, the slow elimination rate poses some potential for tissue accumulation.

Reviews of risk assessment studies related to the use of nano-sized pigments are rare. Overall, it can be said that the quantity of data available on risk assessment of nano-sized pigments related to consumer and professional use in the open literature is low.

6.3 Summary of published data on Life Cycle Assessment

Seven references were found and marked as life cycle studies relevant to nanopigments. The studies mainly refer to the release of nano-sized particle from different lifecycle stages, but were assessed to be of low quality because they did not fulfill the quality criteria for lifecycle assessment (Appendix 8), e.g. presence of release factors, stated scope of life cycle assessment, nanomaterial assessed within entire life cycle etc). The studies were mainly focused on the release factors proven by experimental studies under laboratory conditions.

Release is the starting-point for a particulate exposure by nanomaterials. During release, nanomaterials may be separated from a matrix by external forces, for example abrasion, and can be released into the environment (Rommert et al., 2017). Most publications regarding nanomaterial release are focusing on widely used nanomaterials, such as Ag, TiO_2 CNTs, and SiO_2, and only a few product groups, namely fabrics, paints or coatings, and polymers (Mackevica and Hansen, 2016) are addressed. The studies were focused on the following phases of nano-pigment life cycle: the use of product containing nano-pigment, service life and the end of life (landfilling) of the product containing nano-pigments. The nano-pigments assessed by these studies were: TiO_2, SiO_2, Fe_2O_3, and nanoparticles in general (NPs released from paints containing pigments).

Use of product

Use of the product containing nano-pigment was mainly related to paints, in particular emission of nanoparticles from the weathering of the paint. The measurement of nano-sized emission from indoor paints was investigated by Jorgensen et al. (2017) in an experimental study in a test chamber. Nanoparticle emission was studied for base and full-pigmented versions of three water-borne acrylic paints and one solvent-borne alkyd paint. All experiments were performed in a stainless-steel test chamber under standardized conditions. Emissions (the number concentration and size distribution of nanoparticles) were measured during the paint-drying period. The results from this study showed that interior paints are probably not

very important when it comes to identifying products that release nanoparticles into indoor environments. Specific nano-pigments were not specified or characterized in this study.

Another study by Al-Kattan et al. 2013 looked at weathering-related releases of nano-TiO_2 (50% nano-TiO_2, anatase) from paints containing pigment-TiO_2 and nano-TiO_2. Panels covered with paint with and without nano-TiO_2 were exposed to simulated weathering by sunlight and rain in climate chambers. The same paints were also studied in small-scale leaching tests to elucidate the influence of various parameters on the release such as composition of water, type of support and UV-light. Under all conditions a very low release close to background values (only 0.007% of the total Ti) was observed. This study indicated that paints containing nano-TiO_2 may release only very limited amounts of materials into the environment, at least over the time-scale (3 weeks) investigated in this work.

A study by Zuin et al. 2014 has been carried out for waterborne paints with integrated nanoparticles containing the same amount of silicon dioxide (SiO_2) nanoparticles but differing in the pigment volume concentration (PVC) and in amount and type of binder and pigment. The paints were studied through leaching tests to investigate the influence of these parameters on release of Si from paint. The results indicated greater release of Si, about 1.7 wt.% of the SiO_2 nanoparticles in the paint, for paint formulated with higher PVC value (63%), suggesting that the pigment volume concentration is a crucial factor for release of SiO_2 nanoparticles from paints. The agglomerates of SiO_2 nanoparticles were only found in leachates from paint with higher pigment volume concentration. A paint sample with the highest amount of binder and less calcite filler exhibited a lower release of Si among the paints with a low pigment volume concentration value (35%). No SiO_2 particles were detected in leachates collected from this paint. This suggests that no or low leaching of NP from waterborne paints depends on pigments concentration (the lower the better) but also an important role is played by the amount of binder (as high as possible) and filler (less amounts).

Service life

The service life of a product is a life cycle stage (period of time) when the applied product stays unchanged for the proposed use until removed, degraded.

A service life release study has been carried out for Fe_2O_3 by Neubauer et al. (2017). This study investigated spontaneous and induced release due to mechanical stress during/after simulated sunlight and rain degradation of polyethylene (PE) with organic and inorganic pigments. Additionally, primary leaching from the food contact material (food packaging) and secondary leaching from nanocomposite fragments with an increased surface into environmental media was examined. In all investigated scenarios, the detectable particulate releases were attributed primarily to contaminations from handling and machining of the plastics by standard processing equipment, and were not identified with the pigments. This is the first holistic confirmation that pigment nanomaterials remain strongly bound in a polymer matrix with low diffusion and high persistence such as High Density Polyethylene (HDPE).

The study of Kaegi et al. (2008) presented evidence for the release of synthetic nanoparticles from urban applications into the aquatic environment. The study was focused on TiO_2. Centrifugation-based sample preparation was performed in order to recover TiO_2 particles between roughly 20 and 300 nm. Analytical electron microscopy revealed that TiO_2 particles are detached from new- and aged- facade paints by natural weather conditions and are then transported by facade runoff and are discharged into natural, receiving waters. It was shown that TiO_2 particles are released in significant amounts to the aquatic environment. The buildings in this work did not contain specific nano-paint. The released nanoparticles were originated from normal paint with pigment-TiO_2. This could be a reason for different findings of the Al-Kattan et al., (2013) study. Other exterior applications, such as nano-silver in paints, exposed to natural weather conditions may release nanoparticles in a similar way. The rather fast surface runoff under heavy rainfall conditions may transport nanoparticles without significant retention mechanisms, which inevitably coudl lead to a discharge of synthetic nanoparticles into surface waters.

End of life

Only one study has been identified with the focus on nano-pigments in the end-of-life (waste phase). The study done by Kaegi et al. (2017) assessed the potential release of TiO_2 from construction and demolition (C & D) landfill sites. The leachate samples were collected from a landfill over one year and analysed by complementary analytical techniques to quantify TiO_2 particles in landfill leachates. Total elemental Ti contents were mostly around a few tens of $\mu g/L^{-1}$ and were strongly correlated with total suspended solids. Based on the volumetric discharge of the landfill leachate water from the landfill, it was estimated that a total amount of ~0.5 kg of TiO_2 particles are released annually from the landfill. The measurements dominantly revealed nanoscale TiO_2 particles with a spherical shape typically observed for TiO_2 particles used as white pigments. In addition, angular TiO_2 particles were detected, suggesting that also natural TiO_2 particles of comparable sizes are present in the landfill leachates. The results from this study indicate that (nanoscale) TiO_2 particles are released from C & D landfill sites (~ 5 g/year). The amount of TiO_2 particles released from C & D landfill sites may still be rather low, but it may become relevant as an increasing use of nanomaterial is predicted for construction materials in general.

The review of the life cycle assessment studies related to nano-pigment showed that there is no single study focused on the whole (entire) LC of a particular nano-pigment. Overall, it can be said that the quantity of data available on life cycle of nano-sized pigments related to consumer and professional use in the open literature is very low.

Nevertheless, the experimental case studies reported in the literature provide solid evidence for the release of nanopigments from some consumer products. There are limitations regarding the analytical techniques available to quantify and characterize the particles released. However,it has been shown that the particles that are released are incidental and usually very different from the nanomaterials that have been embedded in the product, and it is more likely that nanoparticles are still embedded in the matrix of the product rather than released as single particles (Mackevica and Hansen, 2016), thereby significantly reducing the potential for exposure.

7. Exposure Scenarios for identified uses of nano-sized pigments

There is relatively little empirical data to support quantitative exposure assessment to nanopigments, i.e. to compare the dose-response data or hygienic limits with the exposure data. Within this project a small number of data-drive exposure scenarios (ES), based on published data, was developed. To supplement this, a list of generic exposure scenarios, not specifically supported by published data, have been envisaged and further elaborated (see chapter 7.4). Analysis of the potential for exposure to nanosized pigments from identified consumer and professional products throughout their entire lifecycles was assessed. Assessment of how such exposures may be impacted by the properties of various nanoparticles, such as their tendency to agglomerate, and the prevailing conditions that may modify such behaviour, and thus affect exposure potential, is often not possible and it is subject to assumptions.

Exposure scenarios for nanomaterials have been developed in several research projects (e.g. NANEX, MARINA, GUIDEnano, SUN). The first ES library for nanomaterials was developed within the NANEX FP7-project (Development of Exposure Scenarios for Manufactured Nanomaterials; http://nanex-project.eu/).

NANEX was focussed mainly on carbon nanotubes (CNTs), nano-sized titanium dioxide (nano-TiO_2) and nano-sized silver (nano-Ag). In total, 62 exposure scenarios (57 occupational and 5 consumer ES) were developed using publicly available data and data collected in several large-

scale sampling campaigns (NANOSH project – FP6, NanoINNOV project – CEA). Only nine occupational ES were complete enough to be included in NANEX Exposure scenario data library (http://nanex-project.eu/mainpages/exposure-scenarios-db.html).

ES identified by NANEX have been further elaborated within the MARINA FP7-project (Managing risks of nanomaterials; http://www.marina-fp7.eu/project/).

The MARINA ES library includes occupational ES for a range of nanomaterials (Carbon Nanotubes, CeO_2, CrO_3, TiO_2, ZrO_2, nano-Ag, nano-Cu, nano-Fe, Quantum Dots) and consumer and professional use of various nano enabled products (textiles, deodorant, paints, mortar, dental restoration material). The MARINA library has been incorporated into the GUIDEnano library.

The FP7 project GUIDEnano (http://www.guidenano.eu/) takes the concept of ES library a step further. It includes an algorithm to quantify the similarity between the ES in a library and the scenario under investigation and gathers data collected within different FP7 projects. The GUIDEnano library (incl. ES from the MARINA library) is available at http://guidenano.iom-world.co.uk/.

We have used the NANEX template of exposure scenario for industrial and professional uses. MARINA/GUIDEnano template requires very detailed inputs, and so not useful for our purposes due to low data availability. The information presented in each scenario is related to the conditions of nanopigment use, the characteristics of product and the measured (estimated) exposure data found within the literature searching.

The ES developed within this survey were further used for preliminary health risk assessment by Control banding tool (CB tool), namely Stoffenmanager Nano Module. This module allows a qualitative assessment of occupational health risks from inhalation exposure to Manufactured Nano Objects (MNOs). Just like the control banding module, hazard properties and information on exposure are combined to derive a risk score. Risk management measures can be selected to control exposure.

Rsk assessment for consumers was not possible with the use of Stoffenmanager Nano Module or other CB tools. The aplicability domain of existing CB tools is covering consumer exposures to a very limited extent. Only the ConsExpo nano tool considers exposure scenarios for consumers but is limited to spray applications only. ConsExpo was used for one generic ES. Other ES on consumer uses of nanopigments were aseessed qualitatively based on expert judgement. Only one scenario (Consumer Scenario 6 – Exposure to TiO_2 via chewing gums) was assessed based on the exposure published in the literature and compared to the available DNEL found.

7.1 Exposure Scenarios from published data

This chapter is presenting the exposure scenarios based on published data and final conclusions and recommendations for each scenario selected for the risk assessment. Uncertainties and knowledge gaps related to risk assessment to identified uses of nanopigments are discussed as part of the Uncertainty analysis (see Chapter 9).

7.1.1 Industrial Scenario 1 – Production of TiO_2

The basic steps within the production of TiO_2 are:

- the process of calcination
- splitting and classifying titanium dioxide
- removing the scattered salt particles from the substance
- the crude form of the pigment is milled (micronisa- tion process) to produce particles in a controlled size distribution
- smashing the substance to obtain smaller particles

- transporting the titanium dioxide pigment to the pigment bagging area for packaging
- packing into small bags
- packing into large bags
- cleaning with pressurized air the automized packing machinery

The study by Huang et al. (2010) compared respirable dust and nanoparticle concentrations measured by different sampling devices at a TiO_2 pigment factory. Respirable particle mass concentrations (MCs), nanoparticle number concentrations, particle size distribution and particle metallic content were measured. The workplace measurement was in the range $4.9 \cdot 10^4$ - $1.1 \cdot 10^5$ particles/cm^3 (number concentration) or 0.050 µg/m^3 (mass concentration). The highest levels of respirable particle MCs and nanoparticle number concentrations were detected near the packing site of the factory. The results of exposure were compared with the limits for fine dust (1500 µg/m^3) specified by NIOSH, which was not exceeded in this case.

In another study with 36 TiO_2 production workers exposed to (nano)TiO_2 pigment and 45 controls (Pelclova et al. 2015), the median total mass TiO_2 concentrations were found to be 0.65 and 0.40 mg/m^3, respectively.

The median of particle number concentrations measured by the scanning mobility particle sizer (SMPS) and aerodynamic particle sizer (APS) were 1.98×10^4 and 2.32×10^4 particles/cm^3, respectively; and about 80% of those particles were in the size fraction smaller than 100 nm. In the research workspace (where the sources of potential release of TiO_2 are not present), lower aerosol concentrations (0.16 mg/m^3 and 1.32×10^4 particles/cm^3) were found. The reason why background concentrations were higher than for working zones may be due to the use of local exhaust ventilation or other technical risk management measures to decrease the exposure during the working activity. However, details of the exposure determinants were not described in the study.

Packing of nano-pigment into small bags or large bags using diesel-powered forklifts was measured by Koivisto et al. (2012). Worker task was to operate and clean the automated packing machinery with pressurized air. The number concentrations range between 1.15×10^4 - 20.1×10^4 (particles/cm^3) with more than 90% of particles lower than 100 nm, and the mass concentration was 225 - 700 µg/m^3.

In the study published by Lee et al. (2011), occupational exposure to TiO_2 and silver was measured during manufacture. The release from the manufacturing equipment into the workplace air was measured during two different tasks: powder collection, and manufacturing reaction process. The level of exposure to TiO_2 was in the range 0.1 – 4.99 mg/m^3. The range of particle number concentrations measured within these activities was 11,418 - 45,889 patricles/cm^3 with a particle size range of 15 – 710.5 nm. The level of exposure to Ag was in the range 0.00002 - 0.00118 mg/m^3. The range of particles measured within these activities was 535-25,022 particles/cm^3 with a wide range of particle sizes.

The study performed by Xu et al. (2016) adopted off-line filter-based sampling combined with real-time activity-based monitoring to measure the concentrations in a workplace manufacturing TiO_2 (primary diameter: 194 ± 108 nm). Mass concentrations of aerosol particles in the packaging workshop (total dust: 3.17 mg/m^3, nano dust: 1.22 mg/m^3) were much higher than those in the milling workshop (total dust: 0.79 mg/m^3, nano dust: 0.31 mg/m^3) and executive office (total dust: 0.44 mg/m^3, nano dust: 0.19 mg/m^3). However, the mass concentrations of TiO_2 were at a relatively low level in the packaging workshop (total TiO_2: 46.4 µg/m^3, nano TiO_2: 16.7 µg/m^3) and milling workshop (total TiO_2: 39.4 µg/m^3, nano TiO_2: 19.4 µg/m^3) by ICP-MS.

The number concentration (NC), surface area concentration (SAC) of aerosol particles potentially deposited in alveolar (SAC_A), and tracheobronchial (SAC_{TB}) regions of lungs in the packaging workshop were (1.04 ± 0.89) × 10^5 particles/cm^3, 414.49 ± 395.07 µm^2/cm^3, and 86.01 ± 83.18 µm^2/cm^3, respectively, which were all significantly higher than those of the milling workshop (0.12 ± 0.40) × 10^5 particles/cm^3, 75.38 ± 45.23 µm^2/cm^3, and 17.60 ±

9.22 $\mu m^2/cm^3$, respectively as well as executive office and outdoor background ($p < 0.05$). The measurements for executive office were following: NC: (0.13 ± 0.19) x105 particles/cm^3, SACTB: 22.16 ± 1.98 $\mu m2/cm^3$, and SACA 101.46 ± 9.61 $\mu m2/cm^3$. The background measurements for outdoor were following: NC: (0.32 ± 0.63) x105 particles/cm^3, SACTB: 37.46 ± 12.44 $\mu m2/cm^3$, and SACA 168.06 ± 57.49 $\mu m2/cm^3$.

In the following tables the exposure data available in the scientific literature are summarized in the form of exposure scenarios. The NANEX templates, based on the REACH templates, are used to describe the ES.

7.1.1.1 The calcination process

Table 5: Contributing exposure scenario (CES) 1.1: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 - Calcination process
Substance type	TiO_2 (anatase/rutile)
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Controls the calcination process in the production hall	
Further specification	
PROC4	
Product characteristics	
Not specified	
Amounts used	
not reported	
Frequency and duration of use/exposure	
2.5 hours per task. Frequency unknown.	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	

The workers spent about 40% of their shifts in the close vicinity of particle emitting production units (in the calcination process, in micronisation, in surface coating, in the filtration process and the transport corridors); the remaining time was spent in the control room, separated by closed door, where they checked the production lines remotely.
Technical conditions and measures at process level (source) to prevent release
not reported
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by equipment SMPS (nano Scanning Mobility Particle Sizer): number concentration was 1.97 x10^4 particles/cm3; The Interquartile Rang (IQR): 1.49 - 3.89 particles/cm^3 ;
References:
Pelclova D, Zdimal V, Fenclova Z, Vlckova S et al. (2015x) Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO_2 (nano) particles. *Occup Environ Med*. p. 1-9; doi:10.1136/oemed-2015-103161. Pelclova D, Barosova H, Kukutschova J, Zdimal V et al. (2015xx) Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO_2 particles: a cross-sectional study. J. Breath Res. Vol. 9, p. 1-12. doi:10.1088/1752-7155/9/3/036008.

7.1.1.2 The micronisation process

Table 6: CES 1.2: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 - Micronisation
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Controls the process of micronisation in the production hall	
Further specification	
Milling of the pigment to produce particles in a controled size distribution; PROC14	
Product characteristics	
Powder; 100% product (anatase/rutile)	
Amounts used	
not reported	
Frequency and duration of use/exposure	
3.5 hours per task. Frequency unknown.	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	
The workers spent about 40% of their shifts in the close vicinity of particle emitting production units (in the calcination process, in micronisation, in surface coating, in the filtration process and the transport corridors); the remaining time was spent in the control room, separated by closed door, where they checked the production lines remotely. Closed pipe production system.	
Technical conditions and measures at process level (source) to prevent release	
not reported	
Technical conditions and measures to control dispersion from source towards the worker	

not reported

Organisational measueres to prevent/limit releases, dispersion and exposure

not reported

Conditions and measured related to personal protection, hygiene and health evaluation

Half-mask, protective clothing, gloves

Additional good practice advice (for environment) beyond REACH CSA

not reported

Exposure estimation

Workplace area sampling was measured by equipmnet SMPS (nano Scanning Mobility Particle Sizer): number concentration was 1.42 $x10^4$ particles/cm^3 (; The Interquartile Rang IQR 1.19 - 2.36); total MCs: 0.76 mg/m^3 (IQR 0.67- 0.84)
UPC (ultrafine particle concentration) was measured by P-TRAK: 0.12 ± 0.40 $x10^5$ particles/cm^3;
SAC (surface area contraction was measured by AeroTrak: 75.38 ± 45.23 $\mu m^2/cm^3$ ($SAC_{A\text{ - alveolar}}$); 17.60 ± 9.22 $\mu m^2/cm^3$ ($SAC_{TB\text{ - tracheobronchial}}$) ; total MCs : 19.4 $\mu g/m^3$

References

Pelclova D, Zdimal V, Fenclova Z, Vlckova S et al. (2015x) Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO_2 (nano) particles. Occup Environ Med. p. 1-9; doi:10.1136/oemed-2015-103161.

Pelclova D, Barosova H, Kukutschova J, Zdimal V et al. (2015xx) Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO_2 particles: a cross-sectional study. J. Breath Res. Vol. 9, p. 1-12. doi:10.1088/1752-7155/9/3/036008.

Xu H, Zhao L, Chen Z, Zhou J et al. (2016) Exposure assessment of workplace manufacturing titanium dioxide particles. *J Nanopart Res*. Vol. 18; p. 1-13. doi: 10.1007/s11051-016-3508-9.

7.1.1.3 Laboratory testing

Table 7: CES 1.3: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 - Laboratory testing
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Laboratory tests new production types on a small scale	
Further specification	
PROC15	
Product characteristics	
Powder; 100% product	
Amounts used	
not reported	
Frequency and duration of use/exposure	
3 hours per task. Frequency unknown.	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	
not reported	
Technical conditions and measures at process level (source) to prevent release	
not reported	
Technical conditions and measures to control dispersion from source towards the worker	
not reported	

Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
The exposure was measured by equipmnet SMPS (10-100 nm particle size):concentration was 0.78 x 10^4 particles/cm^3 (The Interquartile Rang IQR 0.64 - 0.92); total MCs: 0.16 mg/m^3 (IQR 0.15 - 0.22)
References
Pelclova D, Zdimal V, Fenclova Z, Vlckova S et al. (2015x) Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO_2 (nano) particles. Occup Environ Med. p. 1-9; doi:10.1136/oemed-2015-103161. Pelclova D, Barosova H, Kukutschova J, Zdimal V et al. (2015xx) Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO_2 particles: a cross-sectional study. J. Breath Res. Vol. 9, p. 1-12. doi:10.1088/1752-7155/9/3/036008.

7.1.1.4 Other jobs: coating, filtration

Table 8: CES 1.4: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 – Coating and filtration
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Works in surface coating and filtration process and in transport corridors	
Further specification	
PROC	
Product characteristics	
Powder; 100% product	
Amounts used	
not reported	
Frequency and duration of use/exposure	
3.7 hours per task. Frequency unknown.	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	
not reported	
Technical conditions and measures at process level (source) to prevent release	
not reported	
Technical conditions and measures to control dispersion from source towards the worker	
not reported	
Organisational measueres to prevent/limit releases, dispersion and exposure	

not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by equipmnet SMPS (10-100 nm particle size): concentration was 1.30 $x10^4$ particles/cm^3 (; The Interquartile Rang IQR 0.97 - 1.60); total MCs: 0.41 mg/m^3 (IQR 0.31 - 0.52)
References
Pelclova D, Zdimal V, Fenclova Z, Vlckova S et al. (2015x) Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO_2 (nano) particles. Occup Environ Med. p. 1-9; doi:10.1136/oemed-2015-103161. Pelclova D, Barosova H, Kukutschova J, Zdimal V et al. (2015xx) Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO_2 particles: a cross-sectional study. *J. Breath Res.* Vol. 9, p. 1-12. doi:10.1088/1752-7155/9/3/036008.

7.1.1.5 Packing into large bags

Table 9: CES 1.5: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 – Packing into large bags
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Packing of TiO_2 into large bags	
Further specification	
PROC8b	
Product characteristics	
Powder; 10-80nm (the majority being close to 30-50 nm; 35 nm average); 194 ± 108 nm (purity 95.5%, coated with inorganic silicon and aluminium; XRD indicated the main crystal form rutile more than 94% with a small percentage of anatase)	
Amounts used	
not reported. Packaging into 500 - 800 kg packages	
Frequency and duration of use/exposure	
5 minutes. Frequency unknown.	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	
Packing in semi-automatic machines; the operations included turning on the instrument, opening the door of packing machine, filling the bag and then repeating the whole process; forklift events	
Technical conditions and measures at process level (source) to prevent release	
Natural ventilation and a floor-mounted fan (the wind speeds were about 0.3-1.4 m/s); semiautomatic packaging of powder	

Technical conditions and measures to control dispersion from source towards the worker
semi-automatic machine
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
Full face mask, protective clothing, gloves
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by equipmnet FMPS (5.6 - 560 nm particle size): concentration was 4.9 $x10^4$ particles/cm^3 - 1.1 $x10^5$particles/cm^3; total MCs: 80-370 mg/m^3 UPC (ultrafine particle concentration) was measured by P-TRAK: 1.04 ± 0.89 $x10^5$ particles/cm^3; SAC (surface area concentration) was measured by AeroTrak: 414.49 ± 395.07 $\mu m^2/cm^3$ ($SAC_{A\ -alveolar}$); 86.01 ± 83.18 $\mu m^2/cm^3$ ($SAC_{TB\ -\ tracheobronchial}$); total MCs : 0.31 mg/m^3 Background measurement: outdoor: NC: (0.32 ± 0.63) $x10^5$ particles/cm^3, SAC_{TB}: 37.46 ± 12.44 $\mu m^2/cm^3$, and SAC_A 168.06 ± 57.49 $\mu m^2/cm^3$ Executive office: NC: (0.13 ± 0.19) $x10^5$ particles/cm^3, SAC_{TB}: 22.16 ± 1.98 $\mu m^2/cm^3$, and SAC_A 101.46 ± 9.61 $\mu m^2/cm^3$
References
Huang C-H, Tai C-Y, Huang C-Y, Tsai C-J et al. (2010) Measurements of respirable dust and nanoparticle concentrations in a titanium dioxide pigment production factory. *Journal of Environmental Science and Health Part A*. Vol. 45; p. 1227-1233. doi:10.1080/10934529.2010.493792. Xu H, Zhao L, Chen Z, Zhou J et al. (2016) Exposure assessment of workplace manufacturing titanium dioxide particles. *J Nanopart Res*. Vol. 18; p. 1-13. doi: 10.1007/s11051-016-3508-9.

7.1.1.6 Packing into small bags

Table 10: CES 1.6: Uses of substances by workers	
Title	Worker exposure during manufacture of TiO_2 – Packing into large bags
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU, PC, PROC 26, PROC 8a/PROC 8b, PROC 14, PROC 15, PROC 28	
Name of contributing exposure	
Packing of TiO_2 into small bags	
Further specification	
PROC8a/8b	
Product characteristics	
Powder.	
Amounts used	
not reported. Packaging into small tens of kg - 25 kg packages	
Frequency and duration of use/exposure	
not reported.	
Human factors not influenced by risk management	
not reported.	
Other given operational conditions affecting workers exposure	
forklift events, cleaning vehicle passing; hall ventilation was not operating properly	
Technical conditions and measures at process level (source) to prevent release	
not reported.	
Technical conditions and measures to control dispersion from source towards the worker	
not reported.	

Organisational measueres to prevent/limit releases, dispersion and exposure
not reported.
Conditions and measured related to personal protection, hygiene and health evaluation
not reported.
Additional good practice advice (for environment) beyond REACH CSA
not reported.
Exposure estimation
Workplace area sampling was measured by SMPS (< 100 nm): concentration was 1.7 x 10^4 particles/cm^3; WRAS (< 100 nm): 1.1 x10^4 particles/cm^3; Background particle concentration varied in between 1× 10^4 and 2 x 10^4 particles/cm^3
References
Koivisto AJ, Lyyranen J, Auvinen A, Venhala E et al. (2012) Industrial worker exposure to airborne particles during the packing of pigment and nanoscale titanium dioxide. Inhalation Toxicology. Vol. 24 (12); p. 839-849. doi:10.3109/08958378.2012.724474.

7.1.1.7 Discussion and general conclusions

In total, six exposure scenarios (ES) could be developed based on the available literature for Industrial Scenario (IS) 1 – Production of TiO_2. However, the completeness of the ES is low.

The following data gaps in ES impede the risk assessment: nanomaterial/nanoproduct characteristics (e.g. particle size, surface area, density, concentration of nanomaterial in the product etc.) are mostly not recorded (only one CES 1.1 – CES 1.5 include more detailed characterization of the nanomaterial); exposure determinants (i.e. activity emission potential, frequency and duration of exposure, room conditions, etc.) are limited to exposure duration; risk management measures in place are recorded in two ES only – in both cases (CES 1.2 and CES 1.5) the PPE are not specified regarding the level of protection (for face masks), only CES 1.5 include at least some information regarding the technical measures.

The background concentration presented in the studies are available in Table 11 to enable comparison with the measured workplace concentrations. The comparison was made only for particle number concentration indicated at CES 1.5 and CES 1.6. The indoor background values were not exceeded in both contributing exposure scenarios.

Table 11: Comparison of workplace number concentrations with the background (IS 1: Production of TiO_2)

Exposure scenario	Measured number concentration	Background concentration		Reference
		Indoor	Outdoor	
CES 1	1.97 (1.49 - 3.89) x10^4 particles/cm^3	NA	NA	
CES 2	1.42 (1.19 - 2.36) x10^4 particles/cm^3			
CES 3	1.2 ± 4 x10^4 particles/cm^3			
CES 4	0.78 (0.64 - 0.92) x10^4 particles/cm^3	NA	NA	
CES 5	1.30 (0.97 - 1.60) x10^4 particles/cm^3	(1.3 ± 1.9) × 10^4 particles/cm^3	(3.2 ± 6.3) × 10^4 particles/cm^3	Xu et al., 2016
CES 6	1.7 x 10^4particles/cm^3 1.1 x10^4 particles/cm^3	1× 10^4 and 2× 10^4 particles/cm^3		Koivisto et al., 2012

NA = not available

There is a concern with respect to potential health risks as the nano-TiO_2 is in the form of powder, which could get easily airborne and so could be inhaled by the workers. The workplace measurements support this concern. Exposure potential for IS 1 is considered high (based on expert judgement) due to substance form (powder) and its availability (direct contact of workers with the powder). The exposure is predominantly by air (i.e. inhalation exposure to nano-TiO_2).

A very rough preliminary risk assessment was carried out within this survey based on two different approaches: (i) comparison of experimental measured data on exposure available in studied literature with proposed exposure limits for nanomaterial of concern; (ii) use of the control banding tool considering the worst case scenario (where the data were missing).Table 12? shows the comparison of measured data with occupational exposure limits (OELs) for nano-TIO_2 - IS 1. Specific OELs for nano-TIO_2 were proposed (e.g. by National Institute for Occupational Safety and Health – NIOSH, New Energy and Industrial Technology Development Organization – NEDO). The comparison is indicative only, since the measurements are not designed for personal exposure monitoring (and so for comparison with the hygienic limits).

Table 12: Pre-comparison of measured data on Industrial scenario 1 and proposed exposure limits

Reference	CES	Number concentraions of particles x10^4/cm^3	Exposure limit (reference)	mg/m^3	Exposure limit (reference)
Pelclova et al. 2015	Calcination (CES 1.1)	1.97 (1.49 - 3.89)	4x10^4 particles/cm^3	0.64 (0.46 - 0.86)	1.5 mg/m^3 for fine particles (NIOSH); 0.1 mg/m^3 for ultrafine particles (NIOSH) OELs TiO_2: 0.3 mg/m^3 (Swidwińska-Gajewska and Czerczak, 2014); 0.6 mg/m^3 (New Energy and Industrial Technology Development Organization – NEDO); Gamo et al., 2011))
Pelclova et al. 2015	Milling (CES 1.2)	1.42 (1.19 - 2.36)		0.76 (0.67-0.84)	
Xu et al. 2016		1.2 ± 4		0,019	
Pelclova et al. 2015	Laboratory (CES 1.3)	0.78 (0.64 - 0.92)		0.16 (0.15 - 0.22)	
Pelclova et al. 2015	Other jobs (CES 1.4)	1.30 (0.97 - 1.60)		0.41 (0.31 - 0.52)	
Huang et al. 2010	Packing large bags (CES 1.5)	4.9 - 11		80-370	
Xu et al. 2016		10.4 ± 8.9		0.31	
Koivisto et al. 2012	Packing small bags (CES 1.6)	1.1 - 1.7			

CES 1.5 (based on Huang et al., 2010 and Xu et al., 2016) is of high concern based on this comparison. The proposed OEL for nano-TiO_2 is exceeded almost three times considering the particle number concentration, the measured MCs is in the worst case scenario more than 1,200 times higher than the proposed OEL.

The reports from the StoffenmanagerNano Control banding (CB) tool are attached (Appendix 5). The risk level for the six ES reach category I, i.e. high priority. The risk level is highly influenced by the ES data incompleteness. The CB tool automatically counts with the worst case scenarios when the input parameter is unknown.

TiO_2 nanoparticles are among the most studied nanomaterials in terms of their potentional hazard, however, the results of toxicological studies are inconsistent. The available *in vivo* toxicological data on nano-TiO_2 support that hypothesis of a secondary genotoxic mechanism involving chronic inflammation, oxidative stress and cell proliferation (NIOSH, 2011) with a typically non-linear dose responce curve and a threshold (ECETOC, 2013) that is typical for low soluble ow toxic particles. Lung tumour development in rats is believed to be a consequence of species–specific lung overload conditions leading to chronic inflammation, and therefore the relevance of these observations for human health is questionable (ILSI, 2000). Nevertheless, increased inflammatory markers are detected in laboratory animals as well as in humans after occupational exposure. Translocation of nano-Ti2 to the systemic circulation and lymphatic

system and secondary organs have been observed in animals. Acummulation of nano-TiO_2 in these organs (mainly in the liver, spleen, kidney) may potentially cause toxic effects. Translocation to the brain via olfactory bulb should be taken into account in low soluble nanomaterials, potential consequences are not clear.

Evidence-based conclusions on the safety of production of nano-TiO_2 cannot be drawn based on the available literature. However, the high potential for inhalation exposure indicates a potential risk for human health from inhalation of TiO_2 nanoparticles. The industrial exposure scenarios dealing with dry and free nanopigments which may become airborne present the highest concern since there is potential for adverse health effects of (nano) particles on human health through inhalation.

7.1.2 Industrial Scenario 2 – Production of Fe_2O_3

Exposure to nanoparticles during production of Fe2O3 was described by Zou et al. 2015. The production of of nano Fe2O3 includes the following steps:

- oxidation reaction of FeSO4 solution colloid preparation of ferric hydroxide (Fe(OH)3);
- synthesis reaction with a part of the $Fe_2(SO_4)_3$ solution, the $Fe(OH)_3$ colloid, H_2SO_4, and an iron sheet;
- surface treatment of a-$Fe_2O_3{\cdot}nH_2O$ crystals using a water-soluble anionic polymer;
- ash evaporation of the wet product of a-$Fe_2O_3{\cdot}nH_2O$ in a ash dryer;
- powder screening: a portion of the a-$Fe_2O_3{\cdot}nH_2O$ product is manually spread onto a plate;
- calcination: the a-Fe_2O_3 product is produced by removing crystal water from a-$Fe_2O_3{\cdot}nH_2O$ in an infrared dryer;
- material feeding: the a-Fe_2O_3 material is manually fed into a semi-open container for washing;
- Fe_2O_3 or a-$Fe_2O_3{\cdot}nH_2O$ packaging.

Three work sites (for packaging, powder screening, and material feeding) were selected as sampling locations, on the basis that there might be airborne nanoparticles generated. Exposure was measured within the following working tasks: manual screening of powder, material feeding, and the packaging area. The measured mass concentrations of Nano-Fe_2O_3 for each task are described in the following scenarios. The indoor background of mean particle number concentration (mean) was1.4 ± 0.31 x 10^4 particles/cm^3; the outdoor background was 1.51 ± 0.50 x 10^4 particles/cm^3.

7.1.2.1 Manual screening of powder in an open process

Table 13: CES 2.1: Uses of substances by workers	
Title	Worker exposure during manufacture of Fe2O3 – Manual screening of powder
Substance type	Fe_2O_3
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
PROC26, PROC8b	
Name of contributing exposure	
Powder screening	
Further specification	
PROC26 manual screening of powder in an open process	
Product characteristics	
Powder	
Amounts used	
not reported	
Frequency and duration of use/exposure	
not reported	
Human factors not influenced by risk management	
not reported	

Other given operational conditions affecting workers exposure
not reported
Technical conditions and measures at process level (source) to prevent release
None
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation

Workplace area sampling was measured by P-Trak ultrafine particle counter (20-1000 nm): concentration was (mean) 5.04 ± (SD) 1.39 x10^4 particles/cm^3 And equipment AeroTrak (SAC 10-1000 nm): (mean) 26.21 ± (SD) 4.38 $\mu m^2/cm^3$; MC was measured for size 100-1000 nm (mean = 0.04 ± (SD) 0.02 $\mu m^2/cm^3$; Indoor background SAC (10-1000 nm): (mean) 15.37 ± (SD) 1.50 $\mu m^2/cm^3$; MCs was measured for size 100-1000 nm (mean = 0.05 ± (SD) 0.01 $\mu m^2/cm^3$ outdoor background SAC (10-1000 nm): (mean) 14.11 ± (SD) 1.64 $\mu m^2/cm^3$; MCs was measured for size 100-1000 nm (mean = 0.04 ± (SD) 0.01 $\mu m^2/cm^3$
References
Zou H, Zhang Q, Xing M, Gao X et al. (2015) Relationships between number, surface area, and MCs of different nanoparticles in workplaces. Environ. Sci.: Processes Impacts. Vol. 17, p. 1470-1481. doi:10.1039/c5em00172b.

7.1.2.2 Material feeding

Table 14: CES 2.2: Uses of substances by workers	
Title	Worker exposure during manufacture of Fe2O3 – Material feeding
Substance type	Fe_2O_3
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
PROC26, PROC8b	
Name of contributing exposure	
Material feeding	
Further specification	
PROC26 manual pouring of powder	

Product characteristics
Powder
Amounts used
not reported
Frequency and duration of use/exposure
not reported
Human factors not influenced by risk management
not reported
Other given operational conditions affecting workers exposure
not reported
Technical conditions and measures at process level (source) to prevent release
Local exhaust hood
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported

Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by P-Trak ultrafine particle counter (20-1000 nm): concentration was 6.68 ± 2.02 $x10^4$ particles /cm^3 And by next equipment AeroTrak (SAC10-1000 nm): 29.54 ± 9.1 $\mu m^2/cm^3$
References
Zou H, Zhang Q, Xing M, Gao X et al. (2015) Relationships between number, surface area, and MCs of different nanoparticles in workplaces. Environ. Sci.: Processes Impacts. Vol. 17, p. 1470-1481. doi:10.1039/c5em00172b.

7.1.2.3 Packaging process

Table 15: CES 2.3: Uses of substances by workers	
Title	Worker exposure during manufacture of Fe2O3 - Packaging
Substance type	Fe_2O_3
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
PROC26, PROC8b	
Name of contributing exposure	
Packaging	
Further specification	
PROC8b	
Product characteristics	
Powder; Mode size: 12.26 ± 1.91 nm	
Amounts used	
not reported	
Frequency and duration of use/exposure	
not reported	
Human factors not influenced by risk management	
not reported	
Other given operational conditions affecting workers exposure	

Semi-automatic packaging of powder
Technical conditions and measures at process level (source) to prevent release
Dust extraction device
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by P-Trak ultrafine particle counter (20-1000 nm): 2.87 ± 1.28 x10^4 particles/cm^3; equipment AeroTrak (SAC10-1000 nm): 22.20 ± 7.50 $\mu m^2/cm^3$; total MCs: 0.04 ± 0.02 mg/m^3 (Exposure measured close to the breathing zone of the workers)
References
Zou H, Zhang Q, Xing M, Gao X et al. (2015) Relationships between number, surface area, and MCs of different nanoparticles in workplaces. *Environ. Sci.: Processes Impacts*. Vol. 17, p. 1470-1481. doi:10.1039/c5em00172b.

7.1.2.4 Discussion and general conclusions

In total, three exposure scenarios (ES) could be developed based on the available literature data for Industrial Scenario 2 – Production of Fe_2O_3. However, the completeness of the ES is again very low. The following data gaps in ES impede the risk assessment: nanomaterial/nanoproduct characteristics (e.g. particle size, density, concentration of

nanomaterial in the product etc.) are mostly not recorded – only CES 2.3 indicate mean particle size of the Fe_2O_3 powder; exposure determinants (i.e. activity emission potential, frequency and duration of exposure, room conditions, etc.) are not reported at all; risk management measures in place are mostly not reported or poorly specified (ES 2 and CES 2.3 indicate technical measures - ventilation device without any specification regarding its efficiency etc.)– . The indoor and outdoor background concentration are presented in the study enabling comparison with the measured workplace concentrations – see Table 16. The indoor background values were exceeded 2 times (the best case scenario) and almost 5 times (the worst case scenario).

Table 16: Comparison of workplace number concentrations with the background (IS 2: Production of Fe_2O_3)

Exposure scenario	Measured number concentration	Background concentration	
		Indoor	**Outdoor**
CES 2.1 – screening	5.04 ± 1.39 x10^4 particles/cm^3	1.4 ± 0.31 x 10^4 particles/cm^3	1.51 ± 0.50 x 10^4 particles/cm^3
CES 2.2 – feeding	6.68 ± 2.02 x10^4 particles /cm^3		
CES 2.3 – packaging	2.87 ± 1.28 x10^4 particles/cm^3		

Despite the limited data on ES, there is a concern with respect to potential health risks as the nano- Fe_2O_3 is in the form of powder, which could get easily airborne and so could be inhaled by the workers. The workplace measurements support this concern. Exposure potential for IS 1 is considered high (based on our simplified expert judgement) due to substance form (powder) and its availability (direct contact of workers with the powder). The exposure is predominantly by air (i.e. inhalation exposure to nano- Fe_2O_3).The very rough preliminary risk assessment was carried out within this survey based on two different approaches: (i) comparison of experimental measured data on exposure available in studied literature with proposed exposure limits for nanomaterial of concern; (ii) use of the control banding tool considering the worst case scenario (where the data were missing).
Table 17 shows the comparison of measured data with OELs proposed for ultrafine particles. No OELs specific for nano- Fe_2O_3 were found in the literature. The comparison is indicative only, since the measurements are not designed for personal exposure monitoring.

Table 17: Pre-comparison of measured data on Industrial scenario 2 and proposed occupational exposure limits

Reference	CES	#particles x10^4/cm^3	Exposure limit (reference)	mg/m^3	Exposure limit (reference)
Zou et al. 2015	Screening	5.04 ± 1.39	2x10^4 particles/cm^3	NA	1.5 mg/m^3 for fine particles (NIOSH); 0.1 mg/m^3 for ultrafine particles (NIOSH)
	Feeding	6.68 ± 2.02		NA	
	Packing	2.87 ± 1.28		0.04 ± 0.02	

NA = not available

CES 2.2 is of the highest concern from the three CES based on this comparison. The proposed OEL for ultra-fine particle number concentration is exceeded 2.5 times (CES 2.1), 3.3 times (CES 2.2), and 1.4 times (CES 2.3).

The reports from the StoffenmanagerNano Control banding (CB) tool are attached (Appendix 5). The risk level for the three ES reach category I and II, i.e. high and middle priority (high priority in case of packaging – CES 2.3). The risk level is highly influenced by the ES data incompleteness. The CB tool automatically counts with the worst case scenarios when the input parameter is unknown.

Evidence based conclusions on the safety of production of nano- Fe_2O_3 cannot be drawn based on the available literature. Iron oxide nanoparticles are low soluble, low toxicity particles (Pauluhn, 2012). The results from exposure to micro-sized particles are in accordance with suggested mechanism of action of other low soluble low toxic particles: impaired lung clearance at higher doses in rats, inflammation, histopathological changes. Elevated markers of oxidative stress were also found in exposed workers (Pelclova et al. 2016). Typical effects of low soluble low toxic (nano)particles such as local effects in lung, oxidative stress, inflammation, translocation to secondary organs, accumulation and non-specific toxicity may be expected. The high potential for inhalation exposure identified in this exposure scenario indicates a potential risk for human health.

7.1.3 Industrial Scenario 3 – Production of printing inks

An exposure scenario detailing exposure to TiO_2 within production of printing inks was taken from the NANEX library of exposure scenarios. Only one CES is available concerning emptying bags in a filling station. No other CES regarding Industrial Scenario (IS) 3 were found in the literature/other publicly available sources.

Table 18: CES 3.1: Uses of substances by workers	
Title	Production of printing inks
Substance type	TiO_2
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
SU 3; PC 18; PROC 9 or 26	
Name of contributing exposure	
Emptying bags in filling station	
Further specification	
Not reported	
Product characteristics	
Powder , no dustiness result available; 100% product	
Amounts used	

40 kg (4 bags of 10 kg) per task
Frequency and duration of use/exposure
5-10 minutes per task. Frequency unknown
Human factors not influenced by risk management
not reported
Other given operational conditions affecting workers exposure
This task was carried out in an area called the penthouse which was separated by stairs from other production areas. Large room (> 100 m^3) with an open connection to the rest of the facility.
Technical conditions and measures at process level (source) to prevent release
LEV present at the filling station. Design of the filling station: only the front of the fillingstation is open.
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
Disposable RPE, gloves and coveralls were worn
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation
Workplace area sampling was measured by CPC: total particle concentration during activity was 16,285 particles/cm^3 with corresponding non-activity period of 16,509 particles/cm^3. Second measurement result during activity was 10,242 particles/cm^3 with corresponding non-activity period of 9,756 particles/cm^3 during non-activity.
References
Ref Title: D2.2 Report of results and implications of main study to measure nanoparticle concentrations in workplaces - Part 1: Main summary Author: NANOSH NANEX database: http://nanex-project.eu/esreports/nanexES9.pdf

7.1.3.1 Discussion and general conclusions

Only one ES for production of printing inks using TiO_2 was possible to be developed based on the available literature. The completeness of the ES is medium. The following data gaps in ES impede the risk assessment: nanomaterial/nanoproduct characteristics (e.g. particle size, density, concentration of nanomaterial in the product etc.) are not recorded – there is only information on the physical state of the substance (powder) and the composition of the product (100% product). Contextual information on exposure and risk management measures is more detailed in comparison with IS 1 and IS 2. The background (non-activity) concentrations are presented in the CES 3.1 enabling comparison with the workplace concentrations measured during the activity with the potential to release nanoparticles to the workplace air. There are no significant differences between during-activity and non-activity particle number concentrations. The release of nanoparticles during the working task of emptying bags in filling station was not reported. However, there is still a concern with respect to potential health risks as the nano-TiO_2 is in the form of powder, which could get easily airborne and so could be inhaled by the workers. Exposure potential for IS 1 is considered high (based on our simplified expert judgement) due to substance form (powder) and its availability (direct contact of workers with the powder). The exposure is predominantly by air (i.e. inhalation exposure to nano- Fe_2O_3).
A rough preliminary risk assessment was carried out within this survey based on two different approaches: (i) comparison of experimental measured data on exposure available in studied literature with proposed exposure limits for nanomaterial of concern; (ii) use of the control banding tool considering the worst case scenario (where the data were missing).
Table 19 shows the comparison of measured data with exposure limits for nano-TiO_2 - IS 3. Table 20 shows the comparison of measured data with OELs for nano-TIO_2 - IS 1. Specific OELs for nano-TIO_2 were proposed (e.g. by National Institute for Occupational Safety and Health – NIOSH, New Energy and Industrial Technology Development Organization – NEDO). The comparison is indicative only, since the measurements are not designed for personal exposure monitoring (and so comparison with the hygienic limits).

Table 19: Pre-comparison of measured data on Industrial scenario 3 and proposed exposure limits

Reference	CES	#particles x10^4/cm^3	Exposure limit (reference)	mg/m^3
NANEX database	Emptying bags in filling station	1.6	4x10^4 particles/cm^3	----
		1		----
total particle concentration during non-activity (measurement 1) 1.7 x10^4/cm^3 particles/cm^3; Second measurement result during non-activity period:1 x10^4/cm^3				

The reports from the StoffenmanagerNano Control banding (CB) tool are attached (Appendix 5). The risk level for this ES reach category II, i.e. medium priority. The risk level is influenced by the ES data incompleteness. The CB tool automatically counts with the worst case scenarios when the input parameter is unknown.

This ES is not of high concern based on the comparison between the measured particles concentration and the recommended reference value. The measured concentrations are 2.5 times and 4 times lower than proposed OEL. Therefore is can be genrally concluded that risks

can be controlled for this use, provided that standard occupational hygiene rules are observed.

7.1.4 Professional Scenario 1 – Use of printing inks - Professional use of photocopiers

This exposure scenario addresses the exposure to incidental/accidental (nano)particles emitted by the use of printing inks.

A common feature shared by all toners used in one study (Martin et al. 2015) was the visible presence of engineered nanoparticles on their surface. Depending on the toner, one to several types of nanomaterial were clearly visible e.g. organic and elemental carbon. Only two studies have been identified with the focus on the professional exposure to printing inks. In these studies, the incidental nanoparticles were assessed in the relation to photocopies used in offices (Martin et al. 2015, Pirela et al., 2017). Weekly geometric meanparticle number concentration ranged between 3,700 and 33,700 particles/cm^3 with transient peaks >1.4 million particles/cm^3 (measured by Fast Mobility Particle Sizer (FMPS) with a size range of 5.6-560 nm); average particle matter (PM 0.1) mass concentrations at each copy center ranged from 1.8 to 6.4 µg/m^3, and the PM2.5 MCs ranged from 0.8 to 10.0 µg/m^3 up to 12 times greater than the background The sample of PM0.1 contained 6–63% organic carbon, <1% elemental carbon, and 2–8% metals, including iron, zinc, titania, chromium, nickel and manganese, typically in the <0.01 – 1% range. Fe, Mg, Si, Cr, Ni, Cu, tin (Sn) and Ti (SF-ICP-MS analysis).

These findings could have been anticipated from a lack of engineering controls, poor ventilation, crowded rooms, and little awareness of exposures from toner-based printing equipment were documented in most of the 15 photocopy centers surveyed by Martin et al. 2017. The estimated total particle deposition in the lungs for this set of parameters varied from 28% to approximately 40%, with increasing gradient of deposition from the head airways to the alveolar region, as follows: head airways (6%), trachea-bronchial region (10%), and distal alveolar region (15 – 20%) (Martin et al. 2015). The total deposition mass flux based on the modeling of actual exposure data from the aforementioned studies was 1.732 lg/min m^2, meaning significant doses per unit surface area of lungs per unit time can be delivered to individuals running laser printers (LP) or photocopiers (PC) (Pirela et al. 2017).

The only relevant exposure route considered for this scenario is inhalation. Adults are taken as the target group. The duration of working exposure is taken 8 hours as the worst case scenario.

Table 20: PW - CES 4.1 : Uses of substances by workers	
Title	Use of printing inks containing nanomaterials
Substance type	titania, chromium, nickel and manganese, typically in the <0.01–1% range
List of all use descriptors related to the life cycle stage and all the uses under it; include market sector (by PC) if relevant:	
Name of contributing exposure	
Professional use of photocopiers	
Further specification	

Not recorded
Product characteristics
several metal oxides nanomaterial: iron oxide, manganese oxide, copper oxide, titania, alumina, etc
Amounts used
100-1000 g
Frequency and duration of use/exposure
8 hours; daily
Human factors not influenced by risk management
not reported
Other given operational conditions affecting workers exposure
< 100 m^3 (e.g. small room); Wet process where conductive ink is applied dropwise on to a substrate; Weekly cleaning of the print head at cleaning station and daily cleaning of print head in printer (Default taken from similar scenario with Ag)
Technical conditions and measures at process level (source) to prevent release
No ventilation
Technical conditions and measures to control dispersion from source towards the worker
not reported
Organisational measueres to prevent/limit releases, dispersion and exposure
not reported
Conditions and measured related to personal protection, hygiene and health evaluation
not reported
Additional good practice advice (for environment) beyond REACH CSA
not reported
Exposure estimation

Workplace area sampling was established by particle number concentration ranged between GM 3,700 and 33,700 particles/cm^3 up to 12 times greater than the background, (GM 880 – 15,900 particles/cm^3) with transient peaks >1.4 million particles/cm^3; weekly average $PM_{0.1}$ MCs at each copy center ranged from 1.8 to 6.4 µg/m^3; the $PM_{2.5}$ MCs ranged from 0.8 to 10.0 µg/m^3

References

Martin J, Bello D, Bunker K, Shafer M, Christiani D, Woskie S, Demokritou P. (2015) Occupational exposure to nanoparticles at commercial photocopy centers. J Hazard Mater. 298:351–360.

Pirela SV, Martin J, Bello D, Demokritou P (2017) Nanoparticle exposures from nano-enabled toner based printing equipment and human health: state of science and future research needs. CRITICAL REVIEWS IN TOXICOLOGY. doi.org/10.1080/10408444.2017.1318354

7.1.4.1 Discussion and general conclusions

One ES (CES 4.1) for professional use of photocopiers was developed based on the available literature. The completeness of the ES is relatively high (relative to previously discussed ISs). However, comprehensive risk assessment is still not possible due to lack of knowledge on product characteristics, contextual information on exposure and risk management measures. Pirela (2017) indicates that printing inks are generally nano-enabled products, that is that they are specifically designed to contan nano-sized pigments to improve the yechnical function of the product (Martin et al., 2015 and Pirela, 2017).

Background concentrations are given enabling comparison with the workplace concentrations measured during the printing. There are significant differences between during-activity and non-activity particle number concentrations (12 times higher particle number concentrations during the printing in comparison with the background, (Martin et al., 2015). The release of the nanoparticles during printing was confirmed. The composition of $PM_{0.1}$ sample corresponds to the printing ink composition indicated the printing ink being the source of the nanoparticles.

There is a concern with respect to potential health risks as the nanoparticles (probably originating from the printing ink) are airborne and so could be inhaled by the workers. Exposure potential is considered high due to direct contact of workers with the aerolised nanoparticles by inhalation. A rough preliminary risk assessment was carried out within this survey based on two different approaches: (i) comparison of experimental measured data on exposure available in studied literature with proposed exposure limits for nanomaterial of concern, and (ii) use of the Stoffenmanager nanomodule

Table 21 shows the comparison of measured data with OELs for ultrafine particles and printing inks.

Table 21: Pre-comparison of measured data on PS 1 and proposed exposure limits

CES	Number concentration (particles/cm^3)	OEL (particles/cm^3)	MCs (µg/m^3)	OEL (µg/m^3)
CES 4.1: Professional use of photocopiers	GM: 3,700-33,700 (with transient peaks >1.4 million particles/cm^3)	40,000 (Pieter van Broekhuizen, 2012)	PM0.1: 1.8-6.4; PM2.5: 0.8-10.0	0.1 mg/m^3 = 100 µg/m^3 for ultrafine particles (NIOSH)

Based on the available measurements, the proposed OELs won't be exceeded considering the GM values. However, the transient peaks are of concern.

Evidence-based conclusions on the safety of professional use of photocopiers cannot be drawn based on the available literature. However, with respect to the precautionary principle, there is a real potential for inhalation exposure to nanoparticles in such cases, indicating a potential risk for professionals.

7.2 General recommendations – Industrial and Professional Uses of nano-sized pigments

Due to gaps in current knowledge regarding hazard and risk assessment of nanomaterials resulting in high levels of uncertainty, there should be an increased emphasis on exposure assessment and control. Current precautionary measures aim to avoid or at least reduce the exposure to nanomaterials as much as possible.

Sharing of exposure data and development of comprehensive, well designed and realistic ES is essential for increasing our knowledge in exposure to nanomaterials in the workplace and among consumers. The ES, providing conceptual information on particular nanomaterial, operating conditions, applied risk management measures and release and/or exposure measurements, represent a valuable tool for exposure estimation. Compiling ES into ES libraries enable to read-across particular exposure situation of interest and to benchmark different process operations and safety measures. The concept of read-across is of high importance, since the measurements of all exposure situations will not be achievable. In a wider context, ES could form a basis for exposure registries and further epidemiological research. Development of ES is an important step within the whole process of safety management of nanomaterials.

Despite the progress in ES building achieved at the EU level, there are still many obstacles and challenges in this field. Some of them arise from scientific reasons, e.g. the lack of knowledge on a relevant exposure metric, difficulties in conducting measurements associated with temporal and spatial variability in both particle size distribution and number concentration, non-existence of standardized protocols for exposure measurements or even the uncertainty in definition of nanomaterials. Others are linked rather to societal situation, especially to willingness of enterprises to share sensitive data regarding their production and processes involving nanomaterials, which are necessary for building real-world ES. (Sikorova et al., 2014)

7.3 Consumer Scenarios

The consumer exposure scenarios are presented in the form of tables only. The ES templates for consumers exposure are available, however, their completion is impeded by the high level of data incompleteness in the reviewed literature. The preliminary risk assessment with the use of CB tools was not possible due to lack of input data and aplicability domain of existing CB tools which is not covering consumer ES developed within this survey. The exposure estimation/risk assessment was not carried out in the tools because only some of them have relevant consumer scenarios included. However, none of these tools consider the exposure to nanomaterials. Only ConsExpo nano tool consider the exposure and scenario for consumers but limited to sprays application (spray use was not included between evaluated scenarios due to a lack of data). Certainly none of these tools are readily applicable to assess the consumer exposure to nano products considered in this report.

7.3.1 Consumer Scenario 1 – Water-borne acrylic paint and solvent-borne alkyd paint

Product category: Paints

In the study of Jorgensen et al. (2017), the nano-sized emission from four indoor paints was investigated. Emissions were measured for both base and full-pigmented versions three water-borne acrylic paints and one solvent-borne alkyd paint. All experiments were performed twice in a 6.783 m^3 stainless-steel test chamber under standardized conditions (22.98 ^oC, 50.08% RH, air exchange rate 0.48 h^{-1}). Emissions during the paint-drying period were measured using a TSI Fast Mobility Particle Sizer (FMPS) measuring the number concentration of nanoparticles and the size distribution in the range 5.6 - 560 nm.

The results from solvent-borne paint showed the highest particle concentration, with a mean concentration of $3.2 \cdot 10^5$ particles/cm^3 and a maximum of $1.4 \cdot 10^6$ particles/cm^3. This paint also had the smallest particle size distribution, with 9.31 nm particles as the most dominant particle size. Nevertheless, the results from this study showed that the exposure to nanoparticles for the residents evaluated over a 7 or 28 day period was low and that interior paints are probably not very important when it comes to identifying products that release nanoparticles into indoor environments. The results of this study (particles distribution) were taken as the possible source of nanomaterial exposure via inhalation route.

Table 22: Exposure scenario addresses the consumer exposure to emitted nanoparticles within paint-drying period.

Product	NM	Exposure scenario	Target group	Nanomaterial exposure			
				Oral	Dermal	Inhalation	Eye
Paint - Water-borne acrylic paint and solvent-borne alkyd paint	Not specified – emitted nanoparticles	Exposure to nanoparticles emitted within the weathering of paint	Adults	NCR	NCR	$3.2*10^5$ - $1.4*10^6$ particles/cm^3	NCR

NCR = Not considered a relevant exposure route

The only relevant exposure route is considered for this scenario inhalation. Adults are taken as the target group.

7.3.1.1 Discussion and general conclusions

Clear evidence-based conclusions on the safety of using paints containing nanomaterials cannot be drawn based on the presented study. The risk assessment in impeded by lack of information on almost all the necessary input data (no information on chemical composition of the paints, characterization of nanomaterials contained in the paint, release factors, daily intake, etc.). However the potential for exposure is considered low-medium as the nanomaterial is suspended in a matrix. Neither the release of nanomaterials nor the inhalation exposure during the drying period was demonstrated.

7.3.2 Consumer Scenario 2 – Use of printer toner cartridges – emitted nanoparticles

Product category: Printing inks

The investigation of engineered nanomaterials emission during the printing was carried out by Pirela et al. 2015, 2017. The presence of metal/metal oxide nanoparticles in the PEPs and the complex chemistry of PM, even at minute concentrations, is of concern because metals/metal oxides have the potential to trigger a toxic response in the lungs and translocate to other organs. Concentration of nanomaterial in the product: a mixture of polymers (55 – 85% by mass) and a small fraction of fillers, such as ferrite and silicon dioxide (<5 w/w%) and carbon black (<10 w/w%). No information was provided by the manufacturers in terms of the use of nanoscale materials in the toner formulation. The release of volatile organic compounds (VOCs) is also a concern when assessing consumer exposure to laser printer emissions. The presence of such gaseous compounds can influence the biological response to inhaled aerosols, for example the presence of metal/metal oxide nanoparticles could have the potential to trigger a toxic reposnse in the lungs and translocate to other organs (Pirela et al. 2015).

Table 23: Exposure scenario addresses the consumer use of printer toner cartridges.

Product	NM	Exposure scenario	Target group	Nanomaterial exposure			
				Oral	Dermal	Inhalation	Eye
Use of printer toner cartridges	Not specified – emitted nanoparticles (size 49 to 208 nm)	Exposure to nanoparticles emitted within use of printer toner cartridges	Children	NCR	NCR	3,000 to 1.3 million particles/cm^3	NCR
			Adults				

NCR = Not considered a relevant exposure route

The only relevant exposure route is considered for this scenario inhalation. Adults are taken as the target group. The duration of working exposure is taken 0.5 hour as the worst case scenario.

7.3.2.1 Discussion and general conclusions

Based on this scenario, consumer exposure can vary from low amount of particles up to more than millions of particles/cm^3. This exposure could likely cause a risk. In line with normal practice, a consumer protection limit should generally be higher than worker protection levels. The recommended limits could be derived based on the REACH guidance from the occupational limits (Danish EPA, 2015), e.g. for OEL for ultrafine particles 0.1 mg/m^3. Based on the available measurements, the mass concentration was not measured (i.e. the modification of OEL for ultrafine particles was not provided because it would not be applicable on the presented data in number of particles concentration). Evidence based conclusions on the safety of consumers using printer toner cartridges cannot be drawn based on the available literature. However, with respect to the precautionary principle, there is a real potential for inhalation exposure to nanoparticles which would indicate a potential risk for human health.

7.3.3 Consumer Scenario 3 – Exposure through Personal Care Products – sunscreens with TiO_2

Product category: Cosmetics

Analysis of several sunscreens indicated that some contained very high amounts of titanium dioxide (14 to 90 µg/mg) (Weir et al. 2012, Schulz et al. 2002,Danish EPA 2015). The three sunscreens with TiO_2 listed as an ingredient had the highest concentrations of any Personal Care Products (PCPs), whereas others that were not labeled as containing titanium dioxide contained less than 0.01 µg/mg, and contained instead an organic sunscreen agent (e.g., benzonates). The only FDA-stipulated limitation for sunscreens is that the TiO_2 concentration must be less than 25%. Most have a lower concentration, between 2% and 15%. The Danish EPA 2015 was assessing the exposure to nano TiO_2 from sunscreens. The quantitative estimation of exposure was carried out for oral and dermal exposure (see following table).

Table 24: Exposure scenario considering the use of sunscreen with nano TiO_2.

Product	NM	Exposure scenario	Target group	Nanomaterial exposure			
				Oral (just taken the data for licking on fingers, Danish EPA 2015)	**Dermal**	**Inhalation**	**Eye**
Sunscreen	TiO_2 (Particle diameter: ~100 nm aggregates; 30–150 nm)	Dermal application of sunscreen containing TiO_2 (25%)	Children	2.9 mg/kg/day	0.51 mg/cm^2; 447 mg/kg/day	NCR	NCR
			Adults	2.9 mg/kg/day	0.51 mg/cm^2; 300 mg/kg/day		

NCR = Not considered a relevant exposure route

It is assumed that the sunscreen would be used by children and adults. The relevant exposure routes are dermal and oral (licking the fingers).

7.3.3.1 Discussion and general conclusions

Oral exposure
SCCS 2014 "*stated that the oral intake is not major route of exposure to TiO_2 nanomaterials from dermal application of formulations, the acute oral toxicity of TiO_2 is unlikely to be of concern*".

The possible DNEL calculated by Danish EPA 2015 was in the range 0.01-0.02 mg nano-TiO_2/kg bw/day. The authors expressed that the main uncertainty is associated with the possible derivation of DNEL based on the LOAEL used for calculation. Risk following oral intake of suncreens (by licking the fingers) might be overestimated as the worst case scenario. This exposure route might not be insignificant considering recent knowledge related to oral toxicity of nano-TiO_2 (Danish EPA, 2015).

The SCCS also considered that, on the basis of available information, the use of Titanium Dioxide nanoparticles in spray products cannot be considered safe. (SCCS, 2018) *" From analysis of the submitted dossier, the SCCS has concluded that the information provided is insufficient to allow assessment of the safety of the use of nano-TiO_2 in spray applications that could lead to exposure of the consumer's lungs."*
Dermal exposure
There is currently no data indicating that the dermal exposure to sunscreen lotion with nano TiO_2 would be associated with any risk based on current knowledge (Danish EPA 2015). It was shown in the studies that nano-TiO_2 particles can penetrate into the outer layers of the stratum corneum, which could possibly generate reactive oxygen species (SCCS, 2014). Very low TiO_2 concentrations were reported in viable dermis (Danish EPA 2015). There is no evidence at present to indicate that this pathway offers a viable mechanism of entry into systemic circulation. Also in line with the SCCS assessment of nano-TiO_2 in sunscreen, the risk following dermal application might be unlikely based on current level of knowledge.

7.3.4 Consumer Scenario 4 – Exposure to nano-sized pigments via tattoo inks

Hogsberg et al. 2011 investigated the size of the pigments in the tattoo inks. The black pigments were the smallest, the white pigments the largest and the coloured pigments had a size in between the two. The vast majority of the tested tattoo inks contained significant amounts of nanoparticles except for the white pigments. The black pigments were almost pure nanoparticles, i.e. particles with at least one dimension < 100 nm. The authors assumed that physical size determined the distributions. Pigments can distribute via the lymph and possibly also directly to the blood, and a minute fraction may over time undergo metabolic breakdown and as hapten(s) induce allergic reactions of red tattoos (Serup et al. 2017). Carbon black of black tattoos has a tendency to agglomerate and form larger bodies that can elicit foreign body reactions in black tattoos and even granuloma formation with overlap to sarcoidosis in the clinic.

Very little is known about the biokinetics and safety profile of the many tattoo pigments in use, and no specific pigment-related chemical of tattoo ink causing specific adverse reactions in humans has been identified. Inks have many ingredients and contaminants. Tattoos are a single-dose exposure. Pigments normally cannot pass the dermis barrier and thus are trapped in the dermis despite their spontaneous tendency to drift out of the skin. According to a study (Serup et al., 2017) on gold particles in mice and rats, some un- known fraction of small-sized particles pass directly to the blood, become circulated, and reach a variety of distant organs. Soluble ingredients of inks have different biokinetics and are distributed directly to the blood. The precise dose of tattoo pigment injected in human skin by tattooing cannot be determined with acceptable precision and thus remains un- known. The percentage of pigment in tattoo ink by typically is in the range of 10–30% w/v. The studies clearly indicate that red and yellow azo pigments are sensitive to photochemical breakdown, and over time, a significant amount of pigment disappears out of the skin. However, there remains a huge knowledge gap regarding the very many aspects of tattoo pigments, chemicals and human skin including the influence of sun, daylight and laser light.

Schreiver et al. 2017 provided analytical evidence of tattoo particles being distributed inside the human body. Tissue samples of four individuals tattooed with orange, red, green or black and two non-tattooed control donors were analyzed for the presence of organic pigments. In this investigation it was found that a broad range of tattoo pigment particles may be present with up to several micrometers in size in human skin but only smaller (nano)particles transported to the lymph nodes. The exact size limit preventing this translocation is unknown yet.

The only relevant exposure route considered for this scenario is the dermal route. Adults would be the target group. Unfortunately, there is a lack of data about the possible level of exposure from the tattoos.

The ECHA restriction proposal for tattoo inks (ECHA 2017a) estimated an exposure of 14.36 mg tattoo ink/cm2 tattooed skin. A tattoo ink containing 25% pigment was considered to be realistic based on market information The exposure was assessed as the exposure from a single tattoo session. The typical maximum area of a full colour tattoo that can be made in one session (in one day) was estimated to be 300 cm2. The corresponding amount of ink containing 25% pigment injected in a single session was estimated to be 14.36 mg ink/cm2, corresponding to exposure to 4 308 mg ink when the tattoo size is 300 cm2. Quantitative risk assessments and derivation of DNELs were made for a number of threshold substances, such as substances toxic to the reproduction and selected impurities with other threshold effects. Some impurities and non-threshold substances were risk assessed in a semi-quantitative way with derivation of DMELs, primarily for the derivation of concentration limits but also for risk characterisation. The assessed substances were not identified as the nano-pigments (or particles with at least one dimension < 100 nm).

Table 25: Scenario addressing exposure to tattoo inks.

Product	NM	Exposure scenario	Target group	Nanomaterial exposure			
				Oral	Dermal	Inhalation	Eye
Tattoo inks	Tattoo pigments (up to 30%)	Exposure to nano-sized pigments via tattoo inks	Adults	NCR	No data	NCR	NCR

NCR = Not considered a relevant exposure route

7.3.4.1 Discussion and general conclusions

Clear evidence based conclusions on the safety of using tattoo containing nanomaterials cannot be drawn based on the presented studies. The risk assessment is impeded by lack of information on almost all the necessary input data (no information on chemical composition of the tattooes, characterization of nanomaterials contained in the tattooes, release factors, etc.).

The potential for exposure is considered high as the nanomaterial is injected directly into the skin, a conclusion supported by the restriction proposal for tattoo inks (ECHA 2017a). The release of nanomaterials through the skin and its migration and possible adverse effect needs to be further investigated, however in the light of the limited available experimental data, precaution should be applied.

Tattoo ink nanoparticles have been found in lymph nodes in humans (Schreiver et al., 2017) as well as in liver macrophages in experimental animals (Sepehri et al., 2017) proving systemic distribution of the tattoo inks injected into the dermis. The health impact or the rate of this translocation have not been elucidated. Although in the limited available literature no systemic effects have been reported yet, the tattoo inks might potentially represent a long-term source of nanoparticles for the body. Schreiver et al. (2017) also demonstrated that ink nanoparticles may cause conformational alterations of biomolecules that likely contribute to cutaneous inflammation and other adversities upon tattooing.

Important factors affecting toxicity of tattoo inks are their chemical composition and the presence of impurities. Pigments used in tattoos are usually primarily manufactured for other applications (e.g. textiles, cars or plastics) and generally lack high purity standards (Piccinini et al., 2015b). Legislative requirements for chemical composition of tattoo inks vary widely among EU countries and a market controls are lacking. Chemical analyses of the selected inks available on the EU market revealed the presence of prohibited substances as well as a high level of contamination (Forte et al., 2009; Niederer et al., 2018). Moreover, the gradual degradation of the tattoo pigments over time as well as intended tattoo removal by laser irradiation generate degradation and intermediate products which may have different effects. Overall, potential health effects of various chemicals present in tattoos have not been studied in more details. Carcinogenic, sensitizing, irritating and other effects cannot be excluded.

Traditionally used dyes and pigments (colourants) are being replaced by new azo colourants. This development coincides with an increase in reports of adverse reactions to tattoo inks. But the exact incidence of adverse reactions related to tattooing is not known. Among the most often reported adverse effects related to tattooing are inflammatory, granuloma formation, phototoxicity, allergic reactions and poorly understood coincidental diseases implying autoimmunity (Piccinini et al., 2016), Possible long-term systemic effects of tattooing are difficult to be tracked and proven. There are a high number of substances used, many of which

are unknown and of the ones known, there is often insufficient information on concentrations in tattoo inks and/or hazard information to allow a traditional quantitative assessment of their risks. Risks of health effects other than dermal effects, such as systemic cancers, reproductive effects etc. cannot be ruled out (ECHA 2017a).

7.4 Generic exposure scenarios

In the absence of data on product-specific nanopigment use and exposure, it is necessary to describe most professional and consumer uses in a generic way. The following list provides a basis for description of *likely* modes of professional and consumer exposures. The generic scenarios presented in this study are indicative only and need to be further developed. A map of hypothetical exposure scenarios based on the lifecycle assessment study of the route and extent of human exposure via different pathways for commercially available naproducts containing nanopigments should be elaborated. This will allow the pre-judgment on expoure potential and so enable the first attemps on health risk prioritization.

In the absence of data on product-specific nano-pigment use and exposure, it is necessary to describe most professional and consumer uses in a generic way. The following list provides a basis for description of *likely* modes of professional and consumer exposures. The scenarios were assessed (if applicable) in CB tool Stoffenmanager as in the previous section. Due to lack of data, the default inputs for worst case scenario were chosen. Where the CB tool was not possible to use (concretely for consumer scenarios) the qualitative description of activity and possible risk is provided.

7.4.1 Professional Generic scenarios

- **Professional Spray application of pigment-containing paints and coatings: Automotive paint application in car workshop (open systems and spray booths)**
    - Pneumatic spraying of pigment-containing paints
    - Target group – Adults
    - Relevant route of exposure –Inhalation

The scenario and results of qualitative assessment using CB tool are presented in the table 32, Appendix 5.

Despite the limited data on ES, there is a concern with respect to potential health risk. The risk level for this scenario reaches the category I, i.e. high risk. The resulted level of risk did not change even for the same scenario withhalf-mask protection of employee. The risk level is highly influenced by the ES data incompleteness, and using the worst-case scenario within the determinants of exposure and unknown nanomaterial and its adverse effect. The use of manufactured nanomaterials is associated with a high degree of uncertainty. This uncertainty is considered in the attribution to certain exposure and hazard bands. The MNOs with an unknown hazardous profile are associated with a high-risk band in case they fall into a relatively high exposure band.

- Professional hand application of pigment-containing paints, coatings, varnishes, lacquers (by brush, roller)
    - Paint with nanomaterial applied to walls/surfaces by rolling
    - Target group – Adults
    - Relevant route of exposure – Inhalation

The scenario and results of qualitative assessment using CB tool are presented in the table 33, Appendix 5.

For this generic ES there is a potential concern. The risk level for this scenario reaches the category I, i.e. high risk. The risk level is highly influenced by the ES data incompleteness, and using the worst-case scenario within the determinants of exposure and unknown nanomaterial and its unknown hazardous properties. The use of MNOs is associated with a high degree of uncertainty. This uncertainty is considered in the attribution to certain exposure and hazard bands. The MNOs with an unknown hazardous profile are associated with a high-risk band in case they fall into a relatively high exposure band. This preliminary risk assessment is dealing with a very high level of uncertainty due to lack of realistic data.

- **Professional formulation activities (mixing/blending of paints for colour combinations)**
    - Manual mixing of colours
    - Target group – Adults
    - Relevant route of exposure – dermal + inhalation

The scenario and results of qualitative assessment using CB tool are presented in the table 34, Appendix 5.

As in the previous cases, it can be said that due to lack of concrete data on ES, there is a concern with respect to potential health risk. The risk level for this scenario reaches category I, i.e. high risk. The risk level is highly influenced by the ES data incompleteness, and using the worst-case scenario within the determinants of exposure and unknown nanomaterial and its unknown hazardous properties. As the MNOs is already dispersed in the paint, it could be expected that the exposure to released MNOs from this activity could be negligible. The use of MNOs is associated with a high degree of uncertainty. This uncertainty is considered in the attribution to certain exposure and hazard bands. The MNOs with an unknown hazardous profile are associated with a high-risk band in case they fall into a relatively high exposure band. This preliminary risk assessment contends with a very high level of uncertainty due to lack of realistic data.

- **Professional formulation activities (mixing/blending of paints for colour combinations)**
  - Semi-automatic mixing of colours
  - Target group – Adults
  - Relevant route of exposure –Inhalation

The scenario and results of qualitative assessment using CB tool are presented in the table 35, Appendix 5.

This scenario is different from the previous one just by using semi-automatic process. The risk level for this scenario reaches also the category I, i.e. high risk. The risk level is highly influenced by the ES data incompleteness, and using the worst-case scenario within the determinants of exposure and unknown nanomaterial and its unknown hazardous properties. As the MNOs is already dispersed in the paint, it could be expected that the exposure to released MNOs from this activity could be negligible. The use of MNOs is associated with a high degree of uncertainty. This uncertainty is considered in the attribution to certain exposure and hazard bands. The MNOs with an unknown hazardous profile are associated with a high-risk band in case they fall into a relatively high exposure band. However, this preliminary risk assessment compromised by the very high level of uncertainty due to lack of realistic data.

7.4.2 Consumer Generic Scenarios

- **Consumer spray application of paints, coatings, varnishes, lacquers**
  - Pneumatic spraying of pigment-containing paints
  - Target group – Adults+Teenagers
  - Relevant route of exposure – Inhalation

The consumer spray application was assessed in the CB tool, ConsExpo nano[24]. The default scenario for pneumatic spraying of painting products was loaded from that tool. Moreover, some information was requested related to parameters of assessed nanomaterial - the density of nanomaterial and particle size. Nano-TiO_2 was taken as the example due to it's wider use.

The density of nano-TiO_2 and particle size were 3.9 g/cm^3 and 18 nm, respectively. The estimated inhaled dose per event using ConsExpo nano was estimated 2.0×10^9 particles/ event; and mass concentration 4.8×10^1 mg per event. The estimation is affected by very high level of uncertainty due to used defaults and the parameters of nano-pigment. More concrete determinants of real exposure, and characterization of nanomaterial would improve

[24] https://www.consexponano.nl

this risk assessment.

- **Consumer application of paints and coating by brush, roller**
  - Roller application of pigment-containing paints
  - Target group – Adults+Teenagers
  - Relevant route of exposure – dermal

To consider the risks from dermal exposure to nanomaterials by roller application of paints, in the first step, release has to be identified in order that the exposure can be conducted. Some studies were previously discussed which focused on the release of the particles within weathering the paints. Theoretically the most relevant route of exposure is dermal contact with a paint matrix that may contain nanoparticles, and consequently the risks are deemed to be low.

- **Consumer use of artistic paints / pastels / chalks**
  - Painting with the pastels or chalks
  - Target group – Adults+Teenagers+Children
  - Relevant route of exposure – oral+dermal

Pigments canbe found in different type of products used by painters: pigments in oil paints, acrylics, watercolor paints, gouache, encaustic, poster paints, casein paints and tempera. Paints are pigments mixed with a vehicle or binder. Both inorganic and organic pigments are used as colourants. Dry pigments could be of concern because they could be easily inhaled and ingested. They are used in encaustic, paper-marbleizing and in the fabrication of paint products. The likely risk in standard painting techniques could be in accidental ingestion of pigments due to eating, drinking or smoking while working, inadvertent hand to mouth contact, or pointing the paint brush with the lips. The oral exposure is more likely between children compared to toher target groups.

7.4.3 General recommendations related to exposure assessment to nano-pigments

Understanding the properties of both nanomaterials and cells or organisms driving the nano-bio interactions is essential. The technical development of more accurate measurements of nanoparticles under realistic exposure condition, *in situ*, and in media (polymeric, liquids) consumer products and food is strongly recommended. Important requirements for the further elucidation of parameters required for risk assessment include:

- Development of guidelines on how to determine a nanomaterial including easy to use instruments and agreed measurement protocols;
- Development of agreed descriptors for complex non-spherical structures;
- Development of instruments to separate engineered from background nanomaterials;
- Development of dose-response relationships for identified relevant descriptors.

Attention should also focus on the development of a wider range of realistic consumer and professional scenarios, to include all the determinants of exposure.

In summary, it is clear that more information on the use of nanomaterials, their potential for release and exposure in occupational, consumer and environmental contexts is needed in order to derive comprehensive and realistic risk assessment to exposure to nano-sized pigments (Savolainen et al., 2013).

8. Conclusions on risks presented by nano-sized pigments

Evidence-based conclusions on the safety of nanopigments uses cannot be drawn based on the available literature. Significant knowledge gaps are presented throughout all the key steps of health risks assessment and management procedures, i.e. substance/product characterization, hazard identification, exposure assessment, risk characterization and risk control.

Regarding exposure to nanopigments, substantial lack of knowledge is observed in contextual information on exposure as well as in experimental data on release and exposure. Nanopigments and products containing nanopigments are poorly characterized. Chemical composition of the products and weight fractions of the nanomaterials are mostly unavailable from public domain sources. Substance characteristsics are very often limited to physical state. Particle size is rarely indicated. Other characteristics (surface area, zero potential, emission potential etc.) are not recorded in almost all cases. Activity emission potential is mostly missing. Information on risk management measures in place are scarce and, if available, are often poorly defined. Release studies are scarce, investigating real-exposure situations to a very limited extent. Workplace and exposure measurements are seen to be carried out using different monitoring and sampling strategies and procedures with the use of various measuring tools. The absence of harmonised standardised operating procedures (SOPs) for conducting exposure measurements as well as the collection of contextual information hinders the comparison of study results and the further use of the experimental data for decision making within the risk management process.

Only very general conclusions on potential exposure could be drawn based on the available literature. The main pathways of potential human exposure to nanopigments and products containing nanopigments identified in the reviewed studies are the following:

- inhalation of powder, or of aerosolised (nano)particles during production of the substances, formulation of products, packaging of the product (workers), and (professional) use of the products;
- dermal exposure via use of the final product (by consumers);
- oral exposure via food (by consumers);

The review of toxicological data on nanomaterials has shown that nano-sized pigments, as with nanomaterials in general, have the potential to cause a wide range of health effects distinct from normal chemical-inducted toxicity. Toxicity of nanoparticles after skin and oral exposure has been demonstrated to be low under conditions relevant to expected low-level human exposures.

However, inhalation of insoluble particles can lead to a range of sequalae in animal toxicology studies, depending on individual particle characteristics, in particular their size and surface area. This provides nano-sized pigments with unique abilities to translocate and interact with organisams at the cellular level, and to cause biochemical perturbances. Absorbed nanoparticles also have the ability to cause immunological effects, through the action of adsorbed impurities or by acting as adjuvants. Although *in vivo* and *in vitro* studies have yet to be standardised for study of the unique characteristics of nanomaterials, it is often observed that they may elicit oxidative stress and chronic inflammation when clearance mechanisms are overloaded, particularly in the lung and liver, leading to secondary genotoxicity and potentially neoplastic and reproductive/developmental effects from the systemic circulation of inflammatory mediators. In the case of lung overload, a species-specific mechanism has been identified in rats, and its significance for extrapolating to other species, including humans is still under debate. As yet, direct or epidemiological evidence for adverse effects from human-

relevant exposures is lacking.

Based on the precautionary principle, when there is a potential for exposure to nanoparticles, a potential risk for human health cannot be excluded. Exposure is considered real when direct contact between a product containing nanopigment in an available form (i.e. that could be inhaled, ingested or absorbed via skin or via different route of entry), cannot be avoided.

The industrial and professional exposure scenarios dealing with dry and free nanopigments which may become airborne present the highest concern since there is evidence from animal studies of potential adverse health effects of (nano) particles on human health through inhalation. Clear exposure potential ha been identified whenever airborne particles are emitted, for example in production activities, but also during the use of printer toner cartridges by professionals and consumers.

The end uses of all pigments necessitates that they are bound in a matrix, for example when used to colour paints, coatings and polymers. These end-uses generally do not present a significant exposure potential. Even after weathering or abrasion/sanding of the final product it has been shown that nanoparticles are still bound in larger matrix particles which precludes the possibility of significant exposure to nano-sized fractions, and that the matrix itself may be a more important determinant of toxic potential.

9. Uncertainty analysis

Risk assessemt of nanosized pigments presents, at the outset, a number of clear uncertainties where assumptions may need to be made. As discussed previously, this includes whether the level of data available allows the proper characterisation of pigments as nanopigments. Much of this is dependent on available techniques to measure nano-sized fractions of commercial pigments, and indeed the ability of a manufacturer and, more critically importers, to describe their products correctly. Further, toxicological studies are often conducted on bulk forms only, thereby missing nano-specific considerations.

In order to reduce these uncertainties, it is necessary to generate sound scientific data on hazard and exposure by means of relevant frameworks and tools.

The uncertainty involved with the evaluation of toxicological parameters, hazards and risks related to nano-pigments is a complex issue and contains several interlinked steps, especially when results are to be used for human health and environment protection decision making to facilitate risk management. A general scheme of involved steps is shown in Figure 4:

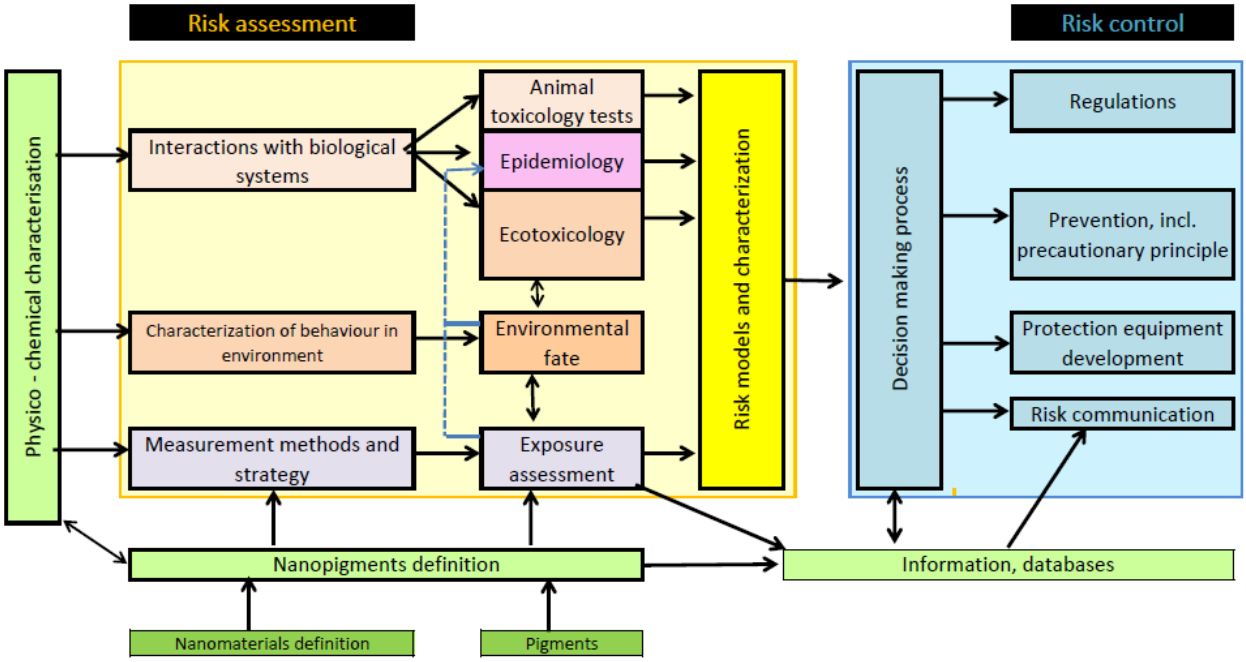

Figure 4: Interlinked steps of risk management of nanoparticles

Uncertainties may be identified and classified by many methods. For the purposes of risk management, two aspects are of particular importance:

- Uncertainty of data generation/collection process, i.e. the availability of robust, reproducible data obtained from independent sources by high-quality methods. In figure 4, this may be may be imagined as "inside the boxes". Frequently, we find a random type of uncertainty here. Standarisation of measurement techniques will give us clear answers about the origin and extent of uncertainty related to the determination of what constitutes a nanopigment for the purpose of risk assessment. Usually this uncertainty refers to the type A of uncertainty accoording to ISO/IEC GUIDE 98-1:2009[25].
- Uncertainties in risk management. Risk management is a process demanding the transfer of proper and relevant data from one domain to another and its overall uncertainty is dependent on the adequacy and reliability of data to be used for decision making. Currently there are not enough scientific data relevant to human toxicity or to uses of nanopigments in products. Referring to figure 4 above, we can imagine this kind of uncertainty as linked to the arrows between boxes, e.g. when knowledge is transferring from one discipline to the other. This type of uncertainty is in many cases type B of uncertainty accoording ISO/IEC GUIDE 98-1:2009. Even when very good quality data is available from one area, high uncertainty may exist in other steps required for risk management. One example is where standard procedures may exist for the testing of media containing nanoparticles that are highly dispersed so as to obtain individual particles. Such data may be reproducible, but the real exposure in working and living environments may be governed by the presence of agglomerates. If the toxicity mechanism is linked to agglomerates more than to individual particles

[25] https://www.iso.org/standard/46383.html

("unknown" at this case), high quality experimental data may not be relevant to the real toxicity effects. Therefore the uncertainty of the decision making process is increased by this fact despite the high quality of primary toxicity tests data.

- Both of the above mentioned types of uncertainty are present to a high extent in the assessment of nanopigments. Even if some uncertainties are not nanopigment-specific, they must be mentioned here to properly understand the overall uncertainty in the nanopigment safety domain.

Individual uncertainties are commented below.

9.1 Uncertainties over the definition of Nano-sized Pigments

Nanopigments may be defined as nanomaterials used as pigments. For overall risk management of nanopigments as a category, it is necessary to have a clear and unambiguous, evidence based, definition of both terms – nanomaterial and pigment. Uncertainty in definition of nanomaterial are directly reflected in uncertainties in the overall risk assessment process.

The main sources of uncertainty with respect to the sound risk management of nanomaterials quickly became evident in this project. In the first instance, the current EU definition of nanomaterial is considered by industry as unsatisfactory in the context of nano-sized pigments[26],[27],[28].

Occupational health and safety as a subject of regulation is based on good definitions of what has to be monitored, controlled and regulated. Therefore, a clear, unambiguous and comprehensible definition of nanomaterial should be agreed. An important requirement is the possibility to measure all definition parameters by accessible technically and economically viable methods.

At least four EU regulatory acts use a definition of nanomaterial adapted from the 2011 EU recommendation (European Union Cosmetic Product Regulation No 1223/2009, Food information to Consumer Regulation No 1169/2011, Biocides Regulation No 528/2012 and Medical Devices Regulation, last amendment 2007/47/EC). The current legal text of the REACH Regulation (No 1907/2006) does not explicitly refer to nanomaterials, however revised Annexes of the REACH Regulation with explicit obligations in the reporting and in the information requirements for nanoforms are to be implemented, subsequent to the agreement of the REACH committee (April 26th 2018[29]) to amend the REACH annexes so as to clarify the reporting requirements for nanomaterials. The EU definition of nanomaterials is currently applied when implementing REACH, yet only a small number of registrations currently indicate nanoforms.

One of the aims of this project was to define the nano-sized pigments currently marketed in the EU. Information needed to be derived from disparate sources which cannot claim to give a

[26] http://www.cepe.org/wp-content/uploads/2018/01/Position-Paper-EU-Commission-Recommendation-on-the-definition-of-nanomaterial-for-regulatory-purposes.pdf

[27] https://www.vdmi.de/englisch/media-library/nano.html?filename=vdmi_factsheet_nanodiscussion_201708.pdf

[28] http://etad.com/en/

[29] http://ec.europa.eu/transparency/regcomitology/index.cfm?do=search.documentdetail&Dos_ID=15915&DS_ID=56122&Version=2

complete overview. It may be suspected that certain pigments may fall under the definition of nanomaterial, either by design or through incidental presence of nano-sized fractions. However, the scope of this project could only include pigments that are *specifically* declared to be marketed as nanoform. Therefore information from national nano inventories, the REACH database, the EU catalogue of nanomaterials used in cosmetic products, and industry data were used to define the list of nanopigments available on the EU market. However, these sources are far from comprehensive, leading to the assumption that the real number of available nanopigments is higher than reported here.

As has been seen, currently REACH database includes only a small number of substances declared as nanoforms, so this cannot be viewed as a comprehensive source. National registries dealing with nanomaterials are currently established in France, Belgium and Denmark, and their databases are confidential. Only data released in public reports in 2015 and 2016 were available for use in this project. More nano-sized pigments may have been declared in the meantime.

Further, currently only the French inventory makes declaration of nano-sized pigment uses compulsory. Both the Danish and Belgian inventories have exemptions to the reporting of pigments in nanoform, however the Belgian inventory does include voluntary declarations. A Swedish nano inventory is currently being established, however pigment uses are also exempted.

On the other hand, the EU catalogue of nanomaterials used in cosmetic products placed on the EU market, as notified through the Cosmetic Products Notification Portal (CPNP), should capture all cosmetic uses up to 2017, as reporting is mandatory.

Industry data with regard to the availability of nano-sized pigments is limited. At this moment, in the framework of this Project, a nano-pigment is a material declared as such by the producer. It means that, in case of doubt, if declaration as nano-sized pigment may lead to additional regulatory burden (e.g. additional testing) a producer may choose not declare it as nanopigment, but as a pigment only. On the other hand, companies marketing pigment may not even know whether their product falls under the definition of nanomaterial. To improve reporting, an objective definition of nanopigment must be linked to the measurable properties and not based on voluntary declaration only.

Therefore, currently in this project, a pigment was definitively confirmed as a nanopigment existing on the EU market when it appeared on either the REACH database and/or in the Danish list and appeared in the French or Belgian nano inventories, or was identified from an industry source.

Uncertainties over the number of nano-sized pigments on the EU market, and their uses, will be reduced if the EU definition is revised, as per recommendation of the Nanodefine (2017) project and through the new REACH reporting requirements for nanomaterials that are addressed in the revised REACH annexes.

9.2 Uncertainties in Hazard Characterisation

Standard testing methods are, to a large extent, applicable to nanopigments as well, nevertheless "nano-toxicity" may differ from the toxicity of substances in bulk form, which are typically used for standard hazard testing:

- Nanoparticles, due to their size and particle characteristics, can translocate within organisms. Nanoparticles may pass biological barriers including cell membranes; enter to the cells as well as accumulate in certain organs. There are only very limited data available for Nanopigments in this respect, with high uncertainty level.

  Nanoparticles of various sizes may exhibit different or enhanced toxicological effects. For

example, for titanium dioxide nanoparticles it has been shown that particles in size range 20 to 30 nm are considerably more toxic when it comes to respiratory health than their microparticle (>100 nm) counterpart. Wide variability in size, shape and coating refers to the extremely high number of different possible nanomaterials with the same basic compound. To test all of them individually would demand immense number of toxicological tests. Therefore procedures to group similar nanomaterials with respect to toxicity, without missing important specific toxicity information, are needed but not yet realised. This phenomenon brings the uncertainty of transferability of data from one nanopigment to another. The uncertainty of transferability of data comes mostly from lack of a minimum standard characterization of the important physicochemical parameters as well as lack of standard methods for nanomaterial testing for many human health endpoints.

- Nanoparticles toxicity is highly influenced by the surface properties, including the "corona" of molecules formed by interaction with biological systems. During the life cycle of nanoparticles, the toxicity may change significantly and the surface modification (coating) of nanoparticles may influence resulting hazard and risk assessments.

- A typical behaviour of nanomaterial is self-assembling, leading to agglomeration, deposition and formation of secondary structures. It presents a principal problem for toxicity testing with regard to how to reach reproducible results. Tests with well-dispersed pristine nanoparticles are preferred but their effects may significantly differ from real exposure situations. Dispersion of nanomaterial in test medium (most frequently solution) is problematic and non-standardised. This creates a high level of uncertainty when translating laboratory results to real-world scenarios. Methods for better understanding of behaviour of nanoparticles in testing media and their potential for interaction are needed.

- The proper metric of dose is uncertain. It is evident that the toxicity of nanoparticles is not only mass-dependent but might also be dependent on physical and chemical properties that are not routinely considered in today's toxicity studies. Some studies found that particle number was the best dose metric; in others, toxicity was related to the surface area or to the number of functional groups on the surface of nanoparticles.

- Beside the "chemical" effect of nanomaterial caused by dissolved species (e.g. Ag^+ ions in the case of nanosilver), secondary toxic effects of nanoparticles are observed, in particular inflammation (caused typically by oxidative stress) leading to genotoxicity, which is often only observed in rats in conditions of biological overload.

9.3 Uncertainties in Exposure Characterisation

There are several problems in exposure assessment. First of all, the metric is uncertain; without knowing which dose metric is used to express toxicity hazard (number or mass concentration, active surface, etc.) it is difficult to assess real-world exposure. The "standard" metric in exposure measurement for standard chemicals is the mass concentration, whilst for nanomaterials a more appropriate approach is to use the number concentration.

Currently available techniques and instrumentation for field measurement of exposure to nanomaterials are complicated and expensive. Simple, low-cost and robust measurement techniques need to be developed and standardised. Moreover, it is not easy to distinguish between background (natural, incidental) nanoparticles and their engineered counterparts. Further, the majority of measurements do not consider background concentrations.

The result is that better standardisation of exposure assessment for nanomaterials through development is needed as well as an universal adoption of libraries of typical exposure scenarios, which need to be led by industry initiatives and harmonized between countries. This would facilitate sharing of experience and transfer of data and knowledge. Such libraries are already in development various research projects (see Section 7 above) and thus, the adoption

by industry and harmonisation must be priority Another difficulty is the high degree of concentration distribution in working areas, which is reflected in high uncertainty of individual exposure results. Moreover, different sizes of nanomaterials are distributed differently in space and agglomeration process influences distribution as well.

Despite the limited data on nanopigments exposure, we can still base future measurement strategies on the wide spectrum of projects that have been devoted to nanomaterial exposure testing in general.

Although efforts have been made to collect as much and as specific data as possible on the possibilities of consumer and professional exposure for the 20 scenarios covered by this report, many uncertainties pertain to several key parameters important for the reliability of the assessment. The overall degree of uncertainty may be seen in the context of the lack of data that is important to address when establishing an exposure scenario. These are listed below:

- The current EU definition of nanomaterial is disputed by industry. In the absence of agreed standard measurement techniques, the industry position is to assume that all pigments should fall under the current EC definition, since it is currently not possible to conclusively prove that they are not nanomaterials. In this respect, a number of recommendations have been made in the NanoDefine project in order to clarify the criteria of what is and what is not a nanomaterial.
- There is very little public-domain data on the use of nanopigments in consumer products and there for it is difficult to accurately gauge exposure/risks to nanopigments specifically.
- Most published data lack adequate dose and exposure metrics. Estimations of consumer / professional exposure must be based on highly conservative assumptions, often based on mass metrics, which are unsuitable for the assessment of nanomaterial exposure.
- The open literature does not allow for the development of an inventory of marketed nanopigments.
- Nanopigments are generally exempt from reporting on the national inventories, however many pigments are still identified this way. There are questions over the comprehensiveness of the inventories.
- The ECHA REACH database, as of April 2018, lists only 23 registrations where a nanoform is identified. This number will rise as the REACH Annexes are updated to explicitly include new requirements for the reporting of substance nanoforms.
- Generally, the chemical identity and/or characterisation of nanomaterials applied in consumer products are not well described, if at all.
- Importers/Distributors generally may not have the technical means to measure nano-sized fractions, and therefore may be unaware that their product is defined as a nanomaterial according to the current definition.
- Pigments are most often often supplied in liquid or solid matrix rather than as powder and therefore the potential for direct exposure is uncertain for most uses.
- Toxicological methods used to test bulk form substances do not translate well to the requirements for testing nanoforms. There is a need for standardisation across all endpoints, including a need to adequately characterise the nanoforms being tested.
- Toxicological studies on nanoforms rarely employ human-relevant exposures.

- Exposure measurements complicated by lack of methods / equipment and the expense involved.
- Mostly, available data and models only allow exposure estimation as mass metric. In general, current exposure estimation models are not designed for estimating exposure to nanomaterials.
- Characterisation of nano-sized fraction of pigments is frequently not detailed enough, increasing the degree of uncertainty in exposure measurements and their applicability for risk assessment.

10. Recommendations for further work

Due to gaps in current knowledge regarding hazard and risk assessment of nanopigments (and nanomaterials in general) resulting in high levels of uncertainty, there should be an increased emphasis on exposure assessment and control. Current precautionary measures aim to avoid or at least reduce the exposure to nanomaterials as much as possible.

Sharing of exposure data and development of comprehensive, well designed and realistic Exposure Scenarios (ES) is essential for increasing our knowledge in exposure to nanomaterials in the workplace and among consumers. Scenarios providing conceptual information on particular nanomaterials, operating conditions, applied risk management measures and release and/or exposure measurements, present a valuable tool for exposure estimation, however there is a high level of assumption involved Compilation of ES into libraries enables extrapolation to particular exposure situation of interest and to benchmark different process operations and safety measures. Use of standard libraries and extrapolation is of high importance, since the measurements of all exposure situations is not achievable. In a wider context, ES could form a basis for exposure registries and further epidemiological research. Development of standard ES libraries is an important step within the whole process of safety management of nanomaterials.

Despite the progress in ES building achieved at the EU level, there are still many obstacles and challenges in this field. Some of them arise from scientific reasons, e.g. the lack of knowledge on a relevant exposure metric, difficulties in conducting measurements associated with temporal and spatial variability in both particle size distribution and number concentration, non-existence of standardized protocols for exposure measurements or even the uncertainty in definition of nanomaterials.

There is a need for enterprises to share sensitive data regarding their production and processes involving nanomaterials, which are necessary for building real-world ES. Further support of harmonized approaches in measuring and gathering knowledge on exposure (e.g. through the NECID database - Nano Exposure & Contextual Information Database, MARINA/GUIDEnano library on exposure scenarios, etc.) is of high importance.

A map of hypothetical exposure scenarios based on the lifecycle assessment study of the route and extent of human exposure via different pathways for commercially available products containing nanopigments should be elaborated. This will allow pre-judgment on exposure potential and so enable the first attemps on health risk prioritization.

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Appendix 1. Nano-sized pigments identified on the EU market (April 2018)

	Identification				REACH Data					Data Source					
No.	**Name**	**CAS No**	**EC No**	**C.I. Colour Index**	**REACH tonnage (tpa)**	**Prof. Use**	**Consumer Use**	**Nano - Form In REACH?**	**Nano-sized Pigment reported on EU Market ?**	**ECHA**	**DK**	**France**	**BE**	**Industry**	**Other**
1	Titanium dioxide	13463-67-7	236-675-5	C.I. Pigment White 6	1,000,000 +	Y	Y	Y	Y	Y	Y	Y		Y	CPNP
2	Rutile (TiO2)	1317-80-2	215-282-2		10,000 - 100,000	Y		N	Y		Y			Y	
3	Iron hydroxide oxide yellow	51274-00-1	257-098-5	C.I. Pigment Yellow 42	100,000 - 1,000,000	Y	Y	Y	Y	Y	Y	Y	Y		
4	Diiron trioxide / Iron (III) oxide	1309-37-1	215-168-2	C.I. Pigment Ref 101 / 77491	100,000 - 1,000,000	Y	Y	Y	Y	Y	Y	Y	Y	Y	CPNP
5	Zinc oxide	1314-13-2	215-222-5	C.I. Pigment White 4	100,000 - 1,000,000	Y	Y	Y	Y	Y	Y	Y		Y	
6	Manganese ferrite black spinel	68186-94-7	269-056-3	C.I.Pigment Black 26	1,000 - 10,000	Y	Y	Y	Y	Y	Y	Y			
7	Silicon Dioxide	7631-86-9, 112926-00-8	231-545-4		1,000,000 +	Y	Y	Y	Y	Y		Y	Y		Y
8	Iron manganese trioxide	12062-81-6	235-049-9	C.I. Pigment Brown 43	1,000 - 10,000	Y	Y	Y	Y	Y	Y	Y	Y		
9	Aluminium Oxide	1344-28-1	215-691-6		10,000,000+	Y	Y	Y	Y	Y		Y	Y		Y
10	(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.04,9]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	NA (DK 68511-62-6)	939-379-0	C.I. Pigment Yellow 150	100 - 1,000	Y	Y	Y	Y	Y	Y		Y	Y	
11	Calcium carbonate	471-34-1, 7440-70-2	207-439-9		1,000,000 - 10,000,000	Y	Y	Y	Y	Y		Y	Y		Y
12	Barium sulphate	7727-43-7	231-784-4		10,000 - 100,000	Y	Y	Y	Y	Y		Y	Y		Y
13	Carbon black	1333-86-4	215-609-9	C.I. Pigment Back 6	1,000,000 +	Y	Y	N	Y		Y	Y		Y	CPNP
14	Cobalt aluminate blue spinel	1345-16-0	310-193-6	C.I. Pigment Blue 28	1,000 - 10,000	Y		N	Y		Y	Y			
15	Bismuth vanadium tetraoxide	14059-33-7	237-898-0	C.I. Pigment Yellow 184	1,000 - 10,000	Y	Y	N	Y		Y	Y			
16	29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	147-14-8	205-685-1	C.I. Pigment Blue 15	10,000 - 100,000	Y	Y	N	Y		Y	Y	Y	Y	
17	5,12-dihydro-2,9-dimethylquino-[2,3-b]acridine-7,14-dione	980-26-7	213-561-3	C.I. Pigment Red 122	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y	Y	
18	5,12-dihydroquino[2,3-b]acridine-7,14-dione	1047-16-1	213-879-2	C.I. Pigment Violet 19	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y		
19	C.I. Pigment Violet 3	1325-82-2	603-635-7	C.I. Pigment Violet 3	Not Registered			N	Y		Y	Y			
20	Polychloro copper phthalocyanine	1328-53-6	215-524-7	C.I. Pigment Green 7	1,000 - 10,000	Y	Y	N	Y		Y	Y		Y	
21	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	2512-29-0	219-730-8	C.I. Pigment Yellow 1	100 - 1,000	Y	Y	N	Y		Y	Y			
22	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	2786-76-7	220-509-3	C.I. Pigment Red 170	1,000 - 10,000	Y	Y	N	Y		Y	Y			
23	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol	2814-77-9	220-562-2	C.I. Pigment Red 4	100 - 1,000	Y	Y	N	Y		Y	Y			

	Identification				REACH Data					Data Source					
No.	Name	CAS No	EC No	C.I. Colour Index	REACH tonnage (tpa)	Prof. Use	Consumer Use	Nano - Form In REACH?	Nano-sized Pigment reported on EU Market ?	ECHA	DK	France	BE	Industry	Other
24	1-[(2,4-dinitrophenyl)azo]-2-naphthol	3468-63-1	222-429-4	C.I. Pigment Orange 5	100 - 1,000	Y	Y	N	Y		Y	Y			
25	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]	3520-72-7	222-530-3	C.I. Pigment Orange 13	100 - 1,000	Y	Y	N	Y		Y	Y	Y		
26	4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone	4051-63-2	223-754-4	C.I. Pigment Red 177	100 - 1,000	Y	Y	N	Y		Y		Y		
27	bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	4424-06-0	224-597-4	C.I. Pigment Orange 43	10 – 100	Y		N	Y		Y	Y			
28	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]	5102-83-0	225-822-9	C.I. Pigment Yellow 13	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y	Y	
29	manganese, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex	5280-66-0	226-102-7	C.I. Pigment Red 48:4	100- 1,000	Y	Y	N	Y		Y	Y	Y		
30	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	5280-68-2	226-103-2	C.I. Pigment Red 146	100 - 1,000	Y	Y	N	Y		Y	Y	Y		
31	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	5468-75-7	226-789-3	C.I. Pigment Yellow 14	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y		
32	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide]	5567-15-7	226-939-8	C.I. Pigment Yellow 83	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y	Y	
33	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]	5580-57-4	226-970-7	C.I. Pigment Yellow 93	100 - 1,000	Y	Y	N	Y		Y		Y		
34	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	6041-94-7	227-930-1	C.I. Pigment Red 2	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y		
35	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene	215247-95-3	613-252-7	C.I. Pigment Violet 23	1,000 - 10,000	Y	Y	N	Y		Y		Y	Y	
36	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	6358-31-2	228-768-4	C.I. Pigment Yellow 74	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y		
37	3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	6410-32-8	229-102-5	C.I. Pigment Red 12	10 – 100	No Use reported	No uses reported	N	Y		Y	Y			
38	N-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	6410-41-9	229-107-2	C.I. Pigment Red 5	10 – 100	No Use reported	No Use reported	N	Y		Y	Y			
39	3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	6535-46-2	229-440-3	C.I. Pigment Red 112	1,000 - 10,000	Y	Y	N	Y		Y	Y			
40	4-[(2,5-dichlorophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxynaphthalene-2-carboxamide	6992-11-6	230-258-1	C.I. Pigment Brown 25	10 – 100	No Use reported	No Use reported	N	Y		Y	Y		Y	
41	Calcium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7023-61-2	230-303-5	C.I. Pigment Red 48:2	1,000 - 10,000	Y	Y	N	Y		Y	Y	Y	Y	
42	Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-	7585-41-3	231-494-8	C.I. Pigment Red 48:1	100 - 1,000	Y	Y	N	Y		Y	Y	Y		

	Identification				REACH Data					Data Source					
No.	**Name**	**CAS No**	**EC No**	**C.I. Colour Index**	**REACH tonnage (tpa)**	**Prof. Use**	**Consumer Use**	**Nano - Form In REACH?**	**Nano-sized Pigment reported on EU Market ?**	**ECHA**	**DK**	**France**	**BE**	**Industry**	**Other**
	naphthoate														
43	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	13515-40-7	236-852-7	C.I. Pigment Yellow 73	100 - 1,000	Y	Y	N	Y		Y	Y	Y		
44	Strontium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (1:1)	15782-05-5	239-879-2	C.I. Pigment Red 48:3	100 - 1,000	Y	Y	N	Y		Y		Y		
45	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]	15793-73-4	239-898-6	C.I. Pigment Orange 34	100 - 1,000	Y	Y	N	Y		Y	Y	Y		
46	3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide	30125-47-4	250-063-5	C.I Pigment Yellow 138	100 - 1,000	Y	Y	N	Y		Y		Y		
47	Dimethyl 2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]terephthalate	35636-63-6	252-650-1	C.I Pigment Yellow 175	10 - 100	No Use reported	No Use reported	N	Y		Y	Y			
48	5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid	36888-99-0	253-256-2	C.I Pigment Yellow 139	1,000 - 10,000	Y	Y	N	Y		Y	Y		Y	
49	4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	36968-27-1	253-292-9	C.I Pigment Red 266	10 - 100	Y	Y	N	Y		Y	Y			
50	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(4-nitrophenyl)azo]-3-oxobutyramide	52846-56-7	258-221-5	C.I Pigment Orange 62	10 - 100	No Use reported	No Use reported	N	Y		Y	Y			
51	Methyl 4-[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[(2-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]benzoate	61847-48-1	263-272-1	C.I. Pigment Red 188	100 - 1,000	Y	Y	N	Y		Y	Y			
52	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide	68134-22-5	268-734-6	C.I Pigment Yellow 154	100 - 1,000	Y	Y	N	Y		Y	Y			
53	Tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate	68516-73-4	271-176-6	C.I Pigment Yellow 155	100 - 1,000	Y	Y	N	Y		Y	Y	Y		
54	5-[(2,3-dihydro-6-methyl-2-oxo-1H-benzimidazol-5-yl)azo]barbituric acid	72102-84-2	276-344-2	C.I. Pigment Orange 64	100 - 1,000	Y	Y	N	Y		Y		Y		
55	N-[4-(aminocarbonyl)phenyl]-4-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzamide	74441-05-7	277-873-1	C.I Pigment Yellow 181	100 - 1,000	Y	Y	N	Y		Y		Y		
56	2,2'-[ethylenebis(oxyphenyl-2,1-eneazo)]bis[N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide	77804-81-0	278-770-4	C.I Pigment Yellow 180	100 - 1,000	Y	Y	N	Y		Y	Y		Y	
57	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-[2-(4-chlorophenoxy)-5-(trifluoromethyl)phenyl]benzamide]	79953-85-8	279-356-6	C.I Pigment Yellow 128	10 - 100	Y	Y	N	Y		Y		Y		
58	3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	84632-65-5 (DK 84632-65-5)	617-603-5 (DK617-603-5)	C.I. Pigment Red 254	1,000 - 10,000.	Y	Y	N	Y		Y		Y		
59	3,6-bis-biphenyl-4-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	NA (DK - 88949-33-1)	413-920-6 (DK 618-223-2)	C.I. Pigment Red 264	10-100	Y	Y	N	Y		Y	Y	Y		
60	Reaction mass of N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-	NA (DK - 99402-80-9)	911-739-1 (DK - 619-430-0)	C.I. Pigment Red 184	100 - 1,000	No Use reported	No Use reported	N	Y		Y	Y	Y		

	Identification				REACH Data					Data Source					
No.	**Name**	**CAS No**	**EC No**	**C.I. Colour Index**	**REACH tonnage (tpa)**	**Prof. Use**	**Consumer Use**	**Nano - Form In REACH?**	**Nano-sized Pigment reported on EU Market ?**	**ECHA**	**DK**	**France**	**BE**	**Industry**	**Other**
	[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide and N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide														
61	Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-Nethyl-, molybdatetungstatephosphate	1325-87-7	215-410-7	Pigment Blue 1	Not Registered				Y			Y	Y		CPNP
62	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	2465-29-4	219-568-8	Pigment Red 209	Not Registered				Y			Y			
63	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	67892-50-6	267-583-3	Pigment Red 53:1	Not Registered				Y			Y			
64	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, molybdatesilicate	75627-12-2	278-270-6	Pigment Red 81:5	Not Registered				Y			Y	Y		
65	Benzenamine, N,N-dimethyl-, oxidized, molybdatetungstatephosphates	101357-19-1	309-916-8	Pigment Violet 3	Not Registered				Y			Y	Y		
66	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	6410-38-4	229-104-6	Pigment Red 9	0 - 10	No Use reported	No Use reported	N	Y			Y			
67	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	6486-23-3	229-355-1	Pigment Yellow 3	100 - 1,000	Y	Y	N	Y			Y			
68	benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-, N-Me derivatives,molybdatephosphates	67989-22-4	268-006-8	Pigment Violet 3:4	Not Registered				Y			Y			
69	Butanamide, 2,2'-((3,3'-dichloro(1,1'-biphenyl)-4,4'-diyl)bis(2,1-diazenediyl))bis(N-(2,3-dihydro-2-oxo-1Hbenzimidazol-5-yl)-3-oxo-	78245-94-0	616-600-6	C.I. Pigment Orange 72	Not Registered				Y			Y			
70	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	12225-06-8	235-425-2	C.I. Pigment Red 176	100 - 1,000	Y	Y	N	Y				Y		
71	Bis[[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthyl]methylene]cyclohexa-2,5-dien-1-ylidene]diethylammonium] dicopper(1+) hexa(cyano-C)ferrate(4-)	82338-76-9	279-935-3	C.I. Pigment Blue 62	10 - 100			N	Y				Y		
72	N,N'-(2-chloro-1,4-phenylene)bis[4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	35869-64-8	252-772-5	C.I. Pigment Brown 23	100 - 1,000	Y	Y	N	Y				Y		
73	Copper(II)-hydroxycarbonate	12069-69-1	235-113-6	Pigment Green 39	1,000 - 10,000	Y	Y	N	Y					Y	
74	Chromium(III) oxide	1308-38-9	215-160-9	Pigment Green 17	10,000 -100,000	Y	Y	N	Y						CPNP
75	Disodium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5858-81-1	227-497-9	Pigment Red 57	10-100	Y	Y	N	Y						CPNP
76	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate			Pigment Red 57:1											CPNP
77	Prussian blue	14038-43-8	237-875-5	Pigment Blue 27	100-1,000	Y	Y	N	Y						CPNP

	Identification				REACH Data					Data Source					
No.	**Name**	**CAS No**	**EC No**	**C.I. Colour Index**	**REACH tonnage (tpa)**	**Prof. Use**	**Consumer Use**	**Nano - Form In REACH?**	**Nano-sized Pigment reported on EU Market ?**	**ECHA**	**DK**	**France**	**BE**	**Industry**	**Other**
78	Triiron tetraoxide	1317-61-9	215-277-5	Pigment black 11	1,000 - 10,000	Y	Y	N	Y						CPNP
79	Silver (Ag) (see notes)	7440-22-4	231-131-3	CI 77820	100,000 - 1,000,000	Y		Y	Y	Y					CPNP
80	Gold	7440-57-5	231-165-9	Pigment Metal 3	100 - 1,000	Y	Y	N	Y						CPNP
81	Copper	7440-50-8	231-159-6	Pigment Metal 2	1,000,000 - 10,000,000	Y	Y	N							CPNP

Legend:

	'True' pigment use
	Significant volume Filler/Extender pigment / co-formulant / similar use

*CPNP = Cosmetic Product Notification Portal

Notes:

Silver is used in specialised coatings as an antimicrobial agent, however it is also identified as a colourant in the catalogue of nanomaterials used in cosmetic products, and is therefore included.
Similarly, gold and copper are metallic pigments also identified as marketed in cosmetics.
Barium sulphate, a common extender pigment, is identified as a colourant in the cosmetics catalogue.

Appendix 2. Mapped REACH Consumer uses of identified nano-sized pigments

No.	Name	CAS No	EC No	C.I. Colour Index	PC1 Adhesives, sealants	PC9a Coatings and paints	PC9b Fillers, putties, plasters etc.	PC9c Finger paints	PC15 Non-metal-surface treatment	PC18 Ink and toners	PC23 Leather treatment	PC24 Lubricants, greases	PC26 Paper / board treatment	PC31 Polishes and waxes	PC32 Polymers	PC34 Textiles	PC35 Cleaning products	PC39 Cosmetics, personal care	Food And Food Contact[30]	PC0 Other	Service Life
1	Titanium dioxide	13463-67-7	236-675-5	C.I. Pigment White 6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2	Iron hydroxide oxide yellow	51274-00-1	257-098-5	C.I. Pigment Yellow 42	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
3	Diiron trioxide / Iron (III) oxide	1309-37-1	215-168-2	C.I. Pigment Red 101 / 77491	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
4	Zinc oxide	1314-13-2	215-222-5	C.I. Pigment White 4	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■
5	Manganese ferrite black spinel	68186-94-7	269-056-3	C.I.Pigment Black 26	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
6	Silicon Dioxide	7631-86-9, 112926-00-8	231-545-4			■	■	■		■			■		■	■			■		■
7	Iron manganese trioxide	12062-81-6	235-049-9	C.I. Pigment Brown 43	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
8	Aluminium Oxide	1344-28-1	215-691-6			■	■											■			■
9	(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.04,9]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	NA	939-379-0	C.I. Pigment Yellow 150		■				■					■					■	■
10	Calcium titanium trioxide	12049-50-2	234-988-1							■											■
11	Calcium carbonate	471-34-1, 7440-70-2	207-439-9		■	■	■	■			■	■	■	■	■	■	■	■			■
12	Barium sulphate	7727-43-7	231-784-4		■	■	■	■		■					■	■		■			■
13	Carbon black	1333-86-4	215-609-9	C.I. Pigment Back 6	■	■	■	■			■	■	■	■	■	■		■	■		■
14	Bismuth vanadium tetraoxide	14059-33-7	237-898-0	C.I. Pigment Yellow 184		■	■		■	■											■
15	29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	147-14-8	205-685-1	C.I. Pigment Blue 15		■	■	■		■					■					■	
16	5,12-dihydro-2,9-dimethylquino-[2,3-b]acridine-7,14-dione	980-26-7	213-561-3	C.I. Pigment Red 122		■	■	■		■			■	■	■	■					■
17	5,12-dihydroquino[2,3-b]acridine-7,14-dione	1047-16-1	213-879-2	C.I. Pigment Violet 19		■	■	■		■	■		■		■	■					■

[30] From: https://www.foodpackagingforum.org/food-packaging-health/nanomaterials
Note that Zinc Oxide is currently not authorised as a food contact material in the EU.

No.	Name	CAS No	EC No	C.I. Colour Index	PC1 Adhesives, sealants	PC9a Coatings and paints	PC9b Fillers, putties, plasters etc.	PC9c Finger paints	PC15 Non-metal-surface treatment	PC18 Ink and toners	PC23 Leather treatment	PC24 Lubricants, greases	PC26 Paper / board treatment	PC31 Polishes and waxes	PC32 Polymers	PC34 Textiles	PC35 Cleaning products	PC39 Cosmetics, personal care	Food And Food Contact[30]	PC0 Other	Service Life
18	Polychloro copper phthalocyanine	1328-53-6	215-524-7	C.I. Pigment Green 7		■	■	■		■					■	■					■
19	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	2512-29-0	219-730-8	C.I. Pigment Yellow 1		■	■	■		■					■						■
20	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	2786-76-7	220-509-3	C.I. Pigment Red 170		■	■	■		■					■						■
21	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol	2814-77-9	220-562-2	C.I. Pigment Red 4		■				■					■						■
22	1-[(2,4-dinitrophenyl)azo]-2-naphthol	3468-63-1	222-429-4	C.I. Pigment Orange 5		■				■					■						■
23	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]	3520-72-7	222-530-3	C.I. Pigment Orange 13	■	■	■	■		■		■		■	■	■					■
24	4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone	4051-63-2	223-754-4	C.I. Pigment Red 177		■	■	■		■	■				■					■	■
25	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]	5102-83-0	225-822-9	C.I. Pigment Yellow 13	■	■	■	■		■			■	■	■		■			■	■
26	manganese, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex	5280-66-0	226-102-7	C.I. Pigment Red 48:4		■				■					■					■	■
27	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	5280-68-2	226-103-2	C.I. Pigment Red 146		■				■					■	■					■
28	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	5468-75-7	226-789-3	C.I. Pigment Yellow 14	■	■	■	■		■			■	■	■	■	■			■	■
29	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide]	5567-15-7	226-939-8	C.I. Pigment Yellow 83		■	■	■		■					■					■	■
30	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]	5580-57-4	226-970-7	C.I. Pigment Yellow 93		■		■		■					■					■	■
31	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	6041-94-7	227-930-1	C.I. Pigment Red 2		■	■			■					■	■					
32	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene	215247-95-3	613-252-7	C.I. Pigment Violet 23		■				■					■	■				■	■
33	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	6358-31-2	228-768-4	C.I. Pigment Yellow 74		■		■		■					■					■	■
34	3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	6535-46-2	229-440-3	C.I. Pigment Red 112		■				■					■	■					■
35	Calcium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7023-61-2	230-303-5	C.I. Pigment Red 48:2		■	■			■					■					■	■
36	Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7585-41-3	231-494-8	C.I. Pigment Red 48:1		■	■														■
37	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	13515-40-7	236-852-7	C.I. Pigment Yellow 73		■	■			■											■
38	Strontium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (1:1)	15782-05-5	239-879-2	C.I. Pigment Red 48:3		■				■					■						■
39	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]	15793-73-4	239-898-6	C.I. Pigment Orange 34		■				■					■	■					■
40	3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide	30125-47-4	250-063-5	C.I Pigment Yellow 138		■				■					■					■	
41	5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid	36888-99-0	253-256-2	C.I Pigment Yellow 139		■				■					■					■	■

No.	Name	CAS No	EC No	C.I. Colour Index	PC1 Adhesives, sealants	PC9a Coatings and paints	PC9b Fillers, putties, plasters etc.	PC9c Finger paints	PC15 Non-metal-surface treatment	PC18 Ink and toners	PC23 Leather treatment	PC24 Lubricants, greases	PC26 Paper / board treatment	PC31 Polishes and waxes	PC32 Polymers	PC34 Textiles	PC35 Cleaning products	PC39 Cosmetics, personal care	Food And Food Contact[30]	PC0 Other	Service Life
42	4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	36968-27-1	253-292-9	C.I Pigment Red 266		■	■			■					■						■
43	Methyl 4-[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[(2-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]benzoate	61847-48-1	263-272-1	C.I. Pigment Red 188		■		■		■					■						■
44	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide	68134-22-5	268-734-6	C.I Pigment Yellow 154	■	■	■	■		■		■		■	■	■		■		■	■
45	Tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate	68516-73-4	271-176-6	C.I Pigment Yellow 155		■	■			■		■									■
46	5-[(2,3-dihydro-6-methyl-2-oxo-1H-benzimidazol-5-yl)azo]barbituric acid	72102-84-2	276-344-2	C.I. Pigment Orange 64		■	■			■					■						■
47	N-[4-(aminocarbonyl)phenyl]-4-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzamide	74441-05-7	277-873-1	C.I Pigment Yellow 181		■	■			■					■						
48	2,2'-[ethylenebis(oxyphenyl-2,1-eneazo)]bis[N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide	77804-81-0	278-770-4	C.I Pigment Yellow 180		■	■	■	■	■	■	■	■	■	■	■		■		■	■
49	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-[2-(4-chlorophenoxy)-5-(trifluoromethyl)phenyl]benzamide]	79953-85-8	279-356-6	C.I Pigment Yellow 128		■		■		■					■					■	
50	3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	NA	401-540-3	C.I. Pigment Red 254	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■
51	3,6-bis-biphenyl-4-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	NA	413-920-6	C.I. Pigment Red 264		■	■	■		■					■						■
52	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	6486-23-3	229-355-1	Pigment Yellow 3		■	■	■		■					■						■
53	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	12225-06-8	235-425-2	C.I. Pigment Red 176	■	■	■	■		■	■	■	■	■	■	■					■
54	N,N'-(2-chloro-1,4-phenylene)bis[4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	35869-64-8	252-772-5	C.I. Pigment Brown 23		■				■					■					■	
55	Copper(II)-hydroxycarbonate	12069-69-1	235-113-6	Pigment Green 39		■	■			■	■	■		■				■			
56	Chromium(III) oxide	1308-38-9	215-160-9	Pigment Green 17		■	■	■										■			
57	Disodium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5858-81-1	227-497-9	Pigment Red 57														■			
58	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9	226-109-5	Pigment Red 57:1		■				■	■				■	■		■		■	
59	Triiron tetraoxide	1317-61-9	215-277-5	Pigment black 11	■	■	■	■	■	■	■	■	■	■	■	■		■		■	
60	Prussian blue	14038-43-8	237-875-5	Pigment Blue 27														■			
61	Gold	7440-57-5	231-165-9	Pigment Metal 3														■			
62	Copper	7440-50-8	231-159-6	Pigment Metal 2		■	■	■		■		■	■	■	■			■			
63	Silver	7440-22-4	231-131-3	CI 77820		■												■			
64	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	3089-17-6	221-424-4	Pigment Red 202		■				■					■						
65	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	5160-02-1	225-935-3	Pigment Red 53:1	■	■	■	■	■	■	■	■	■	■	■	■					■
66	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9	226-109-5	Pigment Red 57:1		■				■	■				■	■		■		■	■

Appendix 3. Mapped REACH Professional uses of identified nano-sized pigments

No.	Name	CAS No	EC No	C.I. Colour Index	PROC 5	PROC 6	PROC 8a	PROC 10	PROC 11	PROC 13	PROC 14	PROC 19	PROC 21	PROC 22	PROC 24	PROC 26	PROC 0 other	Service Life
1	Titanium dioxide	13463-67-7	236-675-5	C.I. Pigment White 6	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2	Rutile (TiO2)	1317-80-2	215-282-2		■	■	■	■	■	■	■	■	■	■	■	■	■	■
3	Iron hydroxide oxide yellow	51274-00-1	257-098-5	C.I. Pigment Yellow 42	■	■	■	■	■	■	■	■	■	■	■	■		■
4	Diiron trioxide / Iron (III) oxide	1309-37-1	215-168-2	C.I. Pigment Ref 101 / 77491	■	■	■	■	■	■	■	■	■	■	■	■	■	■
5	Zinc oxide	1314-13-2	215-222-5	C.I. Pigment White 4	■	■	■	■	■	■	■	■	■	■	■	■	■	■
6	Manganese ferrite black spinel	68186-94-7	269-056-3	C.I.Pigment Black 26	■	■	■	■	■	■	■	■	■	■	■	■		■
7	Silicon Dioxide	7631-86-9, 112926-00-8	231-545-4		■			■	■	■								
8	Iron manganese trioxide	12062-81-6	235-049-9	C.I. Pigment Brown 43	■		■	■	■	■	■	■	■	■	■	■	■	
9	Aluminium Oxide	1344-28-1	215-691-6															
10	(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.04,9]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	NA (DK 68511-62-6)	939-379-0	C.I. Pigment Yellow 150	■		■	■	■	■		■			■			■
11	Calcium carbonate	471-34-1, 7440-70-2	207-439-9		■	■	■	■	■	■	■	■						■
12	Barium sulphate	7727-43-7	231-784-4		■		■	■	■	■		■	■		■			■
13	Carbon black	1333-86-4	215-609-9	C.I. Pigment Back 6	■		■	■	■	■	■		■		■			■
14	Cobalt aluminate blue spinel	1345-16-0	310-193-6	C.I. Pigment Blue 28	■		■	■	■	■		■		■	■	■		
15	Bismuth vanadium tetraoxide	14059-33-7	237-898-0	C.I. Pigment Yellow 184	■		■	■	■	■	■	■	■		■			■
16	29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	147-14-8	205-685-1	C.I. Pigment Blue 15	■													
17	5,12-dihydro-2,9-dimethylquino-[2,3-b]acridine-7,14-dione	980-26-7	213-561-3	C.I. Pigment Red 122	■	■	■	■	■	■	■	■	■		■			■
18	5,12-dihydroquino[2,3-b]acridine-7,14-dione	1047-16-1	213-879-2	C.I. Pigment Violet 19	■		■	■	■	■		■			■			■
19	Polychloro copper phthalocyanine	1328-53-6	215-524-7	C.I. Pigment Green 7	■		■	■	■	■		■	■		■			■
20	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	2512-29-0	219-730-8	C.I. Pigment Yellow 1	■		■	■	■	■		■	■		■			■
21	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	2786-76-7	220-509-3	C.I. Pigment Red 170	■		■	■	■	■		■	■		■			■
22	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol	2814-77-9	220-562-2	C.I. Pigment Red 4	■		■	■	■	■		■			■			■
23	1-[(2,4-dinitrophenyl)azo]-2-naphthol	3468-63-1	222-429-4	C.I. Pigment Orange 5	■		■	■	■	■		■			■			
24	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]	3520-72-7	222-530-3	C.I. Pigment Orange 13	■		■	■	■	■		■			■			■
25	4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone	4051-63-2	223-754-4	C.I. Pigment Red 177	■		■	■	■	■		■	■		■			■
26	bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	4424-06-0	224-597-4	C.I. Pigment Orange 43		■	■	■		■								
27	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]	5102-83-0	225-822-9	C.I. Pigment Yellow 13	■		■	■	■	■		■	■		■			■

No.	Name	CAS No	EC No	C.I. Colour Index	PROC 5	PROC 6	PROC 8a	PROC 10	PROC 11	PROC 13	PROC 14	PROC 19	PROC 21	PROC 22	PROC 24	PROC 26	PROC 0 other	Service Life
28	manganese, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex	5280-66-0	226-102-7	C.I. Pigment Red 48:4														
29	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	5280-68-2	226-103-2	C.I. Pigment Red 146														
30	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	5468-75-7	226-789-3	C.I. Pigment Yellow 14														
31	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide]	5567-15-7	226-939-8	C.I. Pigment Yellow 83														
32	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]	5580-57-4	226-970-7	C.I. Pigment Yellow 93														
33	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	6041-94-7	227-930-1	C.I. Pigment Red 2														
34	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene	215247-95-3	613-252-7	C.I. Pigment Violet 23														
35	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	6358-31-2	228-768-4	C.I. Pigment Yellow 74														
36	3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	6535-46-2	229-440-3	C.I. Pigment Red 112														
37	Calcium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7023-61-2	230-303-5	C.I. Pigment Red 48:2														
38	Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7585-41-3	231-494-8	C.I. Pigment Red 48:1														
39	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	13515-40-7	236-852-7	C.I. Pigment Yellow 73														
40	Strontium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (1:1)	15782-05-5	239-879-2	C.I. Pigment Red 48:3														
41	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]	15793-73-4	239-898-6	C.I. Pigment Orange 34														
42	3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide	30125-47-4	250-063-5	C.I Pigment Yellow 138														
43	5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid	36888-99-0	253-256-2	C.I Pigment Yellow 139														
44	4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	36968-27-1	253-292-9	C.I Pigment Red 266														
45	Methyl 4-[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[(2-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]benzoate	61847-48-1	263-272-1	C.I. Pigment Red 188														
46	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide	68134-22-5	268-734-6	C.I Pigment Yellow 154														
47	Tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate	68516-73-4	271-176-6	C.I Pigment Yellow 155														
48	5-[(2,3-dihydro-6-methyl-2-oxo-1H-benzimidazol-5-yl)azo]barbituric acid	72102-84-2	276-344-2	C.I. Pigment Orange 64														
49	N-[4-(aminocarbonyl)phenyl]-4-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzamide	74441-05-7	277-873-1	C.I Pigment Yellow 181														
50	2,2'-[ethylenebis(oxyphenyl-2,1-eneazo)]bis[N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide	77804-81-0	278-770-4	C.I Pigment Yellow 180														
51	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-[2-(4-chlorophenoxy)-5-	79953-85-8	279-356-6	C.I Pigment Yellow 128														

No.	Name	CAS No	EC No	C.I. Colour Index	PROC 5	PROC 6	PROC 8a	PROC 10	PROC 11	PROC 13	PROC 14	PROC 19	PROC 21	PROC 22	PROC 24	PROC 26	PROC 0 other	Service Life
	(trifluoromethyl)phenyl]benzamide]				■		■	■	■	■		■			■			
52	3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	NA (DK 84632-65-5)	401-540-3 (DK617-603-5)	C.I. Pigment Red 254	■		■	■	■	■		■	■		■			■
53	3,6-bis-biphenyl-4-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	NA (DK - 88949-33-1)	413-920-6 (DK 618-223-2)	C.I. Pigment Red 264	■		■	■	■	■			■		■			■
54	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	6486-23-3	229-355-1	Pigment Yellow 3														
55	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	12225-06-8	235-425-2	C.I. Pigment Red 176	■		■	■		■	■							■
56	N,N'-(2-chloro-1,4-phenylene)bis[4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	35869-64-8	252-772-5	C.I. Pigment Brown 23	■		■	■	■	■		■			■			
57	Copper(II)-hydroxycarbonate	12069-69-1	235-113-6	Pigment Green 39	■		■	■	■	■		■	■					
58	Chromium(III) oxide	1308-38-9	215-160-9	Pigment Green 17	■			■				■						
59	Disodium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5858-81-1	227-497-9	Pigment Red 57	■		■											
60	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9	226-109-5	Pigment Red 57:1	■	■	■	■	■	■	■	■	■	■	■			
61	Triiron tetraoxide	1317-61-9	215-277-5	Pigment black 11	■	■	■	■	■	■	■	■	■	■	■			
62	Prussian blue	14038-43-8	237-875-5	Pigment Blue 27	■		■											
63	Gold	7440-57-5	231-165-9	Pigment Metal 3	■		■						■	■	■			
64	Copper	7440-50-8	231-159-6	Pigment Metal 2			■	■	■	■		■						
65	Silver	7440-22-4	231-131-3	CI 77820				■				■	■					
66	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	3089-17-6	221-424-4	Pigment Red 202	■		■	■	■	■		■			■			■
67	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	5160-02-1	225-935-3	Pigment Red 53:1	■		■	■	■	■	■	■			■			■
68	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9	226-109-5	Pigment Red 57:1	■	■	■	■	■	■	■	■			■			■

Legend: List of Pigment-relevant Process Categories (PROCs) where professional uses are reported, based on the REACH Use Descriptors [31]

PROC5 Mixing or blending in batch processes
PROC6 Calendering operations
PROC8a Transfer of substance or mixture (charging and discharging) at non-dedicated facilities
PROC10 Roller application or brushing
PROC11 Non industrial spraying
PROC13 Treatment of articles by dipping and pouring
PROC14 Tabletting, compression, extrusion, pelletisation, granulation
PROC19 Manual activities involving hand contact
PROC21 Low energy manipulation and handling of substances bound in/on materials or articles
PROC24 High (mechanical) energy work-up of substances bound in /on materials and/or articles

[31] https://echa.europa.eu/documents/10162/13632/information_requirements_r12_en.pdf

Appendix 4. Master list of all Pigments and Extenders/Fillers identified from all sources

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
1	Titanium dioxide	13463-67-7	236-675-5	C.I. Pigment White 6	1,000,000 +
2	Rutile (TiO2)	1317-80-2	215-282-2		10,000 - 100,000
3	Iron hydroxide oxide yellow	51274-00-1	257-098-5	C.I. Pigment Yellow 42	100,000 - 1,000,000
4	Diiron trioxide / Iron (III) oxide	1309-37-1	215-168-2	C.I. Pigment Ref 101 / 77491	100,000 - 1,000,000
5	Zinc oxide	1314-13-2	215-222-5	C.I. Pigment White 4	100,000 - 1,000,000
6	Manganese ferrite black spinel	68186-94-7	269-056-3	C.I.Pigment Black 26	1,000 - 10,000
7	Silicon Dioxide	7631-86-9, 112926-00-8	231-545-4		1,000,000 +
8	Iron manganese trioxide	12062-81-6	235-049-9	C.I. Pigment Brown 43	1,000 - 10,000
9	Aluminium Oxide	1344-28-1	215-691-6		10,000,000+
10	Silver (Ag)	7440-22-4	231-131-3		100,000 - 1,000,000
11	(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.04,9]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	NA (DK 68511-62-6)	939-379-0	C.I. Pigment Yellow 150	100 - 1,000
12	Calcium titanium trioxide	12049-50-2	234-988-1		0 - 10
13	Chromium (III) oxide	1308-38-9	215-160-9	C.I. Pigment Green 17	10,000 - 100,000
14	Orange lead	1314-41-6	215-235-6		10,000 - 100,000
15	Zinc sulphide	1314-98-3	215-251-3	C.I. Pigment White 7	100,000 - 1,000,000
16	Lead monoxide	1317-36-8	215-267-0	C.I. Pigment Yellow 46	100,000 - 1,000,000
17	Hematite (Fe2O3)	1317-60-8	215-275-4		10,000 - 100,000
18	Triiron tetraoxide	1317-61-9	215-277-5	C.I. Pigment Black 11	100,000 - 1,000,000
19	Calcium carbonate	471-34-1, 7440-70-2	207-439-9		1,000,000 - 10,000,000
20	Barium sulphate	7727-43-7	231-784-4		10,000 - 100,000
21	Cerium Oxide	1306-38-3	215-150-4		1,000 - 10,000
22	Carbon black	1333-86-4	215-609-9	C.I. Pigment Back 6	1,000,000 +
23	Cobalt aluminate blue spinel	1345-16-0	310-193-6	C.I. Pigment Blue 28	1,000 - 10,000
24	Antimony nickel titanium oxide yellow	8007-18-9	232-353-3	C.I. Pigment Yellow 53	1,000 - 10,000
25	Ammonium manganese(3+) diphosphate+C138	10101-66-3	233-257-4		10 - 100
26	Lead chromate molybdate sulfate red	12656-85-8	235-759-9	C.I. Pigment Red 104	1,000 - 10,000
27	Chromium iron oxide	12737-27-8	235-790-8	C.I. Pigment Brown 29	10,000 - 100,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
28	Bismuth vanadium tetraoxide	14059-33-7	237-898-0	C.I. Pigment Yellow 184	1,000 - 10,000
29	Cobalt titanite green spinel	68186-85-6	269-047-4	C.I. Pigment Green 50	10 - 100
30	Cobalt zinc aluminate blue spinel	68186-87-8	269-049-5	C.I. Pigment Blue 72	100 - 1,000
31	Chrome antimony titanium buff rutile	68186-90-3	269-052-1	C.I. Pigment Brown 24	10,000 - 100,000
32	Copper chromite black spinel	68186-91-4	269-053-7	C.I. Pigment Black 28	1,000 - 10,000
33	Cobalt chromite blue green spinel	68187-11-1	269-072-0	C.I. Pigment Blue 36	100 - 1,000
34	Cadmium sulfoselenide red	58339-34-7	261-218-1	C.I. Pigment Red 108	100 - 1,000
35	Olivine, cobalt silicate blue	68187-40-6	269-093-5	C.I. Pigment Blue 73	1,000 - 10,000
36	Cobalt chromite green spinel	68187-49-5	269-101-7	C.I. Pigment Green 26	10 - 100
37	Zinc ferrite brown spinel	68187-51-9	269-103-8	C.I. Pigment Yellow 119	1,000 - 10,000
38	Tin antimony grey cassiterite	68187-54-2	269-105-9	C.I. Pigment Black 23	10 - 100
39	Cobalt zinc silicate blue phenacite	68412-74-8	270-208-6	C.I. Pigment Blue 74	0 - 10
40	Silicic acid, aluminum sodium	101357-30-6	309-928-3	C.I. Pigment Blue 29	10,000 - 100,000
41	CHECK	57455-37-5	309-928-3	C.I. Pigment Blue 29	Not Registered
42	Silicic acid, zirconium salt, cadmium pigment-encapsulated	102184-95-2	310-077-5		100 - 1,000
43	29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	147-14-8	205-685-1	C.I. Pigment Blue 15	10,000 - 100,000
44	5,12-dihydro-2,9-dimethylquino-[2,3-b]acridine-7,14-dione	980-26-7	213-561-3	C.I. Pigment Red 122	1,000 - 10,000
45	5,12-dihydroquino[2,3-b]acridine-7,14-dione	1047-16-1	213-879-2	C.I. Pigment Violet 19	1,000 - 10,000
46	Barium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate]	1103-38-4	214-160-6	C.I. Pigment Red 49	0 - 10
47	[[4-[[4-(anilino)phenyl][4-(phenylimino)-2,5-cyclohexadien-1-ylidene]methyl]phenyl]amino]benzenesulphonic acid	1324-76-1	215-385-2	C.I. Pigment Blue 61	100 - 1,000
48	C.I. Pigment Green 1	1325-75-3	215-406-5	C.I. Pigment Green 1	Not Registered
49	C.I. Pigment Violet 3	1325-82-2	603-635-7	C.I. Pigment Violet 3	Not Registered
50	Xanthylium, 9-(2-carboxyphenyl)-3,6-bis(diethylamino)-, molybdatetungstatephosphate	1326-03-0	215-413-3	C.I. Pigment Violet 1	0 - 10
51	C.I. Pigment Violet 2	1326-04-1	215-414-9	C.I. Pigment Violet 2	Not Registered
52	Polychloro copper phthalocyanine	1328-53-6	215-524-7	C.I. Pigment Green 7	1,000 - 10,000
53	Quino(2,3-b)acridine-6,7,13,14(5h,12h)-tetrone	1503-48-6	216-125-0		0 - 10
54	6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one	2379-74-0	219-163-6	C.I. Pigment Red 181	10 - 100
55	2-hydroxynaphthalene-1-carbaldehyde [(2-hydroxy-1-naphthyl)methylene]hydrazone	2387-03-3	219-210-0	C.I. Pigment Yellow 101	0 - 10
56	1-(4-methyl-2-nitrophenylazo)-2-naphthol	2425-85-6	219-372-2	C.I. Pigment Red 3	10 - 100

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
57	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	2512-29-0	219-730-8	C.I. Pigment Yellow 1	100 - 1,000
58	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	2786-76-7	220-509-3	C.I. Pigment Red 170	1,000 - 10,000
59	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol	2814-77-9	220-562-2	C.I. Pigment Red 4	100 - 1,000
60	2,9-bis[4-(phenylazo)phenyl]anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone	3049-71-6	221-264-5	C.I. Pigment Red 178	100 - 1,000
61	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	3089-17-6	221-424-4	C.I. Pigment Red 202	100 - 1,000
62	1-[(2,4-dinitrophenyl)azo]-2-naphthol	3468-63-1	222-429-4	C.I. Pigment Orange 5	100 - 1,000
63	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]	3520-72-7	222-530-3	C.I. Pigment Orange 13	100 - 1,000
64	C.I. Pigment Red 18	3564-22-5	222-643-8	C.I. Pigment Red 18	Not Registered
65	N,N'-phenylene-1,4-bis[4-[(2,5-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	3905-19-9	223-460-6	C.I. Pigment Red 166	100 - 1,000
66	4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone	4051-63-2	223-754-4	C.I. Pigment Red 177	100 - 1,000
67	C.I. Pigment Yellow 108	4216-01-7	224-151-9	C.I. Pigment Yellow 108	Not Registered
68	Bisbenzimidazo[2,1-b:1',2'-j]benzo[lmn][3,8]phenanthroline-6,9-dione	4216-02-8	224-152-4	C.I. Pigment Red 194	10 - 100
69	4,10-dibromodibenzo[def,mno]chrysene-6,12-dione	4378-61-4	224-481-3	C.I. Pigment Red 168	10 - 100
70	bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	4424-06-0	224-597-4	C.I. Pigment Orange 43	10 - 100
71	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methoxyphenyl)-3-oxobutyramide]	4531-49-1	224-867-1	C.I. Pigment Yellow 17	100 - 1,000
72	2,9-bis(3,5-dimethylphenyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone	4948-15-6	225-590-9	C.I. Pigment Red 149	100 - 1,000
73	C.I. Pigment Yellow 109	5045-40-9	225-744-5	C.I. Pigment Yellow 109	Not Registered
74	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]	5102-83-0	225-822-9	C.I. Pigment Yellow 13	1,000 - 10,000
75	Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	5160-02-1	225-935-3	C.I. Pigment Red 53	1,000 - 10,000
76	manganese, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex	5280-66-0	226-102-7	C.I. Pigment Red 48:4	100- 1,000
77	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	5280-68-2	226-103-2	C.I. Pigment Red 146	100 - 1,000
78	3,3'-[(2,5-dimethyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(5-chloro-o-tolyl)benzamide]	5280-80-8	226-107-4	C.I. Pigment Yellow 95	100 - 1,000
79	Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9	226-109-5	C.I. Pigment Red 57:1	10,000 - 100,000
80	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-	5468-75-7	226-789-3	C.I. Pigment Yellow 14	1,000 - 10,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	methylphenyl)-3-oxobutyramide]				
81	2,9-dimethylanthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone	5521-31-3	226-866-1	C.I. Pigment Red 179	100 - 1,000
82	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide]	5567-15-7	226-939-8	C.I. Pigment Yellow 83	1,000 - 10,000
83	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]	5580-57-4	226-970-7	C.I. Pigment Yellow 93	100 - 1,000
84	Pigment Yellow 110	5590-18-1	226-999-5	Pigment Yellow 110	10 - 100
85	C.I. Pigment Yellow 16	5979-28-2	227-783-3	C.I. Pigment Yellow 16	Not Registered
86	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	6041-94-7	227-930-1	C.I. Pigment Red 2	1,000 - 10,000
87	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene	215247-95-3	613-252-7	C.I. Pigment Violet 23	1,000 - 10,000 2 x full registrations
88	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	6358-31-2	228-768-4	C.I. Pigment Yellow 74	1,000 - 10,000
89	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-methylphenyl)-3-oxobutyramide]	6358-37-8	228-771-0	C.I. Pigment Yellow 55	0 – 10
90	2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutyramide]	6358-85-6	228-787-8	C.I. Pigment Yellow 12	10,000 - 100,000
91	Diethyl 4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxylate]	6358-87-8	228-788-3	C.I. Pigment Red 38	10 – 100
92	Barium bis[2-[(2-hydroxy-1-naphthyl)azo]benzoate]	6372-81-2	228-906-3	C.I. Pigment Red 50	0 – 10
93	4-[(2,5-Dichlorophenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one	6407-75-6	229-040-9		Not Registered
94	C.I. Pigment Red 21	6410-26-0	229-096-4	C.I. Pigment Red 21	Not Registered
95	3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	6410-32-8	229-102-5	C.I. Pigment Red 12	10 - 100
96	N-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	6410-41-9	229-107-2	C.I. Pigment Red 5	10 - 100
97	C.I. Pigment Red 22	6448-95-9	229-245-3	C.I. Pigment Red 22	0 - 10
98	3-hydroxy-4-[(2-methoxy-5-nitrophenyl)azo]-N-(3-nitrophenyl)naphthalene-2-carboxamide	6471-49-4	229-313-2	C.I. Pigment Red 23	0 - 10
99	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	6486-23-3	229-355-1	C.I. Pigment Yellow 3	100 - 1,000
100	2-[(4-methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	6528-34-3	229-419-9	C.I. Pigment Yellow 65	100 - 1,000
101	3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	6535-46-2	229-440-3	C.I. Pigment Red 112	1,000 - 10,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
102	C.I. Pigment Red 175	6985-92-8	230-249-2	C.I. Pigment Red 175	Not Registered
103	4-[(2,5-dichlorophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxynaphthalene-2-carboxamide	6992-11-6	230-258-1	C.I. Pigment Brown 25	10 - 100
104	Calcium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7023-61-2	230-303-5	C.I. Pigment Red 48:2	1,000 - 10,000
105	Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	7585-41-3	231-494-8	C.I. Pigment Red 48:1	100 - 1,000
106	C.I. pigment red 117	10142-77-5	600-210-8	C.I. pigment red 117	Not Registered
107	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, molybdatetungstatephosphate	12224-98-5	235-424-7	C.I. Pigment Red 81	0 - 10
108	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2,5-dimethoxy-4-[(methylamino)sulphonyl]phenyl]azo]naphthalene-2-carboxamide	12225-08-0	235-426-8	C.I. Pigment Violet 32	10 - 100
109	N-(4-chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl]phenyl]azo]-3-oxobutyramide	12225-18-2	235-427-3	C.I. Pigment Yellow 97	100 - 1,000
110	2-[(4-chloro-2-nitrophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide	12236-62-3	235-462-4	C.I. Pigment Orange 36	100 - 1,000
111	Ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts	12237-62-6	235-468-7	C.I. Pigment Violet 27	100 - 1,000
112	Manganese, 4-[(4-chloro-5-methyl-2-sulfophenyl)azo]-3-hydroxy-2-naphthalenecarboxylic acid complex	12238-31-2	235-471-3	C.I. Pigment Red 52:2	0 - 10
113	Copper chlorophthalocyanine	12239-87-1	235-476-0		1,000 - 10,000
114	C.I. Pigment Blue 27	12240-15-2	602-780-3	C.I. Pigment Blue 27	Not Registered
115	Calcium bis[3-nitro-4-[[2-oxo-1-[(phenylamino)carbonyl]propyl]azo]benzenesulphonate]	12286-65-6	235-557-0	C.I. Pigment Yellow 61	10 - 100
116	C.I. Pigment Orange 42	12768-99-9	603-227-9	C.I. Pigment Orange 42	Not Registered
117	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	13515-40-7	236-852-7	C.I. Pigment Yellow 73	100 - 1,000
118	Chloro[29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]aluminium	14154-42-8	237-998-4	C.I. Pigment Blue 79	Intermediate
119	C.I. Pigment Red 88	14295-43-3	238-222-7	C.I. Pigment Red 88	Not Registered
120	[1,3,8,16,18,24-hexabromo-2,4,9,10,11,15,17,22,23,25-decachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper	14302-13-7	238-238-4	C.I. Pigment Green 36	100 - 1,000
121	C.I. Pigment Yellow 63	14569-54-1	238-611-1	C.I. Pigment Yellow 63	Not Registered
122	[1-[[(2-hydroxyphenyl)imino]methyl]-2-naphtholato(2-)-N,O,O']copper	15680-42-9	239-763-1	C.I Pigment Yellow 129	10 - 100
123	Strontium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (1:1)	15782-05-5	239-879-2	C.I. Pigment Red 48:3	100 - 1,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
124	C.I. Pigment Yellow 104	15790-07-5	239-888-1	C.I. Pigment Yellow 104	10 - 100
125	4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]	15793-73-4	239-898-6	C.I. Pigment Orange 34	100 - 1,000
126	N-(5-chloro-2-methoxyphenyl)-2-[(2-methoxy-4-nitrophenyl)azo]-3-oxobutyramide	15993-42-7	240-131-2		10 - 100
127	Quino[2,3-b]acridine-7,14-dione, 5,12-dihydro-3,10-dimethyl-	16043-40-6	605-208-0	C.I. Pigment Red 122	Not Registered
128	C.I. Pigment Blue 63	16521-38-3	240-589-3	C.I. Pigment Blue 63	0 - 10
129	1-Butanol, 4-(ethenyloxy)-	17832-28-9	241-793-5		10 - 100
130	2,2'-[(2,2',5,5'-tetrachloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2,4-dimethylphenyl)-3-oxobutyramide]	22094-93-5	244-776-0	C.I. Pigment Yellow 81	10 - 100
131	Copper, (tetrachloro-29h,31h-phthalocyaninato(2-)-n29,n30,n31,n32)-	27614-71-7	248-573-8		100 - 1,000
132	Nickel, bis(2,3-bis(hydroxyimino)-n-phenylbutanamidato-n2,n3)-	29204-84-0	249-503-9	C.I. Pigment Yellow 153	Not Registered
133	Dimethyl 5-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azoterephthalate	29920-31-8	249-955-7	C.I. Pigment Yellow 120	10 - 100
134	3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide	30125-47-4	250-063-5	C.I Pigment Yellow 138	100 - 1,000
135	2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-methoxyphenyl)-3-oxobutyramide]	31775-16-3	250-797-6	C.I. Pigment Yellow 170	10 - 100
136	Butyl 2-[[3-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-hydroxy-1-naphthyl]azo]benzoate	31778-10-6	250-800-0	C.I Pigment Red 208	10 - 100
137	2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzoic acid	31837-42-0	250-830-4	C.I. Pigment Yellow 151	100 - 1,000
138	Manganese, 3-hydroxy-4-[(1-sulfo-2-naphthalenyl)azo]-2-naphthalenecarboxylic acid complex	35355-77-2	252-525-1	C.I. Pigment Red 63:2	0 - 10
139	Dimethyl 2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]terephthalate	35636-63-6	252-650-1	C.I Pigment Yellow 175	10 - 100
140	5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid	36888-99-0	253-256-2	C.I Pigment Yellow 139	1,000 - 10,000
141	4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	36968-27-1	253-292-9	C.I Pigment Red 266	10 - 100
142	C.I. Pigment Yellow 36	37300-23-5	609-398-6	C.I. Pigment Yellow 36	Not Registered
143	[1,3-dihydro-5,6-bis[[(2-hydroxy-1-naph-hyl)methylene]amino]-2H-benzimidazol-2-onato(2-)-N5,N6,O5,O6]nickel	42844-93-9	255-965-2		0 - 10
144	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(4-nitrophenyl)azo]-3-oxobutyramide	52846-56-7	258-221-5	C.I Pigment Orange 62	10 - 100
145	C.I. Pigment Red 255	54660-00-3	611-183-7	C.I. Pigment Red 255	10 - 100
146	4-[[5-[[[4-(aminocarbonyl)phenyl]amino]carbon	59487-23-9	261-785-5	C.I. Pigment Red 187	10 - 100

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	yl]-2-methoxyphenyl]azo]-N-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide				
147	C.I. Pigment Orange 51	61512-61-6	612-162-5	C.I. Pigment Orange 51	Not Registered
148	Methyl 4-[[(2,5-dichlorophenyl)amino]carbonyl]-2-[[2-hydroxy-3-[[(2-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]benzoate	61847-48-1	263-272-1	C.I. Pigment Red 188	100 - 1,000
149	C.I. Pigment Red 185	61951-98-2	263-353-1	C.I. Pigment Red 185	Not Registered
150	Calcium 4,5-dichloro-2-[[4,5-dihydro-3-methyl-5-oxo-1-(3-sulphonatophenyl)-1H-pyrazol-4-yl]azo]benzenesulphonate	65212-77-3	265-634-4	C.I Pigment Yellow 183	100 - 1,000
151	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide	68134-22-5	268-734-6	C.I Pigment Yellow 154	100 - 1,000
152	N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	68227-78-1	269-389-4	C.I. Pigment Red 147	10 - 100
153	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, brominated chlorinated	68512-13-0	270-958-4	C.I. Pigment Green 36	100 - 1,000
154	Tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate	68516-73-4	271-176-6	C.I Pigment Yellow 155	100 - 1,000
155	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(o-anisyl and 2,4-xylyl) derivs.	68610-86-6	271-878-2	C.I. Pigment Yellow 127	100 - 1,000
156	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, chlorinated	68987-63-3	273-501-7	C.I. Pigment Blue 76	1,000 - 10,000
157	Calcium bis[4-[[1-[[(2-chlorophenyl)amino]carbonyl]-2-oxopropyl]azo]-3-nitrobenzenesulphonate]	71832-85-4	276-057-2	C.I Pigment Yellow 168	10 - 100
158	C.I. Pigment Red 222	71872-63-4	615-664-2	C.I. Pigment Red 222	Not Registered
159	5-[(2,3-dihydro-6-methyl-2-oxo-1H-benzimidazol-5-yl)azo]barbituric acid	72102-84-2	276-344-2	C.I. Pigment Orange 64	100 - 1,000
160	Nitrophenyl 3-[[2-hydroxy-3-[(2-methylphenyl)carbamoyl]-1-naphthyl]azo]-4-methoxybenzenesulphonate	72639-39-5	276-755-7		Not Registered
161	3-[(4-chloro-2-nitrophenyl)azo]-2-methylpyrazolo[5,1-b]quinazolin-9(1H)-one	74336-59-7	277-823-9	C.I Pigment Orange 67	100 - 1,000
162	1-[(5,7-Dichloro-1,9-dihydro-2-methyl-9-oxopyrazolo[5,1-b]quinazolin-3-yl)azo]anthraquinone	74336-60-0	277-824-4	C.I. Pigment Red 251	Not Registered
163	N-[4-(aminocarbonyl)phenyl]-4-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzamide	74441-05-7	277-873-1	C.I Pigment Yellow 181	100 - 1,000
164	2,2'-[ethylenebis(oxyphenyl-2,1-eneazo)]bis[N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide	77804-81-0	278-770-4	C.I Pigment Yellow 180	100 - 1,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
165	6-[[(4-Methylphenyl)sulphonyl]amino]hexanoic acid	78521-39-8	278-934-5		100 - 1,000
166	2-[[3,3'-Dichloro-4'-[[1-[[(2,4-dimethylphenyl)amino]carbonyl]-2-oxopropyl]azo][1,1'- biphenyl]-4-yl]azo]-3-oxo-N- (o-tolyl)butyramide	78952-72-4	279-017-2		Not Registered
167	3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-[2-(4-chlorophenoxy)-5-(trifluoromethyl)phenyl]benzamide]	79953-85-8	279-356-6	C.I Pigment Yellow 128	10 - 100
168	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(2-methoxyphenyl)azo]-3-oxobutyramide	82199-12-0	279-914-9	C.I Pigment Yellow 194	100 - 1,000
169	2,9-bis(p-methoxybenzyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone	83524-75-8	280-472-4	C.I. Pigment Black 32	10 - 100
170	Benzonitrile, 3,3'-(2,3,5,6-tetrahydro-3,6-dioxopyrrolo[3,4-c]pyrrole-1,4-diyl)bis-	84632-50-8	617-600-9	C.I Pigment Ornage 72	Not Registered
171	C.I. Pigment Orange 73	84632-59-7	617-601-4	C.I. Pigment Orange 73	Not Registered
172	3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	84632-65-5 (DK 84632-65-5)	617-603-5 (DK617-603-5)	C.I. Pigment Red 254	1,000 - 10,000 7 registrations, 6 intermediate
173	2,5-Dihydro-3,6-bis(4-methylphenyl)-pyrrolo[3,4-c]pyrrole-1,4-dione	84632-66-6	617-604-0		Not Registered
174	C.I. Pigment Red 253	85776-13-2	N/A	C.I. Pigment Red 253	Not Registered
175	2-Naphthalenecarboxamide, n-(4-chlorophenyl)-4-((2,5-dichloro-4 ((dimethylamino)sulfonyl)phenyl)azo) -3-hydroxy-	85776-14-3	N/A		Not Registered
176	[[3-[1-Cyano-2-(methylamino)-2-oxoethylidene]-2,3-dihydro-1H-isoindol-1-ylidene](salicylic)hydrazidato(2-)]nickel	85958-80-1	288-967-7		Not Registered
177	C.I. Pigment Orange 69	85959-60-0	289-055-1	C.I. Pigment Orange 69	0 - 10
178	3,6-bis-biphenyl-4-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	NA (DK - 88949-33-1)	413-920-6 (DK 618-223-2)	C.I. Pigment Red 264	10-100 5 Registrations
179	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(p-anisyl and Ph) derivs.	90268-23-8	290-823-3	C.I. Pigment Yellow 126	10 - 100
180	Reaction mass of N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide and N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	NA (DK - 99402-80-9)	911-739-1 (DK - 619-430-0)	C.I. Pigment Red 184	100 - 1,000
181	Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with p-phenylenediamine	106276-80-6	600-736-8	C.I Pigment Yellow 110	100 - 1,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	and sodium methoxide				
182	2,20-dichloro-13,31-diethyl-4,22-dioxa-13,18,31,36-tetraazanonacyclo[19.15.0.0^{3},^{19}.0^{5},^{17}.0^{6},^{14}.0^{7},^{12}.0^{23},^{35}.0^{24},^{32}.0^{25},^{30}]hexatriaconta-1(36),2,5,7(12),8,10,14,16,18,20,23(35),24(32),25,27,29,33-hexadecaene	215247-95-3	606-790-9	C.I. Pigment Violet 23	1,000 - 10,000
183	Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-Nethyl-, molybdatetungstatephosphate	1325-87-7	215-410-7	Pigment Blue 1	Not Registered
184	2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	2465-29-4	219-568-8	Pigment Red 209	Not Registered
185	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	67892-50-6	267-583-3	Pigment Red 53:1	Not Registered
186	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, molybdatesilicate	75627-12-2	278-270-6	Pigment Red 81:5	Not Registered
187	Benzenamine, N,N-dimethyl-, oxidized, molybdatetungstatephosphates	101357-19-1	309-916-8	Pigment Violet 3	Not Registered
188	4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide	6410-38-4	229-104-6	Pigment Red 9	0 - 10
189	2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	6486-23-3	229-355-1	Pigment Yellow 3	100 - 1,000
190	benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-, N-Me derivatives,molybdatephosphates	67989-22-4	268-006-8	Pigment Violet 3:4	Not Registered
191	Butanamide, 2,2'-((3,3'-dichloro(1,1'-biphenyl)-4,4'-diyl)bis(2,1-diazenediyl))bis(N-(2,3-dihydro-2-oxo-1Hbenzimidazol-5-yl)-3-oxo-	78245-94-0	616-600-6	C.I. Pigment Orange 72	Not Registered
192	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	12225-06-8	235-425-2	C.I. Pigment Red 176	100 - 1,000
193	Bis[[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthyl]methylene]cyclohexa-2,5-dien-1-ylidene]diethylammonium] dicopper(1+) hexa(cyano-C)ferrate(4-)	82338-76-9	279-935-3	C.I. Pigment Blue 62	10 - 100
194	N,N'-(2-chloro-1,4-phenylene)bis[4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	35869-64-8	252-772-5	C.I. Pigment Brown 23	100 - 1,000
195	Copper(II)-hydroxycarbonate	12069-69-1	235-113-6	Pigment Green 39	1,000 - 10,000
196	(2E)-10,12-dioxa-2,3,6,8,14,16-hexaaza-11-nickelatricyclo[11.4.0.0^{4},^{9}]heptadeca-1(13),2,4(9)-triene-5,7,15,17-tetrone; 1,3,5-triazine-2,4,6-triamine	-	939-379-0		100 - 1,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
197	[2,2'-[1,2-phenylenebis(nitrilomethylidyne)]-bis(phenolato)]-N,N',O,O'-nickel(II)	-	400-870-5		10 - 100
198	[2,9,16,23-tetrachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper	16040-69-0	240-183-6		10 - 100
199	[N,N,N',N',N'',N''-hexaethyl-29H,31H-phthalocyaninetrimethylaminato(2-)-N29,N30,N31,N32]copper tris(dodecylbenzenesulphonate)	75247-18-6	278-150-3		10 - 100
200	1,1'-[(6-phenyl-1,3,5-triazine-2,4-diyl)diimino]bisanthraquinone	4118-16-5	223-912-2		100 - 1,000
201	2-(4-(diethylaminopropylcarbamoyl)phenylazo)-3-oxo-N-(2,3-dihydro-2-oxobenzimidazol-5-yl)butyramide	164578-14-7	404-910-2		Confidential
202	2,2'-[(3,3'-dimethoxy[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutyramide]	6505-28-8	229-388-1		10 - 100
203	2,6,8-triamino-4H,10H-pyrimido[5,4-g]pteridin-4-one; pyrimido[5,4-g]pteridine-2,4,6,8-tetramine	346709-25-9	440-560-7		10 - 100
204	2,9-bis(2-phenylethyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone	67075-37-0	266-564-7		10 - 100
205	2,9-bis[N-[3-(diethylamino)propyl]sulfamoyl]-5H,12H-quino[2,3-b]acridin-7,14-dione	168754-51-6	404-230-6		Confidential
206	2,9-dichloro-5,7,12,14-tetrahydro-5,12-diazapentacene-7,14-dione; 2,9-dimethoxy-5,7,12,14-tetrahydro-5,12-diazapentacene-7,14-dione	-	941-220-5		0 - 10
207	2,9-dimethyl-5,7,12,14-tetrahydro-5,12-diazapentacene-7,14-dione; 2-methyl-5,7,12,14-tetrahydro-5,12-diazapentacene-7,14-dione; 5,7,12,14-tetrahydro-5,12-diazapentacene-7,14-dione	-	909-082-0		10 - 100
208	2-[(E)-2-(2,4-diamino-6-hydroxypyrimidin-5-yl)diazen-1-yl]-5-methylbenzene-1-sulfonic acid	-	700-002-8		100 - 1,000
209	2-[(E)-2-(2-methoxy-5-nitrophenyl)diazen-1-yl]-N-(2-methoxyphenyl)-3-oxobutanamide	80675-49-6	617-143-5		10 - 100
210	2-[[2-chloro-4-[3-chloro-4-[[1-(2,4-dimethylphenylamino)-1,3-dioxobutan- 2-yl]diazenyl]phenyl]phenyl]diazenyl]-N-(2-methylphenyl)-3-oxobutanamide	-	911-715-0		1,000 - 10,000
211	29H,31H-phthalocyanine	574-93-6	209-378-3		100 - 1,000
212	2-cyano-2-[2,3-dihydro-3-(tetrahydro-2,4,6-trioxo-5(2H)-pyrimidinylidene)-1H-isoindol-1-ylidene]-N-methylacetamide	76199-85-4	278-388-8		100 - 1,000
213	3,3'-[(2,5-dichloro-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(5-chloro-o-tolyl)benzamide]	5580-58-5	226-971-2		0 - 10
214	3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	-	401-540-3		1,000 - 10,000

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215	3,6-bis(4-phenylphenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	-	413-920-6		100 - 1,000
216	3,6-bis(4-tert-butylphenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	-	416-250-2		100 - 1,000
217	3,6-diphenyl-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione	-	402-400-4		100 - 1,000
218	4,11-diamino-2-(3-methoxypropyl)-1H-naphth[2,3-f]isoindol-1,3,5,10(2H)-tetrone	12217-80-0	235-402-7		10 - 100
219	4-[(1-butyl-5-cyano-1,6-dihydro-2-hydroxy-4-methyl-6-oxo-3-pyridyl)azo]-N-(2-ethylhexyl)benzenesulphonamide	55290-62-5	259-571-1		10 - 100
220	4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide	6471-50-7	229-314-8		0 - 10
221	4-[(E)-2-(4-carbamoylphenyl)diazen-1-yl]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide; 4-[(E)-2-(4-carbamoylphenyl)diazen-1-yl]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	-	911-436-4		10 - 100
222	6,15-dihydroanthrazine-5,9,14,18-tetrone	81-77-6	201-375-5		100 - 1,000
223	9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium bis[3-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-4-hydroxy-N-3-(isopropoxypropyl)benzenesulphonamidato(2-)]cobaltate(1-)	71566-55-7	275-640-9		0 - 10
224	A mixture of: 2-(9-methyl-1,3,8,10-tetraoxo-2,3,9,10-tetrahydro-(1H,8H)-anthra[2,1,9-def: 6,5,10-d'e'f']diisoquinolin-2-ylethansulfonic acid; potassium 2-(9-methyl-1,3,8,10-tetraoxo-2,3,9,10-tetrahydro-(1H,8H)-anthra[2,1,9-def: 6,5,10-d'e'f']diisoquinolin-2-ylethansulfate	-	411-310-4		Confidential
225	A mixture of: N-(4-chlorophenyl)-4-(2,5-dichloro-4-(dimethylsulfamoyl)phenylazo)-3-hydroxy-2-naphthalenecarboxamide; N-(4-chlorophenyl)-4-(2,5-dichloro-4-(methylsulfamoyl)phenylazo)-3-hydroxy-2-naphthalenecarboxamide	-	412-550-2		10 - 100
226	Aluminium oxide	1344-28-1	215-691-6		10000000+
227	Aluminium sulphate	7784-31-8, 10043-01-3	233-135-0		100,000 - 1,000,000
228	Amines, C10-14-branched and linear alkyl, bis[2-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]benzoato(2-)]chromate(1-)	85029-58-9	285-083-3		0 - 10
229	Amines, rosin, compds. with 9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride and disodium hydrogen bis[4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-3-hydroxy-1-naphthalenesulfonato(3-	97862-65-2	308-114-5		0 - 10

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	)]chromate(3-)				
230	Ammonium iron(3+) hexakis(cyano-C)ferrate(4-)	25869-00-5	247-304-1		1,000 - 10,000
231	Barium bis[5-chloro-4-ethyl-2-[(2-hydroxy-1-naphthyl)azo]benzenesulphonate]	67801-01-8	267-122-6		0 - 10
232	Barium bis[6-chloro-4-[(2-hydroxy-1-naphthyl)azo]toluene-3-sulphonate]	73612-34-7	277-553-1		0 - 10
233	Benzenamine, oxidized	13007-86-8	235-850-3		10 - 100
234	Benzenesulfonic acid, 4-chloro-2-[2-[4,5-dihydro-3-methyl-5-oxo-1-(3-sulfophenyl)-1H-pyrazol-4-yl]diazenyl]-5-methyl-, ammonium salt (1:2)	-	416-730-1		10 - 100
235	Bis(2-chloroethyl) 3,3'-[(2,5-dimethyl-p-phenylene)bis[iminocarbonyl(2-hydroxy-1,3-naphthylene)azo]]di-p-toluate	68259-05-2	269-507-4		10 - 100
236	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(4-chloro-2,5-dimethoxyphenyl and 2,4-xylyl) derivs.	90268-24-9	290-824-9		100 - 1,000
237	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(phenyl and 2,4-xylyl) derivs.	72207-62-6	276-461-9		0 - 10
238	Butanamide, 2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-, N,N'-bis(phenyl and o-tolyl) derivs.	68910-13-4	272-732-0		0 - 10
239	Cadmium sulfoselenide	-	701-229-5		10 - 100
240	Cadmium zinc sulfide yellow	8048-07-5	232-466-8		100 - 1,000
241	Calcium 3-hydroxy-4-[(1-sulphonato-2-naphthyl)azo]-2-naphthoate	6417-83-0	229-142-3		0 - 10
242	Calcium 4-chloro-2-(5-hydroxy-3-methyl-1-(3-sulfonatophenyl)pyrazol-4-ylazo)-5-methylbenzenesulfonate	-	403-530-4		10 - 100
243	Calcium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate]	1103-39-5	214-161-1		10 - 100
244	Calcium bis[4-[[1-[[(2-methylphenyl)amino]carbonyl]-2-oxopropyl]azo]-3-nitrobenzenesulphonate]	12286-66-7	235-558-6		100 - 1,000
245	Calcium bis[4-[[3-[[2-hydroxy-3-[[(4-methoxyphenyl)amino]carbonyl]-1-naphthyl]azo]-4-methylbenzoyl]amino]benzenesulphonate]	43035-18-3	256-050-0		10 - 100
246	Chromate(1-), bis[4-[[4-(ethylsulfonyl)-2-hydroxyphenyl]azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-onato(2-)]-, compd. with 1,6-hexanediamine (2:1)	69997-91-7	310-133-9		10 - 100
247	Chrome tin orchid cassiterite	68187-53-1	269-104-3		10 - 100
248	Chrome tin pink sphene	68187-12-2	269-073-6		1,000 - 10,000

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249	Chrome tungsten titanium buff rutile	68186-92-5	269-054-2		100 - 1,000
250	Chromium (III) hydroxide	1308-14-1	215-158-8		100 - 1,000
251	Chromium acetate	17593-70-3	241-562-9		10 - 100
252	Copper dichloride	7447-39-4, 10125-13-0	231-210-2		100 - 1,000
253	Copper dihydroxide	20427-59-2	243-815-9		100 - 1,000
254	Copper dinitrate	3251-23-8, 10031-43-3	221-838-5		1,000 - 10,000
255	Copper oxide	1317-38-0, 1344-70-3	215-269-1		10,000 - 100,000
256	Copper sulphate	7758-98-7, 7758-99-8	231-847-6		10,000 - 100,000
257	Copper thiocyanate	1111-67-7	214-183-1		0 - 10
258	Copper(II) carbonate--copper(II) hydroxide (1:1)	12069-69-1	235-113-6		1,000 - 10,000
259	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, (1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl derivs.	68411-06-3	270-099-5		100 - 1,000
260	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, [[3-(1-methylethoxy)propyl]amino]sulfonyl derivs.	81457-65-0	279-767-0		100 - 1,000
261	Dialuminium tris[2-(2,4,5,7-tetrabromo-6-oxido-3-oxoxanthen-9-yl)-3,4,5,6-tetrachlorobenzoate]	15876-58-1	240-012-5		10 - 100
262	Dialuminium tris[2-(2,4,5,7-tetrabromo-6-oxido-3-oxoxanthen-9-yl)benzoate]	15876-39-8	240-005-7		10 - 100
263	Diammonium hexanitratocerate	16774-21-3	240-827-6		0 - 10
264	Dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione	38720-66-0	254-100-6		10 - 100
265	Dichromium trioxalate	30737-19-0	250-317-5		10 - 100
266	dichromium(3+) ion diiron(3+) ion trizinc(2+) ion λ²-iron(2+) ion octaoxidandiide silicate	-	936-897-9		100 - 1,000
267	Dicopper chloride trihydroxide	1332-40-7, 1332-65-6	215-572-9		1,000 - 10,000
268	Dicopper oxide	1317-39-1	215-270-7		1,000 - 10,000
269	Dierbium trioxide	12061-16-4	235-045-7		10 - 100
270	Diindium trioxide	1312-43-2	215-193-9		0 - 10
271	Diisopropyl 3,3'-[(2,5-dichloro-1,4-phenylene)bis[iminocarbonyl(2-hydroxy-3,1-naphthylene)azo]]bis[4-methylbenzoate]	71566-54-6	275-639-3		10 - 100
272	Dimethyl succinate	106-65-0	203-419-9		1,000 - 10,000
273	Dineodymium tricarbonate	5895-46-5	227-579-4		10 - 100
274	Dinitrogen tetraoxide	10544-72-6	234-126-4		100 - 1,000

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
275	Disodium 4,5-dichloro-2-[[4,5-dihydro-3-methyl-5-oxo-1-(3-sulphonatophenyl)-1H-pyrazol-4-yl]azo]benzenesulphonate	65212-76-2	265-633-9		100 - 1,000
276	Disodium molybdate	7631-95-0	231-551-7		1,000 - 10,000
277	Dodecan-1-ol, ethoxylated	9002-92-0	500-002-6		100 - 1,000
278	Feldspar minerals, hematite and quartz, calcination products of copper mining residues	-	701-090-0		100 - 1,000
279	Feldspar minerals, magnetite and quartz, calcination products of copper mining residues.	-	944-188-0		0 - 10
280	Ferrate(4-), hexakis(cyano-C)-, Et 2-[6-(ethylamino)-3-(ethylimino)-2,7-dimethyl-3H-xanthen-9-yl]benzoate copper(2+) salts	12237-63-7	235-469-2		100 - 1,000
281	Hematite, chromium green black	68909-79-5	272-713-7		1,000 - 10,000
282	Hydrogen [29H,31H-phthalocyaninesulphonato(3-)-N29,N30,N31,N32]cuprate(1-), compound with dodecylamine (1:1)	73455-75-1	277-475-8		10 - 100
283	Hydrogen [tris[[[3-[(2-ethylhexyl)oxy]propyl]amino]sulphonyl]-29H,31H-phthalocyaninesulphonato(3-)-N29,N30,N31,N32]cuprate(1-), compound with 3-[(2-ethylhexyl)oxy]propylamine (1:1)	94277-77-7	304-661-9		10 - 100
284	HYPERSOL YELLOW PIGMENT	-	401-880-2		Confidential
285	Iron cobalt chromite black spinel	68186-97-0	269-060-5		1,000 - 10,000
286	iron(3+) ion ammonium bis(1-[2-(5-chloro-2-oxidophenyl)diazen-1-yl]-3-(phenylcarbamoyl)naphthalen-2-olate)	-	403-590-1		0 - 10
287	Lanthanum oxide	1312-81-8, 7439-91-0	215-200-5		1,000 - 10,000
288	LC-SILICON-PIGMENT	-	418-890-8		Confidential
289	Lead sulfochromate yellow	1344-37-2	215-693-7		1,000 - 10,000
290	Lexmark Black Pigment	-	431-150-9		Confidential
291	Manganese alumina pink corundum	68186-99-2	269-061-0		1,000 - 10,000
292	Manganese antimony titanium buff rutile	68412-38-4	270-185-2		100 - 1,000
293	Manganese dioxide	1313-13-9	215-202-6		10,000 - 100,000
294	Mixture of 4,5,6,7-tetrachloro-3-{[3-methyl-4-({4-[(4,5,6,7-tetrachloro-1-oxo-1H-isoindol-3-yl)amino]phenyl}diazenyl)phenyl]amino}-1H-isoindol-1-one and monomethoxy-heptachloro derivative of 3-[[3-methyl-4-[[4-[(1-oxo-1H-isoindol-3-yl)amino]phenyl]azo]phenyl]amino]-1H-isoindol-1-one and N-[4-[[2-methyl-4-[(4,5,6,7-tetrachloro-3-oxo-isoindolin-1-	106276-78-2	600-734-7		10 - 100

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	ylidene)amino]phenyl]azo]phenyl]acetamide and 4,5,6,7-tetrachloro-3-[3-methyl-4-[m-tolylazo]phenyl]imino-isoindolin-1-one				
295	Mixture of octachloro, monomethoxy-heptachloro and bismethoxy-hexachloro derivatives of 3,3'-[(2-methyl-1,3-phenylene)diimino]bis[2,3-dihydro-1H-isoindol-1-one]	106276-79-3	600-735-2		10 - 100
296	Molybdenum dioxide	18868-43-4	242-637-9		1,000 - 10,000
297	N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-4-[[2-methoxy-5-methyl-4-[(methylamino)sulphonyl]phenyl]azo]naphthalene-2-carboxamide	51920-12-8	257-515-0		10 - 100
298	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[(E)-2-[2-methoxy-5-(phenylcarbamoyl)phenyl]diazen-1-yl]naphthalene-2-carboxamide; N-(5-chloro-2-methylphenyl)-3-hydroxy-4-[(E)-2-[2-methoxy-5-(phenylcarbamoyl)phenyl]diazen-1-yl]naphthalene-2-carboxamide	-	911-739-1		100 - 1,000
299	N-(5-chloro-2-methoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	67990-05-0	268-028-8		10 - 100
300	N,N'-(2,5-dichloro-1,4-phenylene)bis[4-[(2,5-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	40618-31-3	255-005-2		100 - 1,000
301	N,N'-(2,5-dichloro-1,4-phenylene)bis[4-[[2-chloro-5-(trifluoromethyl)phenyl]azo]-3-hydroxynaphthalene-2-carboxamide]	52238-92-3	257-776-0		10 - 100
302	N,N'-(2,5-dimethyl-1,4-phenylene)bis[4-[(5-chloro-2-methylphenyl)azo]-3-hydroxynaphthalene-2-carboxamide	79665-24-0	279-211-7		0 - 10
303	N,N'-(2-chloro-1,4-phenylene)bis[4-[(2,5-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	5280-78-4	226-106-9		100 - 1,000
304	N,N'-[6,13-diacetamido-2,9-diethoxy-3,10-triphenodioxazinediyl]bis(benzamide)	17741-63-8	241-734-3		10 - 100
305	N,N'-1,4-phenylenebis(2-((2-methoxy-4-nitrophenyl)azo)-3-oxobutyramide)	83372-55-8	411-840-6		Confidential
306	N,N'-naphthalene-1,5-diylbis[4-[(2,3-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]	68516-75-6	271-178-7		10 - 100
307	N,N''-naphthalene-1,5-diylbis[N'-[3-[(2-ethylhexyl)oxy]propyl]urea]	71216-01-8	275-276-0		10 - 100
308	N-[4-(acetylamino)phenyl]-4-[[5-(aminocarbonyl)-2-chlorophenyl]azo]-3-hydroxynaphthalene-2-carboxamide	12236-64-5	235-464-5		10 - 100
309	N-C16-18-alkyl-(evennumbered) C18 unsaturated) propane-1,3-diamine	1219010-04-4	629-719-3		1,000 - 10,000
310	Neodymium oxide	1313-97-9	215-214-1		100 - 1,000
311	Nickel iron chromite black spinel	71631-15-7	275-738-1		1,000 - 10,000
312	pentasodium 3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-	-	940-265-8		0 - 10

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	1,4-dione 4-[3,6-dioxo-4-(4-sulfonatophenyl)-2H,3H,5H,6H-pyrrolo[3,4-c]pyrrol-1-yl]benzene-1-sulfonate 4-[4-(4-chlorophenyl)-3,6-dioxo-2H,3H,5H,6H-pyrrolo[3,4-c]pyrrol-1-yl]benzene-1-sulfonate sulfate				
313	Perylene-3,4:9,10-tetracarboxydiimide	81-33-4	201-344-6		100 - 1,000
314	PHTHALOCYANINE PIGMENT	-	424-350-2		Confidential
315	Phthalocyanine-N-[3-(diethylamino)propyl]sulfonamide copper complex	93971-95-0	413-650-9		0 - 10
316	PIGMENT 11990	-	404-460-7		Confidential
317	PIGMENT ADDITIVE 009	-	432-260-1		Confidential
318	Pigment Orange 79	250640-08-5	607-520-2		0 - 10
319	PIGMENT RED 3092C	-	419-370-3		0 - 10
320	Pigment Red 5021B	-	410-210-8		0 - 10
321	Pigment Yellow 214	-	433-240-3		10 - 100
322	Pigment Yellow FC 26290	-	411-080-5		10 - 100
323	PIGMENTADDITIV RL	-	404-110-3		10 - 100
324	PIGMENTGELB P-13456	-	424-630-4		Confidential
325	Prussian blue	14038-43-8	237-875-5		10 - 100
326	pyrimido[5,4-g]pteridine-2,4,6,8-tetramine 4-methylbenzenesulfonate (1:1)	-	700-589-0		Intermediate Only
327	Reaction mass of 3,6-Bis(3-chlorophenyl)-2,5-dihydro-pyrrolo[3,4-c]pyrrole-1,4-dione, 3-(3-Chlorophenyl)-6-(4-chlorophenyl)-2,5-dihydro- pyrrolo[3,4-c]pyrrole-1,4-dione and 3,6-Bis(4-chlorophenyl)-2,5-dihydro-pyrrolo[3,4-c]pyrrole-1,4-dione	-	465-080-5		0 - 10
328	Reaction mass of Amines, C10-14-branched and linear alkyl, bis[2,4-dihydro-4-[(2-hydroxy-5-nitrophenyl)azo]-5-methyl-2-phenyl-3H-pyrazol-3-onato(2-)] chromate(1-) (1:1) and Amines,C10-14-branched and linear alkyl, bis[2,4-dihydro-4-[(2-hydroxy-4-nitrophenyl)azo]-5-methyl-2-phenyl-3H-pyrazol-3-onato(2-)] chromate(1-)	-	943-145-3		0 - 10
329	Reaction mass of copper and iron and zinc	-	912-666-8		0 - 10
330	Reaction mass ofPyrrolo[3,4-c]pyrrole-1,4-dione, 3,6-bis(4-chlorophenyl)-2,5-dihydro-,Pyrrolo[3,4-c]pyrrole-1,4-dione, 3-(4-chlorophenyl)-2,5-dihydro-6-phenyl-, monosulfo deriv., monosodium saltandBenzenesulfonic acid, 4,4'-(2,3,5,6-tetrahydro-3,6-	-	460-020-4		Confidential

No	Name	CAS No	EC No	C.I. Colour Index	REACH registered tonnage (tpa)
	dioxopyrrolo[3,4-c]pyrrole-1,4-diyl)bis-, sodium salt (1:2)				
331	Resin acids and Rosin acids, calcium salts	9007-13-0	232-694-8		1,000 - 10,000
332	Rosin	8050-09-7	232-475-7		100,000 - 1,000,000
333	Solsperse 5000	-	404-170-0		10 - 100
334	Spinels, chromium iron manganese brown	68555-06-6	271-411-2		100 - 1,000
335	Strontium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	73612-29-0	277-552-6		100 - 1,000
336	Strontium 4-[(4-chloro-5-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (1:1)	67828-72-2	267-291-6		0 - 10
337	Strontium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	73263-40-8	277-335-6		0 - 10
338	Strontium chromate	7789-06-2	232-142-6		1,000 - 10,000
339	Tetraammonium hexamolybdate	12411-64-2	235-650-6		10 - 100
340	Tin dioxide	18282-10-5	242-159-0		1,000 - 10,000
341	Trizinc bis(orthophosphate)	7779-90-0	231-944-3		10,000 - 100,000
342	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, molybdatesilicate	63022-06-0	263-793-4		0 - 10
343	Zinc	7440-66-6	231-175-3		1,000,000 - 10,000,000
344	Zinc bis(dihydrogen phosphate)	13598-37-3	237-067-2		1,000 - 10,000
345	Zinc iron chromite brown spinel	68186-88-9	269-050-0		1,000 - 10,000
346	Zirconium iron pink zircon	68412-79-3	270-210-7		1,000 - 10,000
347	Zirconium praseodymium yellow zircon	68187-15-5	269-075-7		1,000 - 10,000
348	Zirconium vanadium blue zircon	68186-95-8	269-057-9		100 - 1,000

Appendix 5. Professional exposure models (Stoffenmanager)

Table 26: Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.1

General data: IS1- CES 1.1		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 – Calcination process	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	2
risk score	I	II
Question	**Answer**	
entered data	Release of primary particles during actual synthesis	
source domain	-	
Appearance	-	
product dustiness	-	
product moisture content	-	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Wet Chemistry (Synthesis – into solution)	
duration of the task	2 to 4 hours a day	
frequency of the task	4 to 5 days a week	
task in the breathing zone.	Yes	
Multiple employees	Yes	

regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	Mechanical and or natural ventilation
protection of the employee.	None

Table 27: Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.2

General data – IS1 – CES 1.2		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 – Micronisation	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Release of primary particles during actual synthesis	
source domain	-	
Appearance	Powder	
product dustiness	-	
product moisture content	-	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Mechanical Reduction (Machining)	
duration of the task	2 to 4 hours a day	
frequency of the task	4 to 5 days a week	
task in the breathing zone.	No	

Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	Half mask respirator with filter, type P2L

Table 28:Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.3

General data IS1 – CES 1.3		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 - Laboratory testing	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of products in small amounts (up to 100 gram) or in situations where only low quantities of products are likely to be released.	
duration of the task	2 to 4 hours a day	

frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	None

Table 29: Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.4

General data – IS1 – CES 1.4		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 – coating and filtration	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Release of primary particles during actual synthesis	
source domain	-	
Appearance	Powder	
product dustiness	-	
product moisture content	-	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Wet Chemistry (Functionalization)	
duration of the task	2 to 4 hours a day	

frequency of the task	4 to 5 days a week
task in the breathing zone.	No
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	None

Table 30: Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.5

General data – IS1 – CES 1.5		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 - packing into large bags	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	4	3
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of products with a relatively high speed/force which leads to dispersion of dust	

duration of the task	2 to 4 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	Mechanical and or natural ventilation
protection of the employee.	Full face respirator with filter, type P2L

Table 31: Stoffenmanager nano risk assessment report for exposure scenario IS1 – CES 1.6

General data – IS1 – CES 1.6		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of TiO2 - packing into small bags	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of products with low speed or little force or in medium quantities	

	(several kilograms).
duration of the task	2 to 4 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	Mechanical and or natural ventilation
protection of the employee.	None

Table 32: Stoffenmanager nano risk assessment report for exposure scenario IS2 – CES 2.1

General data – IS2 – CES 2.1		
product	Fe2O3	
nano particle	Fe2O3	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of Fe2O3 - Powder control	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	C	C
exposure class	3	3
risk score	II	II
Question	**Answer**	
entered data	Release of primary particles during actual synthesis	
source domain	-	
Appearance	-	
product dustiness	-	
product moisture content	-	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	FeO (Iron oxides)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	

task	Mechanical Reduction (Machining)
duration of the task	4 to 8 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	None

Table 33: Stoffenmanager nano risk assessment report for exposure scenario IS2 – CES 2.2

General data – IS2 – CES 2.2		
product	Fe2O3	
nano particle	Fe2O3	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of Fe2O3 – Material feeding	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	C	C
exposure class	3	3
risk score	II	II
Question	**Answer**	
entered data	Release of primary particles during actual synthesis	
source domain	-	
Appearance	-	
product dustiness	-	
product moisture content	-	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	FeO (Iron oxides)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	

task	Manual pouring of powder
duration of the task	4 to 8 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	Local exhaust ventilation
Engineering control measures	No general ventilation
protection of the employee.	None

Table 34: Stoffenmanager nano risk assessment report for exposure scenario IS2 – CES 2.3

General data – IS2 – CES 2.3		
product	Fe2O3	
nano particle	Fe2O3	
concentration of the nano particle in the product	100	
name risk assessment.	Worker exposure during manufacture of Fe2O3 - packing	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	C	C
exposure class	4	4
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	FeO (Iron oxides)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	

task	Handling of products with a relatively high speed/force which leads to dispersion of dust
duration of the task	4 to 8 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	Local exhaust ventilation
Engineering control measures	No general ventilation
protection of the employee.	None

Table 35: Stoffenmanager nano risk assessment report for exposure scenario IS3 – CES 3.1

General data – IS3 – CES 3.1		
product	TiO2	
nano particle	TiO2	
concentration of the nano particle in the product	100	
name risk assessment.	Production of printing inks	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	D	D
exposure class	3	2
risk score	I	II
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	TiO2 (Titanium dioxide)	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	

Actualisation date	-
task	Handling of products with low speed or little force or in medium quantities (several kilograms).
duration of the task	0.5 to 2 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	Local exhaust ventilation
Engineering control measures	Mechanical and or natural ventilation
protection of the employee.	Filter mask P2 (FFP2)

Table 36: Stoffenmanager nano risk assessment report for exposure scenario PW1 – CES 4.1

General data PW – CES 4.1		
Product	Printing ink	
nano particle	MNOs	
concentration of the nano particle in the product	Small (1-10%)	
name risk assessment.	Proffesional use of printers	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	E	E
exposure class	2	2
risk score	I	I
Question	**Answer**	
entered data	Spraying or dispersion of a ready-to-use nanoproduct	
source domain	Ready-to-use-product	
Appearance	Particles dispersed in a liquid	
product dustiness	-	
product moisture content	-	
Dilution	Onverdund	
Visocity	Liquids with medium viscosity (like oil)	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Other MNOs	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	

End date of product work period	-
Actualisation date	-
task	Handling of liquids using low pressure, low speed with large or medium quantities.
duration of the task	4 to 8 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	No
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	None

Table 37: Stoffenmanager nano risk assessment report for exposure scenario PW2 – CES 5.1

General data PW2 – CES 5.1		
product	Ink	
nano particle	Ag	
concentration of the nano particle in the product	Very small (0.01-1%)	
name risk assessment.	Inkjet Printing Of Nano Ag Ink On To Paper	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	C	C
exposure class	1	1
risk score	III	III
Question	**Answer**	
entered data	Spraying or dispersion of a ready-to-use nanoproduct	
source domain	Ready-to-use-product	
Appearance	Particles dispersed in a liquid	
product dustiness	-	
product moisture content	-	
dilution	Onverdund	
visocity	Liquids with medium viscosity (like oil)	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Ag (nano Silver)	
Number of employees that can be exposed	-	
Production or usage volume	-	

Start date of product work period	-
End date of product work period	-
Actualisation date	-
task	Handling of liquids using low pressure, low speed with large or medium quantities.
duration of the task	1 to 30 minutes a day
frequency of the task	Approximately 1 day a week
task in the breathing zone.	No
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	Local exhaust ventilation
Engineering control measures	No general ventilation
protection of the employee.	None

Table 38: Stoffenmanager nano risk assessment of professional exposure scenario within pneumatic spraying of pigment-containing paints

General data		
product	Paints	
nano particle	NM in general	
concentration of the nano particle in the product	Substantial (10-50%)	
name risk assessment.	Automotive paint application in car workshop - spray booths	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	**E**	**E**
exposure class	**2**	**2**
risk score	**I**	**I**
Question	**Answer**	
entered data	Spraying or dispersion of a ready-to-use nanoproduct	
source domain	Ready-to-use-product	
Appearance	Particles dispersed in a liquid	
product dustiness	-	
product moisture content	-	
dilution	Onverdund	
visocity	Liquids with medium viscosity (like oil)	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Other MNOs	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	

task	Handling of liquids on large surfaces or large workpieces
duration of the task	4 to 8 hours a day
frequency of the task	4 to 5 days a week
task in the breathing zone.	Yes
Multiple employees	Yes
regular cleaning of the working room	Yes
regular inspections and maintenance	Yes
control measures at the source	Use of a product that limits the emission
Engineering control measures	Spraying booth
protection of the employee.	Half mask respirator with filter, type P2L

Table 39: Stoffenmanager nano risk assessment of professional exposure scenario within hand application of pigment-containing paints on the walls by rolling

General data		
product	Paints	
nano particle	NM in general	
concentration of the nano particle in the product	Substantial (10-50%)	
name risk assessment.	Professional hand application of pigment-containing paints on the walls by rolling	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	E	E
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Spraying or dispersion of a ready-to-use nanoproduct	
source domain	Ready-to-use-product	
Appearance	Particles dispersed in a liquid	
product dustiness	-	
product moisture content	-	
dilution	Onverdund	
visocity	Liquids with medium viscosity (like oil)	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Other MNOs	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of liquids on large surfaces or large workpieces	
duration of the task	4 to 8 hours a day	
frequency of the task	4 to 5 days a week	
task in the breathing zone.	No	
Multiple employees	Yes	
regular cleaning of the working room	Yes	

regular inspections and maintenance	Yes
control measures at the source	No control measures at the source
Engineering control measures	No general ventilation
protection of the employee.	None

Table 40: Stoffenmanager nano risk assessment of professional exposure scenario within hand application of manual mixing of paints for color

General data		
product	Paints	
nano particle	NM in general	
concentration of the nano particle in the product	Substantial (10-50%)	
name risk assessment.	Professional formulation activities - manual mixing of paints for colour	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	E	E
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Other MNOs	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of products with low speed or little force, which leads to some dispersion of dust.	
duration of the task	4 to 8 hours a day	
frequency of the task	4 to 5 days a week	
task in the breathing zone.	Yes	
Multiple employees	Yes	
regular cleaning of the working room	No	
regular inspections and maintenance	No	
control measures at the source	No control measures at the source	
Engineering control measures	No general ventilation	
protection of the employee.	None	

Table 41: Stoffenmanager nano risk assessment of professional formulation activities - semi-automatic mixing of paints for colour

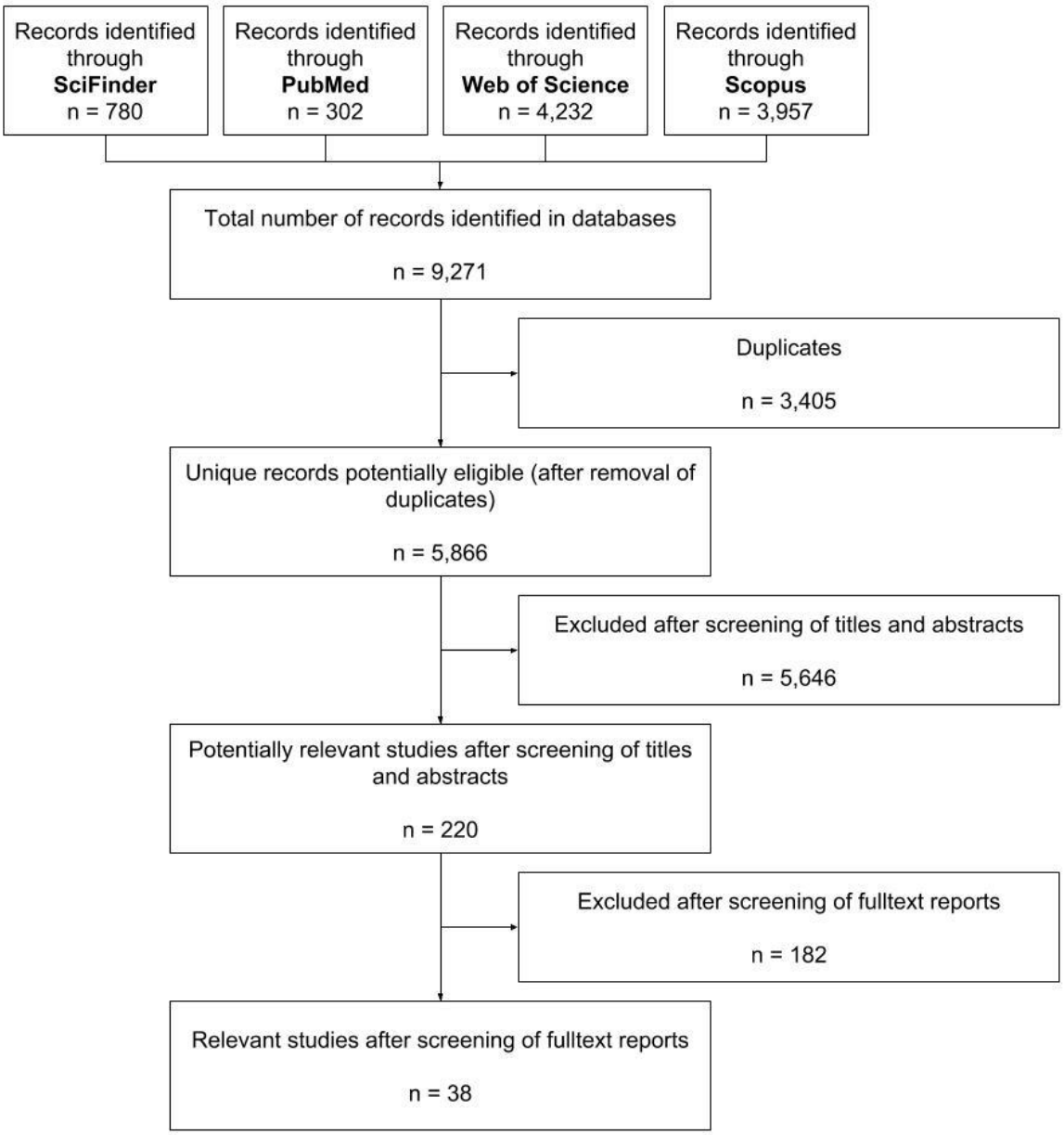
General data		
product	Paints	
nano particle	NM in general	
concentration of the nano particle in the product	Substantial (10-50%)	
name risk assessment.	Professional formulation activities - semi-automatic mixing of paints for colour	
Result risk assessment		
	task weighted	**time and frequency weighted**
hazard class	E	E
exposure class	3	3
risk score	I	I
Question	**Answer**	
entered data	Handling of bulk aggregated/agglomerated nanopowders	
source domain	-	
Appearance	Powder	
product dustiness	Unknown	
product moisture content	Dry product (< 5% moisture content)	
dilution	-	
visocity	-	
fibers	No	
fiber size.	No	
Hazardous properties	Unknown	
nano particle type	Other MNOs	
Number of employees that can be exposed	-	
Production or usage volume	-	
Start date of product work period	-	
End date of product work period	-	
Actualisation date	-	
task	Handling of products with medium speed or force, which leads to some dispersion of dust.	
duration of the task	4 to 8 hours a day	
frequency of the task	4 to 5 days a week	
task in the breathing zone.	Yes	
Multiple employees	Yes	
regular cleaning of the working room	No	
regular inspections and maintenance	No	
control measures at the source	No control measures at the source	
Engineering control measures	No general ventilation	
protection of the employee.	None	

Appendix 6. Literature Search Protocols and Statistics

The literature search protocols used are provided as a separate document due to size.

10.1 Search no. 1

General search on nano-sized pigments (using keywords "nano* AND pigm* OR nanopigm*)



Breakdown of potentially relevant publications (after screening titles and abstracts) – by substance

Substance	No. of potentially relevant publications
Titanium dioxide	69
Zinc oxide	9
Carbon black	4
Barium sulphate	1
Ferric oxide	5
Calcium carbonate	3
Others	25
Unspecified (nanopigments in general)	104

Breakdown of potentially relevant publications (after screening titles and abstracts) – by product categories

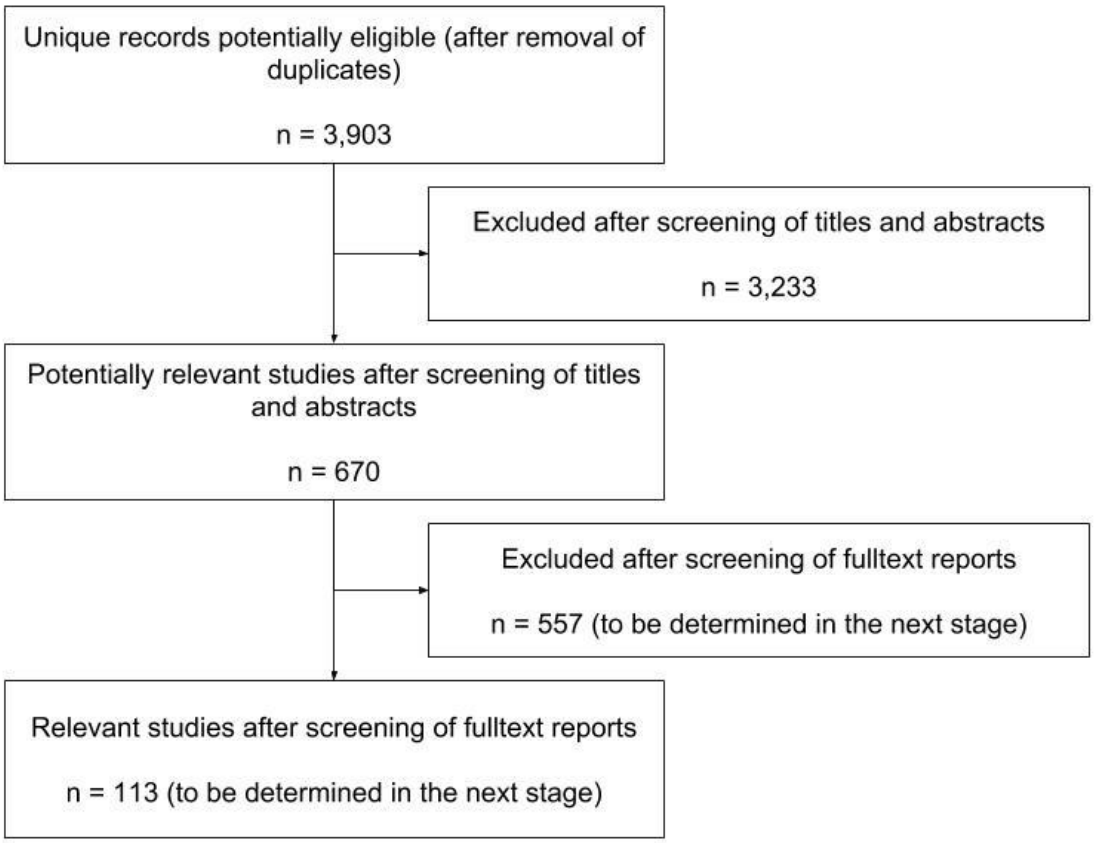
Product category	No. of potentially relevant publications
Paints, inks, etc.	20
Food	5
Other	10
Unspecified	185

Breakdown of potentially relevant publications (after screening titles and abstracts) – by research field (data included in the publications)

Research field (data indicated to be included in the publications)	No. of potentially relevant publications
Substance characterization data	41
Exposure data	31
Toxicological data	51
Epidemiological data	4
Risk assessment data	10
Life cycle data	12

10.2 Search No. 2

Substance-specific searches on toxicological and epidemiological studies of pigments known to be marketed in the EU in their nanoform.



Breakdown of potentially relevant publications (after screening titles and abstracts) – by substance

Substance	No. of potentially relevant publications
Titanium dioxide	399
Zinc oxide	85
Carbon black	20
Barium sulphate	5
Ferric oxide	5
Calcium carbonate	16
Others	76
Unspecified (nanopigments in general)	64

Breakdown of potentially relevant publications (after screening titles and abstracts) – by research field (data included in the publications)

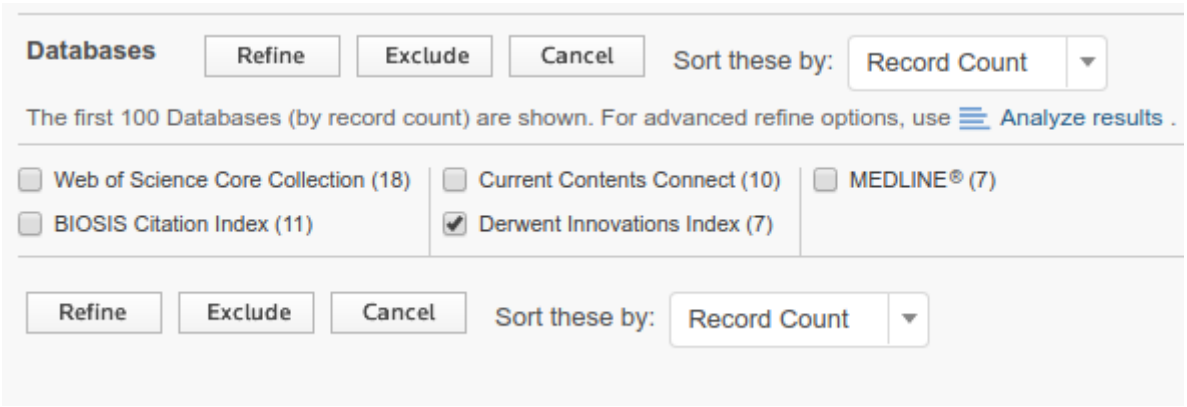
Research field (data indicated to be included in the publications)	No. of potentially relevant publications
Exposure data	57
Toxicological data	515
Epidemiological data	6

10.3 Search no. 3

Search methodology
Web of Science
Web of Science was used for pigment names searches (not searchable by CAS number)
The searches were run across all databases, i.e.:

- Web Science Core Collection (1945-present)
- BIOSIS Citation Index (1994-present)
- Data Citation index (1994 - present)
- MEDLINE (1950 - present)
- Russian Science Citation Index (2005 - present)
- Derwent Innovations Index (1994-present)
    - Patents,

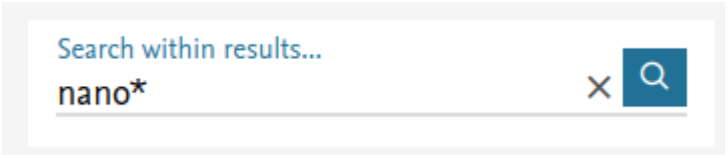
- This database is not of interest but as it is not conveniently removable at the selection stage, the search has been run for all databases and the Derwent hits were removed in Refine stage using Exclude:



- Since the numbers of hits were not prohibitively large, all references were exported, without limiting the search results by the nano* limiter

Scopus

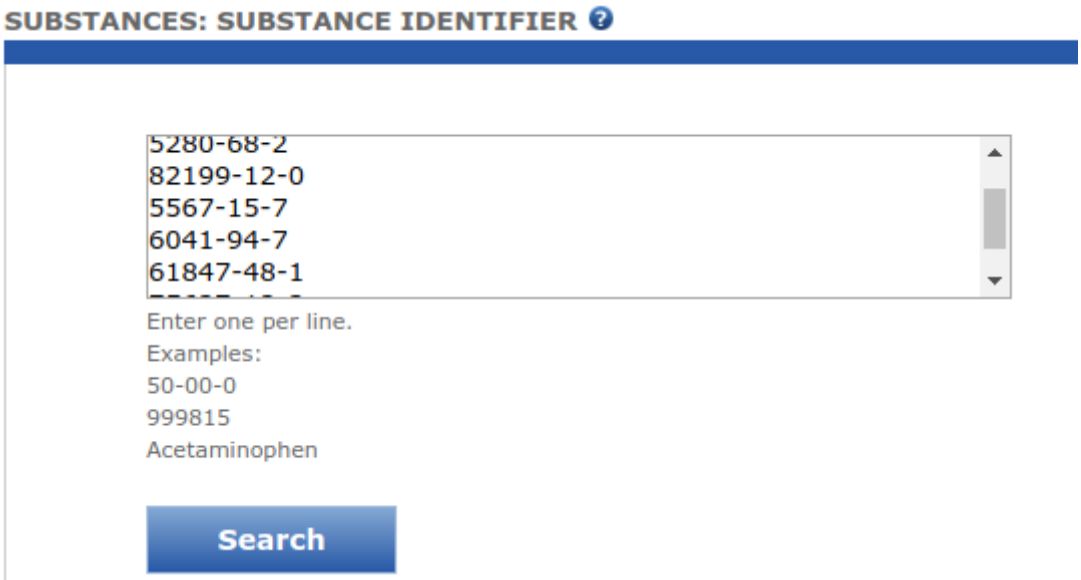
- Scopus at this moment lists 37,956 different sources
- Patents are listed independently and so they did not need to be removed in a step
- Search by CAS number was selected, for efficiency reasons CAS numbers were searched in groups of 10 with the exception of "12240-15-2" which was searched for by an individual search due to a large number of hits
- All searches were further restricted by nano* filter:



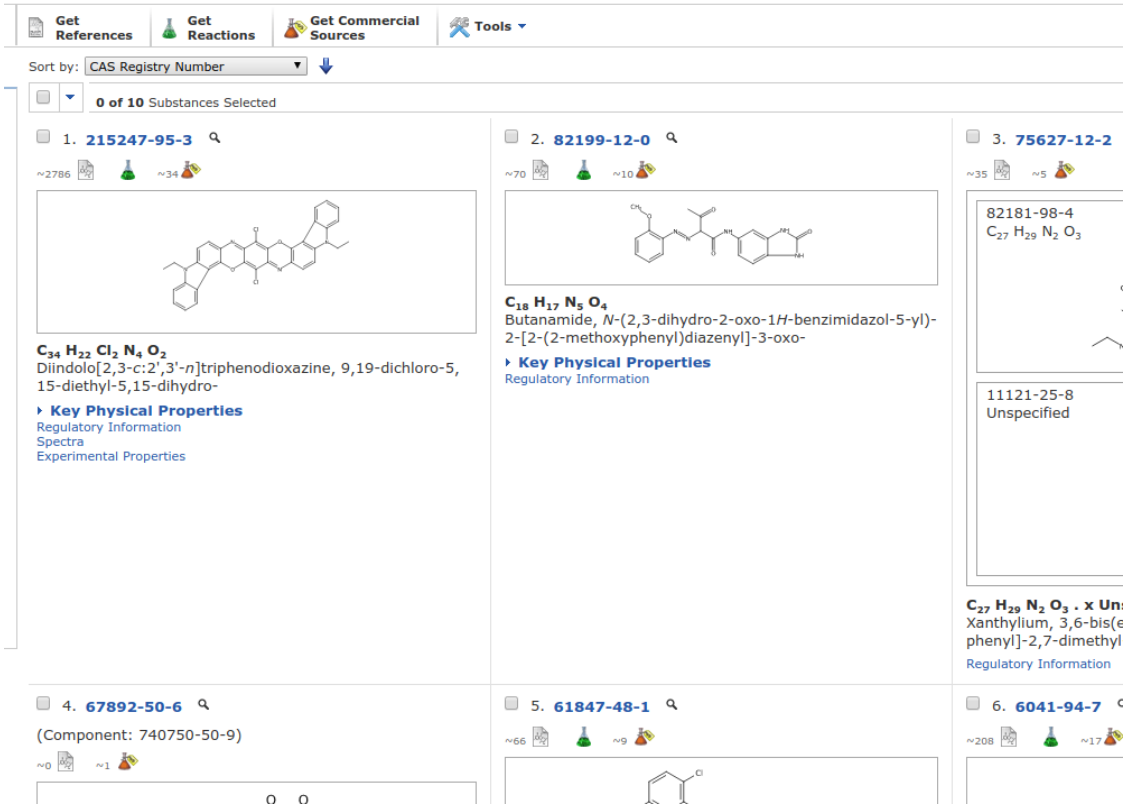
- All nano* restricted numbers are reported in the table, but in cases where the number of hits was not prohibitively large (total hits < 120), all records were exported from the full list, not from the filtered list after nano* restriction

Scifinder

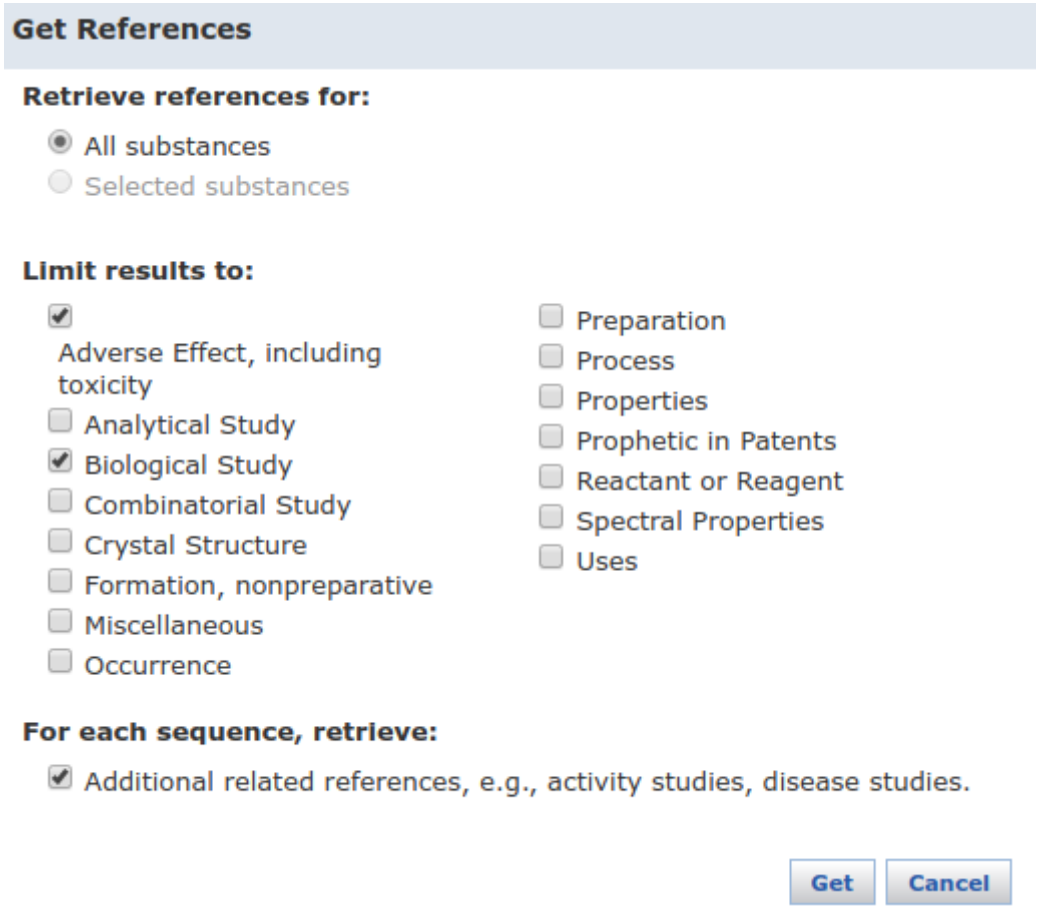
- Scifinder allows searching by a substance identifier - CAS number:



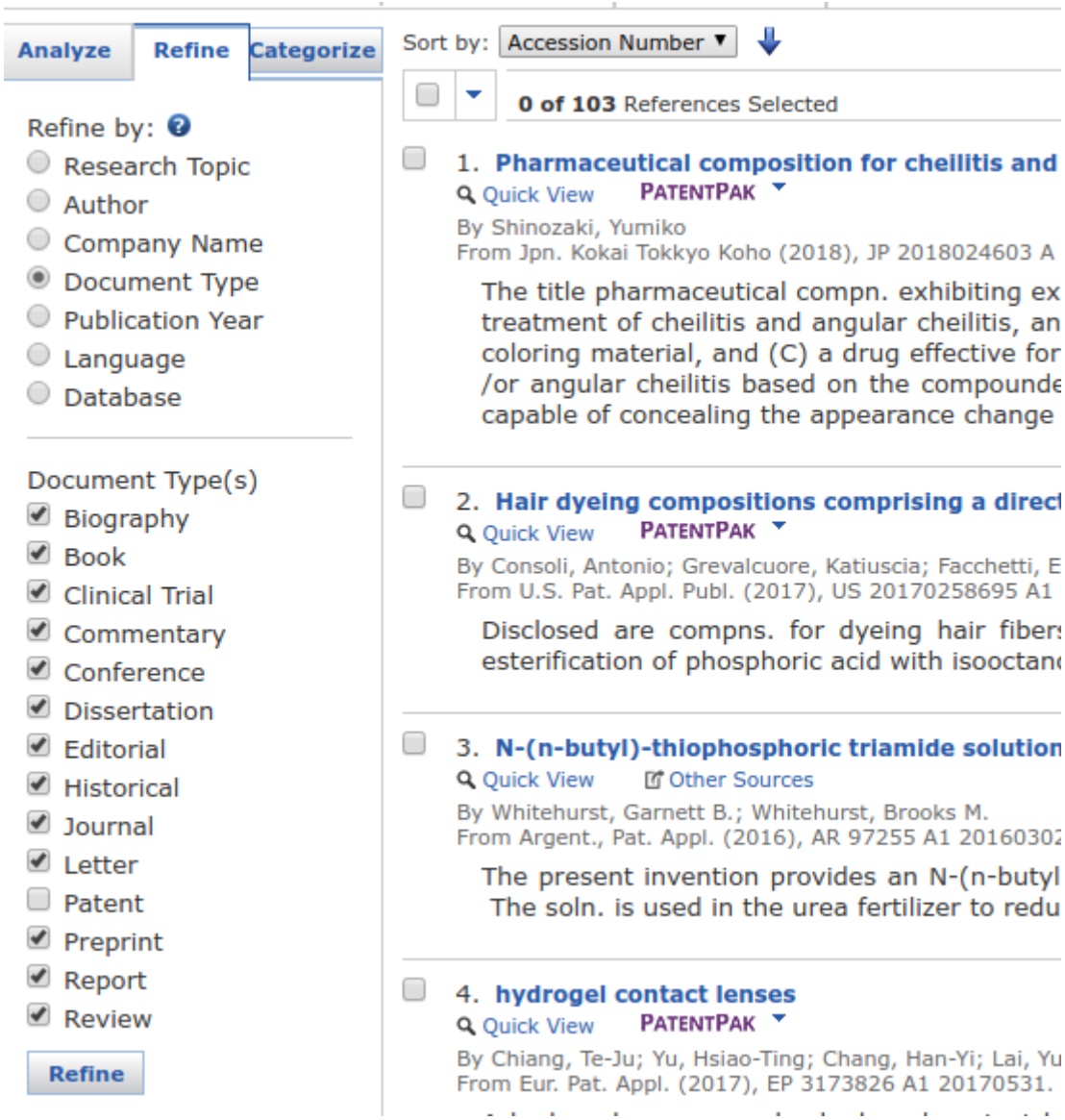
- The search retrieves a list of identified substances:



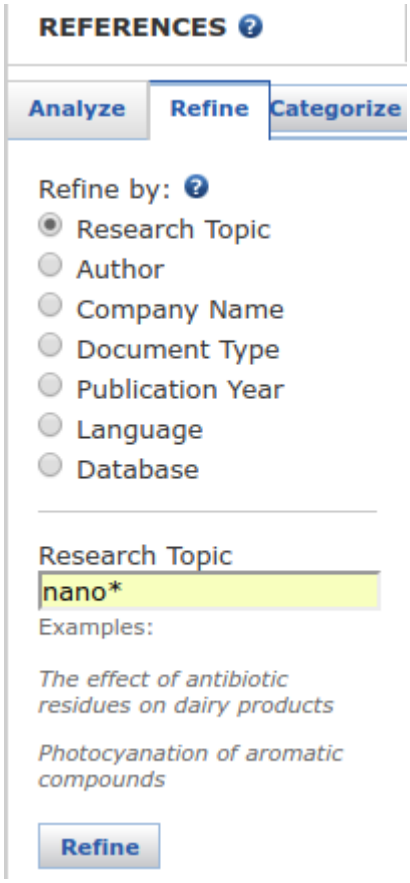
- In the following step "Get references" option offers a selection of articles to be selected. The selection used for the report is shown in the following screenshot:



- Patents were removed in the following step via the "Refine" function:



- and the results were fthen urther refined by nano* research topic:



- All nano* restricted numbers are reported in the table, but in the cases where the number of hits was not prohibitively large (total hits < 140), records were exported from the full hits, not from the filtered list after the nano* restriction

Search results

Web of Science

Information source: Web of Science

Data collections: All collections

Timespan: All years

Date accessed: 9-11 March 2018

Search terms: TOPIC: ("Basic Violet 1")

Total hits: 26

After patent removal: 19

Search terms: TOPIC: ("Pigment Blue 1")

Total hits: 5

After patent removal: 0

Search terms: TOPIC: ("Pigment Blue 15")

Total hits: 255

After patent removal: 23

Search terms: TOPIC: ("Pigment Blue 27")

Total hits: 4

After patent removal: 0

Search terms: TOPIC: ("Pigment Blue 28")

Total hits: 4

After patent removal: 2

Search terms: TOPIC: ("Pigment Blue 61")

Total hits: 6

After patent removal: 1

Search terms: TOPIC: ("Pigment Blue 63")

Total hits: 1

After patent removal: 1

Search terms: TOPIC: ("Pigment Blue 76")

Total hits: 3

After patent removal: 0

Search terms: TOPIC: ("Pigment Green 1")

Total hits: 4

After patent removal: 0

Search terms: TOPIC: ("Pigment Green 36")

Total hits: 85

After patent removal: 5

Search terms: TOPIC: ("Pigment Green 7")

Total hits: 213

After patent removal: 10

Search terms: TOPIC: ("Pigment Orange 13")

Total hits: 13

After patent removal: 3

Search terms: TOPIC: ("Pigment Orange 34")

Total hits: 11

After patent removal: 3

Search terms: TOPIC: ("Pigment Orange 36")

Total hits: 9

After patent removal: 2

Search terms: TOPIC: ("Pigment Orange 42")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Orange 43")

Total hits: 21

After patent removal: 1

Search terms: TOPIC: ("Pigment Orange 5")

Total hits: 23

After patent removal: 7

Search terms: TOPIC: ("Pigment Orange 51")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Orange 62")

Total hits: 3

After patent removal: 1

Search terms: TOPIC: ("Pigment Orange 64")

Total hits: 11

After patent removal: 0

Search terms: TOPIC: ("Pigment Orange 67")

Total hits: 19

After patent removal: 0

Search terms: TOPIC: ("Pigment Orange 69")

Total hits: 2

After patent removal: 0

Search terms: TOPIC: ("Pigment Orange 73")

Total hits: 35

After patent removal: 2

Search terms: TOPIC: ("Pigment Red 112")

Total hits: 35

After patent removal: 5

Search terms: TOPIC: ("Pigment Red 117")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 12")

Total hits: 2

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 146")

Total hits: 18

After patent removal: 2

Search terms: TOPIC: ("Pigment Red 147")

Total hits: 2

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 149")

Total hits: 17

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 166")

Total hits: 11

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 168")

Total hits: 5

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 170")

Total hits: 31

After patent removal: 9

Search terms: TOPIC: ("Pigment Red 175")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 177")

Total hits: 97

After patent removal: 5

Search terms: TOPIC: ("Pigment Red 178")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 179")

Total hits: 13

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 18")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 181")

Total hits: 3

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 185")

Total hits: 21

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 187")

Total hits: 2

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 188")

Total hits: 3

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 194")

Total hits: 2

After patent removal: 2

Search terms: TOPIC: ("Pigment Red 2")

Total hits: 12

After patent removal: 4

Search terms: TOPIC: ("Pigment Red 202")

Total hits: 33

After patent removal: 2

Search terms: TOPIC: ("Pigment Red 208")

Total hits: 8

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 209")

Total hits: 24

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 21")

Total hits: 2

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 22")

Total hits: 16

After patent removal: 10

Search terms: TOPIC: ("Pigment Red 222")

Total hits: 2

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 23")

Total hits: 10

After patent removal: 7

Search terms: TOPIC: ("Pigment Red 253")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 254")

Total hits: 173

After patent removal: 13

Search terms: TOPIC: ("Pigment Red 255")

Total hits: 22

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 266")

Total hits: 2

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 3")

Total hits: 14

After patent removal: 10

Search terms: TOPIC: ("Pigment Red 38")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 4")

Total hits: 4

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 48")

Total hits: 43

After patent removal: 5

Search terms: TOPIC: ("Pigment Red 48:4")

Total hits: 7

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 49")

Total hits: 4

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 5")

Total hits: 3

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 52:2")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 53")

Total hits: 23

After patent removal: 10

Search terms: TOPIC: ("Pigment Red 53:1")

Total hits: 12

After patent removal: 6

Search terms: TOPIC: ("Pigment Red 57:1")

Total hits: 51

After patent removal: 10

Search terms: TOPIC: ("Pigment Red 63:2")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 81")

Total hits: 19

After patent removal: 1

Search terms: TOPIC: ("Pigment Red 81:5")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 88")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Red 9")

Total hits: 6

After patent removal: 3

Search terms: TOPIC: ("Pigment Violet 1")

Total hits: 11

After patent removal: 0

Search terms: TOPIC: ("Pigment Violet 19")

Total hits: 104

After patent removal: 7

Search terms: TOPIC: ("Pigment Violet 2")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Violet 23")

Total hits: 159

After patent removal: 7

Search terms: TOPIC: ("Pigment Violet 27")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Violet 3")

Total hits: 3

After patent removal: 0

Search terms: TOPIC: ("Pigment Violet 3:4")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Violet 32")

Total hits: 4

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 1")

Total hits: 14

After patent removal: 7

Search terms: TOPIC: ("Pigment Yellow 101")

Total hits: 24

After patent removal: 21

Search terms: TOPIC: ("Pigment Yellow 104")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 108")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 109")

Total hits: 9

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 110")

Total hits: 35

After patent removal: 3

Search terms: TOPIC: ("Pigment Yellow 111")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 12")

Total hits: 70

After patent removal: 25

Search terms: TOPIC: ("Pigment Yellow 120")

Total hits: 13

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 126")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 127")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 128")

Total hits: 30

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 129")

Total hits: 13

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 13")

Total hits: 38

After patent removal: 14

Search terms: TOPIC: ("Pigment Yellow 138")

Total hits: 92

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 139")

Total hits: 63

After patent removal: 2

Search terms: TOPIC: ("Pigment Yellow 150")

Total hits: 73

After patent removal: 2

Search terms: TOPIC: ("Pigment Yellow 151")

Total hits: 22

After patent removal: 2

Search terms: TOPIC: ("Pigment Yellow 16")

Total hits: 1

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 168")

Total hits: 4

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 17")

Total hits: 22

After patent removal: 5

Search terms: TOPIC: ("Pigment Yellow 175")

Total hits: 2

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 181")

Total hits: 20

After patent removal: 7

Search terms: TOPIC: ("Pigment Yellow 183")

Total hits: 6

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 194")

Total hits: 6

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 3")

Total hits: 14

After patent removal: 8

Search terms: TOPIC: ("Pigment Yellow 36")

Total hits: 1

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 55")

Total hits: 2

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 61")

Total hits: 0

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 63")

Total hits: 2

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 65")

Total hits: 6

After patent removal: 2

Search terms: TOPIC: ("Pigment Yellow 73")

Total hits: 3

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 74")

Total hits: 201

After patent removal: 10

Search terms: TOPIC: ("Pigment Yellow 81")

Total hits: 5

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 83")

Total hits: 34

After patent removal: 5

Search terms: TOPIC: ("Pigment Yellow 93")

Total hits: 19

After patent removal: 1

Search terms: TOPIC: ("Pigment Yellow 95")

Total hits: 6

After patent removal: 0

Search terms: TOPIC: ("Pigment Yellow 97")

Total hits: 9

After patent removal: 1

Scopus

Information source: Scopus

Data collections: All collections

Timespan: All years

Date accessed: 13 March 2018

Search terms: CASREGNUMBER ("1345-16-0" OR "14059-33-7" OR "101357-19-1" OR "1047-16-1" OR "1325-87-7" OR "1328-53-6" OR "2512-29-0" OR "147-14-8" OR "15793-73-4" OR "2512-29-0")

Total hits: 340

After nano* filter: 78

Search terms: CASREGNUMBER ("2512-29-0" OR "2786-76-7" OR "2814-77-9" OR "2465-29-4" OR "3468-63-1" OR "3520-72-7" OR "35636-63-6" OR "36888-99-0" OR "6471-50-7" OR "4424-06-0")

Total hits: 14

After nano* filter: 1

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Total hits: 5

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Total hits: 2

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Total hits: 0

After nano* filter: 0

Search terms: CASREGNUMBER ("12240-15-2")

Total hits: 1588

After nano* filter: 790

Information source: SciFinder

Data collections: All collections

Timespan: All years

Date accessed: 12 March 2018

CAS numbers: 1345-16-0; 14059-33-7; 101357-19-1; 1047-16-1; 1325-87-7; 1328-53-6; 2512-29-0; 147-14-8; 15793-73-4; 2512-29-0

Total hits: 1264

After patent removal: 592

After nano* filter: 6

CAS numbers: 2512-29-0; 2786-76-7; 2814-77-9; 2465-29-4; 3468-63-1; 3520-72-7; 35636-63-6; 36888-99-0; 6471-50-7; 4424-06-0

Total hits: 308

After patent removal: 90

After nano* filter: 1

CAS numbers: 5468-75-7; 67892-50-6; 5280-66-0; 5280-68-2; 82199-12-0; 5567-15-7; 6041-94-7; 61847-48-1; 75627-12-2; 6358-30-1

Total hits: 103

After patent removal: 29

After nano* filter: 0

CAS numbers: 6358-31-2; 6410-32-8; 6041-94-7; 5280-68-2; 6486-23-3; 6535-46-2; 67989-22-4; 8007-18-9; 10101-66-3; 12656-85-8

Total hits: 377

After patent removal: 48

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CAS numbers: 12737-27-8; 68186-85-6; 68186-87-8; 68186-90-3; 68186-91-4; 68187-11-1; 58339-34-7; 68187-40-6; 68187-49-5; 68187-51-9

Total hits: 42

After patent removal: 16

After nano* filter: 0

CAS numbers: 68187-54-2; 68412-74-8; 101357-30-6; 57455-37-5; 102184-95-2; 1103-38-4; 1324-76-1; 1325-75-3; 1326-03-0; 1326-04-1

Total hits: 655

After patent removal: 41

After nano* filter: 0

CAS numbers: 1503-48-6; 2379-74-0; 2387-03-3; 2425-85-6; 3049-71-6; 3089-17-6; 3564-22-5; 3905-19-9; 4051-63-2; 4216-01-7

Total hits: 501

After patent removal: 116

After nano* filter: 1

CAS numbers: 4216-02-8; 4378-61-4; 4531-49-1; 4948-15-6; 5045-40-9; 5160-02-1; 5280-80-8; 5281-04-9; 5521-31-3; 5580-57-4

Total hits: 733

After patent removal: 132

After nano* filter: 0

CAS numbers: 5590-18-1; 5979-28-2; 6358-30-1; 6358-37-8; 6358-85-6; 6358-87-8; 6372-81-2; 6407-75-6; 6410-26-0; 6448-95-9

Total hits: 215

After patent removal: 87

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CAS numbers: 6471-49-4; 6486-23-3; 6528-34-3; 6985-92-8; 10142-77-5; 12224-98-5; 12225-08-0; 12225-18-2; 12236-62-3; 12237-62-6

Total hits: 110

After patent removal: 52

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CAS numbers: 15790-07-5; 15993-42-7; 16043-40-6; 16521-38-3; 17832-28-9; 22094-93-5; 27614-71-7; 29204-84-0; 29920-31-8; 30125-47-4

Total hits: 188

After patent removal: 30

After nano* filter: 0

CAS numbers: 31775-16-3; 31778-10-6; 31837-42-0; 35355-77-2; 37300-23-5; 42844-93-9; 54660-00-3; 59487-23-9; 61512-61-6;

61951-98-2

Total hits: 23

After patent removal: 13

After nano* filter: 0

CAS numbers: 65212-77-3; 67989-22-4; 68227-78-1; 68511-62-6; 68512-13-0; 68610-86-6; 68987-63-3; 71832-85-4; 71872-63-4; 72102-84-2

Total hits: 9

After patent removal: 3

After nano* filter: 0

CAS numbers: 72639-39-5; 74336-59-7; 74336-60-0; 74441-05-7; 78521-39-8; 78952-72-4; 79953-85-8; 82199-12-0; 83524-75-8; 84632-50-8

Total hits: 16

After patent removal: 6

After nano* filter: 0

CAS numbers: 84632-59-7; 84632-65-5; 84632-66-6; 85776-13-2; 85776-14-3; 85958-80-1; 85959-60-0; 90268-23-8; 106276-80-6; 215247-95-3

Total hits: 64

After patent removal: 8

After nano* filter: 0

CAS numbers: 12240-15-2

Total hits: 896

After patent removal: 446

After nano* filter: 23

Appendix 7. Critical analysis of the collected data

The data quality assessment

The **quality of the evaluated literature** (i.e. contextual information provided) to be judged according to the following characteristics:

- ***Transparency* (T)**: level of clarity of the description to be able to answer the key questions.
- ***Completeness (C)***: level of completeness and sufficiency of the description regarding the key questions to be answered.

There are three levels for both characteristics:

1. Low (L)
2. Medium /M)
3. High (H)

Final quality to be gained as a combination of the characteristics according to matrix in Table 1:

Table 1: Data quality matrix

C \ T	H	M	L
H	HH	MH	LH
M	HM	MM	LM
L	HL	ML	LL

HH, MH, HM – high quality

LH, MM, HL – medium quality

LM, ML, LL – low quality

Hazard data

Criteria for data quality assessment of identified studies were based on general criteria used to assess quality of toxicological studies (Klimisch et al., 1997; Maxim et al., 2014) and nano-specific criteria taking into account possible unique nanomaterial properties and behavior (DANA, 2016)

In vitro studies

1. Nanomaterial characterization for in vitro toxicological studies

- Is nanomaterials solubility in the relevant media evaluated? (ion- or molecule-related toxicity in soluble nanomaterials vs. particle-related toxicity in insoluble nanomaterials)
- Is the preparation of nanomaterial dispersion described in details?
- Is nanomaterial size in relevant media evaluated before exposure (Confirmation of particle dispersion in test media, with sufficient description)?

2. Method and test system description

- Is the study performed according to GLP?
- Are OECD methods referenced or any other EU/national guidelines followed?
- Are relevant doses applied (is dosage classified as overload or non-overload)?
- Clear description of exposure procedure?
- Are negative controls included and their results appropriate?
- Are vehicle controls included and their results negative?
- Are positive controls included and their results reported? Is the mechanism of action of the positive control relevant?
- Are controls for evaluation of potential interference of the nanomaterials with the test method included?

3. Evaluation of the results

- Are there independent biological replicates?
- Are statistical methods for data analysis appropriate and described in a transparent manner?

4. Interpretation of the Results

- Is the mode of toxic action explained?
- Is there any dose- or time-dependency of the results?
- Concordance between interpretation of the results (i.e., in terms of level of evidence and conclusiveness) and the raw data?

In vivo

1. Nanomaterial characterization for in vivo toxicological studies

- Is nanomaterials solubility in the relevant media evaluated? (ion- or molecule-related toxicity in soluble nanomaterials vs. particle-related toxicity in insoluble nanomaterials)
- Is the preparation of nanomaterial dispersion described in details?

- Is nanomaterial size in relevant media evaluated before exposure (Confirmation of particle dispersion in test media, with sufficient description)?

2. Method and test system description

- Are studies performed according to GLP?
- OECD methods referenced or any other EU/national guidelines followed?
- Are appropriate species, strain, sex used?
- Is information on experimental animals' handling, housing and feeding conditions provided?
- Are experimental animals' parameters (weight, temperature, highteight of the test organisms at the start of the study given monitored and reported? (only relevant repeated dose toxicity studies)
- Is the selected frequency and duration of exposure explained?
- Are the selected time-points of observations explained?
- Is the exposure route relevant for human exposure?
- Are relevant doses/concentrations used and dosage classified as overload or non-overload (overload condition leads to distinct toxic mechanisms; cytotoxic doses should not be used in other endpoinds, such as genotoxicity)?
- Are negative controls included? Is there no mortality and low frequency of spontaneous occurrence of diseases in negative controls?
- Are vehicle controls included and results reported and negative?
- Is presence of nanomaterials in the exposed animals evaluated? (e.g. TEM of target organs)?
- Is the duration of follow-up sufficient for the disease to develop as an effect of the exposure?

3. Evaluation of the results

- Was number of tested and control animals sufficient?
- Statistical methods for data analysis described in a transparent manner?

4. Interpretation of the Results

- Is the mode of toxic action explained?
- Toxicokinetics data reported?
- Is there any dose- or time-dependency of the results?
- No species-specific modes of action (e.g. carcinogenicity due to lung overload observed in rat exposed to insoluble particles)?

- Is there dose- or time-dependency of the results?
- Interpretation of the relevance of animal data for humans?
- Concordance between interpretation of the results (i.e., in terms of level of evidence and conclusiveness) and the raw data?

DaNa project (2016). Literature Criteria Checklist. Available at: (http://www.nanopartikel.info/en/nanoinfo/methods/991-literature-criteria-checklist [2018-01-17]

Klimisch HJ, Andreae M, Tillmann U (1997). A systematic approach for evaluating the quality of experimental and toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology* **25**:1-5.

Maxim L, Van der Sluijs JP Qualichem In Vivo: A tool for assessing the quality of in vivo studies and its application for bisphenol A. *PLOS one*, ***9***(1), e87738.

Exposure data

For the exposure measurements there is (still) not a recognised method for collection and interpretation of exposure data. Therefore data uncertainty and minimum quality requirements (e.g. repeated measurements, measure of variability, etc..) are usually not present and therefore uncertainty is difficult to estimate. We may have to rely only on the description of the monitoring campaign, which is also essential to judge validity of the results (refer above as the appropriateness of the method). The integrity can be difficult to judge from the ES template as templates usually include summary information. Suggested framework to evaluate the quality of exposure data is presented in Table 47.

Table 42: Suggested framework to evaluate the quality of exposure data

LEVEL 1: Is there sufficient and valid information to describe the use of the substance? (Y/N)				
Core information	Detailed information	Available (Y/N)	Transparency (H, M, L)	Remarks
Substance description	Name			
	Physical form			
Task characteristics	Description			

	Amount of substance used			
	Duration			
	Frequency			
	Containment			
Exposure controls	General ventilation			
	LEV			
	PPE used			
LEVEL 2: Are the exposure measurements valid? (Y/N)				
Core information	Detailed information	Available (Y/N)	Appropriateness (H, M, L)	Remarks
Measurement strategy	Type (release, area, personal)			
	Background data			
	Type of background			
	Size range measured			
	Sampling duration			
	Purpose (routine, risk assessment …,)			
	Strategy (random sample, representative, worst case)			

LEVEL 3: Do the measurement data represent the ES described? (Y/N)				
Core information	Detailed information	(Y/N)	Confidence (H, M, L)	Remarks
Representativeness	Were measurements collected under the same situation described in the scenario?			
	Where appropriate methods used for the assessment?			
	Was the sampling duration representative of the task described?			

Risk assessment data

The quality of risk assessment studies was assessed based on the presence of hazard and exposure in the study. The quality criteria were:

- Peer Reviewing the Science - the credibility and integrity of the scientific information generated, evaluated, and communicated by the authors.
- Transparency, and effectiveness,
- Efficiency, and scientific integrity;
- Objectivity and Reasonableness - study design, data selection, data interpretation, choice of defaults, models, methods

Life cycle assessment data

The quality of LCA studies were assessed in accordance with guidelines for critical review of product LCA. The general requirements of the ISO standards are:

- the phases goal and scope definition;
- inventory analysis;
- impact assessment, and interpretation of results.

The framework to evaluate the quality of exposure data was following:

- The phases goal and scope definition - the systems to be studied, the system boundaries, and criteria used in establishing system boundaries and the justification of these criteria, allocation procedures, initial data and data quality requirements;
- Inventory analysis - the reference unit in relation to which the environmental exchanges are calculated, what the data includes (the beginning and the end of the unit process, its function, and whether shut-down/start-up conditions and emergency situations are included), the source of the data;
- Characterisation of release factor – release factor, impact factor
- Adequacy of data

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