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Integrating a life cycle perspective in the exposure and impact assessment of conventional and emerging pollutants: case studies on biocides and engineered nanomaterials

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Dedicado a mi familia, por su amor incondicional: ellos son el motor que me ha traído hasta aquí

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LIST OF ACRONYMS

ALI	Air Liquid Interface
BPR	Biocidal Products Regulation
BZ	Breathing Zone
CEN	European Committee for Standardization
CENELEC	European Committee for Electrotechnical Standardization
CNT	Carbon Nanotubes
CPC	Condensation Particle Counter
DRI	Direct Reading Instrument
EEA	European Environment Agency
EC	European Commission
ECHA	European Chemicals Agency
ENM	Engineered Nanomaterial
EOL	End of Life
EPA	Environmental Protection Agency
EPLCA	European Platform on Life Cycle Assessment
ETSI	European Telecommunications Standards Institute
EU	European Union
FAO	Food and Agriculture Organization
FMPS	Fast Mobility Particle Spectrometer
INM	Incidental Nanomaterial
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
MWCNTs	Multi-walled carbon nanotubes
NEA	Nanotechnology enabled application

NEAT	Nanoparticle emission assessment technique
NEP	Nanotechnology enabled product
NOAA	Nano-objects and their aggregates and agglomerates
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
PM	Particulate matter
PNEC	Predicted no effect concentration
PPP	Plant Protection Product
RA	Risk Assessment
RQ	Risk Quotient
SDGs	Sustainable Development Goals
SEM	Scanning Electron Microscope
SMPS	Scanning Mobility Particle Sizer
SSbD	Safe and Sustainable by Design
STP	Sewage treatment plant
UFP	Ultrafine particle
UN	United Nations
VOCs	Volatile organic compounds

I. INTRODUCTION

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I.1. Life Cycle Thinking Approaches in the Management of Chemical Pollution from An European Perspective

The global chemical production (excluding pharmaceuticals) has grown by 7% annually since the mid-1980s, reaching €2.4 trillion in 2010. Most of the growth in the past 25 years has been driven by Asia, which now owns almost half of global chemical sales. If current trends continue, global chemical markets are expected to grow an average 3% in the next 20 years (Kearney, 2021). According to the European Chemical Industry Council (CEFIC, 2022), Europe is the second largest producer of chemicals in the world. Figures of chemicals produced and consumed in the EU are provided by Eurostat as (i) total chemicals, (ii) chemicals that are hazardous to health and (iii) chemicals that are hazardous to the environment. According to most recent data (EUROSTAT, 2022), though chemicals hazardous to human health are produced in lower amounts since about 2010 onwards, their consumption in the EU reached 211 million tonnes in 2019. It is also noteworthy that most of the chemicals that are hazardous to human health are also hazardous to the environment.

Pollution can be defined as the introduction of harmful substances or products into the environment. Chemical pollution occurs when chemicals resulting from human activities and/or their degradation, transformation products and/or metabolites contaminate water, air or soil environmental compartments. Sources of pollution can be categorized into (i) point and (ii) non-point sources. Point source pollution is any individual identifiable point or concentrated area that emits pollution whereas non-point source pollution is any dispersed area of pollution that emits pollution which can't be traced to an identifiable point, a single source, or a concentrated area. The OECD (2017b) refers that releases of hazardous chemicals during the usage stage of products can significantly contribute to the total chemical releases to the environment. It also refers that the public and private use of products falls under non-point source (diffuse) release sources.

The term non-point source in water pollution encompasses a large range of diffuse sources (e.g., the runoff of fertilizers from cropland or air pollutants being washed or deposited on

different water bodies e.g., groundwater, rivers or lakes). As an example of non-point sources, several authors (Szoege et al., 1996; Chowdary et al., 2005; Gilliom et al., 2006; Andrade and Stigter, 2009; Sjerps et al., 2017) have reported groundwater aquifers being increasingly affected by nitrates and pesticides. It is noted that *pesticides* are defined in the European Union as (i) plant protection products (PPP) as defined in Regulation (EC) No 1107/2009 and (ii) biocidal products as defined in Directive 98/8/EC concerning the placing on the market of biocidal products, which is currently replaced by the Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (also known as BPR). In this sense, whereas the use of PPP is generally associated to the agri-food sector, biocidal products are commonly used in a variety of sectors including antifouling paints or wood preservatives. Reference is made to the Annex V of the BPR where biocidal products are classified into 22 biocidal product-types, grouped in four main areas: disinfectants, preservatives, pest control and other biocidal products.

Some of the most important air pollutants include sulphur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), ozone (O_3), volatile organic compounds (VOCs), and particulate matter (PM) of different size ranges including PM_{10}; $PM_{2.5}$ and $PM_{0.1}$ (or ultrafine particles (UFP)) with aerodynamic diameters below 10, 2.5 and 0.1 μm, respectively. Air pollutants of anthropogenic origin can be generated in different activities including burning of fossil fuels in electricity generation, transport, industry and households; industrial processes and solvent use, for example in the chemical and mining industries; agriculture or waste treatment (EEA, 2022). Additionally, air pollutants can be released directly into the atmosphere (primary emissions) or can form as a consequence of chemical interaction involving precursor substances (secondary pollutants). As an example of non-point sources of air pollution, Sigsgaard et al. (2015) reported that biomass combustion for residential heating at a domestic scale is increasing and expected to become the major source of primary PM emission over the next 5–15 years.

Diffuse soil pollution is the presence of a substance or agent (chemical) in the soil because of human activities emitted from moving liquid or gaseous sources, covering a large area, or from multiple sources. The three major pathways responsible for the introduction of diffuse

pollutants into soil are (i) atmospheric deposition, (ii) agricultural inputs, and (iii) flood events. Causes of diffuse pollution in the soil compartment tend to be dominated by the transport of pollutants by erosion processes (wind and water erosion and sedimentation), and excessive nutrient and pesticide applications, heavy metals, persistent organic pollutants (POPs) and inorganic pollutants. (FAO, 2018).

Complementary to environmental pollution, indoor chemical pollution is defined as the presence into indoors air of chemical contaminants not normally present in high quality outdoor air. Articles and consumer products used indoors may contain a variety of both well-known chemicals and emerging substances (Harrad et al., 2008; Venier et al., 2016; Zheng et al., 2017). Such chemicals are emitted in the indoor environment; indoor air and dust representing an important pathway of chemical exposure for humans (Dulio et al., 2018).

Concerns regarding the short and long-term detrimental effects of chemicals on human health and ecosystems (which also represent an indirect source of human exposure) have made the minimization of chemical pollution and associated hazards a vitally important issue. If sustainable development is to be achieved, environmentally efficient products (and product life cycles) are essential (Askham, 2011).

In the EU, the 8th Environmental Action Programme (EAP) (EC, 2022) includes *pursuing a zero-pollution ambition for a toxic free-environment, including for air, water and soil and protecting the health and well-being of citizens from environment-related risks and impacts* amongst its six thematic priority objectives. Some of the actions leading to the achievement of the present target have included (i) the adoption of the Chemicals Strategy for Sustainability (EC, 2020; COM 2020/667) and (ii) the publication of the Zero Pollution action plan (EC, 2021; COM 2021/400) in May 2021.

The Chemicals Strategy sets out concrete actions to support the transition towards chemicals, materials and their use in products that are concurrently safe and sustainable starting with the design phase and considering the overall life cycle: production, use and end-of-life. The document includes the following working definition of Safe and Sustainable by Design (SSbD): *'a pre-market approach that focuses on providing a function (or service), while avoiding volumes*

and chemical properties that may be harmful to human health or the environment, in particular groups of chemicals likely to be (eco-)toxic, persistent, bio-accumulative or mobile. Overall sustainability should be ensured by minimising the environmental footprint of chemicals in particular on climate change, resource use, ecosystems and biodiversity, ***from a life cycle perspective***.' Moreover, it announces that the European Commission will develop EU SSbD criteria by 2022.

From a wider context, the Sustainable Development Goals (SDGs), comprising 17 goals and 169 targets and adopted by all United Nations Member States in 2015, include three specific targets in the areas related to the production and consumption of chemicals, as listed in Table 1.

Table 1: Specific targets related to the production and consumption of chemicals (SDG). Adapted from the 2030 Agenda for Sustainable Development. United Nations. Resolution adopted by the General Assembly on 25 September 2015 (UN, 2015).

SDG		Specific target
	Goal 3 "Ensure healthy lives and promote well-being across all ages"	Target 3.9: "By 2030, substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination"
	Goal 6 "Ensure availability and sustainable management of water and sanitation for all"	Target 6.3 "By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and increasing recycling and safe reuse by [x] per cent globally" (where the "x" is to be defined at a later stage).
	Goal 12 "Ensure sustainable consumption and production patterns".	Target 12.4 "By 2020, achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment".

According to Blum et al. (2017), a holistic approach is necessary to realise the benefits of chemicals for societies in reaching the SDGs – and to prevent the negative impacts of chemicals along their life cycle. This holistic approach is referred to as "sustainable chemistry" and should take into account the three dimensions of sustainable development, preventive measures, and the entire life cycle of a chemical (design, production, use and disposal).

I.2. Introductory concepts: Risk Assessment and Life Cycle Assessment of chemicals

Life Cycle Assessment (LCA) and Risk Assessment (RA) are two of the most prominent environmental assessment methods and are established in terms of having agreed-upon frameworks and models (Arvidsson, 2015). Both methods are introduced next.

I.2.1. Framework for Risk Assessment (RA)

The term risk describes the function of the probability of "exposure" to the "hazard" potential a given chemical exhibits:

Risk = f {exposure; hazard}.

The main idea behind the RA method is that a chemical substance does not pose a risk unless the concentration of the substance is high enough. RA framework is generally made up of a few components, sometimes referred to by different terms. The concepts introduced next correspond to the Environmental RA. Figure 1 depicts the general framework for the environmental RA based on the provisions of the Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (BPR).

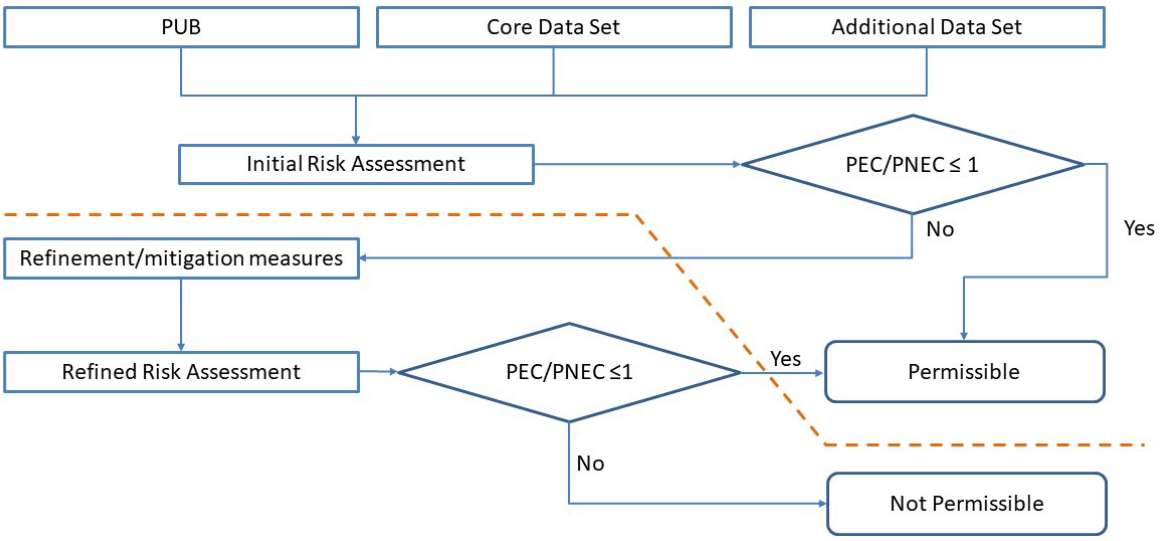

Figure 1: The framework for environmental RA for Biocidal Active Substances in agreement with the BPR has been taken as a reference. An initial RA will account for published data for the biocidal active substance of interest, in addition to the substance's core and the additional data sets required by the BPR. From these data the Predicted Environmental Concentration (PEC) and Predicted No Effect Concentration (PNEC) are calculated. Depending on the risk quotient (RQ), refinement or mitigation measures are implemented and the RQ is calculated again.

(i) Hazard identification (sometimes called problem formulation)

This step consists of identifying potential hazards related to the chemical, such as whether the substance is toxic or bioaccumulative. Potential linkages between the sources of the substance and endpoints are also outlined, endpoints being potentially adversely affected organisms.

(ii) Effect assessment (sometimes called dose-response assessment or hazard assessment)

The environmental hazard assessment focuses on potential effects on ecosystems in any environmental compartment (water - freshwater and marine, including sediments-, air, soil - including groundwater-), predator in the food chain, and microbiological activity of sewage treatment plants (STP). The environmental hazard assessment allows establishing the concentration below which adverse effects in the environmental compartment of concern are not expected to occur (PNEC). For each environmental compartment, PNEC is derived from

the ecotoxicological tests results, which typically deliver parameters such as EC_{50} values, and an assessment factor.

(iii) Exposure assessment

In the case of environmental exposure, this is conducted through detailed modelling of the emissions and environmental fate of the chemical. The modelling is often conducted by dividing the environment into different compartments, generally water, air, soil, and sediment. Mass balance equations describing the fate processes and the transports between different compartments are then employed. The ultimate aim of an environmental exposure assessment is to reach a predicted environmental concentration (PEC) for each compartment.

(iv) Risk characterization

In the last step, called risk characterization, the PEC and PNEC are compared by division to estimate a risk quotient (RQ):

$$RQ = PEC/PNEC$$

When the RQ > 1, it means that the concentration to which the endpoint is exposed is higher than the concentration at which no adverse effects are expected implying that the risk is not controlled. When the RQ ≤ 1, the risk is controlled (Figure 1).

Complementary steps in the RA framework might include uncertainty assessment and conclusions. Whereas the uncertainty assessment describes sources of uncertainty (such as lack of data used in making assumptions of environmental exposure), conclusions integrate the results of the risk characterization and uncertainty assessment, often based on the various outcomes in a systematic weight-of-evidence approach (WoE). WoE in chemicals' RA refers to the process of assembling, weighing, and evaluating evidence, in a RA, to come to a scientifically defensible conclusion. An example would be using chemical concentrations in sediment, water, and plants at a contaminated pond to assess risk to a duck through its diet as one line of evidence and using measured survival of ducklings at the pond as another line of evidence. Another example is represented by the work of De los Ríos et al. (2016) who integrated using multivariate statistics the data obtained in a pollution survey performed in estuarine areas and concluded that contaminants in water were significantly correlated with

contaminants and biomarkers in mussels and with structure of macroinvertebrate benthic communities.

I.2.2. Framework for Life Cycle Assessment (LCA)

LCA is an analytical tool for the (comparative) environmental assessment of products or services in relation to a particular function and generally covers the entire life cycle, or supply chain, of a product or service. Environmentally relevant resource consumption and emissions throughout this life cycle are quantified and the related potential impacts on a number of safeguard subjects (e.g. human health, natural environment, and natural resources) are estimated. It helps identify where improvements can be made in a product's life cycle and helps in designing new products. LCA is one of the methodologies the makes the Life Cycle Thinking (LCT) operational and is primarily used to compare the environmental load of various products, processes, or systems, and a particular product's different life cycle stages.

According to the definition provided in the ISO standards 14040 and14044 (ISO 2006 a,b), an LCA consists of four steps (Figure 2).

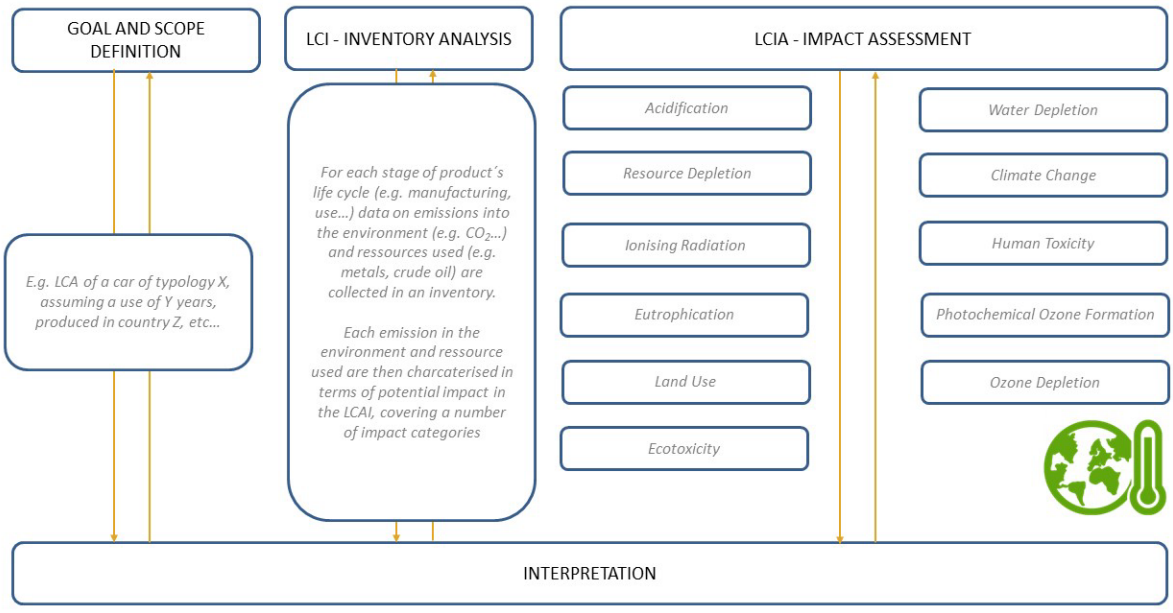

Figure 2: Framework for LCA. Based on ISO, 2006a and Sala et al (2016). The steps of the LCA comprise: goal and scope definition, life cycle inventory analysis, life cycle impact assessment and interpretation.

(i) Goal and scope definition

In this initial step, the actual planning of the study is accomplished. Starting from the objective/aim of the study, adequate system boundaries and the functional unit of the study, that is, the "quantified performance of a product system for use as a reference unit" (ISO, 2006a), are defined to meet the intended purpose of the LCA study. Theoretically, the system boundaries should include all economic processes required to achieve the system function, from cradle to grave (creation to disposal), which generally involves the following phases: extraction of energy and raw materials, manufacturing (infrastructure production, input products, product manufacturing), transportation (e.g., to the consumer), use phase (including maintenance), and the short- and long-term emissions and extractions associated with waste treatment. In this step, one establishes also what information is required, the level of specificity needed, and the best means to organize and present final results (Dicks & Hent, 2015).

(ii) Life cycle inventory (LCI) of extractions and emissions

The actual data collection is accomplished during the inventory analysis. That is, for each step within the system boundaries, its exchanges with nature (i.e., resource consumption/emissions into air, water, and soil) and with other process steps in the technosphere (i.e., energy and material inputs/waste output streams to further treatment) are identified and quantified. As stipulated in Finnveden et al. (2009), this step is "*often challenging due to the lack of appropriate data for the product system under study (e.g. for chemical production)*". Consequently, LCA experts including national authorities, private consultant companies, universities, public research organizations, or industry associations have created large inventory databases (e.g. EcoInvent) that facilitate the realization of the inventory and enable the practitioner to mostly focus on the core manufacturing processes, using existing data for the supply chain in a first screening.

(iii) Life cycle impact assessment (LCIA)

The impact assessment step provides indicators in the form of various LCIA factors and the basis for analyzing the potential contributions of the collected resource extractions and

wastes/emissions to a number of potential environmental impacts, such as climate change, toxicological stress, and land use (Rebitzer et al., 2004).

(iv) Interpretation and uncertainty

In the final interpretation phase of an LCA, the results of all the other steps are discussed to come to the conclusions, recommendations, and the decision-making in accordance with the objective of the study (ISO, 2006a). LCA is meant to be an iterative procedure, performed in at least two iterations. First, a screening should be performed, covering all LCA phases, to assess the orders of magnitude of emissions and related impacts. Then, focusing on the most damaging processes, emissions, and life cycle phases, a more detailed analysis should be carried out to improve the assessment quality. Finally, LCA only deals with the environmental impacts of a product, but sustainability also refers to economic and social aspects, which can be compared with and balanced against the environmental aspects.

I.2.3. Biocidal active substances and products as an example of "conventional chemicals": limitations in the application of RA and LCA approaches

In the European Union, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (EC, 2006) governs the assessment of potential risks from chemicals and their safe use. The REACH regulation requires chemicals to be registered by manufacturers or importers at the European Chemicals Agency (ECHA) before entering the European market. The registration dossiers contain a required standard data set of information on the chemicals. Registrants must provide information on intrinsic physical and chemical properties, fate and behaviour, hazards, exposures, and risks for their substance (details for these data requirements are described in Annexes VII to XI of the REACH regulation). In addition to the horizontal REACH regulation, products (i.e. articles and chemical mixtures and formulations) may be regulated through a number of thematic regulations, which may have specific policy targets. These include, amongst other, the already mentioned BPR and regulations on detergents (EC, 2004), and cosmetics (EC, 2009).

The BPR lays down the rules and procedures for approval of biocidal active substances and biocidal products: the aim of this regulation is to improve the internal market on biocidal products while ensuring a high level of both human and animal health and environmental protection. In order to achieve this objective a RA shall be carried out to determine the acceptability of risks associated with the components of the biocidal product or the substance (and its metabolites). In detail, the ECHA (2018) defines three main types of metabolites:

(i) Major metabolite as that formed in amounts of ≥ 10% of the active substance at any time of the degradation studies under consideration, or
the metabolite appears at two consecutive sampling points at amounts ≥ 5%, or
at the end of the study the maximum of formation is not yet reached but accounts for ≥ 5% of the active substance at the final time point.

(ii) Minor metabolite: metabolites that are not major metabolites.

(iii) Ecotoxicologically relevant metabolite: a metabolite which poses a higher or comparable hazard to any organism as the active substance.

The same guidance document also refers that: *In general, an environmental RA for the relevant compartments needs to be performed for all major metabolites. If there is any reason for concern, a RA also needs to be performed for those ecotoxicologically relevant metabolites which are minor metabolites […].*

Indeed, hazard/risk might be underestimated if chemical metabolites that are more toxic than the parent compound are neglected. Whereas metabolites generated during life cycle stages of biocidal products beyond the manufacturing stage are accounted for in the RA process for the authorization of new biocidal active substances, the BPR does not require to integrate results from LCA studies of biocidal products. However, LCA is considered an essential integrated environmental assessment in support to the EU policy making process and the ambition of many EU strategies such as e.g. the Circular Economy Action Plan (EU, 2022) and the Biodiversity Strategy (EPLCA, 2022; EU, 2022).

Given the importance of eco and human toxicity impact categories in LCA, complete and consistent life cycle inventory data are required for synthetic chemicals such as biocidal active

substances and their metabolites and transformation products when released to the environment throughout their life cycle.

I.3. Nanomaterials as emerging pollutants

According to EPA (2022), an "emerging contaminant" is a chemical or material that is characterized by a perceived, potential, or real threat to human health or the environment or a lack of published health standards. A contaminant also may be "emerging" because of the discovery of a new source or a new pathway to humans. Emerging pollutants can have their origin at the indoor or at the outdoor compartments.

While no definition has been internationally agreed upon (Miernicki et al., 2019), nanomaterials (NMs) are commonly defined as materials having at least one external or internal dimension between 1 and 100 nm. According to the Commission Recommendation (2011) "*NM means a natural, incidental or manufactured materials containing particles, in an unbound state or as an aggregate or agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm-100 nm*". Despite being often composed of known chemicals (e.g. metals, metal oxides or carbon structures), the small size of NMs can lead to behavioural differences in comparison with bulk materials, mostly related to NMs' very high surface-to-volume ratios, quantum effects (Roduner, 2006) and potential to cross biological borders due to their small size.

According to PEN (2020) sources of NMs can be classified into three main categories based on their origin:

(i) engineered nanomaterials (ENMs) defined as manufactured materials with engineered structure between approximately 1 nm and 100 nm

(ii) incidental nanomaterials (INMs) representing materials with a structure between approximately 1 nm and 100 nm that are produced as a by-product of a process of anthropogenic origin (such as welding fumes and diesel emission particulates) and

(iii) natural nanomaterials (NNMs) comprising materials with a structure between approximately 1 nm and 100 nm that are a result of natural processes (examples include particles arising from volcanic emissions, sea spray and atmospheric gas-to-particle conversion).

Additionally, nanoparticles were defined by EPA as particles with at least two dimensions between approximately 1 and 100 nm (2017). Based on such concept, Lespes et al. (2020) introduced the term anthropogenic nanoparticles (ANPs) resulting from human-related activities or processes (e.g., combustion), due to the life cycle of products containing nanoparticles or accidental releases.

In general terms, the stakeholders involved in the Nanotechnology value chain can be classified as (i) Nanotechnology suppliers including entities dealing with nano-research and nano-manufacturers and (ii) Nanotechnology users including entities using Nanotechnology in subsequent processes (in the present text referred to as Nanotechnology enabled applications or NEAs) and consumers of Nanotechnology enabled products (NEPs).

NEAs are used in a variety of industrial sectors including biomedical, diagnosis of diseases, therapeutics, agriculture and food, nanofertilizers, oil, gas, textile and cosmeceuticals and packaging (Singh, 2017). On its side, the number of NEPs, has increased significantly over the last decade: to the day of writing, the revised version of the Nanotechnology Consumer Product Inventory (CPI) by the Woodrow Wilson International Center for Scholars and the Project on Emerging Nanotechnologies (Vance et al., 2015) lists 1833 consumer products whereas the Nanodatabase (2022) accounts for 5224 NEPs.

As the NEAs and NEPs are expanding, unwanted and/or unanticipated exposure to ENMs is becoming inevitable. According to the Royal Academy of Chemistry (2004), one of the difficulties in determining potential future exposure to ENMs of the environment and humans is (i) the lack of information about both the extent to which they will be used in NEPs/NEAs and (ii) the likelihood of such ENMs being released from them in a form or quantity that might cause harm to humans or the environment.

Monitoring the release of ENMs will lead to the identification of their emission sources as emerging contaminants and relevant exposure pathways, and, ultimately, to generate a knowledge base for the prioritization (or not) of ENMs as hazardous chemicals to human health and/or the environment.

I.4. Airborne nanoparticles released throughout the life cycle of Nanotechnology enabled products (NEPs) and applications (NEAs) as potential source of exposure and/or hazard

NEPs and NEAs constitute potential sources of ENMs release and associated worker, consumer and environmental exposure. Current nanosafety research has dedicated large efforts into gaining knowledge on the biological effects of pristine (raw) manufactured NMs and how these may influence human health and the environment. In contrast, research aiming to improve our understanding of the possible exposure under a holistic life cycle perspective covering production, use and disposal of manufactured NMs released from NEPs is far less advanced.

In the present section examples of NEPs and NEAs representing possible sources of released airborne nanoparticles into the outdoor and indoor air (both in workplace and household compartments) are provided according to the current state of the art in the scientific literature. Manufactured NMs have been prioritized versus INMs, whereas NMNs have been intentionally disregarded. Insights are also provided concerning current standards and internationally recognized methodologies and the existing instrumentation for the characterization and measurement of airborne particles.

The information presented in the next sections will need to be updated as new applications and products derived from Nanotechnology are manufactured, used and disposed and as new regulations and standards for exposure assessment to ENMs are published.

I.4.1. Airborne ENMs released from NEPs and NEAs: exposure at the workplace, household and environmental compartments

ENMs (or heterogeneous particles comprising ENMs and the matrix in which those are embedded) can be released during NEP's use to the indoor or outdoor air in form of aerosols of variable size profile and composition. The release of ENMs can occur at any stage along the NEPs/NEAs life cycle. Figure 3 describes the possible sources of human exposure to airborne ENMs released from NEPs or NEAs.

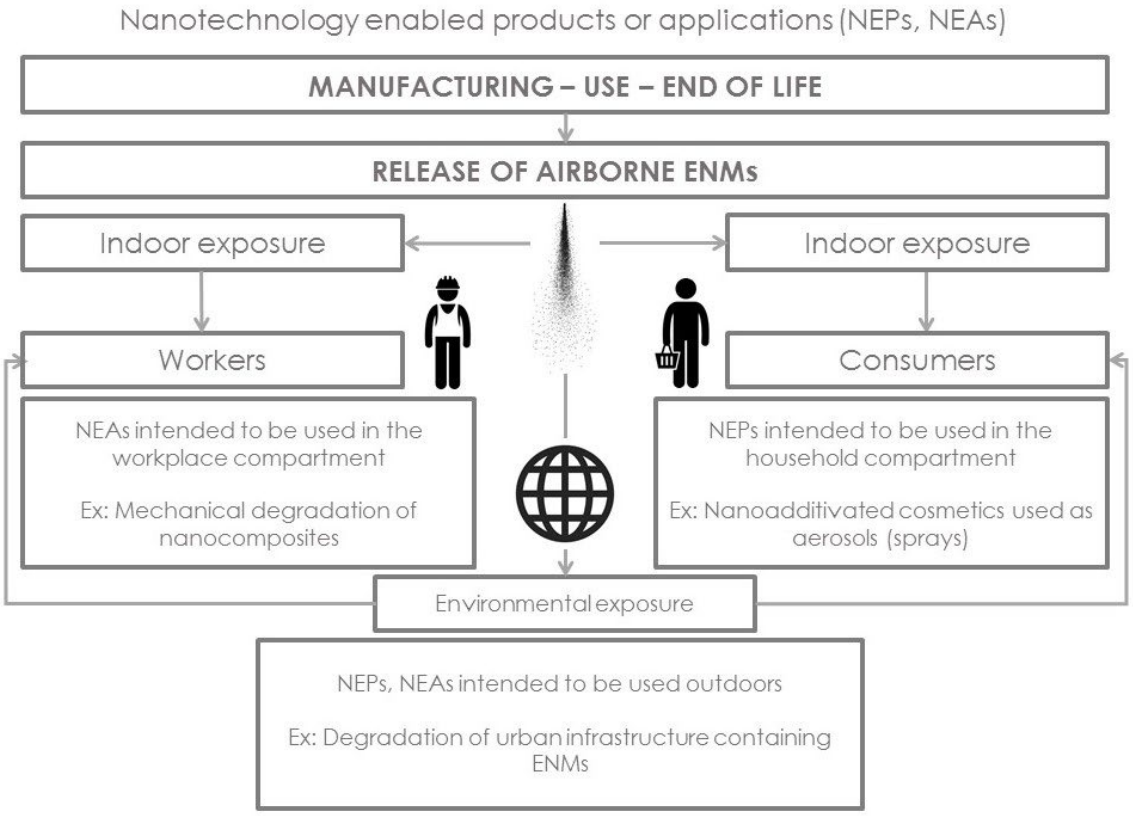

Figure 3: Main sources of human exposure to airborne ENMs released from NEPs. Examples (Ex) of each workplace, household and environmental exposure are given.

Release rates of aerosolized ENMs will vary depending on the specific type of NEP or application, its age and conditions of use. Determining the actual concentrations of ENMs to which human beings are exposed is a difficult task, because the released ENMs often undergo transformations that are not easily measured even in laboratory conditions. In particular,

dispersion forces might take place during release, transport along exposure routes, and during inhalation of agglomerated aerosol particles. Such forces ultimately lead to agglomerated particles breaking up into smaller agglomerates, or even primary particles (Li and Edwards, 1997) which implies a considerable challenge to quantitatively assessing the exposure to airborne released ENMs. Aside from particle size and concentrations, the forms of the ENM to which people may be exposed need to be studied and characterized.

I.4.1.1. Workplace exposure

It is generally thought that exposure of workers to ENMs during the production phase is unlikely because of the controlled conditions applied. However, Asbach (2015) refers that the highest exposure potential exists for workers in workplaces, where these materials are produced, used or handled. Among the routes of exposure, inhalation is the most common pathway for airborne ENPs in the workplace, and the most critical (Niu et al., 2015). In this section possible sources of exposure to professional nanotechnology users have been identified.

Of main concern are the emissions corresponding to NEPs/NEAs that are used in powder or aerosol forms in normal use conditions**.** One example is the recent work by West et al. (2019) measuring and characterizing occupational exposure to airborne nanoscale titanium dioxide (TiO_2) during airless spray painting and subsequent sanding of a nano-enabled paint. According to their findings, task-based exposure measurements collected during the initial airless spray application of the nano-enabled paint suggested a potential for worker exposures to exceed the time-weighted average exposure limit for ultrafine TiO_2 recommended by the National Institute for Occupational Safety and Health (NIOSH). In another study, Cooper et al. (2017) assessed the potential exposure to airborne zinc oxide (ZnO) nanoparticles during spray application and power sanding of a commercially available wood sealant. Authors reported Scanning Electron Microscope (SEM) images illustrating ZnO nanoparticles on the surface of larger airborne particles during spray application.

Concerning incidental workplace exposure, a number of reviews, e.g. Ding et al. (2017), evaluate the research studies addressing the release of aerosolized ENMs from solid polymer

nanocomposites. Some examples describing the assessment of airborne ENMs released incidentally to the indoor air in workplace environments throughout the different stages of the life cycle of solid polymer nanocomposites are provided next. The nanocomposites manufacturing stage, involving the use of ENMs as fillers, has been investigated by Tsai et al. (2008) who used a Fast Mobility Particle Spectrometer (FMPS) to evaluate the exposure to nanoparticles during twin-screw compounding for nanoalumina-containing nanocomposites. According to their results, compounding of nanocomposites easily released and diffused airborne particles of size less than 560 nm. Bello et al. (2009) investigated airborne exposures to nanoscale particles and fibres generated during dry and wet abrasive machining, representing post-manufacturing scenarios, of two three-phase advanced composite systems containing carbon nanotubes (CNTs), micron-diameter continuous fibres (carbon or alumina), and thermoset polymer matrices. Authors concluded that overall particle release levels, particle size distribution, and surface area of the released particles were not significantly different for composites with and without CNTs. Sachse et al. (2013) investigated different factors such as filler type and size and matrix materials on the particle emission from nano and micro reinforced glass fibre-polymer composites during low velocity impact test concluding that, in general, nano and ultrafine airborne particles were emitted from all investigated materials. Jensen et al. (2015) investigated the machining of carbon and glass fibre-reinforced epoxy composite materials at two facilities concluding that machining processes released particles primarily in < 100 nm size range. In relation to the end of life (EOL) stage, Raynor et al. (2012) investigated the potential release of engineered nanoparticles into the air during nanocomposite shredding in a recycling scenario. Test plaques made from polypropylene resin reinforced with either montmorillonite nanoclay or talc and reference polypropylene plaques with no reinforcing material were shredded by a granulator inside a test apparatus. Authors concluded that the particle levels produced during shredding polypropylene resin reinforced with either montmorillonite nanoclay or talc were both stable and lower than those found in some occupational environments and that fewer nanoparticles were generated from the nanocomposite plaques than when the plain resin plaques were shredded. In an incineration EOL scenario, Ounoughene et al. (2015) used nanoclay-based nanocomposites

(nylon-6 incorporating halloysite nanotubes, abbreviated as PA6/HNTs) manufactured at laboratory scale. They collected and characterized combustion residues and aerosol (particulate matter –PM- and gas phase) down-stream of the incinerator furnace concluding that HNTs transformed into other mineral structures which were found in both the aerosol and the residues. In a different study also addressing an incineration scenario, Bouillard et al. (2013) focused on acrylonitrile butadiene styrene (ABS) injection-moulded nanocomposites filled with 3% w/w of multi-walled carbon nanotubes (MWCNTs) that were incinerated at the laboratory scale. The measurement of the ENM fractions sampled in the gas phase revealed nanoemissions that originated from combustion of ABS/MWCNT comprising single as well as bundled CNT fibres in all investigated cases. The detected CNTs had a similar shape compared to their pristine equivalents. Authors of the study demonstrated that not all MWCNTs were combustible and thus can be dispersed in the gas phase although they are defined per se as combustible materials.

Whereas the release of airborne nanosized particles during the mechanical degradation (e.g. drilling) of polymer nanocomposites has been demonstrated, we have found inconclusive results regarding the incidental exposure of workers to freely released ENMs. Regarding EOL stages, complementary studies would be necessary selecting nanocomposites that have been manufactured beyond laboratory scale. Furthermore, without systematic experimental methods, it is challenging to understand how ENM additives modulate the potential nanoparticle release from solid polymeric matrixes when different stress factors (mechanical, thermal) are applied.

I.4.1.2. Consumer exposure

Exposure to ENMs via the respiratory tract is considered as the most likely situation that could lead to hazardous effects for the consumer (Hagendorfer et al., 2010). As in the case of worker exposure, NEPs representing a source of airborne consumer exposure to ENMs at the household compartment are classified in (i) emissions corresponding to NEPs that are used in powder or aerosol forms in normal use conditions and (ii) emissions corresponding to NEPs from which exposure to aerosolized ENMs takes place incidentally.

The first group includes sprays and cosmetic powders. The typical application of cosmetics results in dermal route exposure, but the process of applying cosmetic powders to the skin also results in the release of airborne nanosized particles (Lioy et al., 2010). Nazarenko et al. (2012) quantified exposures to airborne particles ranging from 14 nm to 20 μm due to the use of nanotechnology-based cosmetic powders. Losert et al. (2014) carried out a comprehensive review of the human exposure to conventional and nanotechnology-based sprays. According to their findings, nanoparticles present in the investigated sprays encompass metals and metal oxides such as ZnO, Ag, Cu, Ca, Mg, Zn, SiO_2, MgO and TiO_2, but also nanoparticulate fullerenes (C60) or silanes and siloxanes. However, one relevant conclusion of their study is that due to largely varying experimental setups, to date exposure values for nanosprays are difficult to compare. In this sense, the recent work by Pearce et al. (2019) reports a fully automated exposure platform to examine the aerosol properties of four aerosolized nano-enabled cosmetics using real-time monitoring and sampling instrumentation. Authors used a SEM coupled with energy dispersive x-ray spectroscopy (SEM-EDX) for the physico-chemical characterization of aerosols.

Concerning exposure to ENMs that have been released incidentally, possible sources include construction materials (paints, glass, ceramics, concrete…), printing inks or nanoadditivated textiles. A research study by Wohlleben et al. (2011) reported thermoplastic and cementitious nanocomposite materials being mechanically degraded via sanding. Authors detected surface-structured fragments (but no free CNTs) in a do it yourself sanding scenario on a sample of CNTs containing cement. Conversely, Hirth et al. (2013) concluded that at least 95 wt% of the CNT nanofillers from epoxy and cement composites remain embedded but also observed tubular protrusions corresponding to CNTs on their surface after sanding. Printer ink can also represent a source of ENMs: Pirela et al. (2014, 2015) referred that laser printer toner contains various organic and elemental carbons and metal/metal oxide ENMs, which become airborne and respirable during printing. The health effects of such emissions have been recently studied by Karrasch et al. (2017). Finally, ENMs including Ag, TiO_2 or ZnO, generally used as antimicrobials in textiles, will primarily reach consumers via dermal route. However,

airborne ENMs could incidentally release during abrasion processes or ironing but studies specifically addressing the present knowledge gap have not been traced.

The European Chemicals Agency (ECHA), aiming at assisting users in complying with obligations under the REACH Regulation, published specific guidelines for consumer exposure assessment as a section of the information requirements and chemicals safety assessment. Concerning inhalation exposure, guidelines refer that: "*for a Tier 1 evaluation, it is assumed that 100% of the substance in the consumer product or article will be released at once into the room and there is no ventilation*." Regarding nanomaterials the guidelines also refer that: "*if the product contains releasable nanomaterials then the assumption should be made that it is entirely within the respirable fraction if not otherwise known*" (ECHA, 2016). Still, standard operating protocols to test if an ENM incorporated into a NEP is or not "releasable" (during normal use or in incidental conditions) are lacking.

I.4.1.3. Environmental exposure

The different environmental compartments (air, water, soil and biota) represent an indirect source of human exposure to ENMs. Regarding airborne exposure, ultrafine particles with sizes up to 100 nm (or nanomaterials) are classified $PM_{0.1}$ in air pollution studies. Such classification does not discern within NNMs, INMs or ENMs.

Examples of NEPs potentially leading to human exposure via the respiratory route and having their origin in the environmental compartment are urban infrastructures including building façade paintings, photocatalytic concrete pavements or antireflection layers for road signs and pane. Mohajerani et al. (2019) analyzed the use of ENMs within the construction industry exemplifying their benefits in concrete, asphalt concrete, bricks, timber, and steel. For instance, Mubaraki et al. (2016) added nano-aluminium oxide (Al_2O_3) to asphalt cement concluding that a 5% inclusion of Al_2O_3 in the asphalt mix resulted in a strong resistance to high temperatures. Piccinno et al. (2012) estimated that currently 10-30% of TiO_2, 30% of ZnO, 5- 10% of CeO_2 and 10-30% of Ag ENMs produced worldwide are used in paints and coatings. Concerning release, Künniger et al. (2014) pointed out that large-scale, nano-enabled surfaces of urban buildings and other infrastructures are exposed to wind, rain, ice, and other variable weather

conditions, thus triggering ENM erosion that can lead to air/water transport and deposition of these ENMs into/onto soil, surface water, and impervious surfaces. Nano-additivated lubricants used in road traffic can also represent a source for the release of ENMs: cerium dioxide is used in a number of modes for the control of PM emissions and increase fuel economy from diesel engines. Dale et al. (2017) reported that the combustion process induced significant changes in the size and morphology of cerium oxide particles including, e.g., a volume increase by 2 orders of magnitude. Authors concluded that such changes will very likely alter the reactivity, transport, and/or ultimate fate of these particles in the environment in contrast to the particles as found in the additive. In a different application, if nanocomposites are used as a component of brake or tires, their erosion during use could potentially imply ENMs release. Nanoenabled agro-veterinary chemicals represent another example: though such chemicals primarily affect water and soil, they also represent a source of contamination for the air environmental compartment (Nascimento et al., 2017). Finally, industrial emissions including those of accidental nature (loss of containment; fire and explosions…) can also lead to the presence of ENMs in the environment: Broomfield et al. (2016) referred that the amount of ENMs released to the environment from production sites ranges from 0.7 to 1.6 % of which 6% is estimated to be emitted through air. However, a recent report from the Air Quality Expert Group on ultrafine particles in the UK (2018) refers that none of these processes is expected to give rise to appreciable atmospheric concentrations of nanoparticles, especially compared with the carbonaceous particles that are formed normally in combustion processes.

If released to the atmosphere, environmental factors including atmospheric temperature, relative humidity, and turbulence, govern the size distribution and particle number concentration of ENMs. Moreover, photochemically induced reactions mainly driven by free radicals and UV radiation also mediate the transformation of atmospheric ENMs (Abbas et al., 2020).

While some studies have addressed worker exposure to released ENMs in the outdoor compartment, e.g. in construction sites, we are not aware of any research study on the

secondary human exposure to airborne ENMs released from NEPs from the environmental air compartment.

I.4.2. International guidance and standards and instrumentation for airborne nanoparticle exposure assessment

There are a number of occupational safety agencies carrying out research initiatives related to nanoparticle exposure assessment. Some examples include the German Occupational Safety and Health authority (Institut fur Arbeitsschutz) (IFA, Germany); the French Agency for Food, Environmental and Occupational Health & Safety (L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), (ANSES, France); Safe Work Australia (SWA, Australia); the National Institute of Advanced Industrial Science and Technology (AIST, Japan); the Quebec Occupational Health and Safety Research Institute (Institute de recherche Robert-Sauvé en santé et en sécurité au travail), (IRSST, Canada) and the National Institute for Occupational Safety and Health, (NIOSH, USA).

State of the art initiatives in guidance for exposure assessment, standards and measurement instruments for the determination of airborne (nano-) particles are described next.

I.4.2.1. International guidance

In Europe, a harmonized tiered approach to measure and assess the potential exposure to ENMs and their agglomerates and aggregates at workplaces was elaborated by the Organisation for Economic Co-operation and Development (OECD) in 2015 (OECD, 2015). The methodology is not a Workplace Exposure Standard (WES) and does not provide any specific Occupational Exposure Limit (OEL), but is intended as a pragmatic guidance. In October 2017, a new report on strategies, techniques and sampling protocols for determining the concentration of ENMs in air at the workplace was published (OECD, 2017a). Complementarily, the OECD published in 2010 a compilation and comparison of guidelines related to exposure to nanomaterials exclusively addressing laboratories (OECD; 2010).

The description of the three-tiered assessment process to conduct the evaluation of the exposure by inhalation (OECD, 2015) involves:

• Tier 1. Information gathering to determine potential sources of release of ENMs. Involves a standard industrial hygiene survey of the process area and is predominantly focused on gathering qualitative information, with some quantitative measurement, to identify likely points of particle emission relative to the background.

• Tier 2. Assessment of the release of ENMs into the workplace air form identified sources using direct reading instruments (DRI) for particle number and mass concentration such as Concentration Particle Counters (CPCs), which enable the assessment of particle number concentration in the emission sources, worker's breathing zone (BZ) exposure, incidental and background particles.

• Tier 3. When a release of ENMs has been observed, additional information may need to be determined on whether or not exposure to ENMs can be excluded. In this step, appropriate equipment beyond easy-to-use particle counters must be employed including sampling methods for off-line analysis of particle morphology, chemical composition, and mass or fibre concentration and compared with measurement by real-time instruments. This step generally involves the use of a Scanning Mobility Particle Sizer (SMPS) to assess the particle size distribution, as well as filter based sampling (i.e., elemental mass analysis and particle morphology) in the worker's BZ.

Results from either Tier 2 or Tier 3, or both, can be compared with particle control values for decision-making to assess workplace exposure.

Additional initiatives exist at international level. The NIOSH, for instance, published in 2016 a refined version of the earlier published Nanoparticle Emission Assessment Technique (NEAT 2.0) (Eastlake et al., 2016) comprising four main steps: (i) collection of basic workplace information; (ii) design and implement the sampling plan; (iii) risk assessment and (iv) risk management. NEAT 2.0 places a stronger emphasis in the use of tandem off-line filter-based sampling over the use of direct reading instruments. According to the method, one of such samples should be ideally be used in electron microscopy analyses and another one should be used for elemental mass quantification. These samples are collected in the worker's BZ (samples related to full shift and task specific can be collected), and in a background (far field)

area. The selected background area should be away from the task or processes evaluated and on a different ventilation system.

Both the OECD tiered approach and NEAT 2.0 methods rely on pre-assessment and final confirmation steps, but differ in recommended approaches. When comparing both guidelines, Eastlake et al. (2020) concluded that within the OECD approach, discussion regarding exposure assessment is based on the collection of airborne data from DRIs with the Tier 3 investigation triggered when the difference of the concentrations between background and process data is more than three times the standard deviation for the background. However, there is currently no consensus method on how to statistically analyze and report DRI data. In the NEAT 2.0 method, integrated filter-based sampling is the key step in the exposure assessment process. Subsequent analysis of these samples can confirm the presence or absence of the ENM of interest. As DRIs are unable to effectively identifying the presence or type of ENMs, they are used to support the integrated filter-based results, identify emission sources, and verify the efficacy of engineering controls.

Complementarily, the OECD (2020) compiled the existing risk assessment tools, frameworks and initiatives developed for Safe(r)-by-Design ENMs and NEPS in a report included within its *Series on the Safety of Manufactured Nanomaterials*. Amongst other, this report describes some of the tools developed within EU funded projects that cover exposure assessment to consumers such as LICARA NanoScan (EMPA, 2022), the GUIDEnano tool (GUIDEnano 3.0, 2022) or the Sustainable Nanotechnologies Project Decision Support System (Sunds, 2022). Furthermore, the OECD published three additional documents framed within its *Series on Testing and Assessment* aimed at the evaluation of tools and models for assessment occupational and consumer exposure to manufactured nanomaterials that included (i) a compilation of tools/models and analysis for further evaluation (OECD, 2021a); (ii) performance testing results for occupational exposure tools/models (OECD, 2021b) and performance testing results for consumer exposure/tools models (OECD, 2021c). To date, most of the evaluations with regard to consumers have assessed the performance of models/tools for (quantitative) exposure assessment to ENMs in spray scenarios.

I.4.2.2. Standards

Now twelve years ago the European Commission addressed Mandate M/461 E to the European Committee for Standardization (CEN), the European Committee for Electrotechnical Standardization (CENELEC) and the European Telecommunications Standards Institute (ETSI) for standardization activities regarding nanotechnologies and nanomaterials (EC, 2010). In particular, such mandate refers to a standard method to assess emissions from handling or machining of nanomaterial containing product. Although such standard is not yet available, recent standards such as the CEN: EN 17199-1:2019 for workplace exposure on the measurement of dustiness of bulk materials that contain or release respirable nano-objects and their aggregates and agglomerates (NOAAs) and other respirable particles, represent a significant contribution in the standardization field.

Complementarily, the ISO/TC 229 has published some standards for occupational exposure assessment as listed in Table 2.

Table 2: Standards for occupational exposure assessment (in chronological order, non-exhaustive list)

Standard reference	Title	Contents
ISO/TR 27628:2007	Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment	Contains guidelines on characterizing occupational nanoaerosol exposures and represents the current state-of-the-art, with an emphasis on nanometre-diameter particles, developed by the Technical Committee: ISO/TC 146/SC 2 Workplace atmospheres.
ISO 10808:2010 Nanotechnologies	Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing	Specifies requirements for, and gives guidance on, the characterization of airborne nanoparticles in inhalation exposure chambers for the purpose of inhalation toxicity studies in terms of particle mass, size distribution, number concentration and composition
ISO/TS 12901-2012	Nanotechnologies — Occupational risk management applied to engineered nanomaterials	Provides guidance on occupational health and safety measures relating to ENMs, including the use of engineering controls and appropriate personal protective equipment, guidance on dealing with spills and accidental releases, and guidance on appropriate handling of these materials during disposal
ISO/TR 18637:2016	Nanotechnologies — Overview of available frameworks for the development of occupational exposure limits and bands for NOAAs	Provides an overview of available methods and procedures for the development of occupational exposure limits (OELs) and occupational exposure bands (OEBs) for manufactured NOAAs for use in occupational health risk management decision-making.
ISO/TR 12885:2018	Nanotechnologies — Health and safety practices in occupational settings	This document focuses on the occupational manufacture and use of manufactured nano-objects, and their aggregates and agglomerates greater than 100 nm.

Under the REACH Regulation, companies must provide by 1st January 2020 complementary information on ENMs on the EU market. Such information requirements include physico-chemical properties such as dustiness. However, the dustiness study does not need to be conducted if exposure to the granular form of the substance during its life cycle can be excluded or, in other words, if companies can demonstrate that airborne nanoparticles are not generated and ENM release does not take place (EC, 2018). From the preliminary assessment of currently existing ISO standards and international guidelines we can conclude that the primary focus has been placed on the manufacture and use of ENMs in workplaces whereas exposure from a life cycle perspective has not been addressed. We have not identified standards addressing potential consumer uses and associated exposure to ENMs at all.

I.4.3. Instrumentation for exposure assessment to airborne nanoparticles

This section briefly covers the currently used instruments for the determination of airborne (nano) particles. Not all of the instruments reach the true nanoparticle size range (<100 nm), especially the optical devices, but all can be used during studies of NOAA. It is also noticed that many of the instruments used to detect and measure ENMs are non-specific, i.e. they cannot distinguish ENMs, INMs or NNMs. ENMs release (and associated exposure) requires microscopical/chemical analysis of the released particles in order to determine if and in what form nanomaterials are released.

Data presented have been classified as on-line measuring instruments (stationary in Table 3 and portable in Table 4) and sampling devices for particles (Table 5). For the on-line instruments, the equivalent diameter is indicated in the column of the possible metrics. It must be noted that not all available instruments are listed: in some cases only examples are provided for a class of instruments like the CPC. There are also often different configurations or models of instruments of the same kind available that allow e.g. changing the measurement size range. Measurement instruments have been ordered from lower to upper particle size range.

Table 3: Stationary Instruments. Abbreviations used include: CPC - Condensation particle counter (Instrument that detects particles and that can be used to calculate particle number concentration given the known flow rates into the detector); NC: Number concentration; dp: particle diameter and/or particle number size distribution; n.a.: Not available; TB: Tracheo-Bronchial; A: Alveolar; LDSA: Lung deposited surface area

Measurement principles	Name of instrument Manufacturer	Particle size range (nm)	Concentration range	Metric	Sample flow (lpm)	Time resolution (s)
Ion Mobility + Electrical mobility	Drift Tube Ion Mobility Spectrometer (DTIMS 3006) Kanomax	2 - > 40	1 – 10^5 #/cm³	NC / dp	0.6 – 1.5 (Depending on the particle counter flow, values for fast CPC, model 3650)	60
Electrical mobility	SMPS 3080 (DMA 3081 & 3085) TSI Inc.	2 - 150 10 – 1,000	10^8 #/cm³	NC / dp (electrical mobility diameter)	0 - 3 (depending on particle counter flow)	>120
	DMS500 Cambustion	5-2,500	n.a. (depending on size channel)	NC, dp (electrical mobility diameter)	8	0.1
	U-SMPS 2050 X / 2100 X / 2200 PALAS	8 – 1,200	n.a. (depending on size channel)	NC / dp (electrical mobility diameter)	2.5 – 14	30
	MiniWRAS 1371 Grimm Aerosoltechnik	10 – 35,000	3,000 – 5*10^5 #/cm³ 0 – 3*10^6 #/cm³	NC / dp (electrical mobility diameter and scattered-light diameter)	1.2	60 (electrical) 6 (optical)
	FMPS 3091 TSI Inc.	5.6 - 560	n.a. (depending on size channel)	NC / dp (electrical mobility diameter)	10	1
Particle Condensation	CPC 3750 TSI Inc.	7 - > 3,000	1*10^5 #/cm³	NC	1.0 +- 0.05	1
	CPC 3789 TSI Inc	2.2 / 7 - > 3,000	2*10^5 #/cm³	NC	0.6 / 1.5 / 2.5	1

Measurement principles	Name of instrument Manufacturer	Particle size range (nm)	Concentration range	Metric	Sample flow (lpm)	Time resolution (s)
	GRIMM 5410	4 - > 3,000	$1*10^7$ #/cm³	NC	0.3 / 0.6	3
	UF-CPC 50 PALAS	4 – 10,000	10^4 - 10^7 #/cm3	NC	0.3 – 1 l/min	2
Electrical mobility + inertial size classification	ELPI+ Dekati Ltd.	6-10,000	n.a. (depending on size channel)	NC, dp (aerodynamic diameter)	10	0.1
Electrical diffusion chargers	Aerotrak 9000 TSI Inc.	10 – 1,000	TB: 0 – 2500 µm²/cm³ A: 0 – 10,000 µm²/cm³	LDSA	2.5 / 1.5 / 1	1
	NSAM 3550 TSI Inc.	10 – 1,000	TB: 0 – 2500 µm²/cm³ A: 0 – 10000 µm²/cm³	LDSA	2.5	1
	Welas digital 1000 PALAS GmbH	120 – 3,500 200 – 10,000 300 – 17,000 600 – 40,000	0 – $5*10^5$ #/cm³	NC / dp (scattered-light diameter)	5 / 1.6	> 0.01
Light scattering	OPC 3330 TSI Inc.	300-10,000	4000	NC, dp (scattered-light diameter)	1	1
Light scattering (Time-of-flight)	APS 3321 TSI Inc.	500-20,000	0 – 10000 #/cm³	NC, dp (aerodynamic diameter)	5 (1 if sheath flow is separated)	1

Table 4: Portable Instruments. Abbreviations used include: BC: Black Carbon; d_p: particle diameter and/or particle number size distribution; LDSA: Lung deposited surface area; NC: Number concentration; TSP: Total suspended particles.

Measurement principles	Name of instrument Manufacturer	Particle size range (nm)	Concentration range	Metric	Sample flow (lpm)	Time resolution (s)
Diffusion charge	DISCmini Testo	10 – 300	10^3 - 10^6 #/cm³	NC/dp/LDSA	1	1
	NanoMonitor Oxility	20 – 120 / 10 – 300 (depending on operation mode)	0 – 10^6 #/cm³	NC/dp/LDSA (fast mode only NC)	0.3 – 0.4	3 / 16 (depending on operation mode)
	Partector NANEOS	10 – 10,000	0 – $2*10^4$ #/cm³	LDSA	0.5	1
	Partector 2 NANENOS	20 – 150 (fixed deposition voltage) 10 – 300 (adaptive deposition voltage)	0 – 10^6 #/cm³	NC/dp/LDSA	0.5	1
Condensation	PUFP C100 // C200 Enmont	> 4.5	0 – $2*10^5$ #/cm³	NC	0.3	1
	CPC 3007 TSI Inc.	10 - >1,000	0 - 10^5 #/cm³	NC	0.7	1
	Magic CPC AD	5 - >2,500	0 - 10^5 #/cm³	NC	0.7	1
	NanoWatcher Controlnano	30 - >2,500	0 - 10^6 #/cm³	NC	0.3	1
Change in absorption of transmitted light	MicroAeth AE51 AETHLABS	-	0 – 1 mg BC/m³	BC concentration	0.05/0.1/0.15/0.2	1/10/30/60/300
Light scattering	FIDAS Frog PALAS GmbH	150 – 18,000 / 150 - 93,000	0 – $2*10^5$ #/cm³	PM1, PM2.5, PM4, PM10, TSP. Particle size distribution within size range 0.18 – 100 µm	1.4	1
Electrical mobility	NanoScan SMPS Nanoparticle sizer 3910 TSI Inc.	10 - 420	< $1*10^6$ #/cm³	NC /dp	0.8	60 (1 in single size mode)

Table 5: Sampling Devices. Abbreviations used include: GC: Glassy carbon; TEM: Transmission Electron Microscopy, n.a.: Not available.

Name of instrument Manufacturer	Particle size range (nm)	Sample flow (lpm)	Substrate	Portable
ELPI+ Dekati Ltd.	6-10,000	10	Aluminium or polycarbonate substrates (25 mm)	No
PARTICLEVER Sampler ALCEN	10 – 4.000	1	Polycarbonate track-etched membrane filter quartz filter	Yes
TEM Partector NANEOS	10 – 10,000	0.45	TEM grid	Yes
ESPnano 100 ESPnano	20 nm – supermicron range	0.1	TEM grid metallic/silicon substrate	Yes
TPS100 RJ Lee Group Colorado State University	20 - 600	0.005	Nickel TEM grid	Yes
NRD Zefon International	< 300	2.5	Nylon mesh screens	Yes
miniMOUDI 135 TSI Inc. (10 stage device)	Cut point diameters 56 – 10,000	2	Aluminium foil/ thin plastic films/ membrane filters (37 mm)	No (Yes for 6 and 8 stage device, but higher cut-off diameter)
Quartz crystal microbalance QCM MOUDI	Cut points of 960, 510, 305, 156, 74 and 45 nm	10	Quartz crystal (QMC)	No

As indicated earlier, in addition to the concentration, the forms of the ENM to which people may be exposed need to be characterized. In fact, it has been suggested that ENM toxicity is closely related to surface area and activity, particle number, fiber aspect rather than mass dose (Mark, 2007; Harford et al., 2007). Unfortunately, a thorough evaluation of pertinent ENM properties cannot be obtained using a single instrument or analytical technique (Eastlake et al., 2020). Rather, collection and characterization of released ENMs should be performed via a multifaceted approach involving the use of multiple complementary sampling tools and analytical methods.

In conclusion, a variety of NEPs and NEAs leading to direct human exposure to released ENMs via respiratory route or indirect exposure through outdoor air in the environmental compartment have been reviewed. In the workplace environment, human exposure to free ENMs has not been confirmed for nanocomposites. However, the exposure to aerosolized ENMs-containing fragments of variable size (nano to micron) and composition can take place. Both professional users and consumers of NEPs that are used as aerosols (e.g., nanoenabled paints or cosmetics, respectively) can be exposed to ENMs entering the respiratory tract. Incidental consumer exposure can also take place in the form of aerosolized ENMs-containing matrixes of different size distribution and composition. Regarding the environmental exposure, the outdoor air compartment does not represent a major source of human exposure to airborne ENMs released from NEPs. Standardization bodies have developed a number of standards in the Nanotechnology area; however, internationally recognized protocols to assess the releasability of ENMs and associated human or environmental exposure are lacking. In the area of occupational exposure two main guidelines have been developed: NEAT 2.0 and the OECD tiered approach. A combination of commercially available examples of stationary and portable instrumentation for aerosol particle measurement and aerosol sampling devices for off-line analyses is required for proper exposure assessment and, more precisely, for nano-release assessment.

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II. STATE OF THE ART, HYPOTHESIS AND OBJECTIVES

II.1. State of the art

In 2004, The Royal Society and Royal Academy of Engineering recommended that "*as an integral part of the innovation and design process of products and materials containing nanoparticles or nanotubes, industry should assess the risk of release of these components throughout the life cycle of the product and make this information available to the relevant regulatory authorities*". The same institution anticipated a "*potential low exposure from composites containing nanoparticles and nanotubes, since these typically make up a very small fraction of the final product and the functionality of the material relies on them being retained*" but pointed out the need to test this assumption. However, this might be different in the case of free nanoparticles containing products, such as e.g. cosmetics.

For the implementation of life cycle oriented approaches in the exposure and hazard assessment of NEPs/NEAs, the initial step is to evaluate if ENMs release from the products containing them in different scenarios representing their different life cycle stages with main relevance of those stages where most release can be anticipated. From this assessment three possible outcomes can take place:

- Embedded ENMs do not release throughout product's life cycle and therefore consumer and/or environmental exposure does not take place;
- ENMs are released in free state and the released ENMs can be assimilated to the pristine ENMs upon physico-chemical characterization;
- ENMs are released but differ when compared to pristine ENMs as they have been substantially modified as a result of the NEP/NEA manufacturing process and/or of the different processes responsible for their release in consumer use and/or EOL stages. Those can include dissolution, enzymatic hydrolysis, photodegradation, thermal decomposition or mechanical wear, sometimes in a combined manner.

The three above are possible throughout the different stages of the product's life cycle in normal or accidental conditions and will depend both on the specific NEP/NEA under consideration and also on the specific life cycle process originating (or not) such release.

To date, nano-release assessment from NEPs/NEAs has different constraints that include:

(i) limited standard operating protocols and methodological approaches in order to assess the release of ENMs from NEPs/NEAs, rendering the comparison of the outcomes of the research studies carried out to date a significant challenge;

(ii) depending on the specific scenario and the NEP/NEA under consideration, the quantity of released material in use and EOL simulating approaches is generally low and does not always meet minimum quantity requirements for some characterization techniques and/or for the assessment of the effects associated to released materials through standard toxicity tests;

(iii) difficulties associated to the collection and storage of the released materials. Since direct observation of the physico-chemical profile and/or effects associated to released materials immediately upon their generation is complex, those need to be collected and stored. This implies that released materials might undergo potential changes during these steps potentially interfering with the results of their physico-chemical characterization and/or with the assessment of their (eco)toxicological effects.

In order to enable consumers making informed decisions, industrial stakeholders will need to inform on the presence of ENMs within their products and to demonstrate whether their release takes place or not. Further, if ENMs release takes place, industry will need to demonstrate whether the physico-chemical profile and (eco)toxicological potential of such released materials is comparable to those of the pristine ENMs or if they have been modified throughout different life cycle stages and to what extent. A series of methodological and technical constraints must be overcome in order to comply with the present requirements.

II.2. Hypothesis

A life cycle oriented approach is needed when assessing the risk of both conventional and emerging chemicals hereby represented by biocides and ENMs. The different processes that NEP/NEAs undergo throughout their life cycle -manufacturing, use and EOL- lead to the release of ENMs that, under certain circumstances, have an associated risk towards human health and/or the environment.

II.3. Research objectives

The main objective of the present thesis proposal is to increase the currently existing knowledge in relation to the impacts of conventional and emerging pollutants in life cycle stages beyond manufacturing. We aim at assessing and comparing the ecotoxicological effects of biocides and their corresponding metabolites, degradation and transformation products formed in the aquatic compartment during their use and EOL stages. In the case of emerging pollutants, we aim at evaluating the potential release of ENMs from NEPs/NEAs and, whenever feasible, at deriving characterization factors in order to account for the human respiratory and ecotoxicological impacts of the released forms of ENMs in the Life Cycle Impact Assessment phase of the internationally recognized ISO framework for life cycle assessment (LCA) (ISO 14040:2006). The goal is to follow a holistic, life cycle perspective when the safety of chemicals is considered.

In order to achieve such main target, the next specific objectives have been defined:

- To evaluate the need to implement life cycle oriented approaches in the (eco)toxicological impact assessment of hazardous chemicals, such as biocides, which implies considering life cycle stages beyond manufacturing, ie, targeting metabolites, degradation and transformation products generated during use and EOL by determining the acute toxicological effects of parent biocidal active substances towards trophic levels representative of the freshwater environmental compartment and compare those to their environmentally relevant metabolites.
- To identify different scenarios representing the use and/or EOL phases in the workplace or household compartment of NEPs of different nature for the

implementation of a life cycle oriented approach in the assessment of hazards corresponding to ENMs.

- To simulate the identified scenarios in confined conditions and to characterize the airborne emissions taking place comparing the reference non nano-additivated sample and the NEP in the following two case studies:

  (i) Polypropylene based nanocomposite with potential applications in the automotive sector incorporating different nanosized fillers including Wollastonite (WO) and Montmorillonite (MTT) during 3 different mechanical degradation processes.

  (ii) Cadmium telluride quantum dots (CdTe QDs) containing inkjet printing ink during household printing simulating the use phase.

- To simulate an acute exposure regime to aerosolized CdTe QDs ink as an accidental, worse-case scenario and to use the human pulmonary cell line BEAS-2B cultured at the air liquid interface (ALI) as a model in order to assess the potential health effect of airborne emissions of the nano-additivated ink.

- To integrate the outcomes of the assessments carried out within the traditional framework for LCA, converting the (eco)toxicological effect of the released forms of CdTe QDs into characterization factors (CFs).

Each of these objectives has been addressed in the *Results and Discussion* section of the thesis, in *Chapters 1-4*. Further, all the results obtained have been compiled in the *General Discussion* section and a general approach to integrate the life cycle thinking perspective in the exposure and impact assessment for ENMs embedded into NEPs and NEAS, as emerging pollutants has been proposed.

III. RESULTS

CHAPTER 1. Acute hazard of biocides for the aquatic environmental compartment from a life-cycle perspective

CHAPTER 1. Acute hazard of biocides for the aquatic environmental compartment from a life-cycle perspective

This chapter has been published as:

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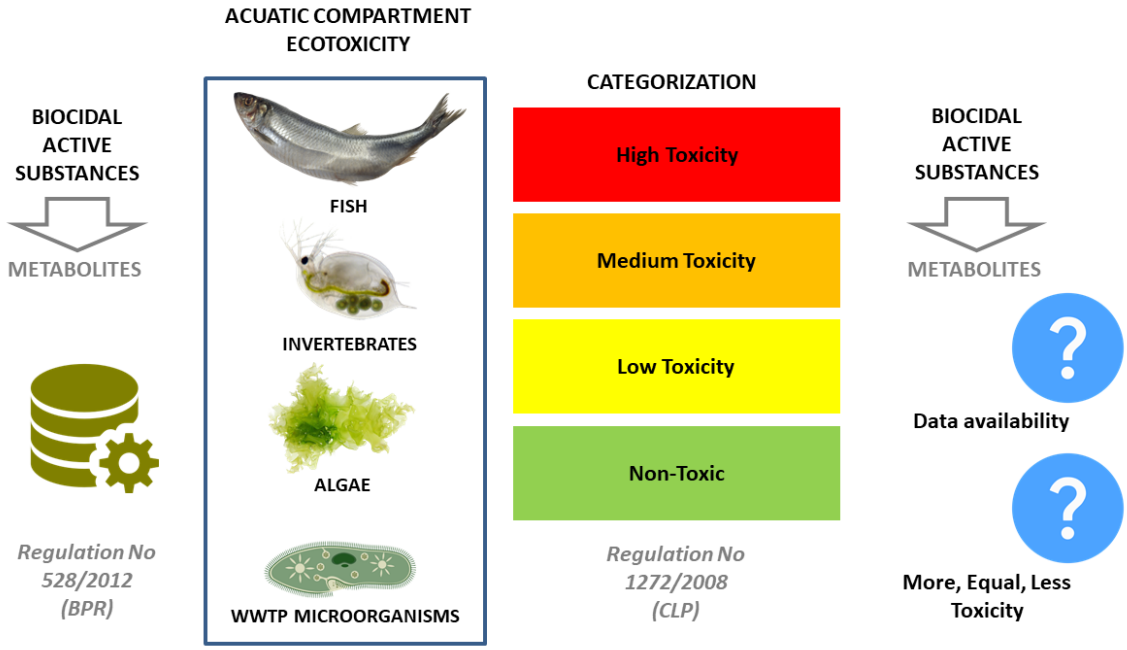
- SETAC Europe 28th Annual Meeting. Rome (Italy) 2018/05/13-17.

Poster communication: Hernández-Moreno, D.; **Blázquez, M.**; Andreu-Sánchez, O.; Bermejo-Nogales, A.; Fernández-Cruz, M.L. Hazard evaluation of biocides and its metabolites for the aquatic compartment.

GRAPHICAL ABSTRACT



ABSTRACT

One of the aims of the European project LIFE-COMBASE is to build a computational tool to predict the acute toxicity for aquatic organisms of biocidal active substances and its environmental degradation products. Within this context, a database was implemented compiling toxicity data for these substances in organisms pertaining to the freshwater/marine and sewage treatment plant (STP) compartments. The goal of this study is to analyze the compiled data to identify the possible hazard of these compounds for the aquatic compartments. Several official and scientific databases were consulted. Data from 196 biocidal substances and 206 environmental metabolites were collected for the taxonomic groups, including fish, invertebrates, algae and STP microorganisms. Substances were categorized for their toxicity in four groups, considering values of $L(E)C_{50}$, according to EU Regulation (EC) No 1272/2008. More than 50% of the parent compounds were located in category 1 ($L(E)C_{50} \leq 1$mg/L) for fish, invertebrates and algae, indicating a high toxicity for the freshwater/marine compartments. However, more than 60% were not toxic for STP microorganisms. Metabolites were mainly less toxic than the parent compounds, but 22–36% presented the same toxicity and ~6% were more toxic. No toxicological information was found for ~50% of the metabolites for fish, invertebrates and algae, reaching the 96% in the case of microorganisms. In addition, information on toxicity to the STP microorganisms was only found for 40% of the parent compounds. The high percentage of toxic metabolites and the scarcity of data for these compounds indicate the need to further study their impact in the aquatic compartments.

KEY WORDS

Biocide; Metabolite; Aquatic compartment; Toxicity category

RESUMEN

Uno de los objetivos del proyecto europeo LIFE-COMBASE es desarrollar una herramienta computacional para predecir la toxicidad aguda para los organismos acuáticos derivada de la exposición a sustancias activas biocidas y sus productos de degradación de relevancia ambiental. En este contexto, se desarrolló una base de datos que recopila datos de

toxicidad para estas sustancias en organismos pertenecientes a los compartimentos de agua dulce/marina y microorganismos de plantas de tratamiento de aguas residuales. La finalidad de este estudio es analizar los datos recopilados para identificar el posible peligro de estos compuestos para los compartimentos acuáticos. Se consultaron varias bases de datos oficiales y científicas. Se recopilaron datos de 196 sustancias biocidas y 206 metabolitos ambientales para los siguientes grupos taxonómicos: peces, invertebrados, algas y microorganismos de plantas de tratamiento de aguas residuales. Los compuestos se clasificaron por su toxicidad en cuatro grupos, considerando sus valores de $L(E)C_{50}$, de acuerdo con el Reglamento de la UE (CE) No 1272/2008. Más del 50% de las sustancias activas biocidas se ubicaron en la categoría 1 ($L(E)C_{50} \leq 1$ mg/L) para peces, invertebrados y algas, lo que indica una alta toxicidad para los compartimentos de agua dulce/marina. Sin embargo, más del 60% no resultaron tóxicos para los microorganismos de plantas de tratamiento de aguas residuales. Los metabolitos fueron mayormente menos tóxicos que las sustancias activas biocidas, pero entre el 22 y el 36% presentaron la misma toxicidad y un ~ 6% resultó más tóxico. No se encontró información toxicológica para ~ 50% de los metabolitos en relación a peces, invertebrados y algas, llegando al 96% en el caso de los microorganismos. Además, solo se encontró información sobre la toxicidad para los microorganismos de plantas de tratamiento de aguas residuales para el 40% de las sustancias activas biocidas. El alto porcentaje de metabolitos tóxicos y la escasez de datos sobre estos compuestos indican la necesidad de estudiar más a fondo su impacto en los compartimentos acuáticos.

PALABRAS CLAVE

Biocida; Metabolito; Compartimento acuático; Categoría de toxicidad

LABURPENA

LIFE-COMBASE europar proiektuaren helburuetako bat tresna konputazional bat garatzea da, substantzia aktibo biozida eta beren ingurumenari dagokionez degradazio produktu garrantzitsuen esposiziotik eratorritako toxikotasun akutua iragartzea uretako organismoetan. Testuinguru horretan, substantzia horien toxikotasun-datuak biltzen

dituen databasea garatu da ur gezako/itsasoko konpartimentuetako organismoetan, baita hondakin-urak arazteko instalazioetako mikroorganismoetan ere. Ikerketa honen xedea da bildutako datuak aztertzea, konposatu horiek uretako konpartimentuetan izan dezaketen arriskua identifikatzeko. Hainbat datu-base ofizial eta zientifiko kontsultatu ziren, eta 196 talde biozidari eta inguruneko 206 metabolitori buruzko datuak bildu ziren talde taxonomiko hauetarako: arrainak, ornogabeak, algak, eta araztegietako mikroorganismoak, besteak beste. Konposatuak lau taldetan sailkatu ziren toxikotasunaren arabera, L(E)C_{50} balioak eta 1272/2008 EBko (CE) araudia kontuan hartuta. Substantzia aktibo bioziden % 50 baino gehiago 1. kategorian (L(E)C_{50} ≤ 1mg/L) kokatu ziren arrain, ornogabe et algen kasuetarako ur gezako/itsasoko konpartimentuetarako toxikotasun altua adierazten duena. Bestalde, % 60 ez ziren toxikoak hondakin-uren araztegietako mikroorganismoentzat. Metabolitoek, oro har, toxikotasun txikiagoa zuten substantzia aktibo biozidek baino, baina % 22-36k toxikotasun bera zuten, eta % 6k, toxikotasun handiagoa. Metabolitoen % 50ean ez da informazio toxikologikorik aurkitu arrainen, ornogabeen eta algen kasuan, eta % 96ra iritsi da mikroorganismoen kasuan. Gainera, araztegietako mikroorganismoekiko toxikotasunari buruzko informazioa substantzia aktibo bioziden % 40rako bakarrik aurkitu da. Metabolito toxikoen portzentai handiak eta konposatu horiei buruzko datuen urritasunak adierazten dute horien eragina uretako konpartimentuetan gehiago aztertzeko beharra.

HITZ GAKOAK

Biozida; Metabolitoa; Uretako konpartimentua; Toxikotasunaren kategoria

1. Introduction

One of the goals of the European project LIFE-COMBASE (www.lifecombase.com) is to build a computational tool to predict the acute toxicity for aquatic organisms of biocidal active substances and their environmental degradation products, to be used mainly for regulatory purposes. Biocides are chemical products employed for eliminating or preventing the action of harmful organisms. Several are their potential applications, including hospital hygiene maintenance, agriculture, disinfecting and preserving quality. During past decades, a wide variety of bioactive organic chemicals have been developed for disinfection, sterilization, and preservation purposes, including quaternary ammonium compounds, alcoholic and phenolic compounds, aldehydes, halogen-containing compounds, quinoline and isoquinoline derivatives, heterocyclic compounds, and peroxygens. Biocides act as poisons or inhibitory agents against a wide range of target organisms, from microbes to plants and vertebrates (Kahkonen et al., 2010; Kahrilas et al., 2015). However, the potentially beneficial effect of biocides has been recognized to be extended beyond the target organisms, with resulting undesired adverse effects to man and the environment. For instance, it is possible to find resistance from bacteria against disinfectants used in hospitals, whereas it has frequently been found non-target harming (Rasmussen et al., 1999). According to the EU Biocides Regulation 528/ 2012 (BPR), biocides can be divided into four main groups (MG) covering disinfectants (MG1), preservatives (MG2), pests' control (MG3) and special biocides (MG4) such as antifouling products and embalming and taxidermy fluids. These four groups are sub-divided into 22 different biocidal product types (PT), depending on the specific uses of the biocide (Table A1, supplementary information). The list of active substance/PT combinations for which an application for approval has been submitted under Directive 98/8/EC (BPD) or Regulation (EU) No 528/2012 (BPR), summarize “existing” active substances already included in the Review Programme and “new” active substances. There are also listed those substances already “approved” and those where the application is on-going (“under review”). In April-2018, the database contained 739 active substance-PT combinations, comprising around 279 biocides in total, number that will be modified along the time as new or existing active substances are being approved or rejected.

Scientific evidence supports the need to undertake a holistic, life cycle perspective when the (eco)toxicological effects associated to chemical substances are of interest. This implies considering the effects associated not just to parent compounds but also to metabolites, degradation and transformation products generated during life cycle stages beyond the manufacturing phase. During the service or end-of-life phases, when released to the different environmental compartments (soil, surface waters, groundwater and air, depending on the actual use of the biocidal product), active substances undergo a series of degradation reactions generating metabolites, transformation or reaction products that might significantly differ from the parent compounds in terms of their associated toxicity. For instance, Nauen et al. (1998) have shown that on the green peach aphid, *Myzus persicae*, and the cotton aphid, *Aphis gossypii*, olefin and the nitroso-derivative, both metabolites fromimidacloprid, showed a 16- and 6-times higher activity than the parent compound (based on 48 h lethal concentration 50% values), respectively. In fact, the European Chemicals Agency (ECHA) refers the concept of ecotoxicologically relevant metabolite and defines it as any minor or major metabolite which e.g. poses a comparable or higher hazard than the active substance (European Chemicals Agency, 2013). Previous studies with plant protection products (Grasso et al., 2002; Sinclair and Boxall, 2003), indicates that in most cases, degradation products have similar toxicity or are less toxic than their parents, although some can be more toxic. For example, 41% of the pesticide degradation products investigated by Sinclair and Boxall (2003) were less toxic than their parent, 39% had a similar toxicity to their parent, 20% of degradation products were N3-times more toxic and some degradation products (9%) were more than an order of magnitude more toxic. Their study was conducted for 89 transformation products arising from 37 pesticide parent compounds. To our knowledge, similar studies are not available for transformation products of biocidal active substances.

The aims of this paper were: 1) to study the acute hazard to aquatic organisms of all the biocidal active substances submitted under BPD or BPR regulations and the related transformation products formed by biological, chemical, and/or physical processes in the environment during the life-cycle of biocidal products; 2) to identify the availability of information about the hazard of these environmental metabolites and 3) to identify the groups of biocides that suppose a higher hazard for the aquatic compartment, based on

their acute toxicity values and potential presence in the aquatic media. This study pretends to be a complete and useful source of knowledge about the acute hazard of active substances and metabolites released from biocidal products in the aquatic compartments fresh/marine water and sewage treatment plants.

Focus has been placed on acute hazard since the acute effects are prioritized in the Biocidal Product Regulation (BPR). These data are required in the "core data set" whereas information related to long term effects is considered "complementary" and is only required in the additional data set. This does not mean that chronic effects are not important. The fact is that the predicted no effect concentration (PNEC) for a given environmental compartment is set following the Technical Guidance Document on Risk Assessment (European Commission, 2003) and covers acute and chronic toxicity. In the setting of the PNEC, different assessment factors are applied depending on the availability of studies of acute or chronic toxicity and taxonomic groups.

2. *Material and methods*

2.1. Data collection and database building

To build the database, which will be publicly available at the LIFE COMBASE project website, the Microsoft Excel 2013 program (Microsoft Office Professional Plus 2013, 2012 Microsoft Corporation) was used to introduce the information. Only biocidal active substances under review and approved were compiled from the ECHA website (https://echa.europa.eu/regulations/biocidal-products-regulation/approval-of-active-substances/list-of-approved-active-substances) since commercially relevant biocidal products were targeted. From these substances, free radicals generated in situ from ambient air or water, microorganisms, inorganic compounds and elements, plant extracts, etc. were disregarded since these are not suitable to be used in *in silico* methods.

Afterwards, for each one of the different selected biocidal active substances and environmental derived metabolites, different blocks of information were included in the database for the substances (parent and metabolite): 1) chemical identification 2) use as biocidal product and state under the BPR for the parent substances, 3) fate parameters

and 4) toxicity data for the aquatic organisms present in the freshwater or sewage treatment plant compartments. For the chemical identification of the biocide or metabolite, the substance name and alternative names, the EC number (European Community number for chemicals within EU regulatory schemes), CAS RN (Chemical Abstracts Service Registry Number), and canonical SMILES (simplified molecular-input line-entry system) of the neutral form were collected. To know the use of the biocide, the MG and related PT where the specific substance is classified according to the categorization described in the BPR were indicated. In addition, information about the state of the substance, if it is under review or is already approved under the BPR and the commercial relevance of the biocide were also introduced in the database. Fate parameters such as n-octanol/water partition coefficient (Log P), half-life in water, degradation pathways and, whenever available, PNEC values (for freshwater and sewage treatment plant) were included. Finally, acute toxicity information was collected for different organisms of the taxonomic groups, including fish, invertebrates, algae and microorganisms. Data were mainly obtained from freshwater species, however for fish and algae some data were also collected for marine species due to the scarcity of data for some of the biocides or metabolites. The reported experimental data includes: 1) purity of the substance used in the assay; 2) the acute hazard value such as lethal or effective concentration (LC or EC) that produces 10%, 50% or 100% of a given endpoint (i.e. mortality, immobilization, growth, etc.) (L(E) C_{10}, L(E)C_{50}, L(E)C_{100} in the organism, non-effective concentrations have been also compiled; 3) the experimental value obtained in the assay; 4) the species; 5) the maturation state; 6) the exposure period and 7) the endpoint of toxicity; 8) the source of the included data.

The information contained on the Excel database has been implemented on an on-line application that constitutes the initial step of the COMBASE Decision Support System. For additional information at the present regard, please visit: www.life-combase.com.

2.2. Sources of data

The information used to build the database has been compiled from an extensive search on toxicological and chemical databases, peer reviewed papers, books and assessment

reports, and other literature regarding the occurrence of contaminants in freshwater. The following databases were the main consulted on-line sources:

- Open Chemistry Database (PubChem),which contains the chemical structures of N300 thousand small organic molecules and information on their biological activities (https://pubchem.ncbi.nlm.nih.gov/).
- ECOTOXicology knowledgebase (ECOTOX), being a source to search for single chemical toxicity data for aquatic life, terrestrial plants and wildlife. This website was created and is maintained by the U.S.EPA, Office of Research and Development (ORD), and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division (MED) (https://cfpub.epa.gov/ecotox/). ECOTOX is directly linked to the Environmental Protection Agency (EPA) OPP Pesticide Ecotoxicity Database, that is updated by the Ecological Fate and Effects Division of the EPA Office of Pesticide Programs which contains all EPA reviewed ecotoxicity endpoints for pesticides registered or previously registered in the U.S. Toxicity data (http://www.ipmcenters.org/ecotox/).
- World Health Organization (WHO). The International Programme on Chemical Safety, through the Environmental Health Criteria (EHC) documents, provides international, critical reviews on the effects of chemicals or their combinations and physical and biological agents on human health and the environment (http://www.who.int/ipcs/publications/ehc/en/).
- The Pesticide Properties Database (PPDB) is a review of pesticide chemical identity, physicochemical, human health and ecotoxicological data. It was created by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire for a variety of end users to support risk assessments and risk management (https://sitem.herts.ac.uk/aeru/ppdb/).
- TOXicology Data NETwork (TOXNET®) is a compilation of databases covering chemicals and drugs, diseases and the environment, environmental health, occupational safety and health, poisoning, risk assessment and regulations, and toxicology. It is managed by the Toxicology and Environmental Health Information Program (TEHIP) in the Division of Specialized Information Services (SIS) of the National Library of Medicine (NLM) (https://toxnet.nlm.nih.gov/).

- The Pesticide Action Network (PAN) Pesticide Database is a location for toxicity and regulatory information for pesticides. There is possible to find out more about insecticides, herbicides and other pesticides selecting different choices from the website. The database and website are updated and enhanced by Pesticide Action Network North America (PANNA) (http://www.pesticideinfo.org/).

Other important sources of data were the assessment reports and scientific outputs, including its scientific opinions, from the European Chemicals Agency (ECHA; https://echa.europa.eu/) and European Food Safety Authority (EFSA; https://www.efsa.europa.eu/) websites. In addition to all these, other websites of different private chemical companies such as Bayer A.G. (https://www.bayer.com/) or BASF Agro B.V. (https://agriculture.basf.com/) were also consulted.

Finally, different academic websites were used to find peer reviewed articles and books reporting toxicological data such as Web of Knowledge, PubMed, Scopus or ScienceDirect. Several terms were displayed in combination to do this search: "name of the substance" OR "CAS NR", "environment", "toxicity", "LC_{50}", "EC_{50}", "acute toxicity", "water", "fish", "*Daphnia*", "mysid", "algae", "sewage treatment plant", "degradation", "metabolite"….

2.3. Data evaluation

The compiled data related to the biocides and metabolites introduced in the database were carefully analyzed and all the reported assays carried out with substances which purity percentage was lower than 80% or tested as an ingredient in a formulation, were excluded for the final database. We acknowledge that a 80% of purity is not the best practice to test the toxicity of a substance, however due to the scarce number of studies generally found, in special for metabolites the threshold of purity for an assayed substance to be selected for the study was moved to >80%. Therefore, taking into account the data obtained with substances (biocides or metabolites) of purity >80%, the different taxonomic groups (microorganisms, algae, aquatic invertebrates and aquatic vertebrates) were studied separately in relation to their sensitivities to the biocides and metabolites. Because the values of $L(E)C_{50}$ for the biocides and metabolites varied for the different species within a same taxonomic group, in order to have a broader idea of their relative toxicities, these data were categorized. For this and following the EU Regulation (EC) No 1272/2008 on

classification, labelling and packaging of substances and mixtures, biocides and metabolites were grouped in four toxicity categories considering the hazard values of L(E)C_{50}. These categories were: category 1, substances with the indicated hazard values ≤1mg/L; category 2, substances with toxicities from >1 to ≤10 mg/L, category 3, substances which toxic L(E)C_{50} were comprised between >10 and ≤ 100 mg/L and; category 4 for non-toxic substances which toxic concentrations were above 100 mg/L. According to the recommendations reported in the above cited regulation, the acute toxicity was considered when the exposure time was stablished as 96 h for fish, 48 and 96 h for invertebrates and 72 and 96 h for algae. Exposure time differences were not considered for microorganisms since most of the experiments were developed for 3 h and there was a limited data on toxic assays reporting EC_{50} values. The grouping of the different results of L(E)C_{50} registered within a taxonomic group for a given substance allowed to classify the toxicity of the substance in a unique category in most of the cases. However, some of them appeared placed in different categories. This applied to 38/170 biocides for fish, 17/168 in invertebrates, 7/117 in algae and 2/72 in microorganisms, and also to 10 of the 206 metabolites identified. In these cases, it was confirmed that the differences in the toxic concentrations found for a selected substance were mainly attributable to the species exposed, whereas the purity of the used substance (taking into account only purities higher than 80%) did not influence the final result. When the results of L(E)C_{50} for a substance within a taxonomic group were grouped in different categories, the criteria followed to conclude on the toxicity of the substance was to place the compound in the lowest category according to the Technical Guidance Document on Risk Assessment (European Commission, 2003). For metabolites, the hazard of each one of them was also classified in less, equal or more toxic than the parent compound within each taxonomic group. In addition, in an attempt to determine which of the MG of biocides included the highest toxic substances, we have studied the percentage of biocidal active substances included in the different toxic categories by MG and taxonomic group.

3. Results and discussion

3.1. Collected data and data evaluation

A total of 279 biocides have been compiled from the ECHA website up to April 2018, however only 196 active substances have been used in the present work after the depuration of data indicated in Section 2.1 (Materials and methods). A total of 206 environmentally relevant metabolites have been identified for the 196 parent compounds. The complete list of chemicals used in this study is shown in Table A2 (supplementary information). All of them were identified in the Table by their specific name and CAS number. Moreover, the MG and PT associated to the biocidal active substances are mentioned. A major goal of this study is the potential hazard by degradation and transformation of the parent compounds during their life-cycle, therefore, all the metabolites identified once the biocides reach the aquatic media are also mentioned in this Table A2. It should be noticed that some metabolites are common for different biocides. This is the case, for example, of 3-phenoxybenzoic acid, which is metabolite of several pyrethroids (cypermethrin, d-phenothrin, cyphenothrin, permethrin…).

The following results were obtained after depurating the database; after maintaining only those results derived from studies developed with high purity substances; after grouping the toxicity values for each biocide and metabolite in the four categories described before, taking into account the lowest L(E)C_{50} value reported for each taxonomic group and; after comparing the toxicity exerted by the biocidal active substances and their related metabolites.

First, it was noticed that no toxicity data for each parent compound or metabolite were available for all the taxonomic groups. Table 1 reports the number of biocidal active substances and metabolites studied for fish, invertebrates, algae and microorganisms, indicating that the taxa for which more data of toxicity exist are fish and invertebrates, followed by algae and few data are available for microorganisms, especially in relation with their metabolites. The low number of studies carried out with microorganisms is surprising, taking into account that most pollutants are from human use and their emissions to the environment are an issue for some wastewater processes, being the study of the fate of the emerging pollutants in wastewater-treatment plants (WWTP) important (Amoros et al.,

2000). Even when the concern about the potential risk of metabolites is increasing (Miyamoto et al., 2013), the number of studies developed with them is still scarce, as the present study is showing (Table 1). Only around half of the identified metabolites (206 in total) were evaluated in fish, invertebrates and algae, and just 9 metabolites in microorganisms. There is a complete lack of studies performed in any of the considered taxonomic groups for 15 biocidal active substances (8%) and 80 metabolites (39%).

Table 1: Number of biocidal active substances and metabolites studied in different representative taxonomic groups of the aquatic compartment.

	Parent	Metabolites
Total number of identified compounds	196	206
Fish	170	106
Invertebrates	167	105
Algae	116	91
Microorganisms	78	9

The toxic categories where parent compounds and metabolites are included are shown in Fig. 1. The number of substances related to each category is presented in terms of percentage, calculated in base to the total number of substances for each taxonomic group. There was a clear difference between the toxic effect of biocidal active substances and metabolites. About 50% of the parent compounds were classified as category 1 (≤1 mg/L) for the aquatic taxonomic groups of fish, invertebrates and algae, indicating a very high toxicity of these compounds for the aquatic compartment. The percentages of parent substances placed in categories 2, 3 and 4 were very similar for these three taxonomic groups and within categories (between 10% and 20%). However, metabolites mainly showed toxicities related to categories 3 and 4. For microorganisms, most of the biocidal active substances (68%) were included into the category 4 (non-toxic), followed by category 3 (21%) and lower percentages in categories 1 and 2. In this taxonomic group, metabolites were mainly classified into category 4 (89%). For all the categories, there was not a great difference among fish, invertebrates and algae in relation to their sensitivities to parent compounds or metabolites. Maybe in category 1, a slightly higher susceptibility could be observed for invertebrates, followed by fish and, at last, by algae.

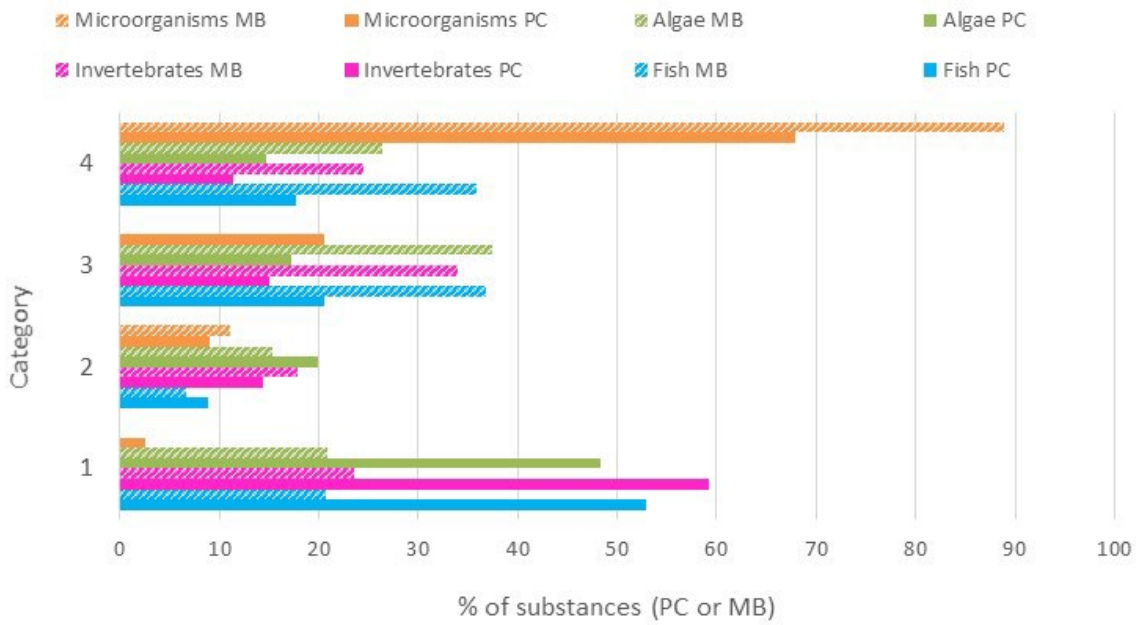


Figure 1. Percentage of biocidal active substances and environmental metabolites included in each of the four categories of toxicity within each taxonomic group. PC: parent compound; MB: metabolite

Fig. 2A presents the toxicity of metabolites in comparison to their parent compounds for each taxonomic group expressed in terms of less, more or equally toxic than the biocides. Percentages are calculated in base to the total number of metabolites for which hazard data were found. For all the organisms, ≤6% of the metabolites presented higher toxicity than their parents. For fish, invertebrates and algae, 34, 36 and 22% of the metabolites showed the same toxicity as their parent compounds, respectively. Finally, 60–72% of the metabolites were less toxic for fish, invertebrates and algae than the biocidal active substances. In the case of microorganisms, the scarce data about toxicity of the metabolites allowed identifying the tested ones as equally or less toxic than the parent compounds (50% each). In general, metabolic transformation of any substance involves either destruction of a toxicophore structure or introduction of a new functional group, generally leading to increased molecular hydrophilicity resulting in a lower toxicity. However, it should be noticed that in some, scarce, cases the first products obtained in the degradation could be as toxic as or more toxic than the initial product as it has been shown for different compounds (Amoros et al., 2000; Farré et al., 2008; Miyamoto et al., 2013; Sinclair and Boxall, 2003). The data presented here indicated a clear pattern relating transformation of products to lower toxicities. However, 28%, 38% and 40% of the studied

metabolites presents a higher or equal hazard than the parent compounds for the taxonomic groups of algae, fish and invertebrates, respectively. These high percentages indicated the need to study the toxicity of the metabolites as much as possible. Due to the actual regulations to avoid animal experimentation, alternative approaches have been proposed. In this sense, Sinclair and Boxall (2003) proposed a flow-chart approach to estimate the acute toxicity to aquatic organisms of transformation products of plant protection product active substances based on chemical structure and data on the toxicity of the parent compound. Within the LIFE-COMBASE project and using the data from the database, *in silico* methods, such as QSAR models, addressing BPR requirements are being developed to predict the toxicity of biocidal active substances and related environmental metabolites. In addition, it should be noticed that many are the metabolites for which no comparisons could be done because of the lack of studies performed with them or their parent compounds in a specific taxonomic group. Indeed, Fig. 2B shows the percentage of metabolites for which toxicity data was available, with respect to the total amount of metabolites identified in the database. Around 45% of the total number of metabolites could not be evaluated during the comparison step in fish, invertebrates and algae. For microorganisms no information on toxicity was available for 96% of the metabolites. In addition, for 39% of the metabolites identified there was no information for any of the taxonomic groups studied. The high percentage of metabolites for which no ecotoxicological data are available at all emphasize the need to further investigate the real risk of these compounds for the aquatic compartment.

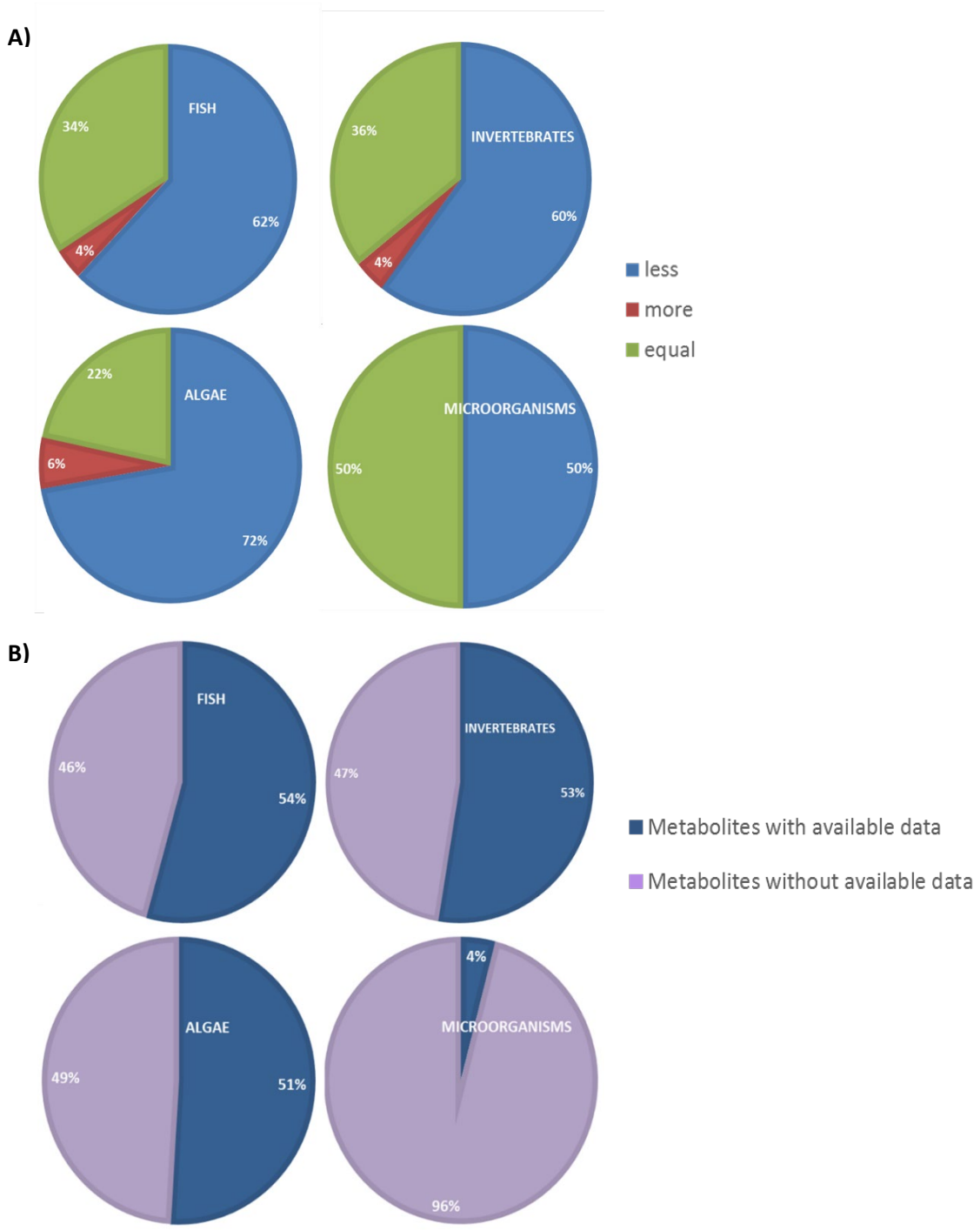


Figure. 2. A) Percentage of metabolites with less, equal or more toxicity than their parent compounds for fish, invertebrates, algae and microorganisms. B) Percentage of metabolites with toxicity data available for each one of the taxonomic groups.

We have tried to determine which of the MG of biocides included the highest toxic substances, therefore, being potentially of higher risk for aquatic organisms once released in the environment. Fig. 3 presents the results regarding toxic categories related to biocidal active substances grouped in the four MG mentioned in Table A1 (supporting information).

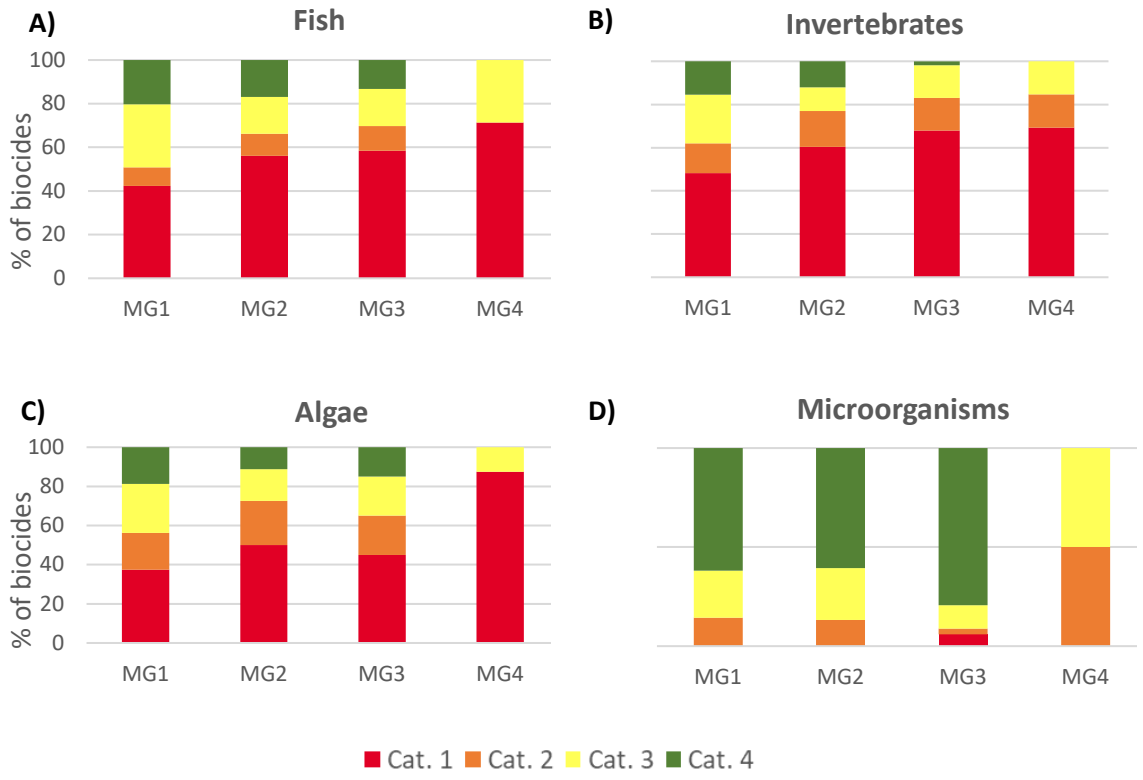


Figure 3. Toxic categorization of biocidal active substances classified by MG for fish (A), invertebrates (B), algae (C) and microorganisms (D).

Substances belonging to MG4 presented higher toxicity. Indeed 71.43%, 69.23% and 87.5% of these biocidal substances where classified in category 1, for fish, invertebrates and algae, respectively. It is worthy to report also the high percentage of substances from the MG3 classified into the category 1, mainly when the effect was evaluated in invertebrates (67.92%), fish (58.49%) and algae (45%). Biocidal substances belonging to MG2 showed similar percentage of substances classified as category 1, presenting 60.44%, 56.18% and 50% for invertebrates, fish and algae, respectively. Finally, 48.28%, 42.37% and 37.5% of the biocidal substances included in the MG1 were categorized as highly toxic for invertebrates, fish and algae, respectively. In general, substances belonging to MG1 presented the lowest toxicity for all the organisms, although the percentage of highly toxic

biocides is still very important. Those substances included into the category 2 of toxicity are also of high concern for the risk assessment once released into the environment. It should be noticed that >50% of substances from MG1 and around 70% from MG2, 3 and 4, for the 3 taxonomic groups, appeared to be included into categories 1 and 2. Once more, the toxic behavior in microorganisms was different to the other organisms, showing the lowest susceptibility to an adverse effect. Most of the assayed biocidal active substances from MG1 (61.90%), MG2 (60.53%) and MG3 (79.41%) presented toxicities that could be considered of no concern (>100mg/L) in the case of microorganisms. Only two active substances from MG4 were used to carry out toxicological experiments with microorganisms, therefore conclusions cannot be extracted.

We have attempted to identify how many metabolites without toxicological data were included in the different MGs. >45% of these non tested metabolites pertained to biocidal active substances belonging to MG2, >30% resulted from MG3 and 11–18% derived from MG1 substances. This means that 75% of these metabolites were related to parent compounds included in MGs that present a high toxicity. Therefore, it should be advisable to recommend a higher surveillance and knowledge of the toxicity of environmental metabolites.

Since the environmental risk of a substance results not only from its hazard, but also the potential exposure is influencing it (Aven, 2016), it is worthy to analyze the probability from the selected biocides to achieve the aquatic compartment. The presence of these substances in the water sources, and therefore the possible exposure of aquatic organisms to them, may be derived from their specific uses. In this sense, biocides from the MG1 (disinfectants) belonging to the PT1, PT2, PT3 and PT5 are susceptible to achieve the water sources by direct immersion of humans in water or surface washings. Our results showed this MG as the one including the biocides with the lower toxicity for the aquatic organisms. However, despite this lower toxicity, the risk of a high probability of exposure should be studied. Less likely is the release of biocides belonging to the MG2 (preservatives) since they are usually used on indoor surfaces. From this group, only those biocides used as wood preservatives (PT8) may be of relevance since they can be used outdoor. Biocides belonging to this PT are usually used to treat wood constructions, especially wood bridges, which are generally located over ponds or rivers, being these compounds potentially

leached on the water (Dubey et al., 2007). Indeed, the presence of some biocides classified into this PT8 and used as fungicides (i.e. propiconazole and tebuconazole) have been reported in water streams from USA, after their increased use in farms (Battaglin et al., 2011). But not only fungicides, some products commonly used as preservatives for protecting wood products such as telephone poles, railroad ties and pier pilings (like creosote) have been reported as contaminants in and around points of large scale production and application (Hale and Aneiro, 1997). The experiments developed in aquatic organisms with these biocides of MG2 denoted high level of toxicity. Therefore, even if their release into the environment can be unexpected in most of the cases, it is encouraged to follow all the recommendations during the manufacturing process and use of these biocides to avoid releases to the environment. Moreover, several biocides are included in more than one single MG, thus presenting a higher risk for the environment, since many potential pathways could lead to their release in the aquatic compartment. Nine of the biocides used as wood preservatives followed this pattern. From them, six were also employed as insecticides, acaricides and products to control other arthropods (PT18) and three as antifouling products (PT21). These usages involve direct contact with water sources. The toxicities of these nine biocides is high (located in categories 1 and 2).

The main groups of biocides presenting higher hazard values for the aquatic compartment were theMG3 (pest control) andMG4 (other biocidal products), being also, as it is discussed below, the PT with the highest probability to be released in the environment and to achieve the natural water sources. Regarding the MG3, it involves some of the most important and potentially dangerous biocides. Those biocides are intended to the pest control, indoor and outdoor, from houses to industries or agronomic lands. They are usually employed on or close to vertebrates and invertebrates, which are in movement and therefore, the possibility to be widespread, is increased. Moreover, their usage outdoor, over fields and even in fisheries facilitates their release in the aquatic media. From all the biocides included in this MG3, ten are used like rodenticides (PT14), thirty nine as insecticides, acaricides and products to control other arthropods (PT18) and eleven like repellents and attractants (PT19). Due to their specific uses, rodenticides dispersed as aerial bait application belonging to this MG3 have been detected in water reservoirs and/or in the organisms inhabiting them (Fisher et al., 2011; Masuda et al., 2015). The case of the biocides from

PT18 is especially important, since this group includes several families of pesticides as avermectins, pyrethroids, carbamates, organophosphates, neonicotinoids, triazines and phenylpyrazoles, all of them with a broad spectrum of use. Other PT potentially supposing a high risk for aquatic organisms is the PT19, involving those biocides used as repellents and attractants, with the clear aim to control harmful organisms (invertebrates such as fleas, vertebrates such as birds, fish, rodents). Due to their use, those products have been detected, for example, in water samples collected from urban and agriculturally dominated aquatic environments, sewage-treatment plant effluents and groundwater systems (Costanzo et al., 2007). Finally, biocides belonging to MG4 (other biocidal products) presented the highest toxicity for aquatic organisms (Fig. 3). This MG includes two different PT, antifouling products (PT21) with eleven biocides and embalming and taxidermist fluids (PT22) with only three biocides included in this group. It is known that the use of biocides as antifouling products in the aquatic environment has proved to be harmful. The presence of these biocides in European ports and marinas has been reported several times. For instance, Sakkas et al. (2002) found concentration levels of different antifouling biocides in Greek ports and marinas. These authors pointed out that the biocides levels detected were not high enough for the aquatic environment, but they showed a seasonal dependence influenced by the boating density, which plays a dominant role. Some of the most common biocides used as antifouling products are DCOIT, zinc pyrithione and zineb, which occurrence and toxic effect in aquatic environments (either marine, estuaries or freshwater) have been demonstrated (Guardiola et al., 2012). All compounds included in this group are of high concern, due to their high toxicity and the high probability of occurrence in the aquatic environment. The present study shows that these compounds are also very toxic for the species present in the freshwater compartment. Only three biocides are included into the PT22, presenting despaired toxicities among them and not homogeneous when the different taxonomic groups are compared. However, they always present high toxicity (categories 1 or 2) for at least one of the taxa, but due to their specific use, it is not really expected to find them in the environment.

3.2. Remarks from data collection and analysis

During the collection of data for each substance and the evaluation process, it was possible to identify several data gaps which were even more evident when the substance was a metabolite. Most of the data collected came from dossiers and official reports, which were compiling data from several articles, books and dossiers from national or international agencies. Some recurrent data gaps were identified. Usually, CAS nr, canonical smiles, type of product, alternative names, log P, bioaccumulation factors or degradation pathways for a given biocidal active substance were reported in the dossiers and assessment reports (ECHA, PubChem…); however, specific information about physicochemical properties was barely reported. The lack of information about the purity of the substance, especially for metabolites, is especially relevant.

Some of the collected studies missed information about basic and important details to perform proper comparisons, like exposure time or toxicity data units; therefore, all these studies were not included in the present study. Another important difficulty was the lack of uniformity related to units, being normally described as mg/L or mg/Kg body weight, but also as ppm or ppb. Direct transformation could seem easy, however in some cases the route of exposure was not described and it was not possible to be sure if the study has been conducted via water or via diet. For algae and microorganisms, it is clear that the exposure medium is aqueous, whereas for invertebrates and fish both are valid. In any case, since the OECD test guidelines for conducting acute toxicity tests in *Daphnia* and fish indicated an exposure through water, unless the substance was not soluble, we considered ppm equal to mg/L.

It was also noticed that some species' nomenclatures have changed along the time. This was taken into account for the interpretation of data.

All the mentioned difficulties were even stronger when the data were related to metabolites: from all the metabolites identified, toxicity tests have only been conducted for around 50% of them. This may be consequence of a lack of commercial availability of the metabolites for testing. Metabolites are only synthetized by manufacturers of biocidal products when data on metabolites are required for the registration of the parental active substance, so that more data could be available in the future during the review program

of the biocidal active substances. In other cases, metabolites may have been produced for research purposes by other scientists but not commercialised, not allowing their use for further studies.

4. Conclusions

50–60% of the biocidal active substances are highly toxic for the freshwater/marine aquatic compartments, especially those belonging to the MG3 and MG4 which, in addition, present a high probability to reach aquatic compartments. In general, metabolites are less toxic than parent compounds for algae, invertebrates and vertebrates, with 4–6% of them presenting higher toxicities, 22–36% equal toxicities and >60% lower toxicities than their parent compounds. It should be noticed that there is no toxicological information for around 50% of the metabolites for fish, invertebrates and algae. There is scarce data of toxicity for STP microorganisms with only available information for 37% of the biocidal substances and 4% of their metabolites, data which indicated that biocides are not very toxic for microorganisms.

In conclusion, biocidal products contain highly toxic active substances which, in general, can easily reach aquatic compartments and can be transformed in metabolites which still present a high toxicity. The development of tools such as QSAR models to predict their acute toxicity is highly encouraged. Other different issue, out of the scope of the present work, that should be envisaged in the future, is the study of the impact of the mixtures of the different substances that could be released in the aquatic media from the biocidal products and the environmental degradation products.

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Supplementary material to Chapter 1

Table A1. Biocides' main groups (MG) and product types (PT) (Annex V of the BPR)

Number	Product-type	Description
MG 1: Disinfectants These product types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.		
PT 1	Human hygiene	Products in this group are biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.
PT 2	Disinfectants and algaecides not intended for direct application to humans or animals	Used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuffs. Usage areas include, inter alia, swimming pools, aquariums, bathing and other waters; air conditioning systems; and walls and floors in private, public, and industrial areas and in other areas for professional activities.
		Used for disinfection of air, water not used for human or animal consumption, chemical toilets, waste water, hospital waste and soil.
		Used as algaecides for treatment of swimming pools, aquariums and other waters and for remedial treatment of construction materials.
		Used to be incorporated in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties.
PT 3	Veterinary hygiene	Used for veterinary hygiene purposes such as disinfectants, disinfecting soaps, oral or corporal hygiene products or with anti-microbial function.
		Used to disinfect the materials and surfaces associated with the housing or transportation of animals.
PT 4	Food and feed area	Used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food or feed (including drinking water) for humans and animals.
		Used to impregnate materials which may enter into contact with food.
PT 5	Drinking water	Used for the disinfection of drinking water for both humans and animals.
MG 2: Preservatives Unless otherwise stated these product-types include only products to prevent microbial and algal development		
PT 6	Preservatives for products during storage	Used for the preservation of manufactured products, other than foodstuffs, feeding stuffs, cosmetics or medicinal products or medical devices by the control of microbial deterioration to ensure their shelf life.

Number	Product-type	Description
		Used as preservatives for the storage or use of rodenticide, insecticide or other baits.
PT 7	Film preservatives	Used for the preservation of films or coatings by the control of microbial deterioration or algal growth in order to protect the initial properties of the surface of materials or objects such as paints, plastics, sealants, wall adhesives, binders, papers, art works.
PT 8	Wood preservatives	Used for the preservation of wood, from and including the saw-mill stage, or wood products by the control of wood-destroying or wood-disfiguring organisms, including insects. This product type includes both preventive and curative products.
PT 9	Fibre, leather, rubber and polymerised materials preservatives	Used for the preservation of fibrous or polymerised materials, such as leather, rubber or paper or textile products by the control of microbiological deterioration.
		This product-type includes biocidal products which antagonise the settlement of micro-organisms on the surface of materials and therefore hamper or prevent the development of odour and/or offer other kinds of benefits.
PT 10	Construction material preservatives	Used for the preservation of masonry, composite materials, or other construction materials other than wood by the control of microbiological and algal attack.
PT 11	Preservatives for liquid-cooling and processing systems	Used for the preservation of water or other liquids used in cooling and processing systems by the control of harmful organisms such as microbes, algae and mussels.
		Products used for the disinfection of drinking water or of water for swimming pools are not included in this product-type.
PT 12	Slimicides	Used for the prevention or control of slime growth on materials, equipment and structures, used in industrial processes, e.g. on wood and paper pulp, porous sand strata in oil extraction.
PT 13	Working or cutting fluid preservatives	Products to control microbial deterioration in fluids used for working or cutting metal, glass or other materials.
MG 3: Pest control		
PT 14	Rodenticides	Used for the control of mice, rats or other rodents, by means other than repulsion or attraction.
PT 15	Avicides	Used for the control of birds, by means other than repulsion or attraction.
PT 16	Molluscicides, vermicides and products to control other invertebrates	Used for the control of molluscs, worms and invertebrates not covered by other product types, by means other than repulsion or attraction.

Number	Product-type	Description
PT 17	Piscicides	Used for the control of fish, by means other than repulsion or attraction.
PT 18	Insecticides, acaricides and products to control other arthropods	Used for the control of arthropods (e.g. insects, arachnids and crustaceans), by means other than repulsion or attraction.
PT 19	Repellents and attractants	Used to control harmful organisms (invertebrates such as fleas, vertebrates such as birds, fish, rodents), by repelling or attracting, including those that are used for human or veterinary hygiene either directly on the skin or indirectly in the environment of humans or animals.
PT 20	Control of other vertebrates	Used for the control of vertebrates other than those already covered by the other product types of this main group, by means other than repulsion or attraction.
MG 4: Other biocidal products		
PT 21	Antifouling products	Used to control the growth and settlement of fouling organisms (microbes and higher forms of plant or animal species) on vessels, aquaculture equipment or other structures used in water.
PT 22	Embalming and taxidermist fluids	Used for the disinfection and preservation of human or animal corpses, or parts thereof.

Table A2. List of chemicals used in this study

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
14548-60-8	(benzyloxy)methanol	2	6, 13	
30507-70-1	(Z,E)-tetradeca-9,12-dienyl acetate	3	19	
26046-85-5	1R-trans phenothrin	3	8	3- phenoxybenzyl (1R, 3R)-2,2-dimethyl-3-[(1RS)-hydroxy-2-methylprop-2enyl]cyclopropanecarboxylate; 3-phenoxybenzyl alcohol; 3-phenoxybenzoic acid
1338-23-4	2-Butanone, peroxide	1	1,2	
122-99-6	2-Phenoxyethanol	1	1,2	
26172-55-4	5-chloro-2-methyl-2H-isothiazol-3-one	2	6	
71751-41-2	Abamectin (avermectin B1a; avermectin B1b)	3	18	[8,9-Z]-avermectin B1a; 8a-oxo-avermectin B1a; 8a-hydroxy-avermectin B1a; 4"-oxo-avermectin B1a; 3"-demethyl-avermectin B1a
160430-64-8	Acetamiprid	3	18	methyl(6-chloro-3-pyridyl)methylamine; IM-1-5 (N-[(6-Chloro-3-pyridyl)methyl]-N-methylacetamidine); IM-1-5 (N-[(6-Chloro-3-pyridyl)methyl]-N-methylacetamidine); IM-1-4 (1-(6-Chloro-3-pyridyl)-N-methylmethanamine); IM-1-2 ((E)-N'-Carbamoyl-N-[(6-chloro-3-pyridyl)methyl]-N-methylacetamidine); IC-0 (6-Chloronicotinic acid)
107-02-8	Acrolein	2	12	3-hydroxypropanol; 3-hydroxypropionic acid; allyl alcohol; acrylic acid
57-06-7	Allyl isothiocyanate	2	9	Allyl allyldithiocarbamate
15879-93-3	alphachloralose	3	14	
67375-30-8	alpha-Cypermethrin	3	18	3-phenoxybenzaldehyde; 3-phenoxybenzoic acid; cis+trans-2,2-dimethyl-3-(2',2'-dichlorovinyl)cyclopropane carboxylic acid
61789-18-2	ATMAC/TMAC	2	8	
35575-96-3	Azamethiphos	3	18	
131860-33-8	Azoxystrobin	2	7,9,10	beta-methoxyacrylic acid; 2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)benzoic acid; 2-(6-hydroxypyrimidin-4-yloxy)benzonitrile
12069-69-1	Basic Copper carbonate	2	8	

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
4299-07-4	BBIT	2	6,7,9,10,13	
32718-18-6	BCDMH	1,3	2,11,12	hypobromous acid; DMH
22781-23-3	Bendiocarb	3	18	NC 7312 (2,2-dimethyl-l,3-benzodioxol-4-ol)
65-85-0	Benzoic acid	1	3,4	hippuric acid; benzoyl glucuronide
100-51-6	Benzyl Alcohol	2	6	
82657-04-3	Bifenthrin	2	8	biphenyl alcohol; biphenyl acid; 4'-OH-bifenthrin
90-43-7	Biphenyl-2-ol	1,2	1,2,3,4,6,7,9,10,13	
2634-33-5	BIT	1,2	2,6,9,10,11,12,13	BIT-S-oxide ; saccharin; o-sulphobenzamide
10043-35-3	Boric acid	2	8	
1303-86-2	Boric oxide	2	8	
56073-10-0	Brodifacoum	3	14	
28772-56-7	Bromadiolone	3	14	
79-08-3	Bromoacetic acid	1	4	bromide ion
52-51-7	Bronopol	1,2,4	2,6,9,11,12,22	2-hydroxymethyl-2-nitropropane-1,3-diol
1305-62-0	Calcium dihydroxide	1	2,3	
7778-54-3	Calcium hypochlorite	1,2	2,3,4,5,11	
37247-91-9	Calcium magnesium oxide/dolomitic lime	1	2,3	
39445-23-3	Calcium magnesium tetrahydroxide/hydrated dolomitic lime	1	2,3	
1305-78-8	Calcium oxide/lime/burnt lime/quicklime	1	2,3	
10605-21-7	Carbendazim	2	7,9,10	2-aminobenzimidazole; methyl (5-hydroxy-1H-benzimidazol-2-yl)-carbamate; 1,2-phenylenediamine; 2-hydroxybenzimidazole; benzimidazole; aniline
18472-51-0	CHDG	1	1, 2, 3	Chlorhexidine
127-52-6	Chloramin B	1	2,3,4,5	benzenesulfonamide; hypochlorous acid
127-65-1	Chloramin T	1	2,3,4,5	
122453-73-0	Chlorfenapyr	2,3	8,18	chlorfenapyr metabolite CL312094; 1H-Pyrrole, potassium salt
7782-50-5	Chlorine	1,2	3, 5, 11	chloramines; chloromethanes
10049-04-4	chlorine dioxide	1,2	2, 3, 4, 5, 11, 12	
59-50-7	Chlorocresol	1,2	1, 2, 3, 6, 9, 13	phenol or carbolic acid
3691-35-8	Chlorophacinone	3	14	

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
67-97-0	Cholecalciferol	3	14	25-hydroxycholecalciferol
104-55-2	Cinnamaldehyde	1	2	
51229-78-8	cis CTAC	2	6, 13	
77-92-9	Citric acid	1	2	
120-32-1	Clorophene	1	2, 3	
210880-92-5	Clothianidin	2,3	8, 18	N-(2-chloro-5-thiazolylmethyl)-N'-nitroguanidine; N-methyl-N'-nitroguanidine; methylguanidine; nitroguanidine
1317-38-0	Copper (II) oxide	2	8	
20427-59-2	Copper hydroxide	2	8	
14915-37-8	Copper pyrithione	4	21	pyrithione disulfide; pyrithione sulfonic acid; pyridine sulfonic acid; 2-mercaptopyridine
7758-99-8	Copper sulphate pentahydrate	1,4	2,21	
1111-67-7	Copper thiocyanate	4	21	
5836-29-3	Coumatetralyl	3	14	
8001-58-9	Creosote	2	8	o-cresol
4080-31-3	CTAC	2	6, 12, 13	
94361-06-5	Cu-HDO	2	8	
420-04-2	Cyanamide	1, 3	3, 18	urea, dicyanamide, thiourea
68359-37-5	Cyfluthrin	3	18	3-(2,2- dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylic acid, Permethric acid; 4-fluoro-3-phenoxy-benzaldehyde, FCR 1260
52315-07-8	Cypermethrin	2,3	8, 18	3-phenoxybenzoic acid; DCVA
39515-40-7	Cyphenothrin	3	18	3-phenoxybenzoic acid
94361-06-5	Cyproconazole	2	8	1,2,4-Triazole
66215-27-8	Cyromazine	3	18	Melamine
231937-89-6	d-Allethrin	3	18	
533-74-4	Dazomet	2	6, 8, 12	MITC (methyl-isothiocyanate)
35691-65-7	DBDCB	2	6	2-methyleneglutaronitrile; 1-bromo-2,4-dicyano-1-butene
10222-01-2	DBNPA	1,2	2,4,6,11,12,13	dibromoacetonitrile; dibromoacetamide; dibromoacetic acid; monobromoacetamide; monobromonitrilo-propionamide; monobromoacetic acid; cyanoacetic acid; cyanoacetamide; oxoacetic acid; oxalic acid; malonic acid
64359-81-5	DCOIT	2,4	7,8,9,10,11,21	NNOMA; NNOA

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
3380-30-1	DCPP	1	1,2,4	methyl-DCPP
7173-51-5	DDAC	1,2	1, 2, 3, 4, 6, 8, 10, 11, 12	
68424-95-3	DDAC (C8-10)	1,2	1, 2, 3, 4, 6, 10, 11, 12	
894406-76-9	DDACarbonate	2	8	
334-48-5	Decanoic acid	1,3	4,18,19	
52918-63-5	deltamethrin	3	18	3-(2,2-dibromovinyl)-2,2-dimethylcyclopro-panecarboxylic acid; 3-phenoxybenzaldehyde
2372-82-9	Diamine	1,2	2, 3, 4, 6, 8, 11, 12, 13	
1085-98-9	Dichlofluanid	2,4	8, 21	N,N'-dimethyl-N-phenylsulphamide
1317-39-1	Dicopper oxide	4	21	
56073-07-5	Difenacoum	3	14	
104653-34-1	Difethialone	3	14	
35367-38-5	diflubenzuron	3	18	
27668-52-6	Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride	1,2	2, 7, 9	
41591-87-1	Dimethyltetradecyl[3-(trimethoxysilyl)propyl]ammonium chloride	2	9	
165252-70-0	Dinotefuran	3	18	
12280-03-4	Disodium octaborate tetrahydrate	2	8	boric acid
7775-27-1	Disodium peroxodisulphate	1	4	
1330-43-4	Disodium tetraborate	2	8	
1303-96-4	Disodium tetraborate decahydrate	2	8	
12179-04-3	Disodium tetraborate pentahydrate	2	8	
330-54-1	Diuron	2	7,10	1-(3,4-dichlorophenyl)-3-methylurea; 3,4-dichlorophenyl urea; 3,4-dichloroaniline
6440-58-0	DMDM Hydantoin	2	2,13	DMH; formaldehyde
13590-97-1	Dodecylguanidine monohydrochloride	2	6, 11	dodecylguanidine
2527-58-4	DTBMA	2	6	
1166-46-7	d-Tetramethrin	3	18	
7747-35-5	EDHO	2	6,13	
3586-55-8	EGForm	1,2	2, 6, 11, 12, 13	formaldehyde; ethylene glycol
54406-48-3	Empenthrin	3	8	

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
64-17-5	Ethanol	1,2	1, 2, 4, 6	
52304-36-6	Ethyl butylacetylaminopropionate	3	19	
75-21-8	Ethylene oxide	1	2	
80844-07-1	etofenprox	2,3	8, 18	
72490-01-8	Fenoxycarb	2	8	phenol or carbolic acid
67564-91-4	fenpropimorph	2	8	fenpropimorph carboxylic acid; cis-2,6-dimethylmorpholine
120068-37-3	fipronil	3	18	RPA 200766; MB45950; MB46136; MB46513; MB46030; MB45897; MB46400; RPA 106889; RPA104615; RPA 105320; RPA 105048; RPA200761
90035-08-8	Flocoumafen	3	14	
131341-86-1	Fludioxonil	2	7,9,10	3-carbamoyl-2-cyano-3-(2,2-difluoro-benzo(1,3)dioxol-4-yl)-oxirane-2-carbocyclic acid; 2,2-difluoro-benzo(1,3)dioxol-4-carbocyclic acid
101463-69-8	flufenoxuron	2	8	N-(4-(2-chloro-4-(trifluoromethyl)phenoxy)-2-fluorophenyl) urea
133-07-3	Folpet	2	6,7,9	Phthalmide; phthalic acid
50-00-0	Formaldehyde	1,4	2, 3, 22	Formic acid
64-18-6	Formic acid	1,2	2, 3, 4, 5, 6	
106-24-1	Geraniol	3	18, 19	
111-30-8	Glutaral	1, 2	1, 2, 3, 6, 11, 12	Carbón dioxide, glutaric acid
79-14-1	Glycolic acid	1	2, 3, 4	
107-22-2	Glyoxal	1	2, 3, 4	
86479-06-3	Hexaflumuron	3	18	2,6-difluorobenzoic acid; 2,6-difluorobenzamide; 3,5-dichloro-4-(1,1,2,2 tetrafluoroethoxy)aniline; 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)urea
4719-04-4	HHT	2	6,11,12,13	benzoic acid
25254-50-6	HPT	1,2	2,6,11,13	
7722-84-1	Hydrogen peroxide	1,2	1, 2, 3, 4, 5, 6, 11, 12	
119515-38-7	Icaridine	3	18	
138261-41-3	imidacloprid	3	18	
72963-72-5	Imiprothrin	3	18	PGH; CPG
7553-56-2	Iodine	1,4	1, 3, 4, 22	Iodate

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
55406-53-6	IPBC	2	6,7,8,9,10,13	propargyl butyl carbamate; 2-propenyl butyl carbamate
34123-59-6	Isoproturon	2	7,10	N-(4-isopropylphenyl)urea; N-(4isopropylphenyl)N'-methylurea; N-(4-(2-hydroxy-1-methylethyl)phenyl)-NP,NP-dimethylurea; N-(4-(1-hydroxy-1-methylethyl)phenyl)-NP,NP-dimethylurea
66603-10-9	K-HDO	2	8	
79-33-4	L-(+)-lactic acid	1,2	1, 2, 3, 4, 6	
91465-08-6	lambda-cyhalothrin	3	18	cyclopropane acid; 3-phenoxybenzoic acid
143-07-7	Lauric acid	3	19	
2527-66-4	MBIT	2	6,13	
5625-90-1	MBM	2	6,13	morpholine; formaldehyde
86347-14-0	Medetomidine	4	21	
3006-10-8	MES	1	1	
137-42-8	Metam-sodium	2	9, 11	
112-12-9	methyl nonyl ketone	3	19	
6317-18-6	Methylene dithiocyanate	2	12	formaldehyde
240494-71-7	Metofluthrin	3	18,19	II; III; IV; V; VI; VII; VIII; IX
2682-20-4	MIT	2	6,11,12,13	N-methyl malonamic acid; N-methyl-acetamide; malonamic acid; 2-(methylcarbamoyl) ethene sulfonic acid; 2-hydroxyethane sulfonic acid; N-methyl-3-hydroxypropionamide; N-methyl-2-oxo-propionamide; N-methyl-3-(methylcarbamoyl)-ethynylsufanyl-acrylamide
84665-66-7	MMPP	1	2	
1746-81-2	Monolinuron	1	2	
27519-02-4	Muscalure	2	10	
134-62-3	N,N-diethyl-meta-toluamide	3	19	
112-05-0	Nonanoic acid, Pelargonic acid	1,3	2, 19	
124-07-2	Octanoic acid	1,3	4, 18	
26530-20-1	OIT	2	6,7,8,9,10,11, 13	
66204-44-2	Oxazolidin/MBO	1,2	2,6,11,12,13	formaldehyde
20018-09-1	p-[(diiodomethyl)sulphonyl]toluene	2	6, 7, 9, 10	
128275-31-0	PAP	1	1,2	

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
494793-67-8	Penflufen	2	8	penflufen pyrazol I-AAP; penflufen-3-hydroxy-butyl
70693-62-8	Pentapotassium bis(peroxymonosulphate) bis(sulphate)	1	2,3, 4, 5	
79-21-0	Peracetic acid	1,2	1, 2, 3, 4, 5, 6, 11, 12	
52645-53-1	Permethrin	2,3	8,18	3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane)carboxylate; 3-phenoxybenzoic acid; 3-phenoxybenzyl alcohol
33734-57-5	Peroxyoctanoic acid	1	2,3,4	
51-03-6	Piperonyl butoxide/PBO	3	8	{2-[(6-propyl-1,3-benzodioxol-5-yl)methoxy]ethoxy}acetic acid; 6-propylbenzo[d][1,3]dioxole-5-carboxylic acid; [(6-propyl-1,3-benzodioxol-5-yl)methoxy]acetic acid
128-03-0	Potassium dimethyldithiocarbamate	2	9,11,12	dimethylamine; carbon disulfide
24634-61-5	Potassium Sorbate	2	6,8	
23031-36-9	Prallethrin	3	18	
71-23-8	Propan-1-ol	1	1,2,4	
67-63-0	Propan-2-ol	1	1,2,4	
60207-90-1	Propiconazole	2	7,8,9	CGA 21795; CGA 91305; CGA 118244; CGA 118245; CGA 136735; 1,2,4-Triazole; 2,4-dichlorobenzoic acid
8003-34-7	Pyrethrins and Pyrethroids	3	18,19	3-phenoxybenzyl alcohol; chrysanthemic acid; chrysanthemic dicarboxylic acid and the mono methyl-ester of the chrysanthemic dicarboxylic acid
95737-68-1	pyriproxyfen	3	18	4'-OH-pyriproxyfen; DPH-Pyr; PYPAC
83-79-4	Rotenone	3	17	rotenolone
609346-29-4	S-156/Momfluorothrin	3	18	
69-72-7	Salicylic acid	1	2,3,4	
7761-88-8	Silver nitrate	1,2	1, 2, 3, 4, 5, 7, 9, 11, 12	
265647-11-8	Silver sodium hydrogen zirconium phosphate	1,2	1, 2, 4, 7, 9	
65733-16-6	S-Methoprene	3	18	7-methoxycitronellic acid; citronellic acid; methoprene acid; methoprene epoxide
132-27-4	Sodium 2-biphenylate	1,2	4, 6, 7, 9, 10, 13	

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
26628-22-8	Sodium Azide	2	6	
51580-86-0	Sodium dichloroisocyanurate dihydrate	1,2	2, 3, 4, 5, 11, 12	
128-04-1	Sodium dimethyldithiocarbamate	2	9, 11, 12	
7681-52-9	Sodium hypochlorite	1,2	1, 2, 3, 4, 5, 11, 12	
7681-57-4	Sodium metabisulfite	2	9	
70161-44-3	Sodium N-(hydroxymethyl)glycinate	2	6	
3811-73-2	Sodium pyrithione	1,2	2,4,7,9,10,13	PSA; OMSA
110-44-1	Sorbic acid	2	6	
168316-95-8	Spinosad	2,3	11,12,18	N-demethylated spinosyn D; spinosyn B; β-13,14-dihydropseudoaglycone of spinosyn A; β-13,14-dihydropseudoaglycone of spinosyn D; Spinosad Spinosyn Factor A pseudoglycon metabolite; Spinosad Spinosyn Factor B; Spinosad N-Demethylated Spinosyn D metabolite
87-90-1	Symclosene	1,2	2, 3, 4, 5,11,12	Isocyanuric acid /Cianuric Acid
21564-17-0	TCMTB	2	9,12	2-mercaptobenzothiazole (2-MBT)
107534-96-3	tebuconazole	2	7, 8, 10	1,2,4-triazole; 5-tert-butyl-5-(1H-1,2,4-triazol-1-ylmethyl)dihydrofuran-2(3H)-one; 4-hydroxy-5,5-dimethyl-4-(1H-1,2,4-triazol-1-ylmethyl)hexanoic acid
886-50-0	Terbutryn	2	7, 9, 10	
7696-12-0	Tetramethrin	3	18	transchrysanthemic acid; 3,4,5,6-tetrahydrophthalimide
148-79-8	Thiabendazole	2	7,8,9,10	5-hydroxythiabendazole; benzimidazole; Benzimidazole-2-carboxamide
111988-49-9	Thiacloprid	2	8	M02; M30
153719-23-4	Thiamethoxam	2,3	8,18	CGA 322704; NOA 407475
137-26-8	Thiram	2	9	
55566-30-8	THPS	2	2, 6, 11, 12	
5395-50-6	TMAD	1,2	2, 6, 11, 12, 13	
731-27-1	Tolylfluanid	2,4	7, 8, 21	N,N-dimethyl-N'-p-tolylsulphamide
122454-29-9	Tralopyril	4	21	CL325,195; CL322,248; CL322,250; R107894

CAS Nr	Biocide	MG	PT	Metabolites in aquatic medium
118712-89-3	Transfluthrin	3	18	NAK 4723; NAK 4452; permethric acid
2893-78-9	Troclosene sodium	1,2	2,3,4,5,11,12	
81-81-2	Warfarin	3	14	
13463-41-7	Zinc pyrithione	1,2,4	2,6,7,9,10,21	
12122-67-7	Zineb	4	21	DIDT; ETU; EU

CHAPTER 2. Particle emission measurements in three scenarios of mechanical degradation of polypropylene-nanoclay nanocomposites

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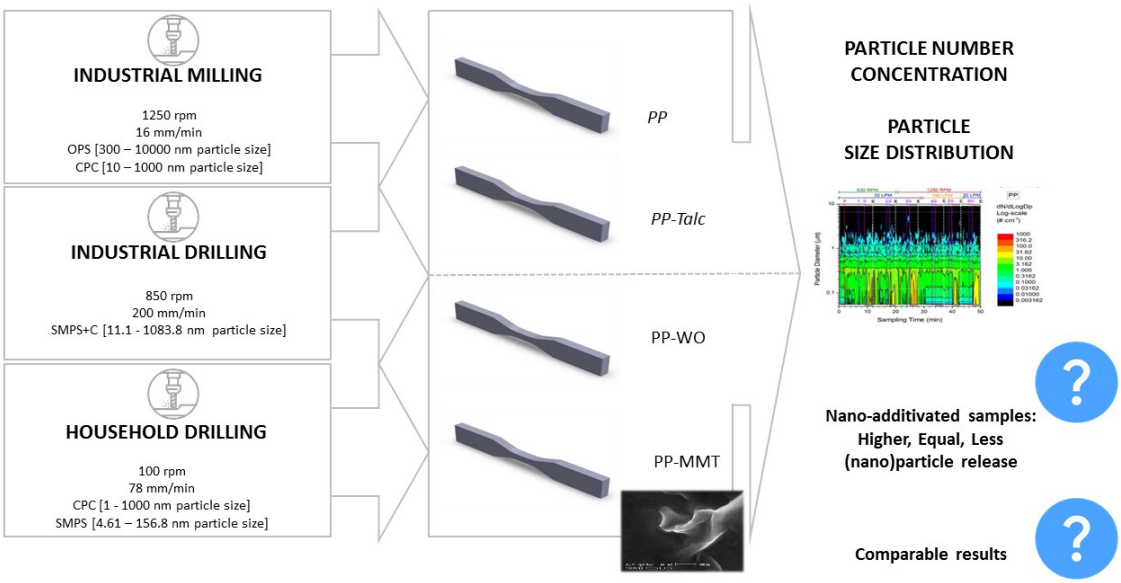
GRAPHICAL ABSTRACT



ABSTRACT

Researchers and legislators have both claimed the necessity to standardize the exposure assessment of polymer nanocomposites throughout their life cycle. In the present study we have developed and compared three different and independent operational protocols to investigate changes in particle emission behavior of mechanically degraded polypropylene (PP) samples containing different fillers, including talc and two types of nanoclays (wollastonite-WO- and montmorillonite-MMT-) relative to not reinforced PP. Our results have shown that the mechanical degradation of PP, PP-Talc, PP-WO and PP-MMT samples causes the release of nano-sized particles. However, the three protocols investigated, simulating industrial milling and drilling and household drilling, have produced different figures for particles generated. Results suggest that it is not possible to describe the effects of adding nano-sized modifiers to PP by a single trend that applies consistently across all different protocols. Differences observed might be attributed to a variety of causes, including the specific operational parameters selected for sample degradation and the instrumentation used for airborne particle release characterization. In particular, a streamlined approach for future assessments providing a measure for released particles as a function of the quantity of removed material would seem useful, which can provide a reference benchmark for the variations in the number of particles emitted across a wider range of different mechanical processes.

KEY WORDS

Polymer nanocomposite; Nanoclays; Mechanical degradation; Particle emission; Exposure assessment

RESUMEN

Tanto los investigadores como los legisladores han afirmado la necesidad de estandarizar la evaluación de la exposición asociada a los nanocomposites poliméricos a lo largo de su ciclo de vida. En el presente estudio, hemos desarrollado y comparado tres protocolos operativos diferentes e independientes para investigar los cambios en la emisión de partículas asociada a la degradación mecánica de muestras de polipropileno (PP) que

contienen diferentes rellenos, incluido el talco y dos tipos de nanoarcillas (wollastonita -WO- y montmorillonita -MMT-) en relación al PP no reforzado. Nuestros resultados han demostrado que los procesos de degradación mecánica de muestras de PP, PP-Talco, PP-WO y PP-MMT provocan la liberación de nanopartículas. Sin embargo, los tres protocolos desarrollados, que simulan el fresado y el taladrado en un entorno industrial y el taladrado en un entorno doméstico, han emitido diferentes cantidades de partículas. Los resultados sugieren que no es posible describir los efectos de añadir rellenos de carácter nanométrico al PP mediante una sola tendencia que se aplique de manera consistente en todos los protocolos diferentes. Las diferencias observadas pueden atribuirse a una variedad de causas, incluidos los parámetros operativos específicamente seleccionados para la degradación de la muestra y la instrumentación utilizada para la caracterización de las partículas emitidas. En particular, parecería útil un enfoque simplificado para evaluaciones futuras que aporte una medida de las partículas emitidas al aire en función de la cantidad total de material removido en el proceso degradación, lo que puede proporcionar un punto de referencia para las variaciones en el número de partículas emitidas en una gama más amplia de procesos mecánicos.

PALABRAS CLAVE

Nanocomposite polimérico, Nanoarcilla, Degradación mecánica, Emisión de partículas, Evaluación de la exposición

LABURPENA

Ikertzaileek zein legegileek adierazi dute nanokonposite polimerikoei lotutako esposizioaren ebaluazioa estandarizatu egin behar dela beren bizi-zikloan zehar. Azterlan honetan, hiru protokolo operatibo desberdin eta independente garatu eta alderatu ditugu, betegarri desberdinak dituzten polipropileno-laginen degradazio mekanikoari (PP) lotutako partikulen emisioaren aldaketak ikertzeko, baita talkoa eta bi nanobuztin-mota ere (wollastonita -WO- eta montmorillonita -MMT-), indartu gabeko PPari dagokionez. Gure emaitzek erakutsi dute PP, PP-Talko, PP-WO eta PP-MMT laginen degradazio mekanikoko prozesuek nanopartikulak askatzea eragiten dutela. Hala ere, garatutako hiru

protokoloek, ingurune industrial batean fresatzea eta zulatzea eta etxeko ingurune batean zulatzea simulatzen dutenek, partikula-kopuru desberdinak igorri dituzte. Emaitzek iradokitzen dute ezinezkoa dela PPri betegarri nanometrikoak eranstearen ondorioak deskribatzea, protokolo guztietan modu sendoan aplikatzen den joera bakar baten bidez. Behatutako aldeak hainbat kausari egotz dakizkioke, lagina degradatzeko berariaz hautatutako parametro operatiboak eta isuritako partikulen karakterizaziorako erabilitako tresneria barne. Bereziki, baliagarria dirudi etorkizuneko ebaluazioetarako ikuspegi sinplifikatu bat, airera igorritako partikulen neurri bat emango lukeena degradazio-prozesuan erauzitako materialaren guztizko kantitatearen arabera, eta horrek erreferentzia-puntu bat eman dezake prozesu mekanikoen gama zabalago batean isuritako partikulen kopuruaren aldaketetarako.

HITZ GAKOAK

Nanokonposite polimerkoa; Nanobuztina; Degradazio mekanikoa; Partikula igortzea; Esposizioaren ebaluazioa

1. Introduction

In material science, the incorporation of nanoclays, comprising e.g. layered mineral silicates, into thermoplastics can lead to improved barrier properties, flame retardance, and mechanical properties, depending on the choice of filler. Wollastonite (WO) and montmorillonite (MTT) represent two types of widely used nanofillers to develop lighter weight structural parts for the automotive industry. WO is an effective and low-cost filler that can improve the tensile modulus, stiffness, hardness and strength of polymers (Ding et al, 2012). Other key characteristics of WO include good thermal stability, low water absorption and relatively low health hazard risk (Balkan et al, 2010). Exfoliated MMT is most popular for use in polymers because of its high surface area and surface reactivity, which both together lead to improved mechanical properties (tensile, hardness, impact, strength, etc.). Although the toxicological potential of this type of materials is generally considered low, Verma et al (2012) have found in their evaluation of the cytotoxicity of nanoclays with different morphologies (platelet and tubular) on human lung epithelial cells A549 the occurrence of varying degrees of dose- and time-dependent cytotoxic effects, which apply to both nanoclay types. Janer et al (2013) compared the *in vitro* effects of commercially available nanoclays for a series of cell lines, including A549, and concluded that toxicological effects were in fact related to the presence of organic modifiers used to exfoliate the platelets. Exposure to nanoclays may therefore cause toxicological effects in pulmonary cell lines, which vary depending on the type of nanoclay used, the presence of organic modifiers and their dosage. As a consequence, it is of utmost importance to evaluate the health risk potential of layered silicates, embedded either in a polymer composite matrix or bound to the material surface, when released into the air during normal wear and tear or as a result of an emergency.

Several studies have adopted a life-cycle oriented approach to address the safety concerns that are attributed to solid nanocomposites. A variety of reviews evaluate the literature investigating the release of ENMs from polymer nanocomposites during product manufacture, consumer use and end of life (EOL) scenarios. Froggett et al (2014) identified fifty-four studies, which evaluated the release of nanomaterials from solid, non-food

nanocomposites, and grouped them according to specific scenarios: machining, weathering, washing, contact and incineration. Authors concluded that release from nanocomposites can take on various forms and highlighted the need to standardize experiments for comparative release assessment. Schlagenhauf et al (2014) summarized investigations concerned with the release of carbon nanotubes (CNTs) from nanocomposites during the service life and concluded that studies investigating the release caused by mechanical impact factors do not provide a consistent picture. Duncan (2015), having evaluated the existing knowledge in relation to the potential release of ENMs from polymer nanocomposites, pointed out a primary limitation of the studies conducted so far as having poorly characterized materials employed in the investigations, hence hindering the reliable formulation of predictive frameworks for ENM release phenomena. More recently, Ding et al (2017) claimed as a limitation in the existing literature the absence of information concerning the properties and amounts of raw materials handled and the need to collect data in an harmonized approach so as to calculate the real release rates of the process concerned.

In the present study we have selected samples from nanocomposite materials that comprise a polypropylene (PP) matrix, which is one of the most widely used composition for commodity thermoplastics in automotive applications. This is because the low density of PP provides weight saving, helps to improve fuel economy and reduces cost (Jansz, 1999). WO and MMT have been reported to be dispersed in PP for the purpose of decreasing density while maintaining favourable mechanical properties (Gonzalez et al, 2014; Salas-Papayanopolos et al, 2014).

The first objective of this investigation has been to develop three different scenarios that simulate the use and EOL phase of PP samples containing different fillers, including talc and two types of nanoclays (WO, MMT). The scenarios include industrial milling and drilling as well as household drilling at spindle speeds of 1250, 8500 and 10000 rpm and feed rates of 16, 200 and 78 mm/min, respectively. All scenarios have been carried out in confined conditions to suppress ambient particle background. A second objective was concerned with comparing particle emission profiles for the nanofilled samples in contrast to the reference samples (neat PP and talc containing PP) within a particular scenario, followed by comparing results among the three scenarios.

The development of scenarios simulating emissions at different life cycle stages is generally regarded as the first step in a broader nano-release investigation, leading the way towards a standardized approach of exposure assessment throughout the life cycle of nanocomposites.

2. Materials and Methods

2.1. Materials and Samples Preparation

Reference materials included a commercially available polypropylene (PP) homopolymer (Moplen HP648T) and a 20% talc filled PP copolymer (Holstacom XM 2416) both purchased from Lyondell Basell Industries (Houston, USA). The reinforcements and concentrations chosen were 5% wt. WO (Harwoll 7ST5) from Nordkalk (Pargas, Finland) and 5% wt. MMT (Nanomer I30T) from Nanocor Corporation (Illinois, USA). The ZSK 26 MEGA compounder twin-screw extruder from Coperion (Stuttgart, Germany) was used for homogenization of the nanocomposite samples. For samples containing WO and MMT, extrusion parameters were 800 rpm screw speed and lateral feeding type. The extruded pellets of the materials were injection moulded by means of an All Rounder 270C-300-100 Injection Moulding Machine from Arburg (Loßburg, Germany). Due to large differences in chemical polarity between PP and MMT or WO, maleic anhydride was mixed into the PP as a coupling agent (MAPP, Polybond 3200 from Addivant, Connecticut, USA). Using MAPP would ensure good interfacial adhesion between the nanofillers and the polymer. Therefore, four sets of samples were fabricated: neat PP, PP with 20% wt. talc (PP-Talc), PP with 5% wt. MMT and 2% wt. coupling agent (PP-MMT), and PP with 5% wt. WO with 2% wt. coupling agent (PP-WO). Pellets from the four samples were injection moulded into standard test specimens for tensile mechanical evaluation according to ISO 527:2012.

Measurements of modulus, strength and density resulted in 2417.6 ± 27 MPa, 25.12 ± 0.4 MPa and 1.035 g/cm^3 for PP-Talc, 2408.8 ± 6.9 MPa, 24.8 ± 0.4 MPa and 0.935 g/cm^3 for PP-WO and 1074.2 ± 16.1 MPa, 23.36 ± 0.3 MPa and 0.942 g/cm^3 for PP-MMT. The characterization of mechanical sample properties revealed that the best performance was obtained by PP-WO, since the addition of only 5 wt.% WO in PP achieved the same tensile

strength to those of a 20 wt.% talc in PP, resulting furthermore in a 10% lower material density saving. This implies that an automotive component can be manufactured with 10% less weight at equivalent mechanical performance. The neat PP sample was used as an additional reference to the PP-Talc sample.

2.2. Experimental set up representing a milling scenario in an industrial environment

The experimental set up corresponding to the industrial milling scenario was partially described in a publication by Schutz and Halliburton (2010). However, while the reported set up was employing an atomizer for the generation of particles, the dust particles from samples investigated in the present study were generated by mechanical degradation using a mill.

(i) Environmental control: A hermetically sealed, torus-shaped circulating Test Duct configuration was used that contained nitrogen as medium for the aerosol. The test duct is a closed circuit where the gas flow inside is driven by a side-channel fan blower (Elektror). Particles released from the PP samples were picked up by the particle-free gas flowing through the duct. All composite samples were tested at a constant flow rate of 32 L/min. The Experiment Section was enclosed on both ends between high efficiency air filters:P3 Sundstroem SR510 upstream and vacuum cleaner S-Class filter (Electrolux) downstream, which remove >99.97% of particles from the airstream before the carrier gas was injected back into the milling chamber.

(ii) Industrial automatic machining system: An Aciera F3 Universal Mill (Anglo-Swiss Tools, UK) with 10 mm diameter High-Speed Steel (HSS) mill-cutter was used to machine a 5 cm long section of the samples. Milling speed of 1250 rpm and feed rate of 16 mm/min were used. The cutting depth was by default 0.5 mm/pass, but the effect of slight deviations from this setting (0.25 or 0.76 mm/pass) was investigated in some milling events for the PP-Talc, PP-WO and PP-MMT samples. These events are marked respectively by labels 0.01" and 0.03" on the upper part of each graph in the results section.

(iii) Particle release measurement: Airborne particles were picked up by sampling tubes and detected by two types of particle counters: a) Optical Particle Sizer (OPS) Model 3330 (TSI Inc., Minnesota, USA) – 16 channels from 300 to 10000 nm particle size and b) a Condensation Particle Counter (CPC) Model 3007 (TSI Inc., Minnesota, USA) – 1 channel from 10 to 1000 nm particle size. The OPS channel configuration "TSI Default" was used (Table S1).

(iv) Data processing: While the OPS 3330 covers all particle size ranges from 300-10000 nm, the CPC 3007 covers particle size ranges from 10-1000 nm. Therefore an overlap takes place in the measurement ranges of these instruments between 300-1000 nm particle size and measurements of both instruments need to be combined in order to manage this overlap.

The OPS channel configuration "TSI Default" (S1) was used, which covers the whole measurement range in 16 channels of equal log-normal width, i.e.:

$$\mathrm{OPS\ Log_{10}}\left(\frac{D_n^u}{D_n^l}\right) = \mathrm{Log_{10}}\left(\frac{D_n^u}{du}\right) - \mathrm{Log_{10}}\left(\frac{D_n^l}{du}\right) = 0.096 \quad (1)$$

for all *n* channels covering specific particles in a range upper and lower size limits given by the interval $[D_n^u, D_n^l]$ and using unit constant d_u = 1 µm.

In order to adjust the scale of the data from the CPC in the range of 10-300 nm and make that data comparable to the OPS data, eq (1) has been used with different values that correspond to properties of the CPC: D_n^u= 0.3 µm, D_n^l= 0.01 µm and using unit constant d_u = 1 µm, which provides a value of 1.477.

$$\mathrm{CPC\ Log_{10}}\left(\frac{D_n^u}{D_n^l}\right) = \mathrm{Log_{10}}\left(\frac{D_n^u}{du}\right) - \mathrm{Log_{10}}\left(\frac{D_n^l}{du}\right) = 1.477 \quad (2)$$

Subsequently, the total CPC measured PNC (10-1000 nm) was scaled by $\frac{1}{\mathrm{Log_{10}}(D_p)}$, multiplying data by 0.096/1.477=0.065.

The next step involved adding up PNC values measured by OPS in a range of 300-1000 nm (number count of channels 1-5 and half the number count of channel 6, see table S1).

Finally, the PNC measured by the CPC for 10-300 nm was calculated by subtracting the PNC measured by the OPS for 300-1000 nm from the PNC measured by the CPC for 10-1000 nm.

This size-normalization is represented in pertinent data plots as 'dN/dlogDp', which includes the PNC for 10-300 nm (CPC scaled data), 300-1000 nm (CPC and OPS combined figure) and 1000–10000 nm (OPS data).

A simplified representation of the Test Duct is provided in Figure 1 (A); the photos in Figure 1 (B) illustrate implementation details. The operational protocol used to mill the nanocomposite samples comprised a series of successive steps, starting from turning on the gas flow and followed by a series of successive milling steps –generally 6-7 passes - taking the specimen in a continuous movement perpendicular to the mill cutter from the Start (S) to the End (E) points at an advance of 16 mm/min.

This represents, to the best of our knowledge, one of the first studies on the emissions associated to polymer nanocomposites milling.

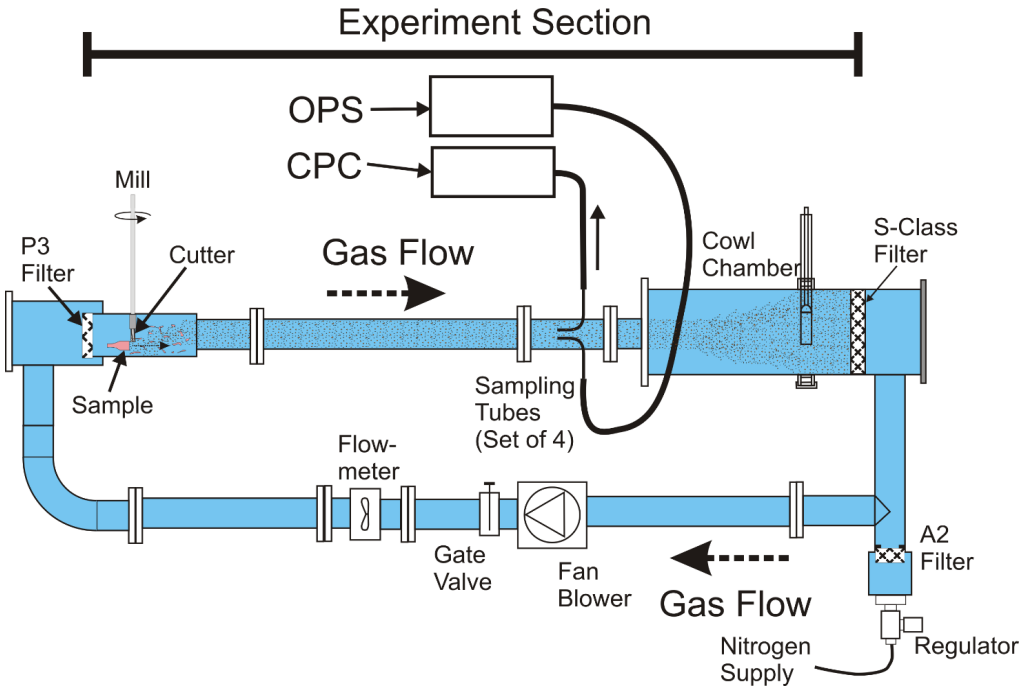


Fig. 1A

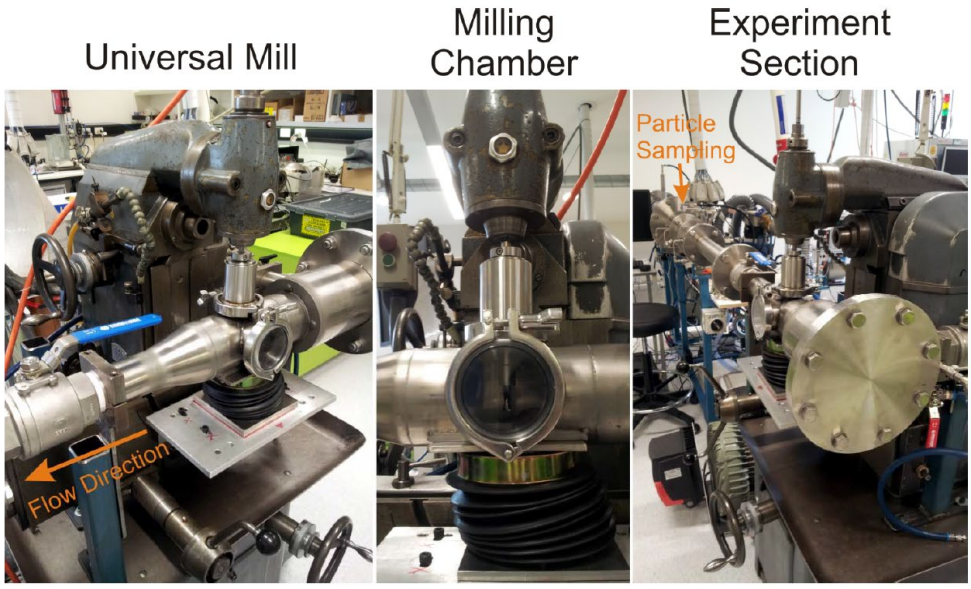


Fig. 1B

Figure 1: A: Schematic diagram of the Test Duct. B: Images of the set-up used to simulate an industrial milling process and characterize the associated particle release.

2.3. Experimental set up representing a drilling scenario in an industrial environment

The experimental set up for industrial drilling was detailed by Gendre et al (2016). Briefly, the main properties of the experimental set up were as follows:

(i) Environmental control: A sealed chamber with a fan, model I100-4 (from BenchVent, Harrogate, UK) was used. The fan recirculated the air inside the chamber and a combination of pre-filter and HEPA filter (category H14) was used to remove particles from the air. An air recirculation system was operating in parallel to control chamber pressure. The spindle drill used for mechanical degrading was water cooled and totally enclosed in order to prevent the generation of background particles by the motor.

(ii) Industrial automatic machining system: A Computer Numerical Control (CNC) machine was designed and built, which allowed precise control of drilling parameters via feed rate, spindle speed, etc. A High-Speed Steel (HSS) plain shank short drill bit of 3.5 mm diameter was used with a spindle speed of 8500 rpm and a feed rate of 200 mm/min.

(iii) Particle release measurement: A scanning mobility particle sizer ('SMPS+C') from Grimm Aerosol (Ainring, Germany) was used to characterize the concentration and particle size distribution (PSD) of airborne nanoparticles released. The 'SMPS+C' comprises of a CPC model 5403 with a Vienna-type classifier, the long U-DMA. This equipment was connected to the chamber using antistatic hoses. The PSD measured ranges from 11.1 nm to 1083.8 nm and distributed across 44 channels.

In relation to the operational protocol, one requirement for the assessment of nanoparticles release was that the total drilling time had to be equal to the time that the SMPS+C needed to complete one full scan, i.e. 7 minutes. Reported data comprises pre-drilling (background), drilling and two successive measurements undertaken after drilling (stabilization). The airborne particle release measurements were replicated a minimum of three times for each sample.

Samples were weighted before and after particle release measurement in order to calculate the amount of material removed by the drilling.

2.4. Experimental set up representing a drilling scenario in a household environment

The experimental set-up used for household drilling was described by Starost et al (2017a and 2017b). Its main properties include:

(i) Environmental control: A closed stainless steel chamber with dimensions of 740 mm x 550 mm x 90 mm was used as containment for the experiment. The chamber was initially cleared of particles before each test through an inflow of clean air with the use of Capsule HEPA Filters from TSI providing >99.97% particle retention.

(ii) Automatic machining system: A drilling tool model 4000 from Dremel (Wisconsin, USA) with a standard stainless steel, 3.5 mm diameter twist drill bit was used at 10000 rpm with a feed rate of 78 mm/min. The tool was manually operated inside the chamber.

(iii) Particle release measurement: The particle number concentration (PNC) was gathered using an Environmental Particle Counter (EPC, a water-based CPC) Model 3783 (TSI Inc., Minnesota, USA) at a flow rate of 0.6 L/min, across a particle range of 1-1000 nm and a concentration range of 0 to 10^6 particles/cm^3 with a reading accuracy of 0.01 particles/cm^3. Using the EPC, the PNC was quantified in situ with a sampling rate of 1 Hz. An SMPS model 3080 (TSI Inc. Minnesota, USA) was used for PSD measurement. The instrument includes an electrostatic classifier utilizing a nano Differential Mobility Analyzer (DMA) with 99 distinct particle diameter channels within a particle range of 4.61 -156.8 nm and a flow rate of 0.31 L/min.

Within the present scenario, particle measurements conducted before drilling was performed revealed the absence of background particles within the detection limits of the EPC. Subsequently, eight holes were drilled within 3 minutes, followed by 1 minute of no drilling to allow particle concentrations to stabilize.

2.5. Statistical analysis

Statistical analyses for the comparison of PNC values of samples tested within each of the operational protocols were performed using the SPSS statistical package v22.0 (SPSS Inc., Microsoft Co, WA, USA). Data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). Then, the non-parametric Kruskal-Wallis test was applied, followed by the Dunn's post hoc test. In all cases, significance was established at $p < 0.05$.

3. Results

3.1. Results of the experimental set up representing a milling scenario in an industrial environment

As an initial step the sensitivity of the experimental set up was characterized. For this purpose, a measurement of the laboratory air was compared in Fig S1 to the release generated by the milling actions on pure PP sample, showing that emissions from milling are below the natural ambient particle concentration levels.

Corresponding PSD contour plots and PNC time-scans from measurements conducted under exclusion of ambient background particles are presented in Figure 2. Results illustrate that particle emissions for all samples increased for particles < 300 nm size during milling actions (sections between labels “S” and “E”, corresponding to those specific moments when the samples were being mechanically degraded). Figure S2 shows details of the mechanically degraded samples.

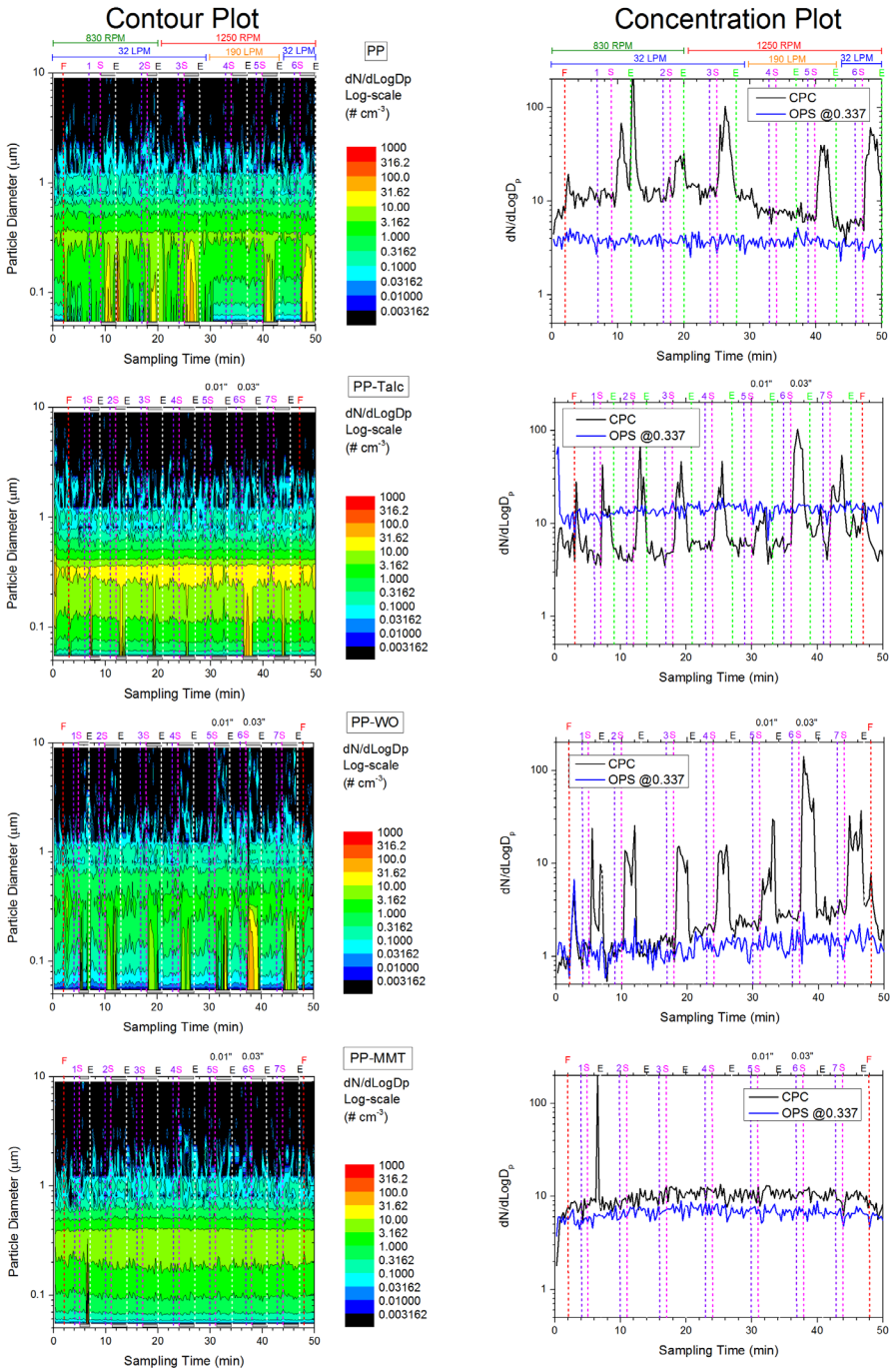


Figure 2: From top to bottom: PSD (left) and PNC (right) expressed as dN/dlogDp (#/cm^3) corresponding to airborne particle release measured using the protocol for simulated industrial milling processes produced by PP, PP-Talc, PP-WO and PP-MMT samples. Results in the contour plots on the left show measured number count concentrations graded according to a colour scale (see legend) as a function of time (abscissa) and particle diameter (ordinate). Temporal cross-sections from these contour plots are shown on the right, where concentration changes over time are shown for given particle sizes: black curves labelled "CPC" hereby refer to particles in a size range from 10 nm to 300 nm and blue curves labelled "OPS @0.337" refer to particles from 300 nm to 374 nm size, according to the respective OPS size-bin. Sections of milling actions are represented as grey bars at the bottom, corresponding to start (S) and end (E) markings at the top of the graph; periods where the mill cutter was not in contact with the sample are the white sections between grey bars.

Since particle distribution results from combined CPC and OPS data show that the only significant increases originate from <300 nm particle size fractions, which corresponds to the range from 10-300 nm that is covered by the CPC, the particle concentration in Table 1 has been calculated using CPC data only at 1250 rpm.

Table 1: PNC (C, particles/cm^3) measured using the industrial milling simulation protocol for 1250 rpm for PP, PP-Talc, PP-WO and PP-MMT samples (CPC Data). Particle levels were determined as an average across all measurements during milling actions of variable durations. The stabilization periods include all particle levels from periods where no milling was conducted and corresponding averages have been calculated. Statistically significant differences have been marked [a] or [b], with the former referring to the reference sample PP and the latter to PP-Talc.

Activity	PP		PP-Talc		PP-WO		PP-MMT	
	Average (n)	SD	Average (n)	SD	Average (n)	SD	Average (n)	SD
C during milling periods	38.98 (23)	24.02	20.27[a] (79)	20.46	21.58[a] (52)	25.48	10.43[a] (66)	1.56
C during stabilization periods	9.65 (76)	7.34	6.23[a] (121)	3.01	2.21[a, b] (148)	1.43	9.42[a, b] (133)	1.95

The nanoparticle-filled composite samples PP-WO and PP-MMT showed a 44.63% decrease and 73.24% decrease, respectively, for the average PNC emissions during milling events when compared to the emissions from the PP sample. The PP-Talc showed a decrease of 47.99% for the same comparison. Differences were significant (p<0.05) in all cases. Significant differences were also observed for the emissions corresponding to all PP-Talc, PP-WO and PP-MMT samples compared with PP sample when no milling was being conducted (stabilization) (p< 0.05). Results therefore suggest that the presence of micro and nanofillers in the PP matrix was decreasing the concentration of emitted particles during mechanical degradation and during subsequent stabilization periods under the conditions tested.

When the comparison was carried out for the emissions of the two nanofilled composite samples relative to those of PP-Talc, the result was a 6.46% increase for PP-WO and a 48.54% decrease for PP-MMT, respectively, during milling actions, differences not being statistically significant. The differences observed during stabilization, however, were significant (p< 0.05).

3.2. Results of the experimental set up representing a drilling scenario in an industrial environment

The SMPS+C 5403 used in the present protocol provides PNC as $dN/dlnD_p$ (cm^{-3}), so it has been converted to $dN/dlogD_p$ (cm^{-3}) by multiplying the values by ln(10) therefore allowing comparisons.

An average value of PSD measurement at peak value of C is presented in Figure 3. PNC shows an increase starting from 62.41 nm with no measured particles for diameters beyond 349.2 nm, approximately, for all samples.

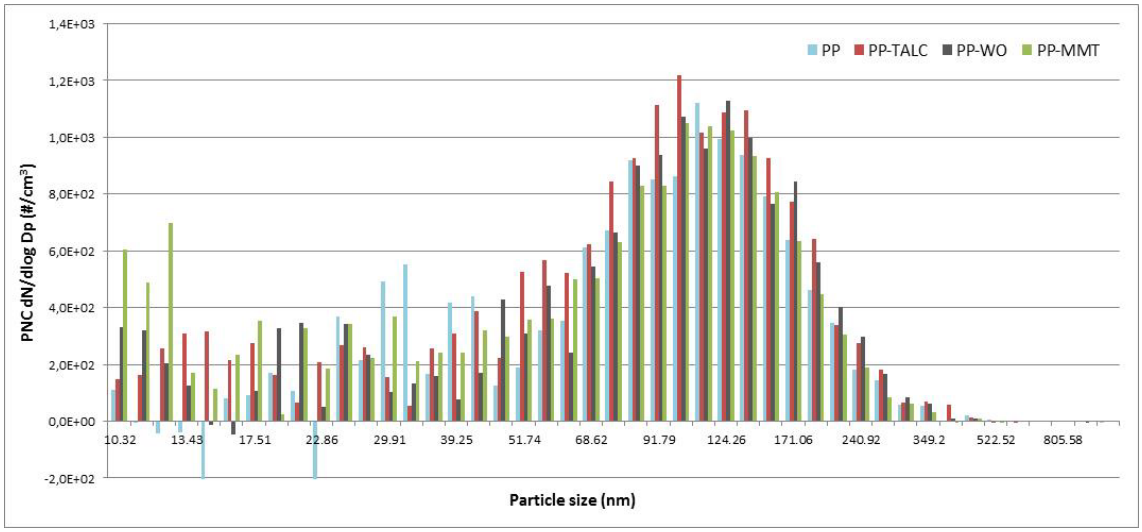


Figure 3: Average of PSD at peak concentration expressed as $dN/dlogDp$ (#/cm^3) having subtracted background concentration corresponding to airborne particle release measurements using the protocol to simulate an industrial drilling process (SMPS+C Data) for PP; PP-Talc; PP-WO and PP-MMT samples.

For the comparison of PNC, values corresponding to the initial scan were considered background and subtracted from successive measurements. PP-Talc, PP-WO and PP-MMT showed an increased PNC of 27.27%, 12.20% and 18.88%, respectively, when compared with the particle concentrations of emissions of PP during drilling but such differences were not statistically significant. The emissions of PP-Talc, PP-MMT and PP-WO during post-drilling periods revealed no significant differences when compared with the emissions of PP sample either (Table 2).

In a second step, PNC data were normalized per quantity of drilled mass. The normalized emissions of PP-Talc and PP-MMT exhibited a 35.73% and 31.03% decrease whereas the emissions of PP-WO sample increased 5.34% in comparison with the emissions of the PP sample during drilling, differences not being statistically significant. No significant differences in PNC were observed either during post drilling periods (Table 2).

Table 2: PNC (C, particles/cm^3) measured in the industrial drilling simulating protocol (SMPS+C Data) for the four samples tested: PP; PP-Talc; PP-WO; PP-MMT. Each scan had a fixed duration of 7 minutes. A total of 4 scans were conducted comprising: an initial scan measuring pre-drilling (background) concentration, a second scan during drilling and two successive scans for particle stabilization.

PP samples

Activity	Scan	Time (min)	Raw Data		Removing Background Values Data		Normalized per Mass Data	
			Average (n=3)	SD	Average (n=3)	SD	Average (n=3)	SD
C pre-drilling (background)	1	0-7	1259.94	123.60				
C during drilling	2	7-14	1834.77	152.87	574.85	264.05	2454.16	1089.37
C during stabilization	3	14-21	2022.87	101.64	762.93	168.45	3266.94	691.09
	4	21-28	1747.65	204.74	293.96	219.40	2096.27	537.32
mass drilled (g)			0.23	0.00				

PP-Talc samples

Activity	Scan	Time (min)	Raw Data		Removing Background Values Data		Normalized per Mass Data	
			Average (n=4)	SD	Average (n=4)	SD	Average (n=4)	SD
C pre-drilling (background)	1	0-7	830.04	271.97				
C during drilling	2	7-14	1561.63	260.96	731.60	80.19	1577.17	148.01
C during stabilization	3	14-21	1828.45	281.15	998.42	92.57	2154.38	185.95
	4	21-28	1749.16	315.91	919.13	123.65	1984.34	264.65
mass drilled (g)			0.46	0.02				

PP -WO samples

Activity	Scan	Time (min)	Raw Data		Removing Background Values Data		Normalized per Mass Data	
			Average (n=4)	SD	Average (n=4)	SD	Average (n=4)	SD
C pre-drilling (background)	1	0-7	901.06	63.57				
C during drilling	2	7-14	1546.02	148.41	644.97	133.76	2585.22	1445.41
C during stabilization	3	14-21	1715.05	156.07	814.00	116.37	3576.66	2644.51
	4	21-28	1637.55	255.93	736.49	251.12	3080.03	1953.81
mass drilled (g)			0.32	0.18				

PP-MMT samples

Activity	Scan	Time (min)	Raw Data		Removing Background Values Data		Normalized per Mass Data	
			Average (n=4)	SD	Average (n=4)	SD	Average (n=4)	SD
C pre-drilling (background)	1	0-7	818.13	254.73				
C during drilling	2	7-14	1501.51	209.33	683.38	184.06	1692.63	324.34
C during stabilization	3	14-21	1642.96	124.37	824.83	173.05	2134.43	790.28
	4	21-28	1504.01	55.49	685.88	251.71	1795.49	793.16
mass drilled (g)			0.43	0.17				

When PP-Talc was taken as reference sample, a 11.84% and 6.59% decrease and a 63.91% and 7.32% increase was observed during drilling for PP-WO and PP-MMT for the not normalized and normalized data, respectively, none of the differences observed being significant. Changes in the PNC values measured during stabilization periods were not significant either.

3.3. Results of the experimental set up representing a drilling scenario in a household environment

The CPC used within the present operational protocol for PNC measurement covers particle size range 1-1000 nm whereas the SMPS covers particle size range 4.61-156.8 nm. This implies that PSD retrieved by the SMPS is just a partial view of the PNC measured by the CPC.

The PSD data obtained by SMPS (Figure 4) displayed a substantial percentage of the particles released from the PP, PP-WO and PP-MMT samples to be between 5-20 nm, whereas the PP-Talc sample emitted larger particle diameters.

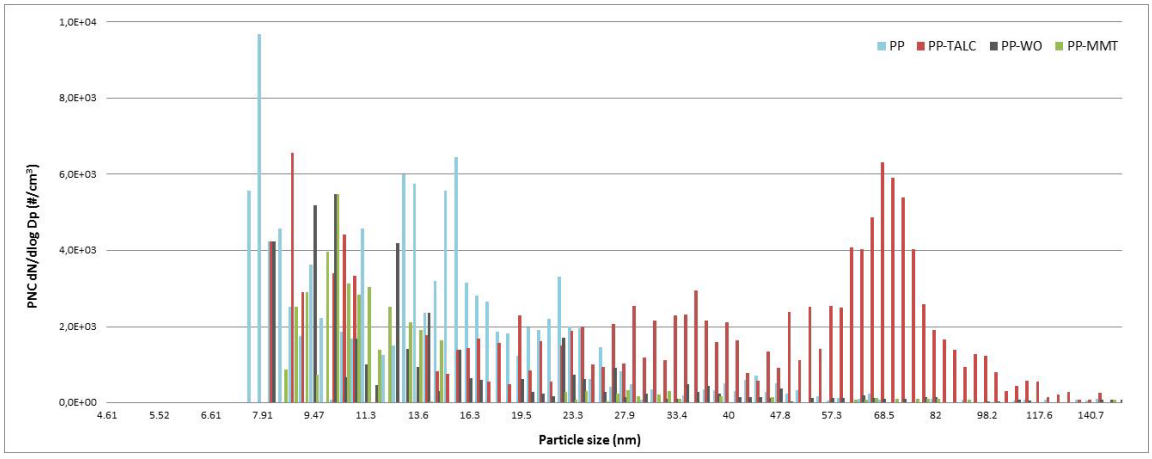


Figure 4: Average of PSD at peak concentration expressed as dN/dlogDp (#/cm^3) of particles emitted during the implementation of a protocol simulating a household drilling process (SMPS Data) for the four samples tested: PP; PP-Talc; PP-WO and PP-MMT.

PNC data was calculated from CPC as average of emission values recorded during 8 holes drilling (3 minutes duration; 180 values, one per second) (Table 3). Average values of the

fourth minute during which drilling was not performed (stabilization) have also been included in Table 3.

Table 3: PNC (C, particles/cm^3) measured in the household drilling protocol (CPC data) for the four samples tested: PP; PP-Talc; PP-WO and PP-MMT. Particle concentration was measured during 8 holes drilling in a period comprising 3 minutes (C during drilling). Subsequently, measurements were conducted during one minute, allowing particles to stabilize (C during stabilization). Statistically significant differences have been indicated as [a] when reference sample was PP or [b] in the case of PP-Talc.

Activity	Scan N // Time (min)	PP		PP-Talc		PP-WO		PP-MMT	
		Average (n)	SD	Average (n)	SD	Average (n)	SD	Average (n)	SD
C during drilling	1-3	501.44 (180)	178.94	199.37[a] (180)	28.68	753.49[a,b] (180)	154.82	352.23[a, b] (180)	80.68
C during stabilization	4	292.46 (60)	24.17	136.32[a] (60)	23.49	97.01[a,b] (60)	61.82	139.89[a] (60)	28.91

Comparison of the particle concentration measured for emissions corresponding to minutes 1-3 (8 holes drilling) of nanofilled samples exhibited a 29.76% decrease (PP-MMT) or a 50.27% increase (PP-WO) in comparison to the neat PP sample. In the case of PP-Talc a considerable decrease of 60.24% was observed. Differences were significant in all cases ($p < 0.05$). Significant differences ($p < 0.05$) were also observed during the stabilization period (minute 4).

In view of the results obtained, the addition of nanofillers to the PP matrix can have an increasing or a decreasing effect (PP-WO and PP-MMT, respectively) in C measured for sample's emissions when compared to the emissions of the neat PP sample under the conditions tested. The addition of talc to the PP matrix implies a decrease in C measured during mechanical degradation under the conditions tested.

When the comparison was carried out taking PP-Talc as reference sample significant differences ($p < 0.05$) were observed in the C of released particles, increasing 277.93% and 76.67% for PP-WO and PP-MMT, respectively. During the stabilization period a significant decrease of 28.83% ($p < 0.05$) was observed for the PP-WO sample only.

4. Discussion

Three operational protocols have been developed to simulate different real-world scenarios for composites and nanocomposites including: industrial milling and drilling and household drilling. Changes in particles emission behavior of mechanically degraded PP samples made of PP only or compounded with different types of fillers, including talc and two types of nanoclays, have been investigated under confined conditions.

No significant differences have been observed in the particle release measurements carried out within the industrial drilling scenario for any of the possible comparisons, both in the case of normalized and not normalized data. The results obtained indicate that all samples released particles in the nanosize range. Bello et al (2010) investigated the generation of nanoscale and submicron particles during solid core drilling of fibre composites containing carbon nanotubes (CNT) and their CNT-free counterparts and concluded that drilling can generate significant exposures to nanoscale and submicron particles for all composites

independently of the presence of CNT, in agreement with the results provided by the industrial drilling scenario. Similarly, Wohlleben et al (2011) reported no significant changes in the concentrations measured during sanding of reference materials without nanofillers and CNT containing nanocomposites.

Both in the industrial milling and household drilling scenarios significant differences were observed when PP sample was taken as a reference, but the tendencies observed were not consistent, being PNC lower for all the comparisons carried out except in the case of the 50.27% increase of PP-WO emissions measured during the household drilling scenario. When PP-Talc was taken as reference, significant differences were observed for the emissions of the two nano-reinforced samples measured during the stabilization period of the industrial milling scenario whereas in the case of the household drilling scenario, significant differences were observed in the concentrations measured during drilling for both PP-WO and PP-MMT and for the PP-WO sample only during the stabilization period. Sachse et al (2012) evaluated the influence of nanoclay on mechanical drilling of polyamide 6 composites in terms of particles generation and concluded that the presence of nanoclay in the composition of the sample led to a significantly lower total particle concentration during drilling, which we have observed as well in the case of the industrial milling (when the PP sample was taken as a reference) and in the household drilling scenarios.

Previous studies concerned with particle release assessment during mechanical degradation of nanocomposites have so far not produced consistent tendencies across different types of degradation. In the present study we have as well not observed a consistent pattern in the particle emissions profile of composite samples that would concisely confirm a significant filler effect. This was true if pure PP was taken as a comparative reference, as well as for PP-Talc.

It is possible that factors other than the presence or absence of micro- or nano-fillers are at the origin of the differences observed, such as the specific scenario, properties of different test equipment or differences in characteristics of instrumentation employed for the measurement of airborne particle release. The operational parameters used for each of the developed scenarios have been summarized in Table 4.

Table 4: Comparison of the three simulated scenarios.

	Industrial Milling	Industrial Drilling	Household Drilling
Machining instrument	Aciera F3 Universal Mill (Anglo-Swiss Tools, UK)	CNC machine (ad hoc designed prototype) Drill bit diameter 3.5 mm	Drill model 4000 from Dremel (Wisconsin, USA) Drill bit diameter 3.5 mm
Spindle speed	1250 rpm	8500 rpm	10000 rpm
Feed rate	16 mm/min	200 mm/min	78 mm/min
Cutting speed	0.654 m/s	1.558 m/s	1.833 m/s
Instrumentation used for airborne particle measurement	OPS Model 3330 from TSI, Inc. 16 Channels [300 – 10000 nm particle size] CPC Model 3007 from TSI, Inc. 1 Channel [10 – 1000 nm particle size]	SMPS+C Model 5403 from Grimm Aerosol (Ainring, Germany). Instrument includes a classifier type Vienna long U-DMA. 44 Channels [11.1 - 1083.8 nm particle size]	CPC Model 3783 from TSI, Inc. [1 - 1000 nm particle size] SMPS Model 3080 from TSI, Inc. Instrument includes an electrostatic classifier with Differential Mobility Analyser (DMA) [4.61 – 156.8 nm particle size]
Background particles	The entrance and exit of the Experiment Section have been equipped with HEPA filters, which each retain >99.97% of all particles	HEPA filters (category H14) were used. Measures undertaken in scans 1 and 2, ie, prior the drilling process had started have been considered background samples. Such particles have been eliminated in the normalization process.	TSI 99.97% retention HEPA Capsule Filters used to attain a negligible background
Additional information	Schutz and Halliburton (2010)	Gendre et al (2016)	Starost et al 2017a and 2017b

Standardized equipment for the physical degradation of nancomposite samples exists e.g. in the case of the Taber Abraser, on the basis of which different wear tests have been developed (e.g., DIN 53754:1977, DIN 68861–2:1981, ISO 5470–1:1999, and ASTM D 4060–95:2007). In fact, this type of device has been used widely in previous life cycle oriented research studies (Schlagenhauf et al, 2012; Wohlleben et al, 2011; Vorbau et al, 2009, to cite a few). In contrast to the drilling/milling approaches discussed here, the Taber Abraser exerts a continuous degradation action on the sample (i.e. particles are emitted in a continuous fashion), whereas milling or drilling generate shorter term peaks of emitted particles that are temporally aligned with respective mechanical degradation events. According to Schutz and Morris (2013), the level of particle release from machining depends on the amount of energy that is invested in the process. In the household drilling scenario, the drill was manually operated hindering the calculation and comparability of the energy input. Furthermore, thermal and mechanical degradation of composite samples are two closely related processes, which means that variations in the measurements of released particles cannot solely be attributed to the mechanical process. Variations in the selected spindle speed and/or feed rate can cause differences in the heat generated by the process, which can cause the material to melt and, consequently, emit particulate vapors that tend to re-condensate and adhere to other material surfaces, hence decreasing the number of emitted particles overall. In fact, Ding et al (2017) having assessed the airborne emissions of drilling and sawing polyurethane nanocomposites referred that the sawing tests generated relatively low particle number concentrations in comparison with the drilling tests. However, the sawing process produced intense heat and, consequently, polymer fumes.

Concerning the instrumentation used for particle release measurements, there were also vast differences in the PSD detection ranges. The SMPS used in the protocol simulating household drilling measured particles up to 156.8 nm, which is in contrast with the instrumentation used by the protocols simulating industrial environments which covered particle sizes up to 1000 nm. This leaves the question if these limited size ranges do only reveal a partial picture of the overall particle release. In fact, the protocol simulating an industrial milling did only detect above-particle-background object concentrations for particles < 300 nm in all samples investigated, which was also confirmed by the results

obtained in the industrial drilling scenario. The limited PSD of the SMPS used in the household drilling protocol is therefore noted as a potential impediment for the comparison of results obtained.

The difficulty of our study relays on the fact that no guidelines or internationally accepted protocols exist to streamline the exposure assessment of ENMs from solid polymer nanocomposites in the framework of a life cycle perspective. Standards should not only pursue how to mechanically degrade the sample, but they should also focus on the exposure assessment that is associated with detecting and characterizing emitted particles. The OECD emission scenario documents on plastic additives (OECD 2014; OECD 2019) represent suitable starting points with this purpose, ENMs being classified as "fillers".

All the three different protocols we have developed for scenario simulation have taught us several lessons. From the milling scenario we have learned that for machining procedures that are not carried out on a continuous approach (several holes drilling, for instance) the exact timing during which the sample is mechanically degraded should be recorded in order to be able to correlate the airborne particle emissions with the sample treatment process. For the same scenario, we have proposed a mathematically sound approach to integrate measurements from two different instruments. From the industrial drilling protocol we have learned that an improved approach to comparing the data obtained from different scenarios would be to normalize the mass of emissions measured in the nanometer range with respect to the total quantity of material removed during the mechanical degradation process. With such purpose, samples should ideally be weighted before and after the mechanical degradation experiment. Finally, from the household drilling process we have learned that a pre-requisite to judge the soundness of the obtained data is that the measurement ranges of the instrumentation used for released particle assessment in different scenarios are similar, so as to be able to retrieve comparable data. An additional lesson we have learned from the household drilling protocol is that automatically operated instruments should be prioritized in contrast to manual instruments with the aim of minimizing possible variations of the energy input applied in the machining process.

The outcomes of the scenarios that we have simulated are transferable to similar processes in realistic environments including the workplace (industrial milling, drilling) or a consumer

use (household drilling). In particular, the drilling process is largely used in composite and nanocomposite materials processing playing a major role in various industries from automotive to aerospace. In this context, Faraz et al. (2009) referred that 55000 holes are generally required to be drilled in a complete single unit production of the Airbus A350 aircraft. The time frames that we have considered in this study range from 3 to 7 minutes in the case of the household and industrial drilling, respectively, and approximately 50 minutes in the case of milling. The exposure to released airborne particles derived from the machining of nanocomposites considerably increases during an average 8 hour workday.

The stability of airborne nanoparticle agglomerates is another important parameter in simulated scenarios for nanomaterial release and associated human exposure. The mechanisms of particle agglomeration include physical interlock (rough surface, entangled surface shapes, or chain-like, branched structure), electric forces (Van der Waal, conductive/non-conductive), magnetic forces (ferromagnetic, induced magnetic) and soft bridging (sticky surface, liquid film, organic functional groups) (Schneider and Jensen, 2009). It is probable that agglomerated airborne particles break up into smaller agglomerates, or even primary particles, when subjected to larger dispersion forces during release, transport along exposure routes, and during inhalation (Li et al., 1996; Li and Edwards, 1997). The three scenarios that we have simulated have been carried out in confined conditions limiting deagglomeration processes which could have potentially led to an increased PNC.

Future studies should ideally complement particle release measurements with particle characterization (e.g. microscopical/chemical analysis) in order to determine if and in what form the nanofillers are released. Furthermore, the (eco)toxicological potential of released nanoobjects should be investigated as it might not correspond to that of the pristine ENM or to that of the matrix in which it is integrated. In fact, Wagner et al (2018) concluded that byproducts generated by the thermal degradation of a polymer polylactic acid-based nanocomposite containing a functionalized MMT could pose a health risk to human lung epithelial cells.

In conclusion, in the present study we have developed three independent protocols with the aim of assessing whether nanoadditivated compositions convey a higher associated

exposure towards released nanoobjects in contrast to the traditionally microreinforced or neat samples used as a reference during machining operations. Our results have shown that mechanical degradation of PP, PP-Talc, PP-WO and PP-MMT samples leads to the release of nanosized particles. Results suggest that it is not possible to describe the effects of adding nano-sized fillers to PP by a single trend that can be applied across a whole range of different scenarios. There is consequently an urgent need to standardize the exposure assessment of ENMs released from nanocomposites when exposed to different wear and tear or machining scenarios, as they emerge at different stages of their life cycle.

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Supplementary Material to Chapter 2

Table S1: Channel configurations of the TSI 3330 OPS: Values for channel lower limits (LL), mean diameter (MD) and geometric mean diameter (GMD) in µm.

Configuration - TSI Default								
Channel	1	2	3	4	5	6	7	8
LL	0.3	0.374	0.465	0.579	0.721	0.897	1.117	1.391
MD	0.337	0.420	0.522	0.650	0.809	1.007	1.254	1.562
GMD	0.335	0.417	0.519	0.646	0.804	1.001	1.246	1.552
Channel	9	10	11	12	13	14	15	16[a]
LL	1.732	2.156	2.685	3.343	4.162	5.182	6.451	8.032
MD	1.944	2.421	3.014	3.753	4.672	5.817	7.242	9.016
GMD	1.932	2.406	2.996	3.730	4.644	5.782	7.198	8.962

[a] The upper limit is approximately 10 µm

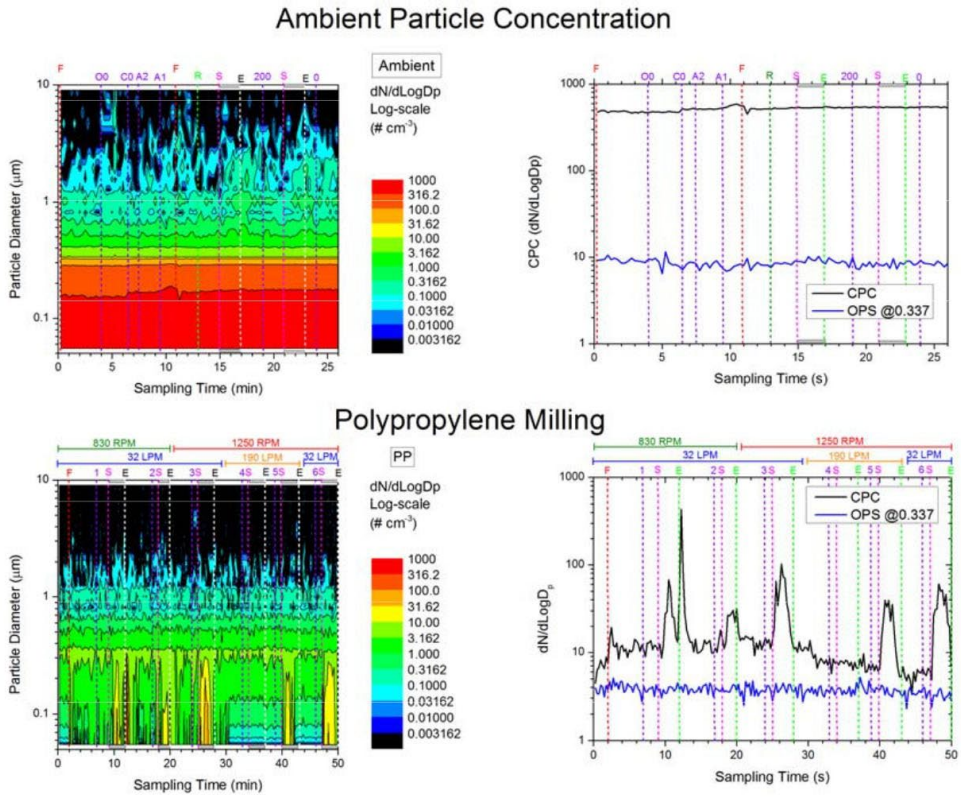

Figure S1: Comparison of particle concentrations of ambient air (top) to particle release generated by milling of PP (without nanoparticles). Markings at the top of each graph refer to specific test parameters at the time of measurement. For ambient particle concentration measurement: F- Start of laboratory air circulation in Test Duct (default: 32 LPM); O0 - OPS: measure ambient particle concentration, rather than concentration in Test Duct; C0 - CPC: measure ambient particle concentration, rather than concentration in Test Duct; A1, A2: Open door to ambient air in corridor (A1) or ante-room (A2) to modify laboratory air; R: Mill-cutter rotating at 1250 RPM, but no advance; S, E: Start / End of milling process at an advance of 16 mm/min (no sample); 200: Increase air circulation flow from 32 LPM to 200 LPM; 0: Stop air circulation (0 LPM). For PP milling changes in rotation speed (RPM) and circulation flow rate (LPM) are indicated at the top of each plot.

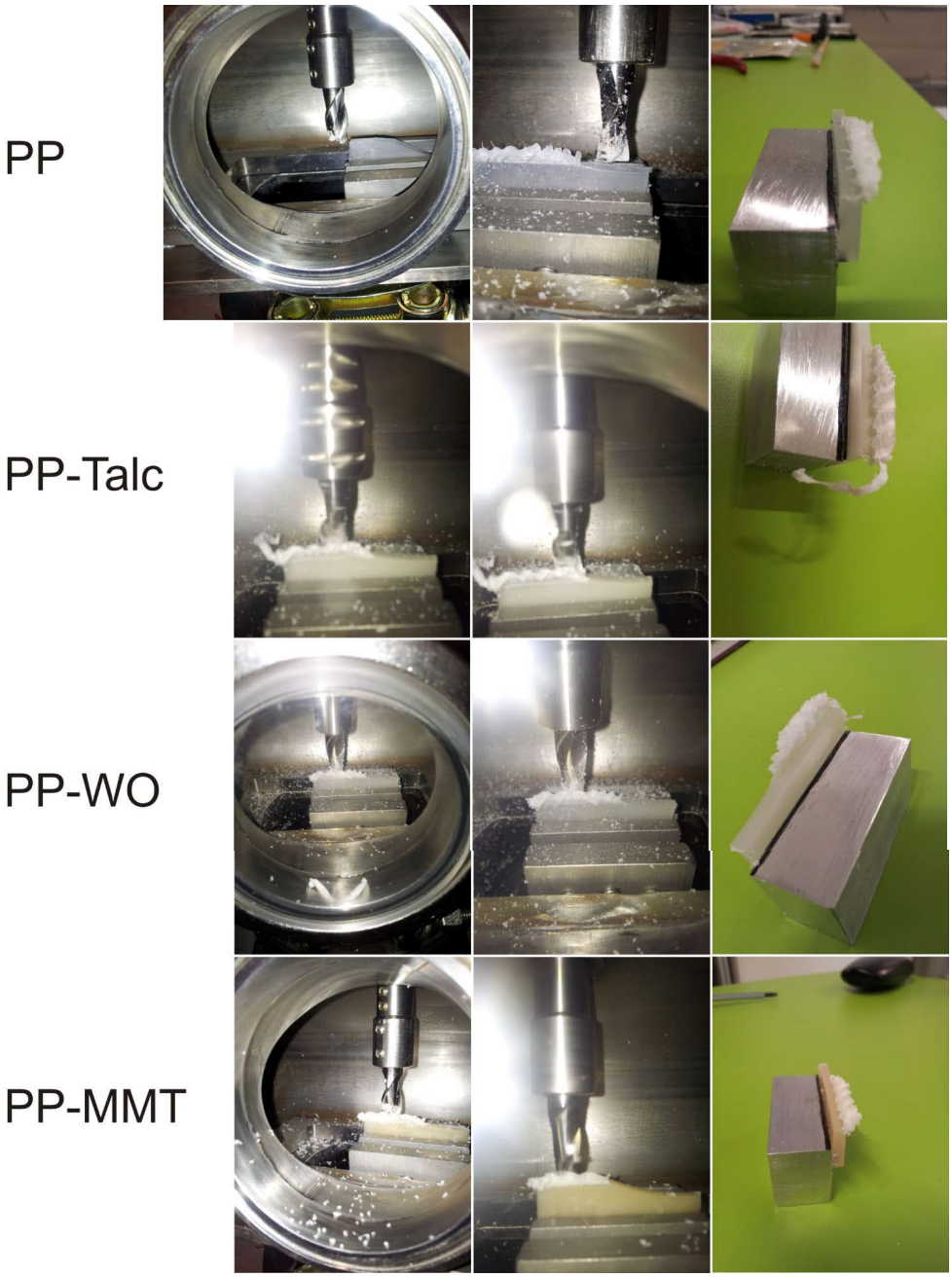

Figure S2: Photos taken during milling of samples and shape after completion of milling.

CHAPTER 3. Release and cytotoxicity screening of the printer emissions of a CdTe Quantum Dots-based fluorescent ink

CHAPTER 3. Release and cytotoxicity screening of the printer emissions of a CdTe Quantum Dots-based fluorescent ink

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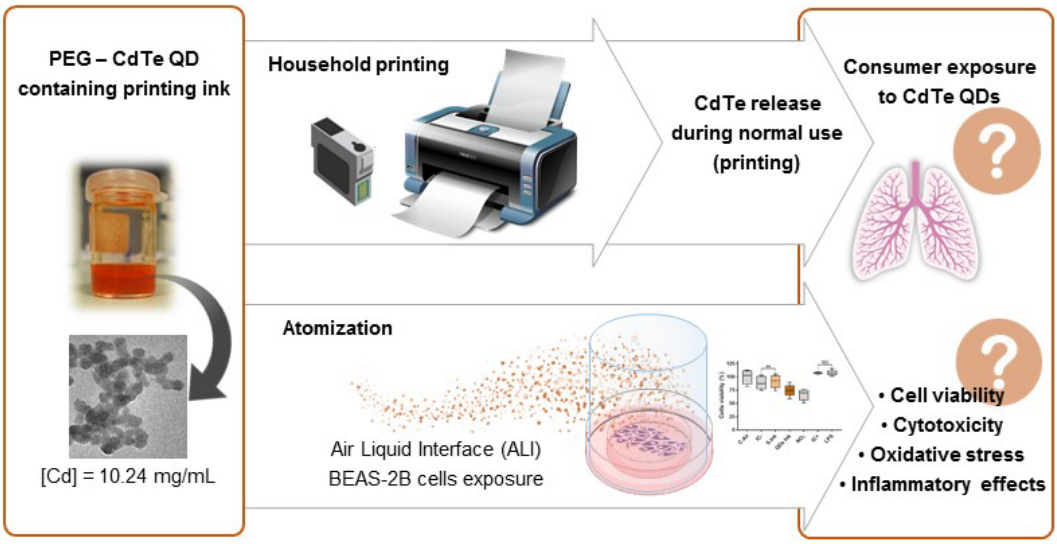
GRAPHICAL ABSTRACT



ABSTRACT

The fluorescent properties of cadmium telluride (CdTe) containing quantum dots (QDs) have led to novel products and applications in the ink and pigment industry. The toxic effects of the emissions associated to the use of printing ink containing CdTe QDs might differ from those of conventional formulations which do not integrate nanoparticles, as CdTe QDs might be emitted. Within this work, the airborne emissions of a water-soluble fluorescent ink containing polyethylene glycol (PEG)-coated CdTe QDs of 3-5 nm diameter have been characterized and studied under controlled conditions during household inkjet printing in a scenario simulating the use phase. Subsequently, the cytotoxicological potential of atomized CdTe QDs ink in an acute exposure regimen simulating an accidental, worse-case scenario has been evaluated *in vitro* at the air-liquid interface (ALI) using the pulmonary cell line BEAS-2B. Endpoints screened included cell viability, oxidative stress and inflammatory effects. We have observed that CdTe QDs ink at 54.7 ng/ml decreased cell viability by 25.6% when compared with clean air after 1h of exposure; a concentration about 65 times higher was needed to observe a similar effect in submerged conditions. However, we did not observe oxidative stress or inflammatory effects. The present study integrates the development of scenarios simulating the use phase of nano-additivated inks and the direct cell exposure for *in vitro* effects assessment, thus implementing a life-cycle oriented approach in the assessment of the toxicity of CdTe QDs.

KEY WORDS

CdTe quantum dots; Printing ink; Release; Airborne exposure; Cytotoxicity

RESUMEN

Las propiedades fluorescentes de los puntos cuánticos (quantum dots, QDs) que contienen teluro de cadmio (CdTe) han dado lugar a nuevos productos y aplicaciones en la industria de tintas y pigmentos. Los efectos tóxicos de las emisiones asociadas al uso de tintas de impresión cuya formulación incluye CdTe QDs pueden diferir de los de las formulaciones convencionales que no integran nanopartículas, ya que podrían emitirse CdTe QDs. En el marco de este trabajo, se han caracterizado y estudiado en condiciones controladas las

emisiones al aire de una tinta fluorescente soluble en agua que contiene CdTe QDs cuya superficie está recubierta de polietilenglicol (PEG) de 3-5 nm de diámetro durante la impresión de inyección de tinta en un escenario que simula la fase de uso a escala doméstica. Posteriormente, se evaluó *in vitro* el potencial citotoxicológico de la tinta que contiene CdTe QDs atomizada en un régimen de exposición aguda que simula un escenario accidental (peor escenario posible), en la interfaz aire-líquido (ALI) utilizando la línea celular pulmonar BEAS-2B. Los parámetros examinados incluyeron viabilidad celular, estrés oxidativo y efectos inflamatorios. Hemos observado que la tinta aditivada con CdTe QDs en una concentración de 54,7 ng/ml disminuyó la viabilidad celular en un 25,6% en comparación con el aire limpio después de 1 h de exposición; siendo necesaria una concentración aproximadamente 65 veces superior para observar un efecto similar en condiciones sumergidas. Sin embargo, no observamos estrés oxidativo ni efectos inflamatorios. El presente estudio integra el desarrollo de escenarios que simulan la fase de uso de tintas nanoaditivadas y la exposición celular directa para la evaluación de efectos *in vitro*, implementando así un enfoque orientado al ciclo de vida en la evaluación de la toxicidad asociada a los CdTe QDs.

PALABRAS CLAVE

CdTe quantum dots; Tinta de impresión; Liberación; Exposición por vía aérea; Citoxocidad

LABURPENA

Kadmio-teluroa (CdTe) duten puntu kuantikoen propietate fluoreszenteek (quantum dots, QDs) produktu eta aplikazio berriak sortu dituzte tinta eta pigmentuen industrian. CdTe QDs formulazioa duten inprimaketa-tinten erabilerari lotutako isurien efektu toxikoak eta nanopartikulak osatzen ez dituzten ohiko formulazioenak desberdinak izan daitezke, CdTe QDs isuri baitaitezke. Lan honen esparruan, CdTe QDs duen tinta fluoreszente disolbagarri batek airera egiten dituen isurketak baldintza kontrolatuetan karakterizatu eta aztertu dira. Tinta horren azalera 3-5 nm-ko diametroko polietilenglikolez (PEG) estalita dago, tinta injektatu bitartean, etxeko eskalako erabilera-fasea simulatzen duen eszenatoki batean. Ondoren, *in vitro* ebaluatu zen CdTe QDs atomizatua duen tintaren potentzial

zitotoxikologikoa, ustekabeko egoera bat simulatzen duen esposizio akutuko erregimen batean (egoera okerragoa), aire likidoko interfazean (ALI), BEAS-2B biriketako zelula-lerroa erabiliz. Aztertutako parametroek bideragarritasun zelularra, estres oxidatiboa eta hantura-efektuak barne hartu zituzten. Ikusi dugunez, CdTe QDs erabiliz gehitutako tintak, 54,7 ng/ml-ko kontzentrazioan, % 25,6 murriztu zuen zelularen bideragarritasuna, esposizioko ordu bat igaro ondoren aire garbiarekin alderatuta; kontzentrazioa 65 aldiz handiagoa izatea beharrezkoa izan zen, urpeko baldintzetan antzeko efektu bat ikusteko. Hala ere, ez dugu estres oxidatiborik ikusten, ezta hantura-efekturik ere. Azterlan honek nanoaditibatutako tinten erabilera-fasea simulatzen duten agertokien garapena eta *in vitro* efektuen ebaluaziorako zuzeneko esposizio zelularra biltzen ditu, eta, horrela, bizi-zikloari zuzendutako ikuspegia inplementatzen du CdTe QDei lotutako toxikotasunaren ebaluazioan.

HITZ GAKOAK

CdTe puntu kuantikoak; Inprimatzeko tinta; Askapena; Aire bidezko esposizioa; Zitotoxikotasuna

1. Introduction

The use of nanoparticles (NPs) in ink formulations is leading to products with new or enhanced properties and applications. This has opened new market opportunities and several companies are taking advantage of these nano-based technologies. Quantum dots (QDs) confer a wide range of optical properties to pigments/inks, covering the most requested needs in applications for the near future. They have a broad absorption and narrow band emission that can be tuned based on their composition and size, change electrical conductivity or improve thermal and photochemical stability (Kshirsagar et al., 2013; Lange and Wedel, 2017). One of the most widely used are cadmium telluride (CdTe) QDs, due to their ease of tunability, high photoluminescence, quantum efficiency and stability in water (Wuister et al., 2003).

Inkjet printing is an attractive technology for microscale patterning since it can eject tiny droplets (with diameters in the range 50–100 μm), which are composed of either solutions or dispersions of functional materials, onto addressable sites on a substrate (Tekin et al., 2007). To date, whereas most of the research studies have focused on emissions from laser printers and their corresponding effects towards human health (Karrasch et al., 2017; Nakadate et al., 2018; Pirela et al., 2015; Terunuma et al., 2019), no solid scientific evidence has been reported in the literature confirming the emission of airborne particles from inkjet printers (Shi et al., 2015; The Danish Environmental Protection Agency, 2015). However, Konga and coworkers concluded that inkjet printer emissions exert a synergistic effect in the presence of environmental tobacco smoke and induce intense damage to the lung mitochondria in an asthmatic murine model by disrupting the structural and functional integrity of the mitochondrial membrane (Konga et al., 2009).

In general, focus of the research so far has been placed on the type of printers and printing parameters, but the emissions and health effects that can be attributed to the nature of the ink have not been investigated. Furthermore, the toxicological effects of the emissions of inkjet printing ink containing NPs, used as additives, might differ from those of conventional formulations. In this regard, the CEPE European Council of the Paint, Printing Ink and Artists' Colours Industry, in a communication from 2012, argued that (i) *NPs used in paints, printing inks and Artists 'Colours are bound either in a liquid matrix (mixture before*

application) or in a solid matrix (final film after application and drying) and that by definition, the term "bound" includes all types of physical/chemical bonds (e.g. covalent, ionic, Van der Waals) which prevent any release of NPs from such matrices and; (ii) *NPs used in paints, printing inks and Artists 'Colours are not likely to be extracted or released (from the mixture or the final film) under normal or reasonably foreseeable conditions of use* (CEPE, 2012).

Cadmium and cadmium-containing compounds have been classified as carcinogenic to humans at the lungs by the International Agency for Research on Cancer (IARC 2012), based on the evidence that ionic cadmium causes genotoxic effects in different types of eukaryotic cells, including human cells. Nevertheless, a number of cadmium-based chemicals along with CdTe have been developed in Europe (ICdA, 2020) and are registered in REACH (ECHA, 2020a, b).

Specifically concerning CdTe QDs, Nguyen et al. investigated their effects on mitochondria in human hepatocellular carcinoma HepG2 cells, concluding that CdTe QDs caused disruption of the mitochondrial membrane potential, increased intracellular calcium levels, impaired cellular respiration, and decreased adenosine triphosphate synthesis (Nguyen et al., 2015). In another study, Su et al. compared the cytotoxicity values of QDs and free cadmium ions, and found that CdTe QDs were more cytotoxic than $CdCl_2$ solutions, even when the intracellular Cd^{2+} concentrations were identical in HEK293 cells (Su et al., 2010). Of main relevance concerning the respiratory exposure route, Zheng et al. (2018) studied the cytotoxicity and carcinogenicity of uncapped CdTe QDs concluding that acute exposure to CdTe QDs with diameter < 5 nm for 24 and 48 h elicited dose-dependent cytotoxicity in BEAS-2B cells, suggesting that CdTe QDs are potent human lung carcinogens. In 2019, Xu et al. characterized the proteome response of BEAS-2B observing that uncapped CdTe QDs with diameter < 5 nm significantly altered the BEAS-2B proteome, inducing oxidative stress (Xu et al., 2019).

The present study is based on the hypothesis that the use of nano-additivated ink in normal or accidental conditions may lead to human exposure and associated health risks. It characterizes the emissions of a product under development consisting in a water-based ink containing PEG-CdTe QDs during household inkjet printing under controlled conditions, representing potential release pathways for the PEG-CdTe QDs during the ink's usage. Subsequently, an acute exposure regimen to high-dose PEG-CdTe QDs aerosol as

an accidental, worse-case scenario is simulated. To attain such purpose, an approach is developed using atomization and *in vitro* exposure at the air liquid interface (ALI) of the human bronchial epithelial cell line BEAS-2B, representing the initial part of the airways in contact with inhaled aerosols, in order to assess the potential health effect of airborne emissions of the nano-additivated ink. Additionally, cell viability is assessed in submerged conditions using the same cell line for comparison. Finally, a selection of genes relevant to screen oxidative stress and inflammatory response are investigated at the ALI.

The approach undertaken, in addition to assuring reproducibility in exposure, is relevant for first toxicity screening purposes in a safer-by-design approach. Furthermore, the present study is useful in other situations potentially conveying an associated release of NPs under normal conditions of use since water-based inks can also be used in alternative printing methods including, amongst others: flexographic, lithographic, gravure or screen-printing, and serves as a model for inkjet printing inks containing other NPs (e.g. Ag NPs). To the best of the authors´ knowledge, no previous studies have been published analyzing the toxicological effect of airborne emissions of CdTe QDs containing inks.

2. Materials and methods

2.1. Ink formulation and characterization

Ink containing CdTe QDs of 3-5 nm diameter capped with PEG (750)-O-C(=O)CH_2CH_2-SH and a λ emission maximum of 750 ± 5 nm was synthesized, manufactured and provided by PLASMACHEM GmbH (Germany) as a prototype. Its composition cannot be detailed for confidentiality issues. The solvent ink formulation without QDs was also provided to be used as control material.

The PEG-CdTe QDs ink was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500, Agilent Technologies) to determine the Cd and Te concentration after digesting the ink with acid in an analytical microwave digestion system (MARS, CEM, 1600W) (for a detailed protocol see supporting information section 1 (SI-S1)). Transmission electron microscopy (TEM, JEOL 1010) and high-resolution TEM (JEM-2011) coupled with energy-dispersive X-ray spectroscopy (EDX) were used to determine the shape and size of

the QDs. The TEM images were analysed using FIJI software (Schindelin *et al* 2012). Thermogravimetric analysis (TGA) was performed on the ink with and without QDs to determine the differences produced by the QDs' incorporation, using a TGA Q500 (TA Instruments) under a N_2 flow of 90 mL·min^{-1} and heating from 25°C to 995°C at a heating rate of 10°C·min^{-1}. Fourier-transform infrared spectroscopy coupled to an Attenuated Total Reflectance (FTIR-ATR, Affinity-1 Shimadzu 8400) was performed to determine the presence of characteristic functional groups of the coating or impurities present in the sample; spectra were recorded in the region of 600-4000 cm^{-1}. Before TGA and FTIR-ATR were performed, the ink was freeze-dried (100-9 PRO Freeze Dryer, CoolSafe) at -95 °C and ~0.3 mbar, to increase the PEG-CdTe QDs signal in the analysis by getting rid of the water.

2.2. QDs release during paper printing process at household scale

For the printing experiment, a household inkjet printer (Pixma P7250, Canon) with refillable ink cartridges PGI-525 and CLI-526, with auto-reset chips (Octopus) was used. The PEG-CdTe QDs ink was manually loaded into the cartridges after gentle shaking. Printing was done at maximum quality on greyscale to assure that the ink was exclusively printed from the refillable black cartridge for PGI-550 black, whereas the rest of the cartridges were inserted in the printer but intentionally left empty. Printing was performed on 100 % recycled paper 80 g/m^2 (Staples) and the printed pattern was maintained during the whole process to minimize variability.

The printer was enclosed in a hermetically closed methacrylate box of 1.00 x 0.60 x 0.40 m (0.24 m^3), which had a clean air polycarbonate filter (225-9542, SKC). Silicon tubes of 6 mm diameter were introduced inside the box and connected to the air measurement instruments to sample the air. Sampling points were located around 5 cm above the output tray of the printer. A schematic representation of the setup has been included in Figure SI1. For particle number concentration (PNC) measurement two different instruments were used: an Scanning Mobility Particle Sizer (SMPS) spectrometer (NanoScan 3910, TSI) with size range of 10 to 420 nm, 60 seconds scan time and 0.75 L/min flow rate, and an Optical Particle Sizer (OPS 3330, TSI) with size range of 300 to 10000 nm, 60 seconds scan time and 1.00 L/min. Measurements of PNC were undertaken before, during and after the household printing process. Once a low background of particles inside the chamber (<150

particles/cm^3) was reached, a 10-minute printing cycle was performed followed by uninterrupted air monitoring for 75 minutes, to observe the evolution of PNC.

A transmission electron microscopy (TEM) sampler device (MPS, Ecomeasure) was connected to the SMPS outlet allowing to collect the released material directly onto copper TEM grids coated with carbon, which were later analyzed. In a different configuration of the setup and with the purpose of collecting particles emitted in each of the stages (i.e., before, during and after printing) a glass fibre filter (Type A/E filter, Pall Corporation) was placed into the OPS to capture the particles passing through it, and runs were conducted changing the filters at the end of each stage. The collected material was further observed using an optical microscope (Axioplan, Zeiss) equipped with epifluorescence and a multi-band fluorescence bandpass filter.

2.3. Ink aerosol generation for *in vitro* cell exposure at the ALI

A new setup based on atomization of the PEG-CdTe QDs ink was designed for ALI exposure of a pulmonary cell line in a Vitrocell® module (Figure SI2) with four compartments. For the atomization experiments, 2 mL of each sample (solvent ink and PEG-CdTe QDs ink) were dispersed in 80 mL ultrapure water, to obtain a 1:40 dilution for an optimal atomization (among the several dilutions tested, this had the smallest particle mode without interference of smaller particles < 10 nm as measured in-line using the 3936 SMPS from TSI at particle size range 15-661 nm; see Figure SI3). These dispersions were atomized in a fume cupboard, using a collision-type atomizer (ATM 220, Topas) and a pressure of 2 bar. The QD-containing air flow leaving the atomizer at 4 lpm was dried using a diffusion dryer (DDU 570, Topas). Using a T-split junction, 0.3 lpm of the aerosol flow entered the dispersion unit of the Vitrocell® exposure module, and the remaining flow of 3.7 lpm was led into the exhaust of the fume hood using a pump connected to a mass flow controller. Each of the four positions of the exposure module extracted 0.003 lpm from the dispersion unit using a pump with a four way flow splitter with, for each flow, adjustable high precision valves.

During the exposure, temperature was controlled through a circulating 37°C water bath and the exposure period was 1 h. Four replicate cultures were treated in parallel in the Vitrocell® system.

2.4. Cell culture settings

The BEAS-2B cell line (CLR-9609, ATCC), originally derived from normal bronchial epithelial cells, was cultured as described by Verstraelen *et al.* (2014). Briefly, cells were seeded at a ratio of 0.2 mL/cm^2 surface area, in growth medium (BEGM), consisting of bronchial epithelial cell basal medium (BEBM, Lonza) supplemented with 0.5 ml retinoic acid, 0.5 ml epinephrine, 0.5 ml triiodothyronine, 0.5 ml human recombinant epidermal growth factor, 0.5 ml insulin, 0.5 ml gentamicin sulfate amphotericin-B, 0.5 ml transferrin, 0.5 ml hydrocortisone, and 2 ml bovine pituitary extract (BulletKit, Lonza). Cells were kept in a humidified atmosphere at 37°C and 5% CO_2, and subcultured before reaching 80% confluence. Medium was refreshed every 2-3 days and cells were subcultured every 4-5 days (1500–3000 cells/cm^2).

For the exposure at the ALI, cells were seeded at a density of 15000 cells/cm^2 on precoated Corning® Transwell® polyester membrane inserts, pore size 0.4 µm as described elesewere (Geys et al., 2006), membrane diameter 24 mm (Sigma-Aldrich Chemie Gmbh). Inserts were placed in a sterile 6-well plate, and BEGM was added to both sides: 2 mL basolateral and 1 mL apical. Plates were incubated for 48 h at 37°C, 5% CO_2 in a humidified incubator before replacing the growth medium by BEBM without growth factors, 16 h prior to ALI exposures to synchronize cell growth. Immediately before exposure, culture medium was completely removed from the apical side of the inserts and cells were washed with sterile phosphate buffered saline (PBS). Next, cells were transferred into the chambers of the *in vitro* exposure module (6PT-CF, Vitrocell® Systems) with 4 compartments to hold 24 mm inserts. The basolateral chambers were filled with 15-16 mL pre-warmed BEBM.

2.5. Deposition efficiency and dose quantification

To calculate the deposition efficiency, aerosolized PEG-CdTe QDs ink generated by the atomizer during 1h was collected in 0.5 ml ultrapure water (quadruplicates, corresponding to the 4 positions in the Vitrocell® module) using the same setup as for the cell exposures at the ALI. Cd and Te concentrations were determined using a quadrupole ICP-MS instrument (Nexion 300s Perkin Elmer) after microwave oven digestion with nitric acid, as described in SI-S2.

To quantify only the ions concentration to which cells had been exposed in the Vitrocell® module, Cd and Te ions were collected in BEGM medium without cells and separated from non-ionic particulate compounds by transferring the medium to centrifuge tubes (Pall Microsep™ Advance with 10 kDa Omega™ membrane) and centrifugation at 4000xg for 10 minutes (Hettich, Rotanta 460R). Ions were quantified using non-destructive ICP-MS analysis. Recovery tests of an internal standard Rhodium (^{103}Rh) showed 20% signal suppression due to matrix interference during sample analysis, and this correction was applied to the results.

2.6. *In vitro* cell exposure at the ALI

BEAS-2B cells were exposed in the Vitrocell® module to PEG-CdTe QDs ink (QDs Ink) and solvent ink (S. Ink). An optimal flow rate of 3 ml/min during 1 h of exposure was selected, based on prior experiments with synthetic clean air, in order to minimise mechanical stress and dehydration of the cells, as well as to maintain cell viability after exposure. As vehicle and positive controls for cell viability and oxidative stress assessment and cytokine production, cells were exposed to synthetic clean air (C. Air, vehicle control) and to 10 ppm NO_2, respectively. Additionally, untreated cells on inserts without apical medium were kept for 1 h in a humidified 37°C incubator with 5% CO_2 were used to control basal cell growth and viability on the inserts (IC-). Finally, as additional negative and positive control conditions for cytokine induction, cells submerged in apical medium for 24 h without treatment (IC+) or containing 20 µg/ml lipopolysaccharide (LPS) from *Escherichia coli* (Sigma-Aldrich Chemie Gmbh) were used, respectively. Two biologically independent runs (2 replicate inserts/run) were performed for each exposure condition (Figure 1).

After exposure, cells were post-incubated by placing the inserts in a new sterile six-well plate with 2 and 1 ml BEGM basolateral and apically, respectively, and allowing cell recovery in a humidified 37°C incubator with 5% CO_2, in agreement with previous works (Persoz et al., 2012). Specifically, cells in two replicate inserts were post-incubated for 23 h along with the incubator controls (without or with apical medium, and LPS-treated cells) for evaluation of cell viability and cytokine secretion, whereas the other two replicate inserts were post-incubated for 1 h and analysed for oxidative stress and inflammatory response by gene expression using RT-qPCR (Figure 1).

Complementarily, and taking into consideration previous studies reporting direct association between Cd ions from CdTe-QDs in the cell culture medium and cytotoxicity in other epithelial cell lines (Du et al., 2019; Su et al., 2010), BEAS-2B was exposed to six serial 1/5 dilutions of PEG-CdTe QDs from 8.6-27000 ng/ml in submerged conditions, and Cd and Te ions were quantified by ICP-MS (see the details in SI-S3). Previous data from our laboratories exposing the same cell line in submerged conditions to $CdCl_2$ were also used with comparison purposes.

2.7. Viability assessment

Cell viability was assessed using the Prestoblue™ Cell Viability Reagent (Life Technologies), according to the manufacturer's instructions. The apical medium was replaced with 1 mL of a 10% Prestoblue™ solution in growth medium to allow living cells to metabolize resazurin. After 2 h of incubation at 37°C, fluorescence of the collected apical solution was measured in a 96-well plate at an excitation wavelength of 530 nm, and an emission wavelength of 620 nm using a Fluoroskan Ascent® reader (ThermoFisher Scientific, Waltham, MA, USA). Cell viability was expressed as the percentage of fluorescence relative to the vehicle control (i.e. C. Air) cells.

In submerged conditions cell viability was assessed by neutral red assay and by trypan blue (vital) staining and counting using a Countess Automated Cell Counter (Invitrogen), for $CdCl_2$ and the solvent and CdTe QDs inks respectively (SI-S4).

2.8. Oxidative stress and inflammatory response

For gene expression analysis, the medium was aspirated from the apical surface of the cells after 1 h post-incubation and cells were lysed with RLT lysis buffer (Qiagen) containing 1% 2-mercaptoethanol (Sigma-Aldrich Chemie Gmbh). Total RNA was isolated using the mini RNeasy RNA isolation kit (Qiagen), according to the manufacturer's specifications. The RNA concentration was determined using a NanoDrop Spectrophotometer (NanoDrop Technologies). RNA was stored in RNase-free water (Qiagen) at −80 °C.

For cDNA synthesis, the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science) was used, based on the use of random hexamer and oligo-dT primers. The procedure is described in the manufacturer's specifications. The amount of starting RNA

in the reverse transcription reaction was 0.5 µg. cDNA samples were further diluted in nuclease-free water and stored at -20°C.

After cDNA synthesis, qPCR was performed in 96-well plates in duplicate for each sample using a LightCycler 480 Probes Master Mix on a LightCycler 480 instrument (Roche Applied Science). Amplification reactions for genes encoding interleukin (IL)-8, IL-6, heme oxygenase (HMOX)1, tumor necrosis factor (TNF)-α and IL-1β proteins, and reference genes Ribosomal Protein Lateral Stalk Subunit P0 (*RPLP0*), Guanine Nucleotide-binding protein subunit Beta-2-like 1 (*GNB2L1*), and Peptidyl-Prolyl cis-trans Isomerase A (*PPIA*) were monitored using double-quenched probes™ designed using PrimerQuest Tool and supplied as PrimeTime qPCR probe assays (Integrated DNA technologies). All samples were measured in the same run for a given target (i.e. sample maximization strategy according to Hellemans et al. (2007)). Gene expression changes were analysed using the qBase software. The expression levels obtained were normalized against the reference genes and fold changes in expression levels of conditions at the ALI (QDs and S. ink and NO_2) were given relative to the vehicle control (i.e. C. Air).

At the protein level, the pro-inflammatory cytokines interferon (IFN)-γ, IL-1β, IL-6 and TNF-α were also measured using a multiplex protein assay. After 23 hours post-incubation apical culture supernatants were collected and centrifuged at 300 x g for 5 min to remove cell debris. Protease inhibitor cocktail (0.2% v/v; Sigma–Aldrich Chemie Gmbh) was added to the samples before freezing at -80° C, and samples were stored until analysis. The cytokines were quantified using the human pro-inflammatory I (4-plex) kit (Meso Scale Discovery) on a MESO QuickPlex SQ120 instrument, according to the manufacturer's instructions. Calibration curves were used to calculate the cytokine concentrations, expressed in ng/ml. Fold changes in of cytokine concentrations measured in the different conditions at the ALI (QDs and S. ink and NO_2) were given relative to the vehicle control (i.e. C. Air).

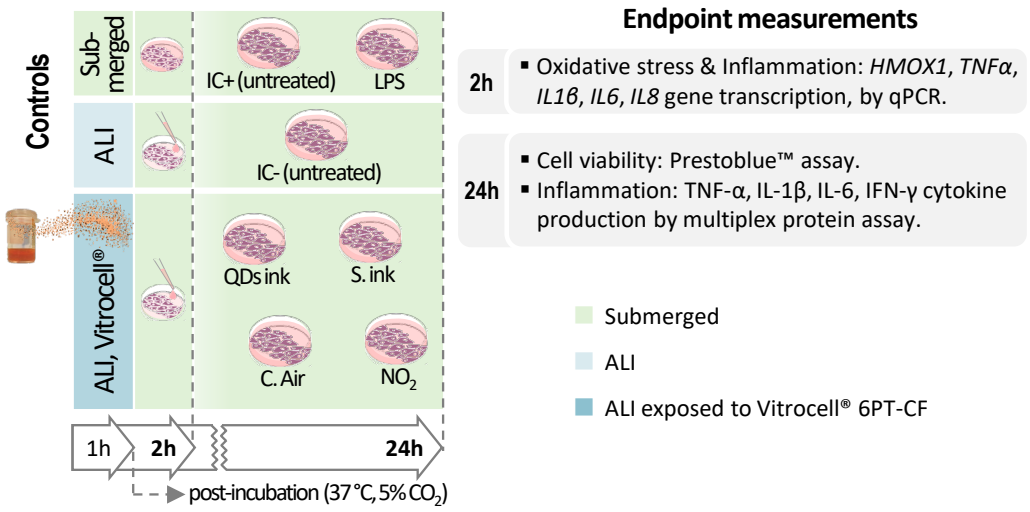


Figure 1: Schematic representation of the different *in vitro* cell conditions, with indication of exposure and post-incubation times, control conditions and the respective endpoint measurements. Abbreviations: IFN: interferon; IL: interleukin; LPS: lipopolysaccharide; TNF: tumour necrosis factor.

2.9. Statistical analysis

The biological response data for ALI experiments are shown as mean values ± standard deviation of duplicate inserts from two independent experiments. For the statistical tests and graphical representations, the GraphPad Prism 7.04 software was used. For the evaluation of the biological data normality was assessed both for cytotoxicity (D'Agostino & Pearson test, K2), inflammation and oxidative stress (Shapiro-Wilk test) datasets before applying a 2-way ANOVA (Tukey's multiple comparison tests).

3. Results

3.1. PEG CdTe-QDs ink characterization

Results from the ICP-MS elemental analysis indicated Cd and Te concentrations in the PEG CdTe-QDs ink of 10.24 (±0.86) mg/mL and 3.53 (±0.18) mg/mL, respectively. Thus, the total concentration of CdTe-QDs in the ink was 13.77 mg/mL. Considering that the atomic mass of Cd is 112.4 u and 127.6 u for Te (Meija et al., 2016) the Cd and Te ratio was around 3:1. The higher Cd content can be explained by the fact that Cd atoms are located around the QDs' surface, as a result of the synthesis method employed. Cadmium is needed in the surface to attach the QDs to the ligand (PEG) through a Cd-S bond. For control samples of

ultrapure water and solvent ink, values under the limit of detection (<0.1 ng/mL) were obtained for both Cd and Te.

From the TEM characterization it can be observed that the particles were slightly aggregated, forming networks (Figure 2A). Particles size measurements (Figure 2B) confirmed that the QDs contained in the ink presented the size claimed by the manufacturer, from 3 to 5 nm. The EDX measurements showed a Cd:Te molar ratio of 2.7 (Figure 2C), a result concordant to the one determined by ICP-MS. At higher magnifications (Figure 2D) the interplanar distances could be observed and measured, resulting in an average distance of 3.53Å, which was close to the expected interplanar distance between (111) planes in CdTe structures (3.75Å) (Guillén-Cervantes et al., 2015).

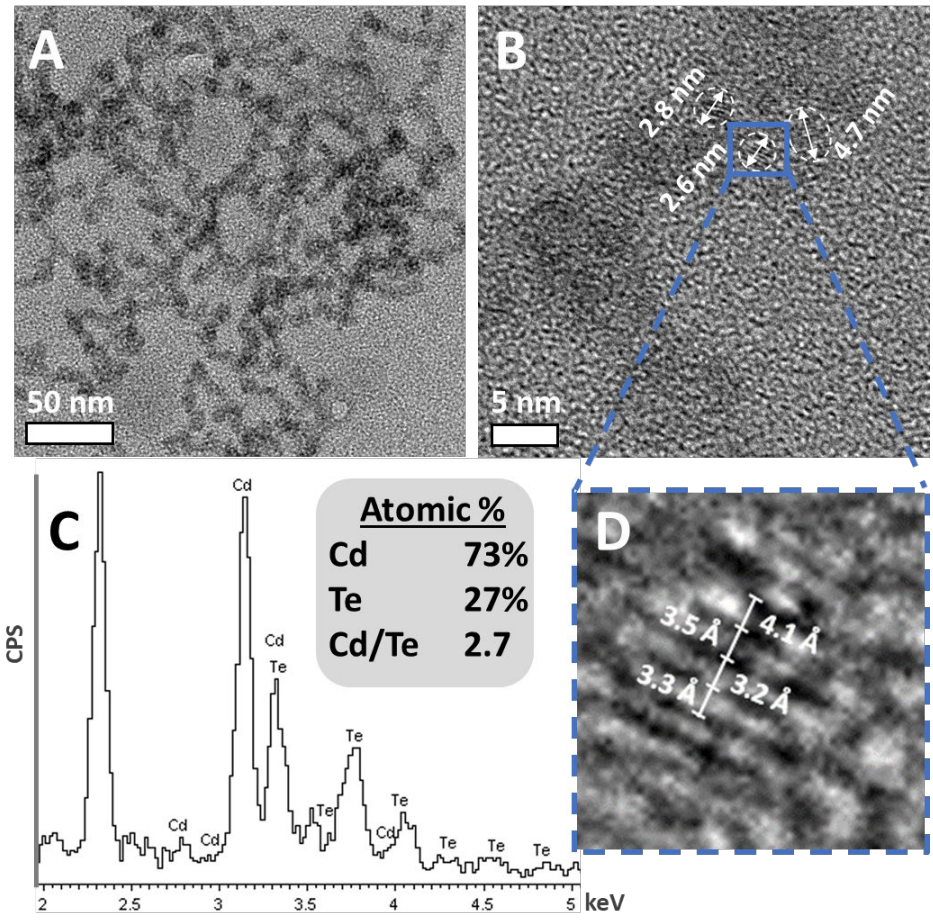


Figure 2 – **A**) TEM image of the ink showing aggregated PEG-CdTe QDs networks. **B**) Section of the PEG-CdTe QDs network where individual QDs are indicated by a white dashed line and their diameter measured. **C**) EDX spectrum of the PEG-CdTe QDs ink showing the atomic percentage of Cadmium and Tellurium: Counts per Second (CPS) are displayed against voltage (keV). **D**) Higher magnification image of a QD where the interplanar distances were measured.

TGA allowed to determine the inorganic mass percentage in the PEG-CdTe QDs ink (Figure SI4), since Cd and Te remained after the temperature raised over 400 ºC while the organic components were volatilized. For the solvent ink almost no residue remained, whilst for the PEG-CdTe QDs ink the residue represented a 6.7% of the weight. Considering that after the freeze-drying process only the 20.9% of the ink mass remained (which was a viscous liquid), the 6.7% measured in the TGA corresponds to the 1.4% of the ink. Since the ink's density is close to 1 g/cm^3, the concentration is equivalent to 14.0 mg/mL, which was close to the results obtained in the ICP-MS measurements (Cd plus Te). This suggests that, as expected, the only inorganic material in the ink (the TGA residue) corresponds to the PEG-CdTe QDs. Moreover, for both the solvent ink and the PEG-CdTe QDs ink, the two main weight losses were observed at the same temperatures, at 200°C and 350℃, suggesting that there were no alterations on the solvent ink after the CdTe QDs addition.

The FTIR-ATR spectrum of the freeze-dried PEG-CdTe QDs ink (Figure SI5) showed the characteristic absorption bands of PEG, at 2800 cm^{-1} (stretching C-H) and at around 1100 cm^{-1} (stretching C-O). Interestingly, another peak was observed at around 1630 cm^{-1} which could be related with thioglycolic acid which is a common reagent employed in CdTe QDs synthesis (Abd El-sadek and Babu, 2011, Arivarasan et al., 2014). The presence of a broad band observed around 3400 cm^{-1} might be related with the presence of residual water.

3.2. QDs release during paper printing process at household scale

Variations of PNC in air before, during and after the household PEG-CdTe QDs ink printing process measured with the SMPS and OPS (size range from 10 to 10000 nm) are shown in Figure 3. Before printing, the pumps incorporated in the air measurement instrument circulated the air outside the chamber allowing new air to enter through the polycarbonate filter and renewing the air inside the chamber. As a result, the PNC decreased along time, going from 350 to 140 particles/cm^3 after 90 minutes. When the printing process started (grey shadowed region), a quick increase in PNC could be clearly observed, reaching a maximum value of 593 particles/cm^3. After the printing stopped, due to the recirculation of air with the instruments' pump, PNC progressively decreased. One hour was needed to reach a PNC around 220 particles/cm^3. As can be seen in the top part of Figure 3, no

fluorescence (related to PEG-CdTe QDs) was observed in the filter corresponding to the background stage. However, intense fluorescence was observed on the filter placed during the printing process, meaning that QDs were released into the air. After printing, fluorescence was also detected (with a lower intensity than in the filter belonging to the printing process), suggesting that some QDs remained in the air after printing.

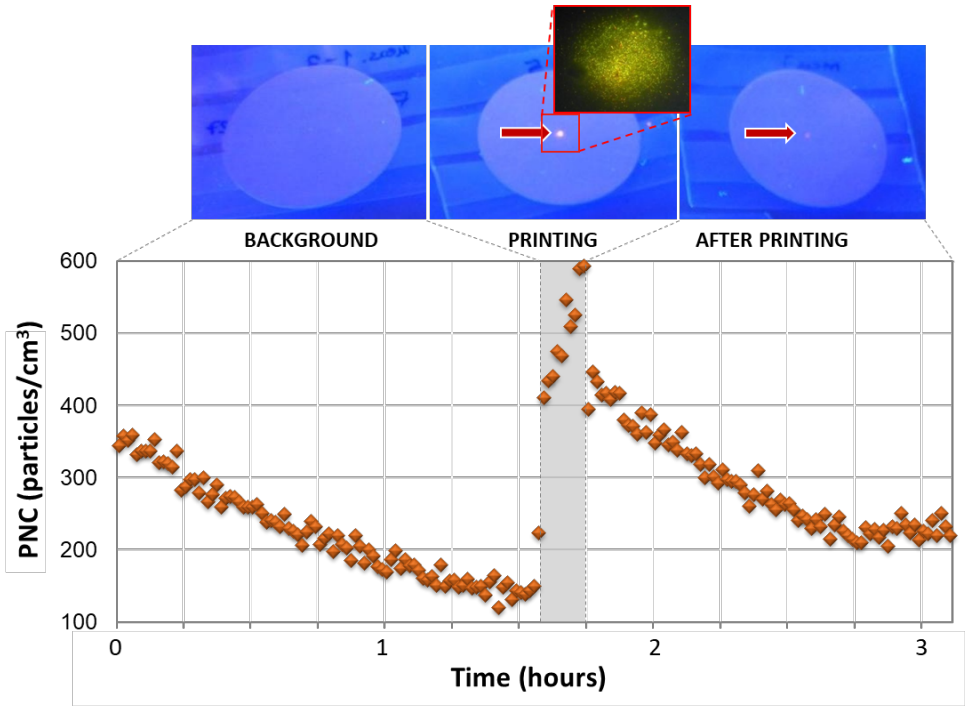


Figure 3 - Particles number concentration (PNC) in air during the PEG-CdTe QDs ink household printing process measured using an SMPS and OPS in the defined setup, and filters (images at the top) containing material collected for 10 min before (background), during and after printing. The insert shown in the image during printing corresponds to an epifluorescence image of the filter.

Electron microscopy images of the particles released during the household printing process and retained on the TEM grid are shown in Figure 4. Evidences of the PEG-CdTe QDs collected during the printing process were found. Similar aggregates to the ones observed in the ink (Figure 2) were observed, suggesting that released materials did not contain isolated PEG-CdTe QDs, but droplets containing PEG-CdTe QDs aggregates instead (Figure 4A-B). At higher magnifications, the crystallographic planes of the PEG-CdTe QDs were observed, presenting an approximate interplanar distance around 3.23 Å, which is very close to the size measured in the original PEG-CdTe QDs ink sample (Figure

4C-D). Measurements of released individual PEG-CdTe QDs provided diameter sizes similar to the ones observed in the ink, 3 to 5 nm, while aggregates presented diameter sizes around 20 nm. EDX measurements (Figure SI6) confirmed that the observed structures contained Cd and Te.

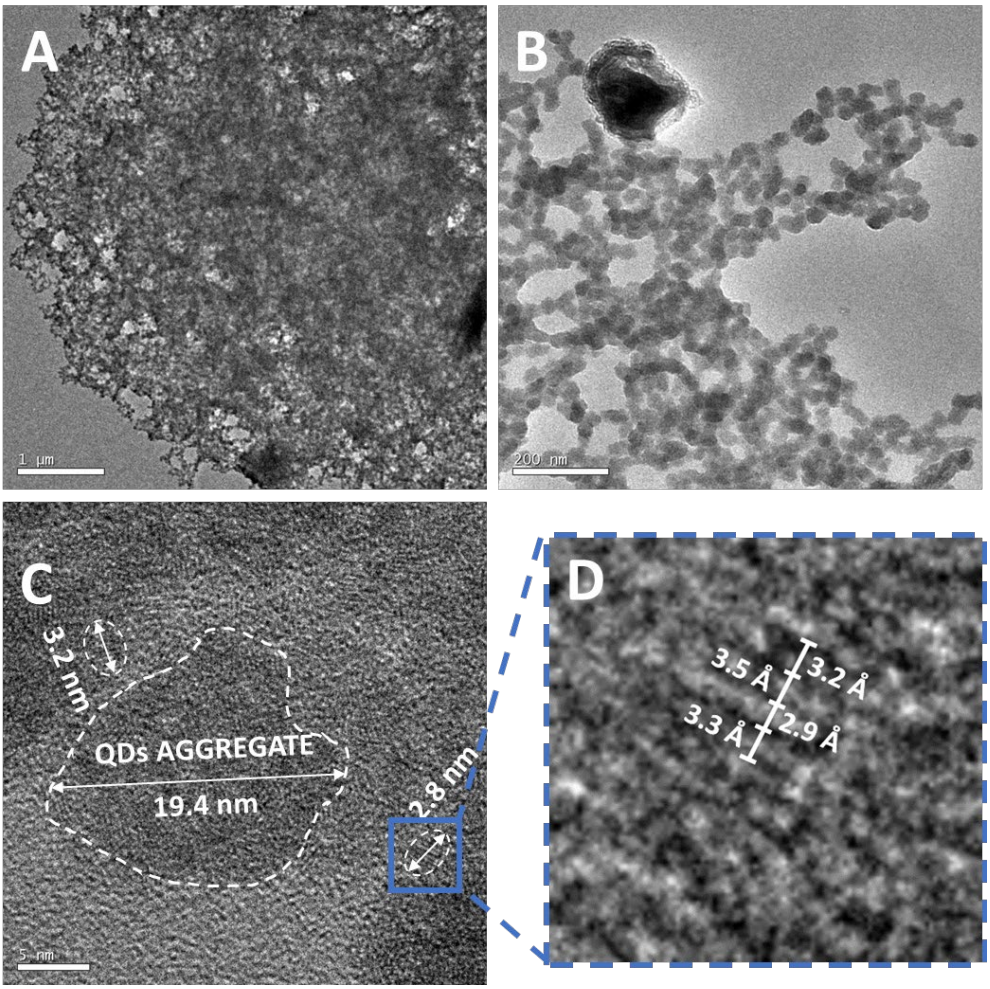


Figure 4: TEM images of the airborne CdTe-QDs emitted during PEG-CdTe QDs ink household printing collected with sampler device. **A**) TEM image of aggregated ink on the TEM grid. **B**) PEG-CdTe QDs networks **C**) Section of the PEG-CdTe QDs network where individual QDs and QDs aggregates are indicated by a white dashed line and their diameter measured. **D**) Higher magnification image of a QD where the interplanar distances were measured.

3.3. Deposition efficiency and dose quantification after *in vitro* ink exposure

The maximum possible dose of 1246.9 ng per well was calculated with the average PNC of $6.74 \cdot 10^6$ particles per cm^3 and the average diameter of 69.5 nm, both determined by SMPS.

Dose quantification by ICP-MS of the aerosolized particles in the Vitrocell® module for each compartment determined that BEAS-2B cells were exposed to a total dose of 37.1 (±6.2) ng/ml of Cd, and 17.6 (±2.6) ng/ml of Te (Table 1). It corresponds to a Cd:Te ratio of 2.4:1 and a total CdTe concentration of 54.7 ng/ml (± 8.6), implying an exposure of 12.1 ng/cm^2. A deposition efficiency of 4.39% was determined by SMPS based on the average particle size. Solvent ink gave values under the limit of detection (<0.1 ng/mL) for both elements in the conditions tested.

Table 6: Dose quantification after 1 h exposure to the aerosolized PEG-CdTe QDs ink in the Vitrocell® module using ICP-MS. Total and ionic doses of CdTe, Cd and Te are indicated.

Total CdTe (ng/ml)	**Total Cd** (ng/ml)	**Ionic Cd**		**Total Te** (ng/ml)	**Ionic Te**	
		(ng/ml)	(%)		(ng/ml)	(%)
54.7± 8.6	37.1±6.2	10.9 ±3.6	29.4	17.6 ±2.6	16.0 ±4.5	90.9

Concerning the exposure in submerged conditions, the percentage of dissolved Cd and Te ions determined was inversely proportional to dose (Table SI1), as could be expected at low concentrations, where the dissolution rate increases to reach an equilibrium. Though, when compared to the dose determined for cells in the Vitrocell® module, the estimated ionic dose was much higher for Cd, with a 63.1% (46.9 ng/ml) present in its ionic form, whilst Te was ionized by 45.0% (15.9 ng/ml).

3.4. Cell viability assessment

Atomized solvent and PEG-CdTe QDs inks decreased cell viability by 9.0% (±11.9%) and 25.6% (±15.7%) respectively, whereas NO_2 –positive control– decreased cell viability by 32.8% (±11.7%) in contrast to clean air condition.

Differences in the cytotoxicological response between all the conditions tested at the ALI were statistically significant, with p-values <0.001 (see Figure 5). Regarding complementary controls, IC- (incubator control at the ALI) showed statistically significant differences ($p<0.0001$) when compared with IC+ (incubator control in submerged conditions); whereas

the cytokine induction control with LPS (performed also in submerged conditions) compared to IC+ did not show significant differences.

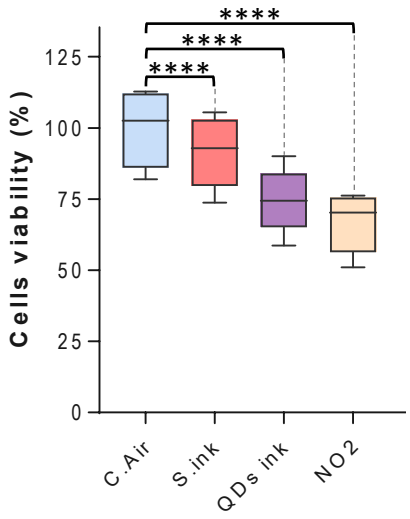


Figure 5 – Box-and-whisker plot showing the mean viability of BEAS-B2 cells exposed for 1 h at the ALI to different conditions, compared to a clean air (C. Air) atmosphere: solvent ink (S. Ink), PEG-CdTe QDs ink (QDs Ink) and nitrogen dioxide (NO_2) as cytotoxicity control for cytokine induction control. Error bars indicate standard deviation. **** = $p<0.001$.

Results in submerged conditions showed that cytotoxicity had a good correlation (r^2 = 0.872) with Cd ionic concentration, whether it had been added as QDs or as a salt. However, no cytotoxicity could be observed below 500 ng/ml of Cd in ionic form ($p > 0.05$). A similar cell survival attributed to PEG-CdTe QDs at the ALI (i.e. 74.4% cell viability compared to vehicle control, 83.4% compared to solvent ink control), with 10.9 ng/ml Cd ions, corresponded to 890 ng/ml of Cd ions in submerged conditions (Figure SI7). This remarkable change in the dose-effect may be due to a differential impact caused by the exposure route, highlighting the importance of the model used for cytotoxicity assessments relevant to airways.

3.5. Oxidative stress and inflammatory response assessment

It has previously been described that QD-induced cytotoxicity can at least partially be explained by oxidative stress (Lovrić et al., 2005), which is accompanied by the induction

of antioxidant mechanisms in the cell. In addition, oxidant air pollutants, such as particulate matter and NO_2, have been shown to induce lung inflammation through stimulation of the oxidative stress process, which is a major pathway leading to pathological conditions in the lungs. Therefore, we investigated possible changes in gene expression of pro-inflammatory (IL-1β, IL-6, IL-8 and TNF-α) and oxidative stress (HMOX1) markers by real-time qPCR after *in vitro* exposure of BEAS-2B cells to both solvent and PEG-CdTe QDs inks at the ALI, and in submerged conditions for comparison.

IL-1β, IL-6, IL-8 and TNFα did not show altered transcription levels after atomized solvent and PEG-CdTe QDs inks exposure at the tested concentration when compared to clean air (Figure 6A); for IL-1β, differences were observed only when comparing C. Air and NO_2 conditions ($p=0.0333$). Similarly, HMOX1 which is a primary enzymatic anti-oxidant in the lungs induced by environmental components (heat stress, hypoxia, metals, endotoxins, hormones), was upregulated only by the positive control (NO_2).

In submerged conditions, transcription levels of HMOX1 were upregulated compared to the solvent ink control after exposure to ≥216 ng/ml of PEG-CdTe QDs ink, while IL-8 and IL-6 were upregulated after exposure to at least 5400 or 27000 ng/mL QDs ink, respectively. IL-1β and TNF-α mRNA levels remained unchanged after QDs ink treatment (Figure SI8A).

The production of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and IFN-γ was also measured at the protein level using a multiplex protein assay. No significant changes in the cytokine levels could be detected in the apical cell medium conditioned with the cells during post-incubation following the solvent and PEG-CdTe QDs inks exposure at the ALI compared to clean air condition (Figure 6B). Concerning complementary controls, no significant differences were observed due to removal of the culture medium at the ALI (IC+ vs IC-) for all selected proteins. LPS showed statistically significant differences ($p<0.0001$) for IL-6 and IFN-g, and for TNFa ($p=0.002$) when compared to IC+.

In submerged BEAS-2B cell cultures exposed to the PEG-CdTe QDs ink, IL-6 was also the only cytokine that was detected, showing increasing levels compared to solvent ink from 216 ng/ml onward (Figure SI8B). The levels of the other 3 cytokines were not induced by the PEG-CdTe QDs ink, as they did not pass the respective detection limits of the assay (IL-6 0.448 pg/ml; IFN-γ 0.924 pg/ml; IL-1β 0.355 pg/ml; TNF-α 0.204 pg/ml).

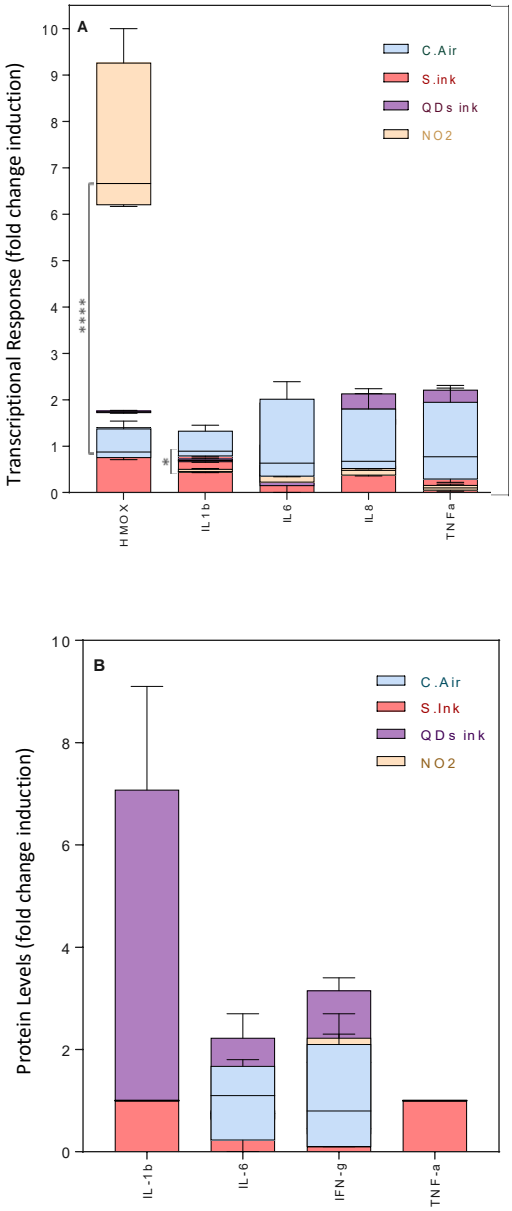


Figure 6: Box-and-whisker plot showing the impact of solvent and PEG-CdTe QDs inks, and nitrogen dioxide (NO_2) in contrast to clean air (C. Air) on BEAS-2B cells after *in vitro* exposure for 1 hour at the ALI, at two regulation levels: **A**) the transcription of oxidative stress (*HMOX1*) and pro-inflammatory cytokines (*IL1b*, *IL6*, *IL8* and *TNFa*) markers; **B**) the protein synthesis for the proinflammatory cytokines IL1-β, IL-6, INF-γ, and TNF-α. In both cases, fold-change was calculated compared to the vehicle control (C. Air). Error bars indicate standard deviation. * = $p<0.05$; **** = $p<0.0001$.

4. Discussion

In this study we report the release of QDs into the air during inkjet printing of a prototype of PEG-CdTe QDs containing ink and the *in vitro* screening of its health effects. We simulated a household printing process and confirmed that airborne PEG-CdTe QDs were released during inkjet printing. Released particle formats were determined by TEM, and revealed aggregated PEG-CdTe QDs forming networks, although their individual identity (with 3 to 5 nm diameter) was not lost. This is in contrast to Shi and colleagues who did not observe NP emission from 5 inkjet printers in a normal office environment (Shi et al., 2015). The discrepancy may be explained by the a priori presence of NPs (PEG-CdTe QDs) in the ink in our study.

The simulated release scenario of nano-ink aerosols may cause relevant human exposure and associated health risks. To assess potential health effects from respiratory exposure to aerosolized PEG-CdTe QDs ink, we performed an *in vitro* investigation using human bronchial epithelial cells that were exposed to PEG-CdTe QDs ink aerosol at the ALI in contrast to the not nano-additivated solvent ink. Such ALI exposure has been proven to provide a controlled, yet more practically and physiologically relevant approach for biological response assessment (Ihalainen et al., 2019; Lacroix et al., 2018), as the cells undergo an acute exposure scenario (1 h) followed by a physiological response time ranging from 1 to 23 h for mRNA expression and protein expression and metabolic response, respectively. To our knowledge this approach has not been applied to QDs toxicity assessment before and studies investigating the inhalation route of exposure are scarce (Zheng et al., 2018).

PEG-CdTe QDs contained in ink was delivered to the cells at a concentration of 37.1 ng/ml Cd and 17.6 ng/ml Te (54.7 ng/ml PEG-CdTe QDs), decreasing cell metabolic activity by 25.6% (±15.7%) in the ALI inhalation simulating conditions. A 9.0% (±11.9%) decrease of cell viability was also observed for atomized solvent ink. Notwithstanding the relevance of the results obtained, 1h cell exposure at relatively high concentrations of aerosolized ink does not reflect long-term exposure to low concentrations of particles resulting from household inkjet printing. We have used the Multiple Particle Path Dosimetry Model (MPPD) (v3.04) (Anjilvel and Asgharian, 1995) to calculate the lung deposition and deposition mass flux for particles emitted during inkjet printing on the human respiratory

system. Values used as input for the model were 500.14 particle/cm^3; 62 nm and 5.85 g/cm^3 for average particle concentration, mean particle diameter and particle density, respectively. The pulmonary deposited mass rate per unit area estimated by the MPPD model corresponded to a concentration of $9.30 \bullet 10^{-16}$ µg/min/cm^2, equivalent to $9.30 \bullet 10^{-12}$ ng/cm^2 for a 10 minutes printing period whereas the dose calculated during 1h atomization (12.1 ng/cm^2) corresponds to 2.02 ng/cm^2 if a 10 minutes period is taken as a reference. Despite the considerable difference with the estimated pulmonary deposited mass, the exact dose delivered to the cells in ALI is difficult to measure or estimate. For instance, Olham and coworkers (2020) have recently reported experimentally measured deposition efficiencies ranging from 0.013 to 0.86% in a Vitrocell® 24/48 ALI *in vitro* exposure system whereas we have reported a 4.39% deposition efficiency. Delivering nanoparticles in the form of suspensions is susceptible to inaccuracies related to dispersion techniques, dispersant media, re-agglomeration and setting behavior. An additional difficulty is related to the deposition rates of nanoparticle aerosols which are generally quite low (Paur et al., 2011), liming the accuracy of the calculated dose. The instrumentation used for dose measurement represents another obstacle: we have derived the dose on the basis of PNC and average particle diameter measured by an SMPS in conjunction with an OPS or by an SMPS only during the inkjet printing scenario and the atomization process, respectively (see M&M sections 2.2. and 2.3.), this implying that the full spectrum of particles generated during the inkjet printing and/or atomization process might not be covered within the particle size range measured by these instruments.

Long-term exposure is not possible with current *in vitro* ALI systems, such as Vitrocell®, since the survival of the cells is low (<4-8 hours) as the models lack culture medium and thus nutrients at the apical side. Alternatively, we have represented an acute worse-case-exposure scenario. In this set-up, aerosol generation by atomization was needed to obtain high nanoparticle levels and reproducibility in exposure. Even if the dose that we have selected is unrealistic, the composition of the aerosolized inks corresponds to that of particles released during inject printing: we have not exclusively focused on the toxicological effects associated to PEG-CdTe QDs used as an additive in inkjet printing ink. In this sense, Pirela et al. (2017) highlighted the use of toner particles instead of "real world"

exposures as a major research gap in a review describing the toxicological effects derived from nanoparticle exposures from toner-based printing.

An additional limitation is related to the short culture times (16h) of the cells before onset of experiments at the ALI restricting their mucociliary differentiation. In fact, our BEAS-2B cell model is only suitable for basal cell toxicity screening purposes. Epithelial cells contribute to lung immunity by secretion of chemokines, cytokines and antimicrobial compounds (e.g. ROS) (Parker and Prince, 2011). For further studies, to observe integrated cell responses such as interactions with mucus-secreting goblet cells and ciliar clearance of foreign particles, more complex models would be needed. In any case, only *in vivo* exposure models would allow to assess effects over the full human airway physiology: bronchus or conductive part and alveoli or terminal branches where gas exchange occurs; vascular endothelium and immune cells (alveolar macrophages being the front line cell type); or systemic effects such as translocation/accumulation of nanoparticles in secondary organs, and the subsequent responses.

Despite the aforementioned, ALI *in vitro* inhalation models mimic more closely the pulmonary region than classic (i.e., submerged) *in vitro* methods. In this sense, in previous studies also based on the assessment of metabolic cell activity, a similar toxicity level was reached around 37.5 nM (9 ng/ml) CdTe QDs in HEK293 cells (Su et al., 2010), 1 µg/ml in MCF-7 cells (Lovrić et al., 2005), and 15 µg/ml in HeLa cells (Du et al., 2019) after 24 hours exposure in submerged culture conditions. Differences of 4 orders of magnitude are observed, which may have several causes related to the key intrinsic QDs properties (including size, surface ligand) and purity of the suspension (Su et al., 2009). Furthermore, exposure time, exposure concentration, assay type, cell anatomical type and cell origin seem to also be major attributes to QD-induced toxicity response (Oh et al., 2016), as is the *in vitro* exposure format (ALI *versus* submerged cultures). The latter highlights the importance of the model used for cytotoxicity assessments related to airways. Of main relevance for nanoadditivated products, the exposure conditions at the ALI prevent from possible changes undergone in NP's physicochemical properties (e.g. dissolution, agglomeration) resulting from interactions with the cell culture medium.

Moreover, the use of ALI models has the potential to reduce the number of animals used in research as they may provide better previous information on *in vivo* processes in

humans, since some specific mechanisms might differ or do not exist in animal models due to physiological differences (Lacroix et al., 2018).

The CdTe QDs' cytotoxicity observed in this study could be due to the eventual entering of these toxic species into the cells, thereby causing a synergistic effect between CdTe-QDs' nanotoxicity and Cd ions-induced cytotoxicity (Zheng et al., 2018). Su et al. also reported a direct association between the total CdTe-QDs ingested by HEK293 cells and cytotoxicity (Su et al., 2010). However, they did not find a direct relationship between intracellular Cd^{2+} ions and CdTe QDs concentration supplied to the cells, and concluded that the nanoscale properties of the QDs played an important role in their cytotoxicity. Since Cd and its compounds are classified as group 1 carcinogens, its dissolution even capped must however be considered of high relevance for human and environmental health. Yet, in a recent study on *in vivo* biodistribution and systemic effects, Nguyen et al. observed that Cd alone cannot fully explain the toxicity of CdTe-QDs (Nguyen et al., 2019). Intrinsic QDs properties can also be major contributors of toxicity including size, surface ligands and residual reagents from the QDs synthesis process (Oh et al., 2016). The size (between 3 and 5 nm) of the PEG-CdTe QDs used in our study, as well as the observed presence of QDs' stabilizing reagent thioglycolic acid in the inks have been related to increased toxicity by others (Liu et al. 2013, Zheng et al. 2018, Xu et al. 2015, Du et al. 2019). In addition, but generally less well considered, tellurium is mildly toxic and thus can be partially responsible for the observed effects. *In vitro* studies by Vij and Hardey (2012) indicated that diphenyl ditelluride and tellurium tetrachloride significantly decreased cell viability in transformed (HT-29, Caco-2) and non-transformed colon cells (CCD-18Co), starting from 31.25 (4.0 µg/ml) and 500 µM (63.8 µg/ml) respectively. In our study, the total aerosol delivered Te dose to BEAS-2B cells was ~113 times lower, and therefore unlikely to contribute to the observed toxicity. Finally, PEG capping was applied to the QDs' surfaces in our study, which is known to reduce their non-specific binding to cells and subsequent uptake, as well as the release of Cd^{2+}, thus resulting in higher biocompatibility. Also, PEG conjugation to CdTe QDs having a similar surface ligand (i.e. thioglycolyl acid/ mercapto-acetohydrazide) and primary size (3.8 nm) as in our study has been shown to significantly reduce their *in vitro* and *in vivo* toxicity (Du et al., 2019). Nevertheless, we have demonstrated that cells exposed at the ALI with aerosolized PEG-CdTe QDs ink at 135

times lower effective dose show residual cellular toxicity. This shift can be attributed to a different reactivity of the PEG-CdTe QDs at a given concentration, as cell exposure in the ALI scenario represents a different, yet more relevant mechanism of cell-particle interaction.

HMOX1 was selected to screen the oxidative potential of PEG-CdTe QDs ink, one of the most important mechanisms of cell toxicity (Lovrić et al., 2005). QDs have been observed to elicit reactive oxygen species (ROS) production and to impact on antioxidant glutathione levels. We investigated whether the PEG-CdTe QDs ink altered antioxidant HMOX1 expression in the cells, but did not measure differential gene regulation. We did not observe either an inflammatory response at the ALI compared to the clean air condition.

5. Conclusions

The emissions of a water-based printing ink containing coated PEG-CdTe QDs during a household inkjet printing scenario simulated under controlled conditions have been characterized, showing the release of PEG-CdTe QDs in the use phase of the ink. Release forms consisted of aggregated PEG-CdTe QDs forming networks, though maintaining their individual identity (with 3 to 5 nm diameter).

An integrated approach using atomization of nano-additivated ink and exposure of BEAS-2B cells at the ALI for the *in vitro* screening of the cytotoxicological effect of airborne emissions has been developed. Using this approach, aerosol of PEG-CdTe QDs ink was delivered to the cells under the ALI inhalation simulating conditions at a concentration of 54.7 ng/ml (37.1 ng/ml Cd and 17.6 ng/ml Te), resulting in decreased cell viability by 25.6% (±15.7%) when compared with the clean air condition. A 9.0% (±11.9%) decrease of cell viability was also observed for atomized solvent ink.

Our *in vitro* basal acute toxicity screening study indicates potential health impact of CdTe-QDs particles derived from inkjet printing emissions. These findings warrant further exploration using more complex *in vitro/ex vivo* models and *in vivo* studies, to enable proper data collection for risk assessment.

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Supplementary material to Chapter 3

Materials and methods

SI-S1. Ink formulation and characterization. Inductively coupled plasma mass spectrometry (ICP-MS) protocol

Samples were digested with an acid solution (10 ml of nitric acid 70%, HNO_3 for trace analysis, Sigma Aldrich) in an analytical microwave digestion system (MARS, CEM). Before the digestion, samples were directly weighed (0.01 g sample) into 55 mL perfluoroalkoxy alkanes (PFA) microwave vessels (MARSXPress, CEM). Then, 10 mL of nitric acid were placed into the vessel to perform the microwave acid digestion program. Specifically, the conditions and procedure of microwave digestions were as follows: heat from room temperature to 200ºC in 10 min, then maintain this temperature for 15 min and, finally, cool down to room temperature to manipulate the samples safely. After having carried out the described digestion program, all samples were filled with ultrapure water to 50 ml. Then, further dilutions were made with 2% HNO_3 aqueous solution to obtain the desired concentrations for ICP-MS characterization. Cadmium (Cd) and Tellurium (Te) analysis were performed by ICP-MS (Agilent 7500, Agilent Technologies). The quantification was done by interpolation in a standard curve obtained from 1000 ppm commercial standard (Sigma Aldrich).

SI-S2. Deposition efficiency and dose quantification after ink exposure at the ALI. ICP-MS protocol

For destructive ICP-MS analysis, an aliquot of 400 µl of the sample was transferred into a polypropylene (PP) 15 ml disposable test tube, and 400 µl of nitric acid (67-69% Fisher scientific Optima grade) was added. The test tubes were closed and transferred to a microwave oven (Milestone mls1200 mega) and heated three times for 2 minutes at 150 Watt. After cooling down 3.2 ml of ultrapure water was added resulting in a 10% (v/v) nitric acid digestion solution.

For non-destructive analysis samples were prepared by diluting them at least 10 times in 2% nitric acid and measured without further digestion.

The (non-)digested samples were measured using a quadrupole ICP-MS instrument (Nexion 300s, Perkin Elmer) equipped with an ESI SC2-DX autosampler with SC-FAST automated sample introduction system. In total 6 calibration standards, all matrix matched, together with a calibration blank were used to calibrate the ICP-MS in standard mode. Three calibration standards (1.0 µg/l, 10 µg/l and 50 µg/l) were used to calibrate for Cd and Te, and three calibration standards (same concentrations) were used for the calibration of tin (Sn) and molybdenum (Mo). The latter was necessary to determine the presence of these elements in the samples, since they are a source of interference on the different masses of Cd. After analysis there appeared no presence of Mo and Sn in the samples. The calibration was verified by independent control samples in the concentration range of 0.1 µg/l, 1.0 µg/l and 10 µg/l, also matrix matched. After the measurement of the samples the calibration blank was re-analysed together with the highest calibration standards. An Internal standard Rhodium (103Rh) was pumped online and mixed with all samples to control for non-spectral interference due to the sample matrix. All results obtained were corrected for this internal standard.

Results

SI-S3. Dose efficiency and quantification after in vitro *ink exposure. Submerged conditions*

To compare total and ionic exposure of cells to Cd and Te ions in the ALI vs. in submerged conditions, six serial 1/5 dilutions of PEG-CdTe QDs ink in BEGM medium were measured after 24 hours of exposure (Table S1). The range covered equitably concentrations above and below the CdTe dose value determined using the Vitrocell® module. ICP-MS readout series indicated a linear pattern of dissolved Te ions (r^2 = 0.846) and Cd ions (r^2 = 0.958) at all the dilutions tested, except for Te at the highest QDs concentration (27 µg/ml) (see next section SI-S4 and Figure SI8). In that case, ionic Te concentration decreased to values detected in the medium at ~1.1 µg/ml QDs, whereas Cd ion concentration increased further. These ionic Cd:Te ratio changes with concentration can be explained by the fact that at that the highest dose only the QDs' surfaces dissolve in the culture media, where Cd atoms dominate.

Table SI7. Cd and Te ionic concentrations detected by ICP-MS in filtered BEGM medium (submerged conditions) after 24 hours incubation with PEG-CdTe QDs ink. Solvent ink was used as negative control. * All the values indicated in ng/ml.

QDs exposure values*	Te ions detected*	Cd ions detected*	QDs ionic % detected*	Cd:Te ions ratio
0.0	<0.1	<0.1	N/A	N/A
8.6	2.3	3.8	71%	1.65
43	7.7	12	46%	1.56
216	29	37	31%	1.23
1080	99	122	20%	1.23
5400	160	427	11%	2.67
27000	102	1050	4,3%	10.29

SI-S4. Cell viability assessment. Submerged conditions

Potential *in vitro* cytotoxic effects of the PEG-CdTe QDs containing ink in submerged conditions was also assessed using the same human bronchial epithelial BEAS-2B cell line. Cells were exposed for 24 hours to the same 1/5 serial dilutions of PEG-CdTe QDs used for the deposition efficiency assay, from 8.6-27000 ng/ml. After submerged exposure of the BEAS-2B cells to the PEG-CdTe QDs ink, they were detached from the well plate by trypsinization, and 10 µl cell suspension was added to 10 µl of 0.4% trypan blue staining solution (Invitrogen) in an Eppendorf tube. The mixture was gently pipetted up and down, and 10 µL was added to a Countess™ Cell Counting Chamber slide (Invitrogen) which was read out on a Countess™ Automated Cell Counter after parameter adjustment. The changes in cell count for live, dead and total cells, and calculated percentage viability, as displayed on the screen, were used as a measure of toxicity in exposed compared to untreated BEAS-2B cells.

Results were compared to previous data from our laboratories, exposing the same cell line in submerged conditions to serial concentrations of $CdCl_2$ salt (190-50000 ng/ml). Briefly, the assay was done following the provider's specifications, and using a Multiskan Ascent

spectrophotometer (Thermo Fisher) at 540 nm for the readouts. In submerged conditions, an exponential decline on cell viability related to Cd ions ($CdCl_2$) concentration within three orders of magnitude was observed (Figure SI8, golden dots), where EC_{50} calculated using neutral red assay was 2000 ng/ml.

Although a different protocol was followed (cell density and cytotoxicity test), highly similar EC_{50} values were obtained, and results relative to Cd ionic doses showed a good exponential correlation (r^2 = 0.8721) between both experiments. Consequently, Cd ions seemed to be the main cause of the observed cytotoxicity. Figure SI-S8 shows a comparison with cytotoxicity observed in ALI exposure.

Cells were not affected by concentrations of Cd ions lower than 500 ng/ml ($p \leq 0.05$), corresponding to a concentration of PEG-CdTe QDs in ink between 5400-27000 ng/ml). The observed IC_{20} was at 1050 ng/mL of Cd^{2+} (27 µg/mL of QDs) compared to the positive control. No cytotoxicity could be attributed to solvent ink in submerged conditions, tested at 0.25% v/v, the same final concentration as for the PEG-CdTe QDs ink.

At the ALI, viability observed for cells exposed to the PEG-CdTe QDs ink (Figure SI8, green square) was two orders of magnitude lower than in submerged conditions, 74.4%, though 2/3 of the cytotoxicity observed (16.6%) could be attributed to PEG-CdTe QDs, and 1/3 (9%) to the presence of solvent ink.

Figures

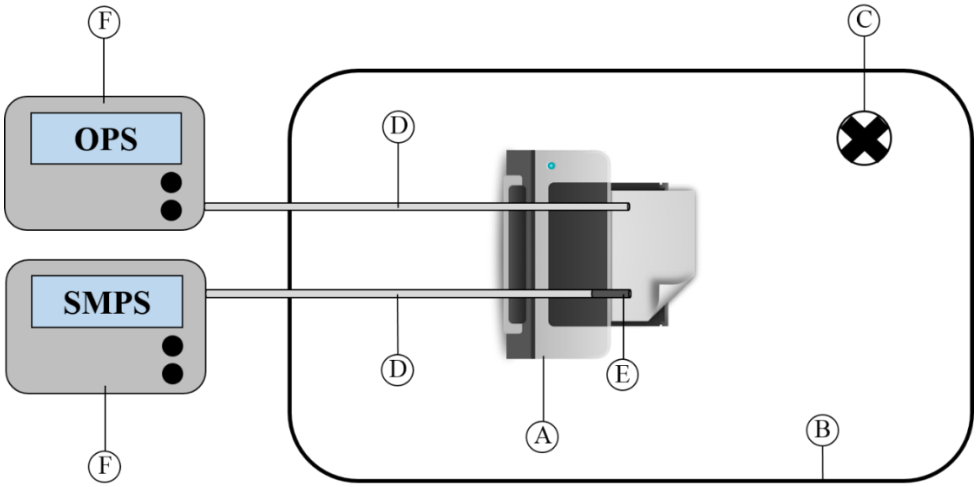

Figure SI1: Schematic representation of the setup used to determine the Particle Number Concentration (PNC) in air during the household printing process using the Optical Particle Sizer (OPS) and Scanning Mobility Particle Sizer (SMPS). A) Printer; B) Methacrylate box; C) Polycarbonate filter; D) Silicon tubes; E) TEM sampler device; F) Air measurement instruments.

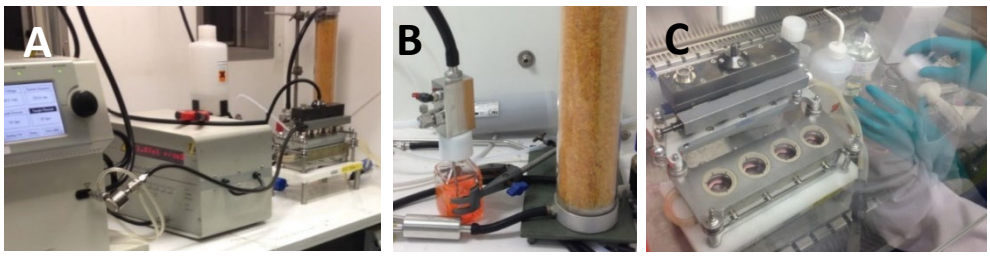

Figure SI2: Experimental set up for the aerosol generation and air-liquid interface (ALI) exposure. A) PEG-CdTe QDs ink sample and diffusion dryer; B) Vitrocell® Module; C) Collison type atomizer; Instrumentation for particle measurement (3936 SMPS).

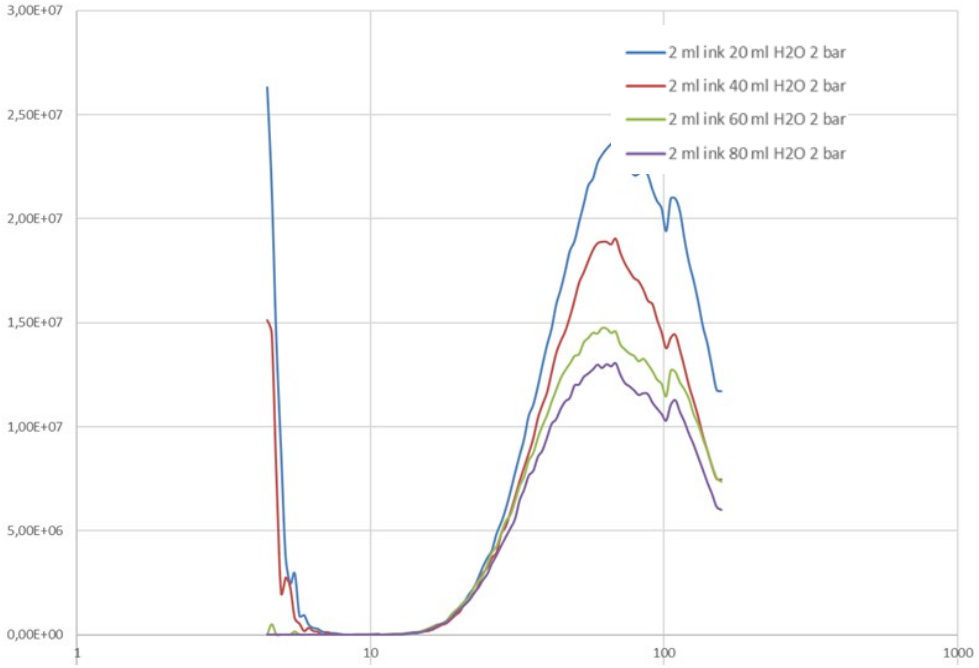

Figure SI3: SMPS graph showing the size distribution measurements of different dilutions of the PEG-CdTe QDs ink tested to determine the most optimal atomization settings, evidencing that the 1:40 dilution (purple curve) has the smallest particle mode and no interference of smaller particles < 10 nm.

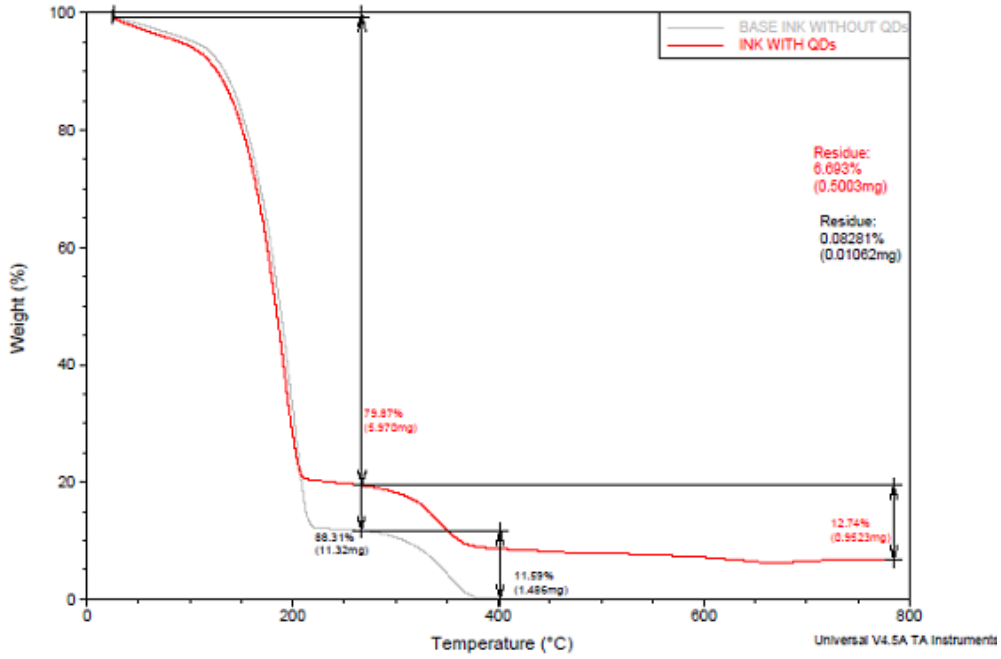

Figure SI4: TGA curves for the base solvent ink (grey) and the PEG-CdTe QDs ink (red).

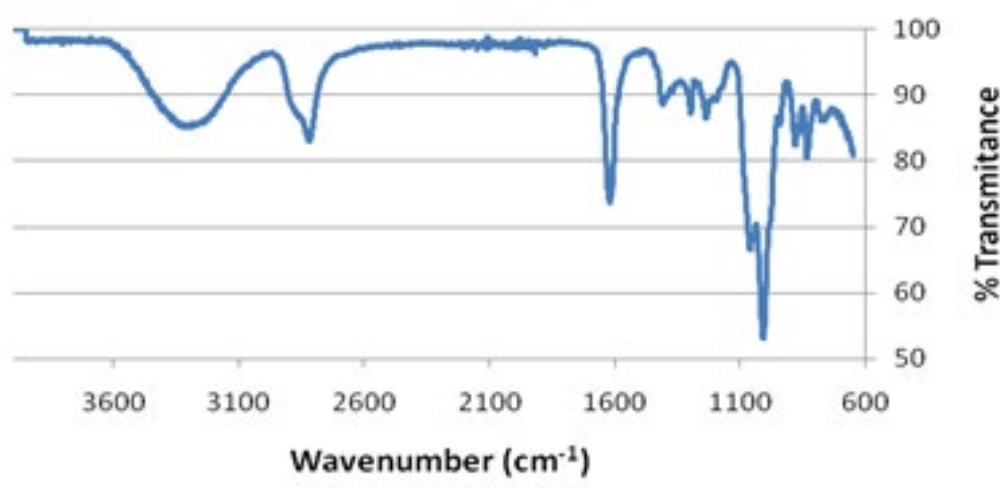

Figure SI5: FTIR-ATR spectrum of PEG-CdTe QDs ink after freeze-drying.

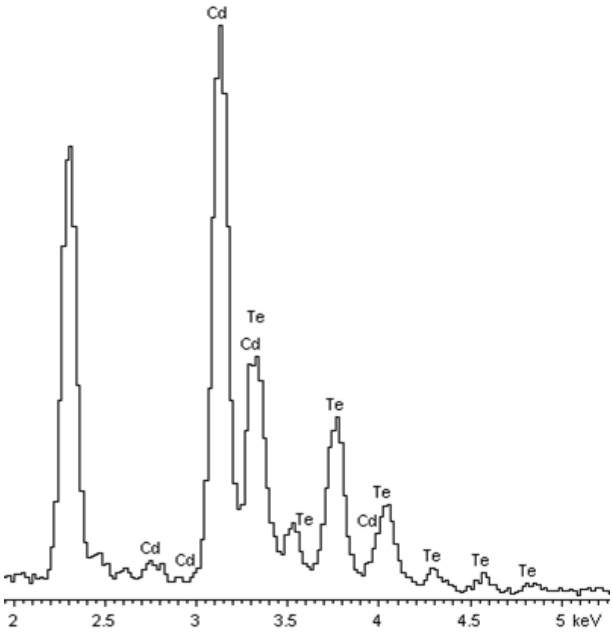

Figure SI6: EDX spectrum of the PEG-CdTe QDs released during the printing process and collected on the TEM grid using the sampler device.

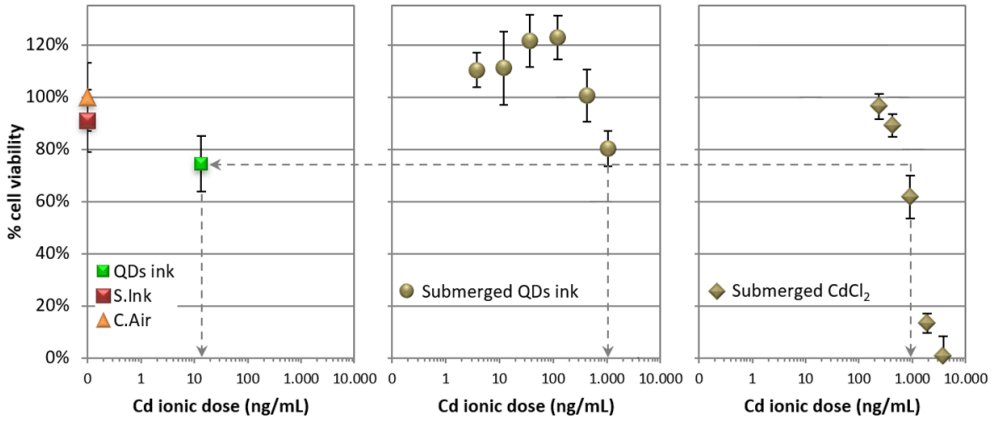

Figure SI7: BEAS-B2 cells viability after 24 hours of exposure to Cd ions from different sources, compared to ALI control exposed to clean air (C.Air). Conditions tested: PEG-CdTe QDs ink at the ALI (QDs ink), solvent ink at the ALI (S.Ink), and Cd ions in submerged conditions (from PEG-CdTe QDs ink and CdCl2). Error bars = SD (standard deviation).

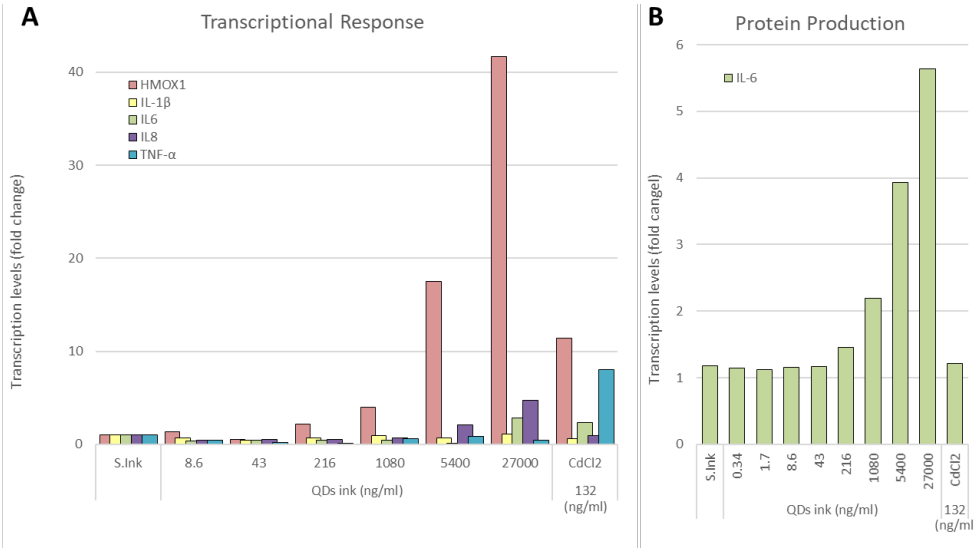

Figure SI8 - Evaluation of oxidative stress and pro-inflammatory cell response in BEAS-2B cells exposed for 24 hours to PEG-CdTe QDs ink (QDs ink) in submerged conditions, compared to the solvent ink control (S.Ink) and to cadmium in ionic form ($CdCl_2$). **A**) Potential alterations in the transcriptional response of HMOX1, IL-1β, IL-6, IL-8 and TNF-α measured by real-time qPCR; **B**) changes in protein production of IL-1β, IL-6, TNF-α and IFN-γ detected using a multiplex cytokine assay, where only IL-6 protein levels were over the detection limit of the assay.

CHAPTER 4. A proposal to derive characterization factors for CdTe Quantum Dots integrating changing particle sizes throughout their life cycle

CHAPTER 4. A proposal for the derivatization of characterization factors for CdTe quantum dots integrating changing particle sizes throughout their life cycle

This chapter has been published as:

Blázquez, M.; Corral, B.; Buist, H.; Ligthart, T.; Henzing, B.; Rosenbaum, R. and Cajaraville, M.P. (2022) On the combination of USEtox® and SimpleBox 4 Nano models for the derivatization of size-dependent characterization factors for engineered nanomaterials. *E3S Web of Conferences 349, 03004* LCM2021 [https://doi.org/10.1051/e3sconf/202234901006]

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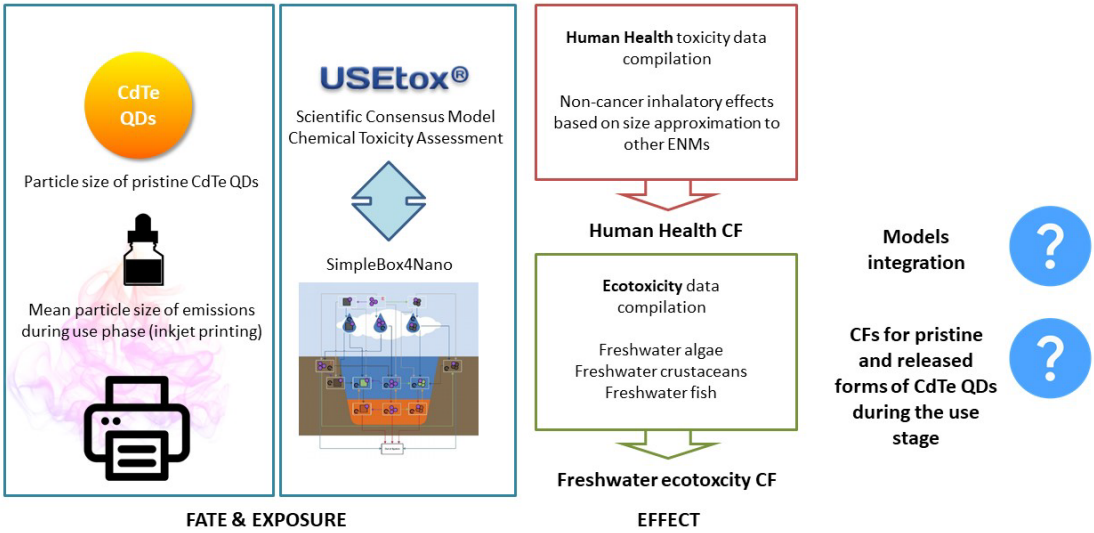
Poster communication: **Blázquez, M.**; Corral, B.; Buist, H.; Ligthart, T.; Henzing, B.; Rosenbaum, R. and Cajaraville, M.P. A proposal for characterization factors derivatization for CdTe Quantum dots integrating changing particle sizes measured throughout their life cycle.

GRAPHICAL ABSTRACT



ABSTRACT

Quantum dots (QDs) confer a wide range of optical properties to pigments/inks. With new products and applications entering the market, the airborne emissions of QDs incorporating inks during usage stage at consumer scale (e.g. household printing) and their corresponding impacts towards human health and the environment need to be investigated. In the present work cadmium telluride (CdTe) QDs have been selected as a case study. The targets of this research have comprised: (i) the characterization under controlled conditions of the emissions during inkjet printing of a prototype of a CdTe QDs nanoadditivated ink, (ii) the derivatization of human health and freshwater effect factors (EF) for potentially released CdTe QDs with the USEtox® consensus model and (iii) the assessment of the potential integration of USEtox® and SimpleBox4Nano models for the calculation of characterization factors (CFs). Mean particle size after 60 minutes inkjet printing in controlled conditions corresponded to 59.52 nm. For human health EF calculation an extrapolation to the human EF of other nanomaterials has been proposed considering CdTe QDs' specific surface area whereas for the calculation of the freshwater EF few of the data available have revealed suitable. A generic constraint to calculate both EFs for CdTe QDs released throughout the life cycle of a product incorporating them is related to the absence of information corresponding to their specific (eco)toxicological impacts. Furthermore, the possibility to combine and integrate USEtox® and SimpleBox4Nano appeared to be limited since there is no absolute correspondence between the two models.

KEY WORDS

Cadmiun telluride, quantum dot, characterization factor, toxicity, fate

RESUMEN

Los puntos cuánticos (QD) confieren una amplia gama de propiedades ópticas a los pigmentos/tintas. Con la entrada en el mercado de nuevos productos y aplicaciones, es necesario investigar las emisiones al aire de QD que incorporan tintas durante la etapa de uso por el consumidor (p. ej., impresión doméstica) y sus correspondientes impactos en la

salud humana y el medio ambiente. En el presente trabajo, hemos seleccionado los QD de telururo de cadmio (CdTe) como caso de estudio. Los objetivos de esta investigación han comprendido: (i) la caracterización en condiciones controladas de las emisiones que tienen lugar durante la impresión por chorro de un prototipo de tinta nanoaditivada con CdTe QDs, (ii) la derivatización de factores de efecto (FE) sobre la salud humana y el agua dulce para las potenciales emisiones de CdTe QDs empleando el modelo de consenso USEtox® y (iii) la evaluación de la posibilidad de integrar los modelos USEtox® y SimpleBox4Nano para el cálculo de factores de caracterización (FCs). El tamaño medio de partícula después de 60 minutos de impresión por chorro de tinta en condiciones controladas correspondió a 59,52 nm. Para el cálculo de FE en salud humana, se ha propuesto una extrapolación a la FE humana de otros nanomateriales considerando el área de superficie específica de los QD de CdTe, mientras que para el cálculo de FE en agua dulce, pocos datos disponibles han resultado adecuados. Una restricción genérica para calcular ambos FE para los QD de CdTe liberados a lo largo del ciclo de vida de un producto que los incorpora está relacionada con la ausencia de información correspondiente a los impactos (eco)toxicológicos específicos de los QDs liberados. Además, la posibilidad de combinar e integrar USEtox® y SimpleBox4Nano resultó limitada ya que no existe una correspondencia absoluta entre ambos modelos.

PALABRAS CLAVE

Telururo de cadmio, punto cuántico, factor de caracterización, toxicidad, destino

LABURPENA

Puntu kuantikoek (QD) propietate optiko ugari ematen dizkiete pigmentu/tintei. Produktu eta aplikazio berriak merkatuan sartzen direnez, kontsumitzaileen eskalan (adibidez, etxeko inprimaketan) tintak txertatzen dituzten QDen aireko isuriak ikertu behar dira (adibidez, etxeko inprimaketak) eta gizakien osasunean eta ingurumenean dagozkien inpaktuak ikertu behar dira. Lan honetan kadmio telururoa (CdTe) QD-ak kasu azterketa gisa hautatu dira. Ikerketa honen helburuak honako hauek izan dira: (i) CdTe QDs tinta nanoaditibo baten prototipo baten tintazko inprimaketan zehar emisioen baldintza

kontrolatuetan karakterizatzea, (ii) potentzialki askatu daitezkeen giza osasunaren eta ur gezako efektu faktoreen (EF) deribatzea. CdTe QDak USEtox® adostasun ereduarekin eta (iii) USEtox® eta SimpleBox4Nano ereduen integrazio potentzialaren ebaluazioa, karakterizazio-faktoreak (CFs) kalkulatzeko. Batez besteko partikulen tamaina 60 minutuko tintazko inprimaketa baldintza kontrolatuetan 59,52 nm-ri dagokio. Giza osasunaren EF kalkulurako, beste nanomaterial batzuen giza EFrako estrapolazioa proposatu da CdTe QD-en azalera espezifikoa kontuan hartuta, eta ur gezako EFaren kalkulurako, eskuragarri dauden datu gutxi batzuk egokiak agertu dira. Horiek txertatzen dituen produktu baten bizi-zikloan zehar askatutako CdTe QD-en bi EFak kalkulatzeko muga generiko bat haien inpaktu (eko)toxikologiko espezifikoei dagokien informaziorik ezarekin lotuta dago. Gainera, USEtox® eta SimpleBox4Nano konbinatzeko eta integratzeko aukera mugatua omen zen, bi ereduen arteko erabateko korrespondentziarik ez dagoelako.

HITZ GAKOAK

Kadmio telururoa, puntu kuantikoa, karakterizazio faktorea, toxikotasuna, patua

1. Introduction

Life cycle assessment (LCA) and its corresponding ISO framework (ISO 2006 a, b) are recognized as suitable tools to identify the potential environmental and human health impacts of nano-enabled applications (NEAs) or nano-enabled products (NEPs) along their complete life cycles covering manufacturing, use and end of life (EOL) stages (Klöpffer et al., 2006). As such, a number of review articles have been published in the past decade that cover the application of LCA to nanotechnology such as the recent work by Salieri et al. (2018).

The LCA methodology comprises four iterative steps: (i) goal and scope definition, (ii) inventory analysis, (iii) impact assessment, and (iv) interpretation. Life cycle impact assessment (LCIA) converts emissions into environmental damages through linked fate-exposure-effect models that require robust experimental data and a mechanistic understanding for each of these components. LCIA methods describe environmental impacts in terms of characterization factors (CFs). A CF is a substance-specific quantitative representation of the (relative) impact of a substance in the environment (LC-Impact, 2020). CFs are based on models of cause-effect chains for a specific impact category.

USEtox® (Rosenbaum et al., 2008; 2011) is a fate-effect model specifically made for LCA applications as it calculates human and eco-toxicity CFs based on the properties of a substance and the environmental compartment of initial release. The model estimates CFs by multiplying three aggregated parameters related to fate (fate factor, FF), exposure (exposure factor, XF), and toxicity (effect factor EF), respectively of a specific chemical. USEtox® operates on four different spatial scales: indoor, urban, continental and global. The indoor and urban scale only have an air compartment, whereas the continental and global scales consist of five compartments: air, agricultural soil, natural soil, freshwater and sea water. USEtox® is recommended by the Life Cycle Initiative for the calculation of CFs for human toxicity and freshwater ecotoxicity in LCIA (Life Cycle Initiative, 2022).

For human health related impact, with the assumption of linear dose-response relationship, USEtox® calculates human health EF as

$$EF = 0.5/ED50 \quad (1)$$

where ED50 is the lifetime daily dose (ED corresponding to effective dose) resulting in 50% probability of effect. USEtox® takes into consideration the inhalation and ingestion exposure routes and differentiates between the contributions of cancer and non-cancer toxicity impacts. Human EF is expressed by a loss of (healthy) life time expressed or disability adjusted life years (DALY) per kg intake. Examples of human EFs (HEFs) have been reported by (Buist et al., 2017) for engineered nanomaterials (ENMs) including Ag, TiO_2, carbon black, high-aspect ratio, rigid and flexible multi-walled carbon nanotubes (MWCNT), all of them in their pristine form.

For ecotoxicological impacts, the freshwater ecotoxicity EF is determined using the linear slope of dose-response curves of the chemical up to the point where 50% of the species are affected. Therefore, the EF for ecotoxicity can be calculated as

$$EF = 0.5/HC50 \quad (2)$$

where HC_{50} is the hazardous concentration at which 50% of the species are affected above their EC_{50}. Freshwater organisms include representatives of the trophic levels of algae, crustaceans and/or fish in order to reflect the variability of the physiology and to ensure a minimum diversity of biological responses. Freshwater EF is expressed as Potentially Affected Fraction (PAF) of freshwater species integrated over the exposed water volume per kg of bioavailable chemical in the aquatic environment (PAF m^3 kg^{-1}). According to Temizel-Sekeryan & Hicks (2021), 29 freshwater CFs for different ENMs including carbon nanotubes (CNT), TiO_2; Ag or Au nanoparticles, amongst other, have been proposed to date.

Though USEtox® has been reported for the calculation of EF for ENMs, this model cannot be directly applied to the LCIAs of NEPs and NEAs since the fate modelling (FF) is not nano-specific. SimpleBox4Nano (SB4N) (Meesters et al., 2014, 2016, 2019), is able to model the fate of ENMs dependent on their size. SB4N has three main compartments: regional, continental and global, but the inner nested compartment, regional, has not only air as a medium but also freshwater (including lake, lake sediment, freshwater and freshwater sediment), seawater (including surface sea, deep sea and marine sediment) and natural, agricultural soil and urban/industrial soil. From all these media transfers to the other

compartments and media are possible. Furthermore, the global compartment is split into three sub-compartments: moderate, arctic and tropical. SB4N models the fate of (i) freely dispersed (pristine) nanoparticle, (ii) nanoparticle hetero-aggregated with natural colloid particles (<450 nm) and (iii) nanoparticle attached to larger natural particles (>450 nm) (Meesters et al., 2014).

Ettrup et al. (2017) adapted the USEtox® 2.0 consensus model to integrate the SB4N for the development of CF of TiO_2 nanoparticles to be incorporated in future LCA studies. Also focusing on TiO_2 nanoparticles, Salieri et al. (2019) presented an integrative approach for USEtox® 2.0 model with SB4N and proposed CFs for the freshwater toxicity impact category. More recently, Temizel-Sekeryan & Hicks (2021) have calculated freshwater ecotoxicity CFs for silver nanoparticles by combining the principles of colloidal science with the USEtox® model using data from published mesocosm conditions.

Finally, the implementation of a life cycle perspective in the design phase of NEAs and NEPs needs to take into consideration releases of ENMs taking place at multiple stages along their life cycle (e.g. during use). The chemical and physical form of the emissions (releases) varies along these processes, as does the potential for human or environmental exposures and associated impacts. This implies that CF calculated for a pristine ENM might need to be adapted to its released form. In this sense, Salieri et al. (2018) identified a total of 92 LCA studies of nanotechnologies out of which only 5 accounted for ENMs releases (Ferrari et al., 2015;, Hischier et al., 2015; OECD, 2014 and Walser et al., 2011).

In the present work we have selected cadmium telluride quantum dots (CdTe QDs) as an example of NEP. CdTe QDs are characterized by their ease of tunability, high photoluminescence, quantum efficiency and stability in water (Wuister et al., 2003) and their use as additive in inkjet printing inks has been reported (Du et al., 2017). Our objectives in the present study have included (i) the simulation of the usage stage (i.e. printing) at consumer scale of a prototype of a water based CdTe QDs additivated inkjet printing ink and the characterization of corresponding airborne emissions, (ii) the compilation of information of CdTe QDs for human toxicity via inhalation route and freshwater ecotoxicity for EF calculation using USEtox® and (iii) to compare the information requirements and outputs by USEtox® and SB4N models in order to integrate

them for the derivatization of size-dependent CFs for the varying sizes of ENMs throughout their life cycle.

To the best of our knowledge, no human health or freshwater ecotoxicity EFs have been proposed in the literature so far for CdTe QDs neither in their pristine nor in their released forms.

2. Materials and Methods

2.1. Experimental assessment of particle size of the emissions during the use of inkjet printing ink incorporating CdTe QDs

The average particle size of the airborne particles emitted during inkjet printing of a prototype of polyethylene glycol (PEG) CdTe QDs additivated water based ink provided by PLASMACHEM GmbH (Germany) was characterized under controlled conditions. The composition of the ink cannot be detailed due to confidentiality issues.

The protocol for the printing process was detailed in chapter 3. Briefly, CdTe QDs containing ink was loaded into refillable ink cartridges which were subsequently inserted in a household inkjet printer (Pixma P7250, Canon). A pattern representing 65.71 % coverage of a A4 paper was printed on recycled paper. The printer was enclosed in an aerosol exposure chamber with approximate dimensions of 0.74 x 0.55 x 0.59 m (0.24 m^3), to which clean air entered through a HEPA 14 capsule filter. The chamber was equipped with a scanning mobility particle sizer (SMPS Model 3936L25, TSI Inc.) measuring in a size range from 15 to 661 nm, a scan time of 100 s, a retrace time of 15 s, a 2 min recurrence interval and the sample flow rate was adjusted to 0.3 L/min. In addition, an ultrafine water-based condensation particle counter (CPC Model 3786, TSI Inc.) was used to measure total particle number concentration (PNC) in a size range from 2.5 to 3000 nm and with a flow rate of 0.6 L/min. A 60 min printing cycle was performed followed by uninterrupted air monitoring during 60 min, to observe the evolution of particle sizes. The experiment was repeated using not-nano additivated base ink with comparison purposes.

2.2. Compilation of (eco)toxicological information for Human Health and Freshwater Effect Factors calculation and calculation of specific surface area

Different academic websites including Web of Knowledge, PubMed, Scopus or ScienceDirect were selected as a source for (eco)toxicological information. Several terms were displayed in combination to perform this search: "CdTe", "Cadmium Telluride", "Quantum Dots", "EC_{50}", "ED_{50}", "carcinogenic", "chronic inhalation toxicity" and "freshwater toxicity". Data corresponding to QD formulations other than CdTe (e.g., CdS, CdSe) were disregarded. Despite that the coating is a particularly critical variable, as it affects solubility and therefore also (eco)toxicity (Katsumiti & Cajaraville, 2019), we have not taken into consideration surface functionalization due to limitations associated to USEtox®. Particle's specific surface area (SSA) was calculated using the Sauter formula (Sauter, 1926, 1928):

$$SSA = 6/\rho L \quad (3)$$

where ρ and L correspond to particle's density and diameter, respectively.

2.3. Comparison and integration of SimpleBox4.0-Nano and USEtox®

SimpleBox4.0-Nano and USEtox® 2.12 versions have been used. The comparison of both models has comprised two steps: (i) definition of the USEtox® air, water and soil scenarios in which the main parameters for the Regional and Continental compartments in SB4N have been set to fit those of USEtox®' Urban and Continental compartments, respectively and (ii) identification of rate constants that are common for both models.

3. *Results and discussion*

For comparison purposes, the size corresponding to the pristine CdTe QDs reported in Chapter 3 has been taken as a reference. Chapter 3 detailed the use of CdTe QDs capped with PEG (750)-O-C(=O)CH2CH2-SH of 3-5 nm size as nano additive in water-based inkjet printing ink. In the present chapter a spherical diameter of 3.6 nm has been assumed for CdTe QDs. Considering density of CdTe QDs (5.83 E+ 06 g/m^3) (ECHA, 2021) and a spherical particle diameter of 3.6 nm, the SSA for pristine CdTe QDs corresponds to 286 m^2/g.

3.1. Characterization of emissions associated to inkjet printing

Particle sizes of the emissions measured in three different moments of the CdTe QDs inkjet printing under controlled conditions (times 0, 60 and 120 min, respectively) have been represented in Figure 1. Mean particle sizes and total particle concentration at t=0 (start of the printing process), t= 60 (ned of printing) and t=120 (60 minutes after the end of printing) corresponded to 61.35, 59.52 and 56.23 nm and to 69.25, 1167.97 and 2196.62 (#/cm^3), respectively. Whereas the mean particle size remained relatively constant, a time-dependent increase in the concentration of particles was observed. The mean particle size of 59.52 nm measured at the end of printing (t=60) has been selected as representative of the emissions associated to the usage stage of the CdTe QDs additivated inkjet printing ink. Figure 2 compares the evolution of particle concentration for CdTe QDs containing ink and for not-nanoadditivated (blank) ink. The emission profiles of both nanoadditivated and blank inks were similar, with some marked peaks observed at the start of the printing process for the CdTe QDs ink that can be attributed to artefacts during the experiment due to the clogging of the cartridges filled with such ink.

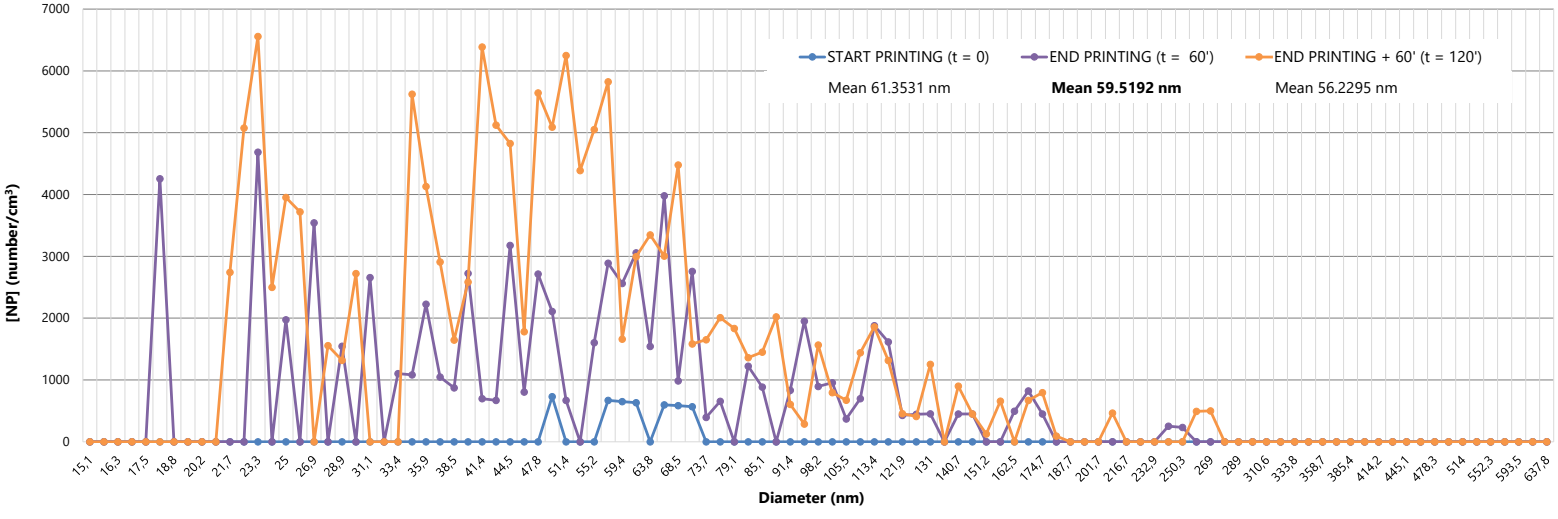


Figure 2: Particle size of emissions measured at the start of the printing process (t=0); at the end of the printing process (t=60'), and 60' starting from the end of the printing process (t=120') measured by the SMPS.

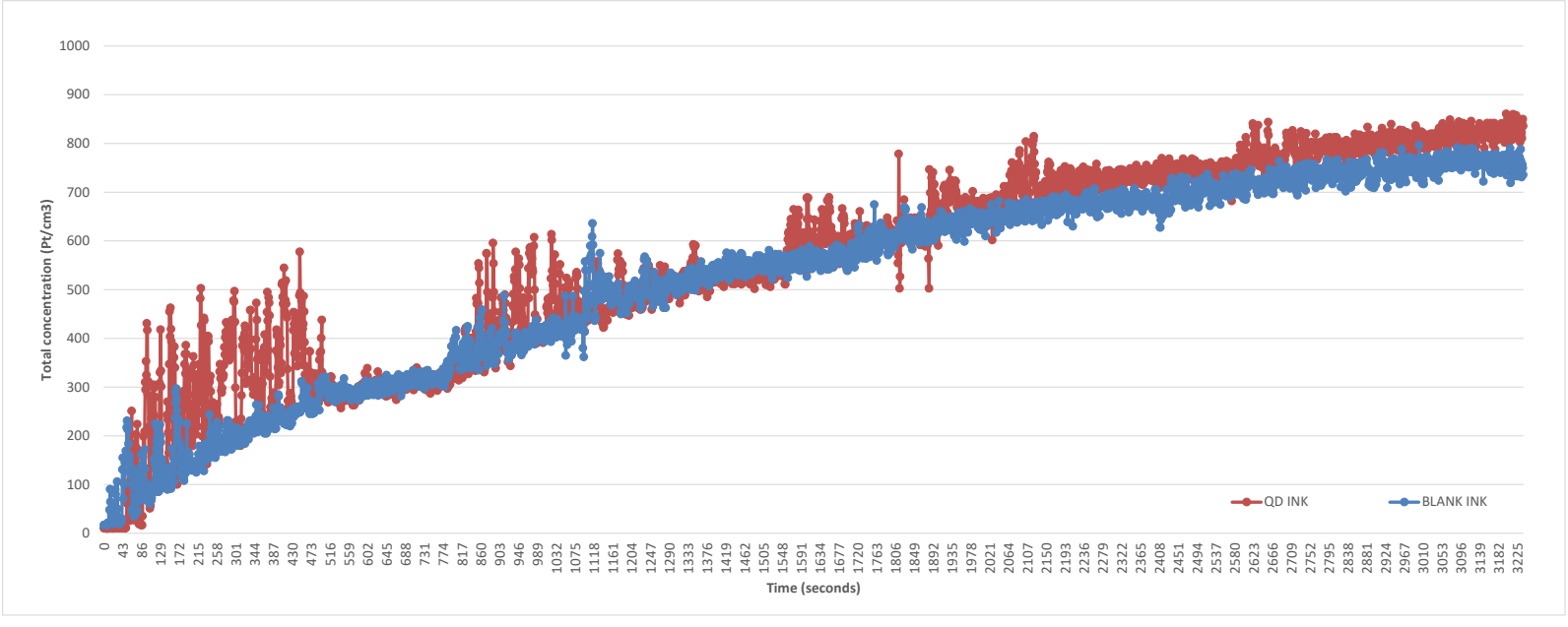


Figure 3: Evolution of particle concentration (Pt/cm^3) measured by the CPC for both blank and CdTe QDs containing inks.

3.2. Compilation of (eco)toxicological information and EF calculation

3.2.1. Human health toxicity data

Based on *in vitro* studies, toxicity of Cd containing QDs seems to be determined by both their nano size as well as by the release of toxic Cd^{2+} ions. The latter effect is confirmed too by *in vivo* studies in mice showing QDs-induced histopathological abnormalities (Wang et al., 2016), which are also observed in studies with soluble bulk cadmium species (see review by ATSDR, 2012). However, it is difficult to compare different studies executed with different Cd containing QDs due to widely differing dose parameters used and insufficient data on Cd release by the different QDs.

Another factor of importance is coating and/or functionalization of CdTe QDs. For instance, Bobine Serum Albumin (BSA) conjugated CdTe QDs are less cytotoxic than bare CdTe QDs (Lovrić et al., 2005). Likewise, their conjugation with polyethylene glycol (or PEGylation) has a mitigating effect on QDs cytotoxicity (Hoshino et al., 2004). In contrast, QDs with carboxylic surface groups are positive in the Comet assay, while QDs with other surface functionalizations are weakly positive or negative at the same dose (Ali et al., 2019).

No *in vivo* studies on CdTe genotoxicity performed in mammals have been encountered in public literature. An *in vitro* study by Wang et al. (2010) using human umbilical vein endothelial cells (HUVECs), showed that a 12 h treatment with CdTe QDs, surface coupled with mercaptopropionic acid, induced DNA damage in a dose-dependent manner (ED50 approx. 50 µg/mL). Based on this limited evidence no firm conclusion can be drawn with respect to CdTe QDs' genotoxicity.

Most *in vivo* toxicity studies with Cd containing QDs encountered in public literature employ a route of exposure not very relevant for respiratory risk assessment, e.g. the intraperitoneal (i.p.) or the intravenous route (i.v.). There is no sufficient data on long-term effects of QDs according to published reviews (Rzigalinski and Strobl, 2009, Wu and Tang, 2014). We found two good quality *in vivo* inhalation studies with Cd containing QDs, performed by Ma-Hock et al. (Ma-Hock et al., 2012, 2014), but those were short-term studies with CdS/Cd $(OH)_2$ nanoparticles, which release high amounts of Cd^{2+} and may be quite different in that respect from PEG-CdTe QDs. Furthermore, they applied only one

concentration, thus no dose-effect relationship can be established (which is necessary to derive a HEF).

As an alternative, the HEFs derived by Buist et al. (2017) have been taken as a reference to calculate a range of values providing an indication of the possible value of the CdTe QDs respiratory human health EF (Table 1). This range of values does not take into consideration the specific effects on human health of CdTe QDs, rather, an approximation to the HEF of other ENMs based on their SSA has been proposed.

Table 1: HEF for LCA, chronic inhalation exposure, for various ENMs derived by Buist et al. (2017) and approximation to CdTe QDs based on calculated particle's SSA. DALYs have been calculated based on a SSA of 286 m^2/g for pristine CdTe QDs. In the case of released forms of CdTe QDs (*in italics*), a SSA corresponding to 17.3 m^2/g has been calculated taking into consideration the selected average size of particles emitted during printing (59.52 nm) whereas the density has been assumed to remain equivalent to that of pristine CdTe QDs. Cases have been calculated based on 0.23 DALYs/case for chronic obstructive pulmonary disease (COPD) (Buist et al. 2017).

Reference ENM from Buist et al. (2017) [Surface area (m^2/g)]	**Values (DALYs) from Buist et al. (2017) [$(m^2/g)^{-1} \cdot kgintake^{-1}$]**	**Equivalent HEF for pristine CdTe QDs [SSA 286 m^2/g]**		***Equivalent HEF for released CdTe QDs [SSA 17.29 m^2/g]***	
		DALYs · $kgintake^{-1}$	**Cases · $kgintake^{-1}$**	***DALYs · $kgintake^{-1}$***	***Cases · $kgintake^{-1}$***
Carbon black [230]	0.0067	1.9	8.3	*0.12*	*0.50*
Ag [20]	0.15	42.9	186.5	*2.60*	*11.28*
TiO_2 [48]	0.013	3.7	16.2	*0.22*	*0.98*

Based on these comparisons, the chronic HEF of pristine CdTe QDs could range between 1.9 and 42.9 DALYs $kgintake^{-1}$ (8.3 and 186.5 cases $kgintake^{-1}$) representing low and high toxicity scenarios and corresponding to carbon black and Ag nanoparticles (NPs), respectively (Table 1).

HEF values adjusted to the SSA calculated for the average size of the particles emitted during the use of CdTe QDs-incorporating ink have also been presented (Table 1). The present approach has not taken into consideration the composition of the released

particles which will be integrated by CdTe QDs and the solvent ink. The proposed HEF for the use stage should ideally be corrected by a quantified proportion of CdTe QDs contained in the total emitted particles. Furthermore, we have assumed that the CdTe QDs are tightly aggregated and do not separate once lodged in the lungs.

Rigid high-aspect ratio carbon nanotubes described by Buist et al. (2017) referred to as MWCNT – Nanocyl NC 7000 are excluded from this comparative study because these properties imply a very different mechanism of toxicity (fibre paradigm). Additionally, these authors proposed for 125 DALYS ($kgintake^{-1}$) as non-carcinogenic respiratory HEF for MWCNT-Baytubes. Such ENMs cannot be used for the hereby proposed extrapolation based on SSA, however, aforementioned DALYs could be used directly if clear indications that CdTe QD toxicity is mass-related instead of SSA-related existed.

It is noteworthy that out of the three ENMs that have been selected as a reference, the closest scenario would be that of Ag nanoparticles since their mechanism of toxicity and that of CdTe QDs are based on the release of ions. The scope of the present work is limited to non-carcinogenic effects derived from the inhalation route.

3.2.2. Freshwater toxicity data

In addition to *in vivo* data reported in Table 2, which were used for freshwater EF calculation, the use of fish cell lines in *in vitro* toxicity studies for CdTe QDs has also been reported. For instance, Gagné et al. (2008) performed an *in vitro* test with primary cultures of hepatocytes of rainbow trout *Oncorhynchus mykiss* (48 h exposure). Though the fish cell line RTgill-W1 has been recently accepted as an alternative to predict fish acute toxicity (OECD, 2021), the currently available version of USEtox® cannot take into consideration results from *in vitro* tests for the calculation of freshwater EF.

Additionally, in the marine mussel *Mytilus galloprovincialis* 14 days of exposure to 10 µg.equivalents Cd/L of carboxylated CdTe QDs did not induce micronuclei or other nuclear abnormalities, but were positive Comet assay for DNA strand breaks (Rocha et al., 2014). Nuclear abnormalities were seen with a comparable exposure to soluble Cd^{2+}, which also caused an increased percentage of tail DNA in the Comet assay, comparable to that of the QDs (Rocha et al., 2014). *M. galloprovincialis*, however does not represent the freshwater

compartment and thus these results have not been accounted for the calculation of freshwater EF.

Table 2: Values accounted for the calculation of the Freshwater Ecotoxicity EF. *In vivo* acute exposure data have been retrieved only: chronic values are calculated by a factor of 0.5 in agreement with the general provisions of USEtox®. Subsequently, (i) log(10) is calculated for each chronic EC_{50} value [corresponding to EC_{50}i], (ii) the average of EC_{50}i values for the three trophic levels considered is calculated [corresponding to the aggregated EC_{50}] and, finally, (iii) the aggregated HC_{50} value is calculated as the antilogarithm of EC_{50}.

Organism	**Effect endpoint**	**Exposure duration (h)**	**EC_{50} (mg/L)**	**Chronic values**	**log(10) of chronic values [EC_{50}i]**	**avlog (EC_{50}i) [EC_{50}]**	**10^EC_{50} [HC_{50}]**
Algae *(Chlamydomonas reinhartii)* (J. Wang et al., 2008)	Growth	72	5.00	2.50	0.40	0.12	1.34
Crustacean *(Daphnia pulex)* (Tang et al., 2015)	Death	48	0.25	0.125	-0.90		
Fish *(Danio rerio)* (Zhang et al., 2012)	Death	120	15.28	7.64	0.88		

Based on data reported in Table 2 a freshwater ecotoxicity EF for CdTe QDs calculated as $0.5/HC_{50}.10^3$ and corresponding to 374.10 Potentially Affected Fraction (PAF) of freshwater species m^3/kg is proposed. Ecotoxicity data used for the calculation of the freshwater EF is limited to one value per trophic level which conveys a high uncertainty in the proposed value. This value is thus preliminary and will need to be updated as more data become available.

3.2.3. General remarks and limitations

Information of (eco)toxicological nature has been retrieved in the absence of clear standard operating protocols for ENMs' (eco)toxicity assessment. Moreover, tests were done with the same compound but different materials (size, shape, functionalization…). Concerning the HEF, an approximation based on SSA only has been considered for CdTe QDs whereas the ENMs taken as a reference have different compositions and, thus,

associated toxic effects. An exception is represented by Ag nanoparticles with toxicity mechanisms associated to solubility, as in the case of CdTe QDs (Su et al., 2010; Katsumiti & Cajaraville, 2019). The absence of dosimetry studies (nominal versus measured exposure levels) represents an additional limitation.

3.3. Integration of USEtox® and SB4N

3.3.1. Common constants for both models

In this section, original USEtox® values were selected to be inserted in the USEtox® Air, Water & Soil scenario(s) defined in SB4N (SB4N - *Scenarios* sheet. Landscape settings). As shown in Table 3, only the *Area land* rate constant from the Urban compartment in USEtox® (USEtox®-DEF values) needs to be fed into the rate constants of the Regional scale of SB4N. As indicated in Table 38, *Area land*, *Area sea*, *Fraction fresh water*, *Fraction natural soil*, *Fraction agricultural soil* and *Depth fresh water* constants' s values in the Continental scale of SB4N need to be adjusted to fit with USEtox® (USEtox®-DEF values).

Table 3: Constants of the Regional scale in the SB4N. Values from the Urban compartment of USEtox® (USEtox®-DEF values) with grey background need to be transferred.

Constant – Regional scale	Variable Name	Units	SB4N Default scenario	USEtox® Air, Water & Soil Scenario
Area land	AREAland.R	[km^2]	2,3E+05	**2,4E+02**
Area sea	AREAsea.R	[km^2]	1,0E+03	1,0E+03
Fraction lake water	FRAClake.R	-	2,5E-03	2,5E-03
Fraction fresh water	FRACfresh.R	-	2,8E-02	2,8E-02
Fraction natural soil	FRACnatsoil.R	-	2,7E-01	2,7E-01
Fraction agricultural soil	FRACagsoil.R	-	6,0E-01	6,0E-01
Fraction urban/industrial soil	FRACothersoil.R	-	1,0E-01	1,0E-01
Temperature	TEMP.R	[oC]	1,2E+01	1,2E+01
Wind speed	WINDspeed.R	$m.s^{-1}$	3,0E+00	3,0E+00
Average precipitation	RAINrate.R	$mm.yr^{-1}$	7,0E+02	7,0E+02
Depth lake water	DEPTHlake.R	m	1,0E+02	1,0E+02
Depth fresh water	DEPTHfreshwater.R	m	3,0E+00	3,0E+00
FRACTION discharge regional fresh water to continental scale	FRAC.w1R.w1C	-	0,0E+00	0,0E+00
Fraction run off	FRACrun.R	-	2,5E-01	2,5E-01
Fraction infiltration	FRACinf.R	-	2,5E-01	2,5E-01
Soil erosion	EROSION.R	$mm.yr^{-1}$	3,0E-02	3,0E-02

Table 4: Constants of the Continental Scale in SB4N. Values from the Continental compartment of USEtox® (USEtox®-DEF values) with grey background need to be transferred.

Constant – Continental scale	Variable Name	Units	SB4N Default scenario	USEtox® Air, Water & Soil Scenario
Area land	AREAland.C	[km^2]	3,7E+06	**9,0E+06**
Area sea	AREAsea.C	[km^2]	3,7E+06	**9,9E+05**
Fraction lake water	FRAClake.C	-	2,5E-03	2,5E-03
Fraction fresh water	FRACfresh.C	-	2,8E-02	**3,0E-02**
Fraction natural soil	FRACnatsoil.C	-	2,7E-01	**4,9E-01**
Fraction agricultural soil	FRACagsoil.C	-	6,0E-01	**4,9E-01**
Fraction urban/industrial soil	FRACothersoil.C	-	1,0E-01	1,0E-01
Temperature	TEMP.C	[oC]	1,2E+01	1,2E+01
Wind speed	WINDspeed.C	$m.s^{-1}$	3,0E+00	3,0E+00
Average precipitation	RAINrate.C	$mm.yr^{-1}$	7,0E+02	7,0E+02
Depth lake water	DEPTHlake.C	m	1,0E+02	1,0E+02
Depth fresh water	DEPTHfreshwater.C	m	3,0E+00	**2,5E+00**
FRACTION discharge continental fresh water to regional scale	FRAC.w1C.w1R	-	0,0E+00	0,0E+00
Fraction infiltration	FRACinf.C	-	2,5E-01	2,5E-01
Fraction run off	FRACrun.C	-	2,5E-01	2,5E-01
Soil erosion	EROSION.C	$mm.yr^{-1}$	3,0E-02	3,0E-02

3.3.2. Identification of mass balance rate constants that are common for both models

USEtox® calculates the fate factors from 17 mass balance rate constants (k) [d^{-1}]. Mass balance rate constants are distributed as follows: (i) 4 for the continental environment, (ii) 11 for the intermedia transfer at continental scale, (iii) 1 for the urban environment and (iv) 1 for the intermedia transfer at urban scale (Tables 5-8). The Excel sheets and cells containing such constants in both USEtox® and SB4N have been indicated.

Table 5: Mass balance rate constants used by USEtox® to calculate the FF: Continental environment. Equivalence in SB4N. (N/A refers to Not Available, it has been indicated in italics)

Constant	**Denomination (*Fate* sheet; USEtox®)**	**Excel cell identification (*Run* sheet; USEtox®)**	**Denomination in SB4N**	**Excel cell identification (*Engine* sheet, SB4N)**	**Excel cell identification (*All species output* sheet, SB4N)**
k.aC.aU	*TRANSFER air - urban scale*	*G25*	*N/A in SB4N*		
k.aC.aG	*TRANSFER air - global scale*	*G31*	*N/A in SB4N*		
k.w1C.w2C	TRANSFER fresh water - coastal seawater	H28	TRANSFER rate continental fresh water - continental sea water (*Continental* sheet, SB4N)	BF67; BG68; BH69; BI70	Not used as a constant in the transport section (Output table 2)
k.w2C.w2G	*TRANSFER coastal seawater* - global *scale*	*I33*	*N/A in SB4N*		

In absence of an Urban compartment and as an alternative to k.aC.aU, SB4N uses k.aC.aR (TRANSFER rate continental air - regional air); Excel cells corresponding to AU9, AV10, AW11 and AX12 (*Engine* sheet, SB4N).

Table 6: Mass balance rate constants used by USEtox® to calculate the FF: Intermedia Transfer at Continental scale. Equivalence in SB4N. (N/A refers to Not Available, it has been indicated in italics)

Constant	**Denomination (*Fate* sheet; USEtox®)**	**Excel cell identification (*Run* sheet; USEtox®)**	**Denomination in SB4N**	**Excel cell identification (*Engine* sheet; SB4N)**
k.aC.w1C	TRANSFER air - fresh water	G27	Not Mentioned	AU63
k.aC.w2C	TRANSFER air - seawater	G28	Not Mentioned	AU67
k.aC.s1C	TRANSFER air - natural soil	G29	Not Mentioned	AU83
k.aC.s2C	TRANSFER air - agricultural soil	G30	Not Mentioned	AU87
k.w1C.aC	TRANSFER fresh water - air	H26	Not Mentioned	BF52
k.w2C.aC	TRANSFER seawater - air	I26	Not Mentioned	BJ52
k.s1C.aC	TRANSFER natural soil - air	J26	Not Mentioned	BZ52
k.s2C.aC	TRANSFER agricultural soil - air	K26	Not Mentioned	CD52
k.s1C.w1C	TRANSFER natural soil - fresh water	J27	TRANSFER rate natural soil – water (*Continental* sheet)	BZ63
k.s2C.w1C	TRANSFER agricultural soil - fresh water	K27	TRANSFER rate agricultural soil – water (*Continental* sheet)	CD63
k.w1C.s2C	*TRANSFER fresh water - agricultural soil*	*H30*	*N/A in SB4N*	

Table 7: Mass balance rate constants used by USEtox® to calculate the FF: Urban environment. Equivalence in SB4N. (N/A refers to Not Available, it has been indicated in italics)

Constant	Denomination (*Fate* sheet; USEtox®)	Excel cell identification (*Run* sheet; USEtox®)	Denomination in SB4N
k.aU.aC	*TRANSFER air - continental scale*	*F26*	*N/A in SB4N*

In absence of an Urban compartment and as an alternative to k.aU.aC, SB4N uses k.aR.aC (TRANSFER rate regional air – continental air); Excel cells corresponding to D52, E53, F54 and G55 (*Engine* sheet, SB4N).

Table 8: Mass balance rate constants used by USEtox® to calculate the FF: Intermedia Transfer at Urban scale. Equivalence in SB4N. (N/A refers to Not Available, it has been indicated in italics)

Constant	Denomination (*Fate* sheet; USEtox®)	Excel cell identification (*Run* sheet; USEtox®)	Denomination in SB4N
k.aU.s3U	*TRANSFER air - continental fresh water*	*F27*	*N/A in SB4N*

3.3.3. Gneral discussion and limitations of the integrative approach proposed

The absence of mass balance rate constants in SB4N related with the urban environment including k.aC.aU [TRANSFER air - urban scale]; k.aU.aC [TRANSFER air - continental scale] and k.aU.s3U [TRANSFER air - continental fresh water] can be anticipated, as the Urban compartment does not exist in SB4N; therefore, in this work we propose to assimilate it to the Regional compartment in SB4N.

However, there are a number of additional constants used in USEtox® that are missing from SB4N, namely: k.aC.aG [TRANSFER air - *global* scale], k.w2C.w2G [TRANSFER coastal seawater - *global* scale] and k.w1C.s2C [TRANSFER fresh water - agricultural soil].

Additionally, k.w1C.w2C [TRANSFER fresh water - coastal seawater] is indeed included in SB4N though it does not appear in the *All species output* sheet in SB4N (Table 5).

Ettrup et al. (2017) reported the integration of USEtox® 2.0 and SB4N and mentioned that "*the USEtox-defined dimensions of the continental and global boxes were thus adapted to the dimensions of the SB4N model*". According to Salieri et al. (2019), this implied to expand the number of environmental compartments considered in USEtox® and to create a version with three separate sub-compartments corresponding to the three species of engineered nanoparticles (free, aggregated and attached). These authors mentioned that "*whilst this approach is relatively straightforward, it generates a large number of compartments that i) may impede interpretation, ii) will increase the number of CFs to be calculated, and iii) requires the introduction of a rain compartment that does not explicitly exist in USEtox*" and proposed an alternative strategy based on the reduction of the number of compartments considered (and excluding the rain compartment) based on which they calculated CFs for nano TiO_2 for the freshwater ecotoxicty impact category. As it can be derived from former research studies, no generally accepted approach exists for the integration of both models. Furthermore, these studies have considered the (eco)toxicological impacts of the pristine forms of ENMs (TiO_2, in particular) for the calculation of the EF.

Concerning the LCA studies that have accounted for the releases of ENMs, the work by Hischier (2015) modelled two different worst case EOL scenarios assuming (i) a 100% release into air of the carbon nanotubes (CNTs) contained in a field emission display (FED) panel in a shredding scenario and (ii) a 100% of the amount of CNTs contained in the slag emitted into the ground-water compartment in an incineration scenario. However, this author mentioned: "*so far none of the LCIA methods contains a CF for the impact of CNT releases on human health or human toxicity*". This statement can also be extended to other LCA studies that have integrated the release of ENMs, since CF that are specific for the released forms of ENMs are not available to date.

4. Conclusions

Since the impacts of CdTe QDs on human health and the environment are not fully understood, in the present work we have proposed an extrapolation to the HEF of better known ENMs considering the SSA of both pristine and released forms of CdTe QDs. For freshwater EF calculation, we have selected one ecotoxicity value per trophic level representative of the freshwater compartment which increases the uncertainty of the proposed value. Future updates of USEtox® should integrate the results from *in vitro* tests for fish acute toxicity for RTgill-W1. A generic constraint to calculate both HEF and freshwater EF of the released forms of CdTe QDs is related to the absence of specific (eco)toxicological information of the emissions taking place throughout the life cycle of products incorporating them such as e.g. during printing (use stage). Hereby proposed EFs will need to be adjusted as new information corresponding to the impacts of the released forms of CdTe QDs becomes available.

The average particle size of the airborne emissions associated to 60 minutes inkjet printing of a CdTe QDs-additivated ink corresponded to 59.52 nm. Our approach to integrate USEtox® and SB4N being widely proposed for the LCIA phase and allowing size-dependent fate calculations, respectively, for the derivatization of the FF which is required for the calculation of CFs has consisted on the identification and assimilation of common mass balance rate constants and aligning common constants that define the environmental compartments. However, this possibility has revealed to be limited since there is no absolute correspondence between the two models. Different approaches to integrate USEtox® and SB4N should be developed and tested in future studies.

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IV. GENERAL DISCUSSION

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Part of this work has been published as:

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Blázquez, M. and Marchante, V. (2021). Scenarios simulation at laboratory scale for the assessment of the release of engineered nanomaterials. [CHAPTER 6; In Health and Environmental Safety of Nanomaterials. Njuguna, J.; Pielichowski, K.; Zhu, H. (eds). 2nd ed.] [Elsevier Ltd., DOI: https://doi.org/10.1016/B978-0-12-820505-1.00015-8].

Life Cycle Thinking is about going beyond the traditional focus on production site and manufacturing processes to include environmental, social and economic impacts of a product over its entire life cycle.

A product life cycle can begin with the extraction of raw materials from natural resources in the ground and the energy generation. Materials and energy are then part of production, packaging, distribution, use, maintenance, and eventually recycling, reuse, recovery or final disposal.

In each life cycle stage there is the potential to reduce resource consumption and improve the performance of products.

What is Life Cycle Thinking? The UNEP SETAC Life Cycle Initiative (2022)

IV.1. On the integration of a life cycle perspective: common approaches for the case studies presented in the results section

The initial case study (Chapter 1) describes the impacts of potential chemical pollution of the (fresh)water environmental compartment derived from the professional or consumer use of biocidal products. Outdoor or indoor use of biocidal products might lead to the environmental exposure to these chemicals. In the case of indoor use, wastewater and landfill are the receiving environmental compartments generally after cleaning operations or as a consequence of EOL processes. Concerning outdoor use, depending on the specific area of use, rainwater and wastewater or soil can be reached in the case of urban and rural areas, respectively.

Data from 196 biocidal substances and 206 environmental metabolites have been collected for four taxonomic groups, including fish, invertebrates, algae and sewage treatment plant (STP) microorganisms and compiled in a database. Subsequently, a categorization of substances according to their toxicity in four groups, considering $L(E)C_{50}$ values in

agreement with the EU Regulation (EC) No 1272/2008 has been proposed. Our results have shown that more than 50% of the parent compounds were located in category 1 ($L(E)C_{50}$ ≤ 1 mg/L) for fish, invertebrates and algae, indicating a high toxicity for the freshwater/marine compartments whereas more than 60% were not toxic for STP microorganisms. However, information on toxicity to the STP microorganisms was only found for 40% of the parent compounds. In general, metabolites were less toxic than the parent compounds, but 22-36% presented the same toxicity, ~6% being more toxic. No toxicological information was found for ~50% of the metabolites for fish, invertebrates and algae, reaching the 96% in the case of microorganisms. We have observed a relatively high percentage of toxic metabolites: the scarcity of data for these compounds, specially in the case of microorganisms, indicate the need to implement life cycle oriented approaches in the study of the impact of biocides.

A life cycle perspective has been integrated by the inclusion of the comparison of hazards to the aquatic compartment associated to environmentally relevant metabolites generated from biocidal active substances during the use or end of life phases of biocidal products (i.e. beyond manufacturing stage) in contrast to those of their parent compounds. Figure 1 is a schematic representation of the life cycle perspective integrated in the environmental hazard assessment of biocides, representative of conventional pollutants.

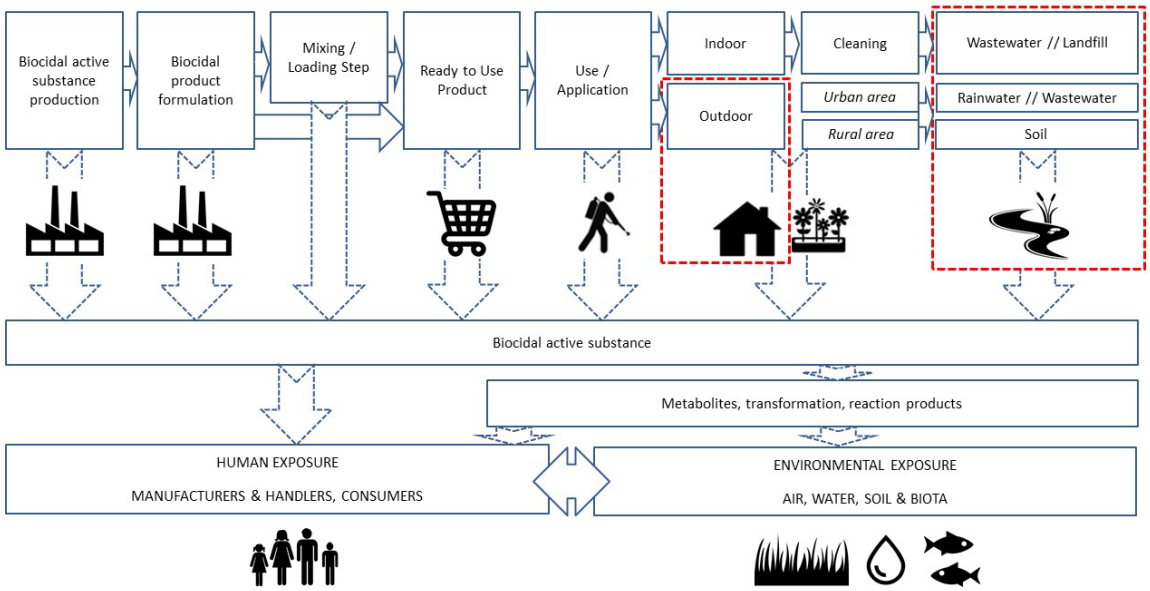

Figure 1: Life cycle perspective in the exposure and impact assessment of biocides. The life cycle stages that have been considered in the present thesis have been framed in red. Metabolites generated in the environmental compartment after the use or application life cycle stages can lead to human and environmental exposure.

In the second case study (Chapter 2), the potential pollution of the indoor workplace and household compartments by ENMs as emerging contaminants (nanoclays, in particular) derived from the mechanical degradation of nanocomposites during post manufacturing machining processes or consumer use has been investigated.

We have developed three different scenarios in confined conditions to investigate changes in particle emission behavior of mechanically degraded polypropylene (PP) samples with different fillers including talc and two types of nanoclays: wollastonite (WO) and montmorillonite (MMT) in contrast to no-nreinforced PP. The presence or absence of EMNs in the nanocomposite formulation has been investigated as the main factor causing differences in the particle emission profile. Airborne particle concentration and particle size distribution profiles obtained within three independent scenarios simulating industrial milling and drilling and household drilling have been compared. All the three scenarios have yielded different figures for particles generation both in absolute and relative terms. Results suggest that it is not possible to describe the effects of adding nano-sized modifiers to PP by a single trend that applies consistently across all different protocols.

Variations in the results observed for airborne released particles during mechanical degradation of solid PP nanocomposites might be attributed to a variety of reasons including the specific operational parameters selected and instrumentation used for airborne particle release measurements. An integrative approach providing released particles as a function of the quantity of removed material has been proposed for future assessments in order to enable the comparison of variations in the number of emitted particles by means of different mechanical processes.

This case study has been carried out from a life cycle perspective by identifying mechanical degradation processes such as drilling and milling as potential life cycle stages leading to the emission of nano-sized fillers used in nanocomposites (i.e. beyond manufacturing stage). Figure 2 is an schematic representation of the implementation of a life cycle perspective in the risk assessment of nanocomposites.

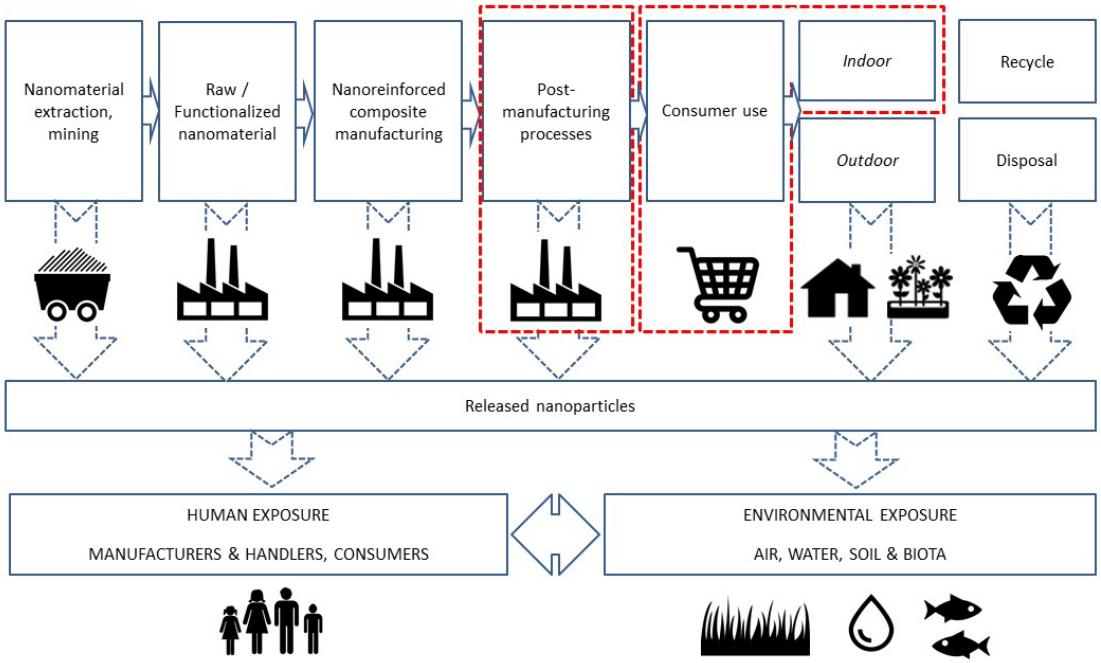

Figure 2: Life cycle perspective in the exposure and impact assessment of nanocomposites. The life cycle stages that have been considered in the present thesis have been framed in red. The main focus of this research has been placed upon post manufacturing processes and consumer use, both in the indoor compartment.

The third case study (Chapter 3) has adressed the potential pollution of the indoor household compartment by ENMs as emerging pollutants (cadmium telluride quantum

dots) derived from the use of a prototype of a nanoadditivated printing ink at consumer scale.

To attain this target, the airborne emissions of a water-soluble fluorescent ink containing polyethylene glycol (PEG)-coated CdTe QDs of 3-5 nm diameter have been characterized and studied under controlled conditions during household inkjet printing in a scenario simulating the use phase. Subsequently, the cytotoxicological potential of atomized CdTe QDs ink in an acute exposure regime simulating an accidental, worse-case scenario has been evaluated *in vitro* at the ALI using the pulmonary cell line BEAS-2B. Endpoints screened included cell viability, oxidative stress and inflammatory effects. We have observed that CdTe QDs ink at 54.7 ng/mL decreased cell viability by 25.6 % when compared with clean air after 1h of exposure; a concentration about 65 times higher was needed to observe a similar effect in submerged conditions. However, we did not observe oxidative stress or inflammatory effects.

Thus, the study described in Chapter 3 integrates the development of scenarios simulating the use phase of nano-additivated inks and the direct cell exposure for *in vitro* effects assessment, thus implementing a life cycle oriented approach in the hazard assessment (cytotoxicity) of CdTe QDs. Figure 3 represents a generic approach to take into consideration the different life cycle stages of a nano-additivated ink.

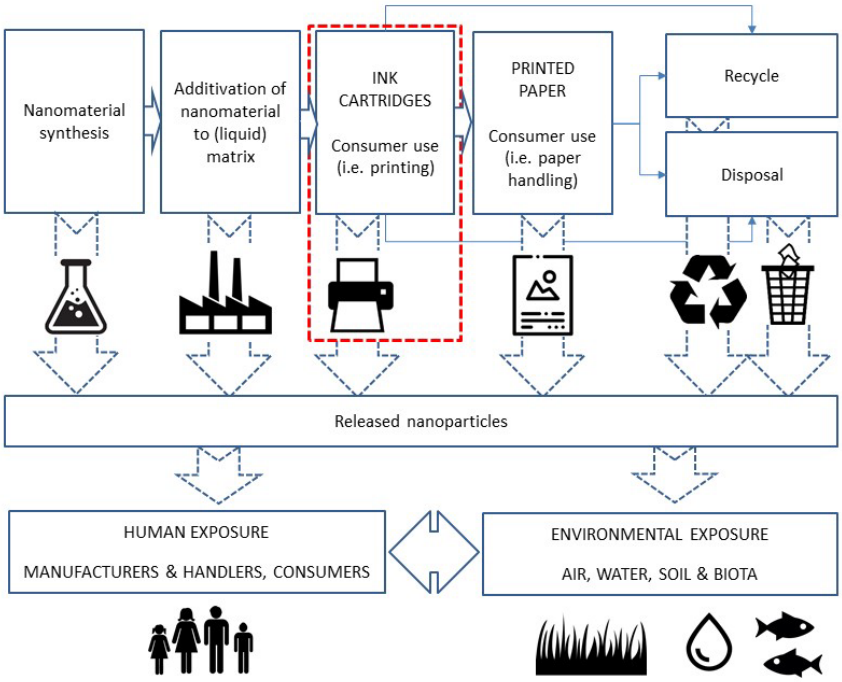

Figure 3: Life cycle perspective in the exposure and impact assessment of a nano-additivated printing ink. The life cycle stages that have been considered in the present thesis have been framed in red. Other life cycle stages potentially leading to human and/or environmental exposure include the manipulation of printed paper by consumers (leading to e.g. dermal exposure) or emissions associated to the EOL processes e.g. emissions associated to landfield.

The final chapter of this thesis stems from the need of characterization factors (CF) for (eco)toxicity effects of emerging pollutants (nanomaterials) for life cycle impact assessment. An attempt is presented to calculate CFs for human toxicity (non-carcinogenic effects) and freshwater toxicity impact categories to be integrated in LCA modelling has been calculated by USEtox® based on the size-depending fate factors from SB4N, which is a nano-specific model. CdTe QDs have been selected as a case study. In order to select particle sizes representative of different life cycle stages, the emissions during printing of a NEP consisting of water-soluble printing ink have been characterized under controlled conditions. In detail, particle size of the emissions have been measured by an SMPS at the start of the printing process (t=0); at the end of the printing process (t=60'), and 60' accounting from the end of the printing process (t=120'). Mean particle size at the end of the printing process (t=60') equivalent to 59.52 nm has been selected as representative

size of the emissions corresponding to the use stage of the NEP in order to propose a fate factor (FF) corresponding to the use phase.

A life cycle perspective has been considered in Chapter 4 by approaching the potential derivatization of CFs corresponding to particle sizes representative of emissions taking place during printing (i.e. beyond those of pristine nanoparticles). The main limitation of the present approach is that the integration of both models cannot be executed straightforward, since the compartments and constants they use differ.

To date, the public literature proposes different values for CF for ENMs (see the review included in the Supporting Information by Temizel-Sekeryan & Hicks, 2021), which implies that there is no widely accepted methodology to calculate such CFs. Efforts are required to integrate the impact of nanomaterials in LCA studies and, in particular, of their released forms.

As a result of the work carried out, we hereby propose a generic approach for the assessment of the release of ENMs from NEPs or NEAs focusing mainly on potential airborne emissions.

IV.2. Generic approach for the assessment of the airborne release of ENMs from NEPs or NEAs

The environment, workplace and household compartments represent a source of airborne exposure to ENMs released from NEPs or NEAs. Taking into consideration the evidences presented for NEPs potentially leading to human and/or environmental exposure, a general approach for nano-release assessment consisting of a series of steps are considered necessary.

Step 1: Evaluate the physico-chemical characteristics and (eco)toxicological hazard of the pristine ENM

Priority should be given to those NEPs or NEAs containing ENMs that raise concern in their pristine form: existing evidence on the possible (eco)toxicological effects of the pristine

ENMs and the physicochemical properties associated to such effects need to be carefully considered and/or investigated.

Step 2: Pre-select the life cycle scenario during which maximum airborne release (and associated direct or indirect human/environmental exposure) is expected.

Realistic exposure scenarios need to be defined considering the different possible uses of the NEP or NEA (e.g. indoor or outdoor use). The use of expert criterion would be desirable in order to identify the most relevant life cycle stage in terms of potential airborne ENMs release.

At this regard, two extreme situations can take place: (i) 100% Release (if all ENMs originally contained in the NEP or NEA are released as airborne particles in the life cycle of interest); (ii) 0% Release (if the release of airborne ENMs originally contained in the NEP or NEA in the life cycle of interest does not take place).

In alignment with the guidelines by the ECHA (2016) regarding consumer exposure assessment, a 100% release of the ENM needs to be anticipated. This approach would allow calculating the theoretical maximum concentration of released ENMs that human beings would be exposed to. Such concentration must be compared with the information compiled within Step 1 (concentration and other dose metrics) in order to have a preliminary indication on the possible effects of the released ENMs.

Step 3: Simulate the selected scenario in confined conditions

An initial simulation step not involving the use of NEPs is recommended. The objective of this step is to precisely discern background particles (attributed to the equipment used for usage simulation, for instance) that could bias further measurements using NEPs. The development of scenarios in confined conditions also serves the purpose of protecting the researchers taking part in the investigation.

3.1. Define the protocol to simulate the life cycle scenario of interest

Whenever available, the defined protocol for the simulations should ideally be based on product-specific already existing standards. Literature refers a number of examples of studies that have used standards to assess product's resistance, simulating the usage stage

such as the use of the Taber Abrader according to the standard conditions NF EN ISO 7784-1 and NF ENISO 7784-2 for the abrasion of surface paints by Golanski et al. (2012). The use of standards was also promoted by Koivisto et al. (2017) who proposed to mimic the stress scenario by following standardized stress test procedures or simulate the process in environmentally relevant and well-controlled conditions.

In absence of standards, the protocol that is closest to the real life cycle scenario should be prioritized.

3.2. Select appropriate samples

Close to market applications need to be prioritized in contrast to applications or products that are available at laboratory scale only. This is due to the fact that optimizations in the manufacturing process may lead to changes in the releasability of the incorporated ENM.

Complementarily, and if available, a product that does not contain ENMs but that has the same functionality should be selected as a reference sample. Such NEP or NEA could contain the bulk counterpart of the ENM of interest (e.g. in the micrometric scale) in order to compare the influence that can be specifically attributed to the nanodimension. Alternatively, a product or application of a completely different composition could be selected as long as its properties are comparable to those of the NEP or NEA of interest.

Step 4: Characterize the human/environmental exposure by means of on-line and off-line technologies

The ultimate purpose of this step is to quantitatively and qualitatively characterize human/environmental exposure to released ENMs during their life cycle. Ideally, ENMs release studies should report mass balance (released airborne ENMs versus originally added ENMs to the NEP/NEA of interest). However, particle number emission rates alone cannot be used to estimate mass flows or linked to hazard.

The characterization of the exposure associated to released ENMs should be carried out by means of direct reading instruments (DRI) with integrated filter or electrostatic precipitator based sampling. Such samples should be used in electron microscopy analyses and elemental mass quantification (e.g. via energy-dispersive x-ray spectrometry) in order to confirm the presence of the ENM of interest, as suggested by NEAT 2.0.

Step 5: Compare the characteristics of the released and pristine ENMs

From the quantitative assessment -and in comparison with the data retrieved within Step 3- the (eco)toxicological effect of the released ENMs could be anticipated. However, the physicochemical characteristics of the particles released from a NEP/NEA during its life cycle are generally not representative of those of the pristine ENMs incorporated into the manufacturing stage as a consequence of the changes undergone during the product manufacturing process and/or during product's use or EOL stages.

In this sense, two extreme scenarios can take place from the comparison: (i) 100% correspondence of the physico-chemical characteristics of pristine and released ENMs; (ii) total absence of correspondence of the physico-chemical characteristics of pristine and released ENMs.

As mentioned earlier, and taking into consideration the examples of potential ENMs releases described in former sections, the existence of 100% correspondence of the physico-chemical characteristics of pristine ENMs and the ENMs released throughout the life cycle of NEPs/NEAs is highly improbable. The implementation of life cycle oriented approaches therefore leads to the generation of a different class of materials that must be assessed from hazard perspective. Since, a priori, and depending on the specific NEP/NEA of interest, the quantities of released ENMs are expected to be low (Giese et al., 2018), particular attention should be given to chronic exposures/effects and to potential synergistic effects.

Depending on the specific test protocol and of the endpoint of interest, the repetition of Step 3 could be required in order to increase the quantity of ENMs released from NEPs or NEAs.

Step 6: Implement a safe(r) by design (SbD) approach

In the present context, the SbD approach aims at minimizing the exposure of ENMs from the design stage of a NEP/NEA, taking into account the entire life cycle. If ENMs released throughout the life cycle of the NEP/NEA represent a hazard, a redesign aiming at preventing the release of ENMs should be considered. Ideally, the present step should therefore be undertaken at pre-market stage.

Complementary to the stepwise approach proposed, practical lessons for the development of scenarios that simulate life cycle stages of NEPs and NEAs beyond the manufacturing stage (Step 3, Figure 4) are presented in the following section.

Figure 4 integrates the aforementioned steps in a comprehensible approach.

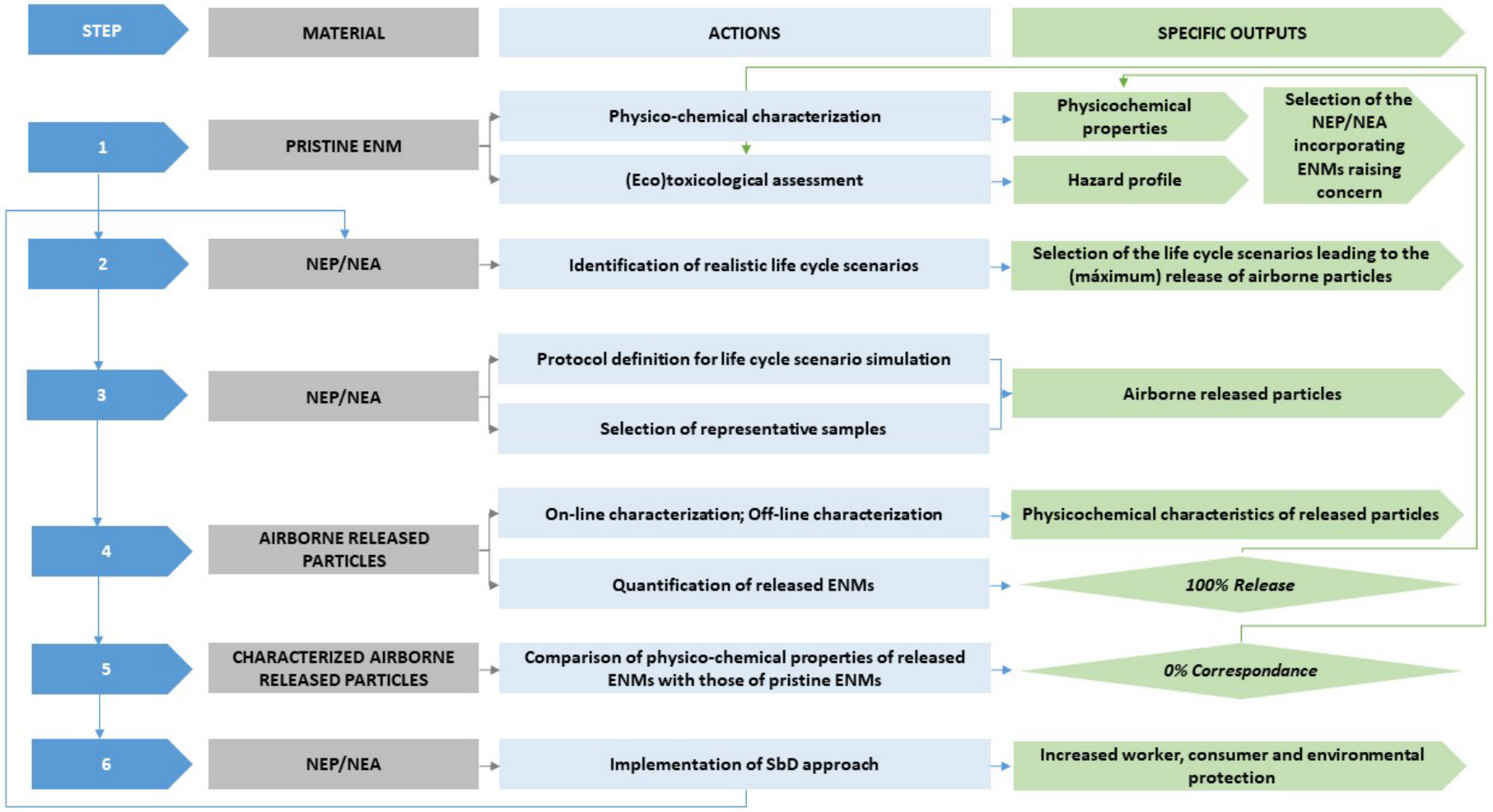

Figure 4: Stepwise approach for the release assessment of airborne ENMs from NEPs or NEAS.

IV.3. Lessons learned from the development of scenarios simulating different life cycle stages of NEPs and NEAs

In the present section reference is made to the VAMAS Guidelines for the Design and Operation of Interlaboratory Comparisons (ILCs) by Roebben (2017). The ISO/IEC 170473:2010 defines ILCs as the organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions (ISO, 2010).

If the objective of the ILC is to quantitatively assess the reproducibility of a new method (e.g. a method to simulate nanocomposites drilling and to characterize released particulate material) some of the basic requirements of the ILC design include: (i) the use of a 'real-life' material (having the main characteristics of the technically or commercially relevant materials); (ii) the use of sample material that is sufficiently homogeneous and stable; (iii) the provision of enough sample material for ≥ 2 measurements per lab; which will enable the separation of the contributions of method repeatability (within laboratories) and reproducibility (between laboratories) to the variance of the results and (iv) the definition of a clear and unambiguous test protocol that should be implemented by all participating laboratories. In the present context, a protocol simulating the release of ENMs from embedding matrices should be defined so that the reproducibility of the results generated (quantity and quality of released particulate material) is assured.

Such premises should be taken into consideration in the development of protocols to simulate life cycle stages undergone by NEPs and NEAs and to assess the associated release of ENMs and especially if the data are generated by different laboratories and need to be compared. In addition to the generic requirements cited above for ILCs, other relevant aspects are discussed in the next sections.

Experimental set-up

The simulation of life cycle scenarios potentially leading to nanoparticle release from NEPs and NEAs must be carried out under controlled conditions for two main reasons: personnel protection and minimization of particle losses. For encapsulation of the release simulation

process, the use of high-quality glass such as grade A glassware, rather than low-grade soda glass is preferable. If plastic ware must be used, its suitability should be assessed first. When sample generation requires the use of a given instrument (as in the case of mechanical stress: a drill), all equipment and materials used should be pre- treated to minimize as far as possible bacterial contamination. In the absence of specific regulatory cleaning methods or recommendations, routine cleaning methods should be used. Possible alternatives include the use of alcohol or autoclaving.

Avoidance of background particles in simulated scenarios at laboratory scale

Release induced particulate material sampling in a given medium (air, water, soil) should always include measurement of the background (via a control sample) by evaluating the naturally occurring background concentration. This information should be regarded as the correct baseline from which particulate release should be monitored. If the air compartment is of interest, quantifying the proportion of released airborne nanoparticles associated with background aerosols is a considerable challenge; therefore, the easiest way to ensure an aerosol free background concentration is to develop simulation scenarios in confined conditions as described by Starost et al. (2017a, 2017b).

The presence of particle emissions generated from concurrent processes (i.e., use of combustion or electro motors in the equipment required for the simulation of the life cycle of interest) as reported by e.g. Van Broekhuizen *et al.* (2011) should be eliminated too.

Different processes potentially leading to the release of ENMs from NEPs and NEAs

Taking (nano)composites as an example, the most frequent stress sources for plastics include mechanical/physical stress, hydrolysis, photolysis, chemical stress, thermal stress or other sources of stress (Andrady, 2011). Mechanical stress refers to the active physical processes that degrade plastics. Examples of these include abrasion, drilling, sawing, and sanding. Photolysis refers to the degradation processes due to the effect of UV radiation, whereas hydrolysis refers to water-d riven degradation mechanisms. Chemical stress degradation processes include such effects as ozonolysis, and other processes that degrade plastics by the action of chemicals. It should be noted that in the different life-cycle stages of plastics, degradation processes generally occur in combination: an active

physical process usually has an associated heat release that causes the degradation of plastics via thermal stress. Finally, other sources of stress include degradation-causing agents of a biological nature (biotic degradation), such as enzyme based degradation processes (Mohanan, 2020).

Such processes might lead to the release of ENMs if those are completely or partially protruding from the surrounding matrixes or to the release of particulate material of different sizes (micro to nano). In this sense, the work by Wohlleben et al. (2014) is of relevance as it reports the first pilot inter-laboratory study detailing the critical parameters for nanoparticle release assessment in a polyamide containing 4% wt. of silica nanoparticles (nanosilica). In particular, authors investigated the release induced by mechanical shear after dry weathering at different UV intensities and spontaneous release during wet weathering. Authors reported that the released fragments were a polydisperse mixture of predominantly composite fragments from the nanometre up to several micrometre diameter, and of clustered or individual nanosilica unbound to polymer. The unbound fraction was microscopically observed but could not be quantified. Finally, the authors included a detailed protocol for the assessment of material released by aging of polymer nanocomposites. To the best of our knowledge, a similar protocol for the assessment of materials released by mechanical/physical stress of polymer nanocomposites has not been proposed to date.

On-line measurement of released (airborne) particles from NEPs and NEAs

A battery of instruments exist for aerosol characterization having their origin in air pollution science; their assessment and comparison is out of the scope of the present discussion. As an example, the recent work by Kangasluoma et al. (2020) examines state of the art methods and instruments for physical characterization of aerosol ultrafine particles (also referred to as particulate matter ≤ 0,1μ ($PM_{0,1}$)). According to the authors, $PM_{0,1}$ particles have short lifetimes and require sufficiently fast-measuring instruments to capture the full dynamics of the particles; and the measurement should take place close to the source to maximize the sampling efficiency. Finally, authors provide some suggestions for the appropriate selection of instruments based on their corresponding response time and limit of detection.

Collection of released airborne particles from NEPs and NEAs for off-line analysis

Nano-release assessment necessarily requires a combined approach of on-line and off-line characterization. The off-line characterization of collected airborne released samples must be carried out immediately after their generation/collection. If samples are delivered to other laboratories, personnel receiving the samples must characterize the samples upon receipt in order to verify if any significant transformation has taken place during transportation.

Electron Microscopy (EM) allows visualization of particles down to the nanometre scale, thereby providing physical information on the single particle level e.g. geometric size, agglomeration state, and shape. The most common EM techniques are Transmission Electron Microscopy (TEM) and SEM. SEM provides a lower resolution than TEM, but recent advances have made it possible to automatically scan large areas of a sample, process the acquired images, locate particles, and perform subsequent energy dispersive x-ray spectroscopy (EDS or EDX) analysis without user intervention except for the initial setup (Margiotta et al., 2015; Arndt et al., 2016). EDS/EDX can be used for both qualitative and quantitative analysis, enabling users to identify both the type of elements that are present as well as the percentage of each element's concentration within individual particles.

In order to use SEM and complementary analysis such as EDS/EDX it is necessary to collect the airborne particles onto a surface. There are currently many collection methods e.g. thermophoretic precipitation, filtering, electrostatic collection, and impaction. Depending on the collection method, samples can then either be inserted directly into the electron microscope or may need additional steps before analysis. If possible, preparatory steps should be avoided since they may alter the state or appearance of the particles before analysis.

IV.4. Considerations for the (eco)toxicological assessment of samples released from nanocomposites

The processes described under the present section apply if the released particles are to be used for (eco)toxicological assessment which represents a considerable challenge since no testing protocols exist for sample materials comprising dust of heterogeneous physico-chemical nature released as a consequence of different stress sources generated during simulated life cycle scenarios of nanocomposites. Hereby described considerations apply mainly to nano-release studies on NEPs/NEAs in solid state (e.g. plastic nanocomposites) and should be taken into consideration for the human health and environmental impact assessment of the airborne particles released in the experimental procedures described in Chapter 2.

Generation of samples released from nanocomposites for (eco)toxicological assessment

An example of an (eco)toxicity assessment using materials released as a consequence of the physical degradation of nanocomposite samples is provided by Schlagenhauf et al. (2015), who abraded multiwalled carbon nanotube (MWCNT) containing epoxy nanocomposite samples and performed *in vitro* toxicity studies for genotoxicity, reactive oxygen species formation, and cell viability assessment using A549 human alveolar epithelial cells and THP-1 monocyte-derived macrophages. A description of the particle size distribution and the microstructure of the abraded particles is included. For cytotoxicity testing, abraded particles or MWCNTs were suspended in liquid medium and sonicated. According to the authors, the toxicity tests using the abraded particles did not induce any acute cytotoxic effects. Interestingly, authors prelabelled MWCNTs with led ions with quantification purposes and used different approaches for PM_1 and ultrafine particles ($PM_{0.1}$) collection generated from the abrasion process.

Another study by Saber et al. (2016) evaluated the toxic effects of dusts generated by sanding of MWCNT containing epoxy composites. To attain this purpose, mice were intratracheally instilled with different quantities of MWCNT and sanding dust from MWCNT containing epoxy and neat epoxy composite (reference) sample specimens and DNA damage in lung and liver, lung inflammation and liver histology were evaluated. Authors

described the generation of the dusts, and the on-line and off-line characterization of the aerosol generated via particle size distribution and SEM images, respectively. Test materials were dispersed forming suspensions in liquid medium prior instillation (dynamic light scattering and SEM were used to characterize MWCNT and dusts in the suspensions). They concluded that pulmonary deposition of epoxy dusts both with and without MWCNT induced inflammation and DNA damage in lung tissue. However, altered effects have been associated to the use of instillation as administration route, e.g. Silva et al. (2014) reported increased inflammatory effects of MWCNT when administered via intratracheal instillation.

In Chapter 3 we have selected a different ENM as a case study: CdTe QDs. Further, such ENMs were added to a consumer product in liquid state (nano-additivated ink). Since the nature of the sample is different, the BEAS-2B cell line was directly exposed to the aerosolized CdTe containing ink avoiding the need to undergo an initial abrasion stage. In contrast with the *in vitro* toxicity data reported earlier for the dusts from abraded nanocomposites containing MWCNTs, we observed a 25.6% reduction in cell viability in comparison with clean air exposure at the air-liquid interface.

Samples released from nanocomposites storage and labelling (prior (eco)toxicological assessment)

As a general rule, released particulate dust samples should not be stored as nanocomposites and plastics in general tend to evolve. A number of challenges are associated with the determination of the most suitable conditions for sample maintenance and storage, especially since such conditions will influence the results obtained. For instance, the physico- chemical properties of ENMs in liquid suspensions tend to change with time and surrounding environment. It is thus desirable to store the samples in a dry state and prepare testing solutions immediately before assays are performed. Any storage conditions should avoid extremes of temperature, sunlight, and moisture and be similar to the general conditions for storing chemical substances.

Relevant information in regard to samples' labelling can be classified into three categories. The first one is information related to the original nanocomposite macrosample. The availability of these data will depend on the nature of the particular nanocomposite: materials can be of a commercial nature or manufactured at a laboratory scale for research purposes. Basic characteristics of the nanocomposite should be compiled, depending on

the intended applications (mechanical properties, permeability, thermal stability, electrical conductivity, chemical resistance, etc.). In the case of commercial products, the acquisition date should be recorded together with all information provided by the manufacturer. The weight percentage of ENMs used in samples manufacturing, if available, is also relevant. The second category is information related to the process of generating released particulate material. Information related to previous sections of this general discussion must be documented, especially that related to sample generation and sample collection. Examples of data to be recorded include: date of sample generation; parameters used in the simulation study; size, weight, and thickness of the test specimen, responsible researcher; interassays equipment cleaning procedure. The final category of information is that related to the characteristics of the sample determined off-line. In addition to the information above, if samples have been received from another laboratory, additional data should be recorded, including (i) date of receipt; (ii) name of operator receiving the sample and (iii) verification of the integrity of the sample (e.g. if there has been any accidental release during transportation).

Pre- treatment of samples released from nanocomposites: use of dispersing agents, sonication, stirring and mixing

ENMs suspended in liquids may form agglomerates. Agglomeration reduces the total number of particles, as well as the total surface area of the suspended ENM available for interaction with cells. Thus, a reproducible protocol for achieving well-dispersed ENM dispersions is a primary requirement for obtaining comparable toxicity data (RISKGONE, 2021). Dispersing agents can be either natural or synthetic, each of which has benefits and drawbacks for (eco)toxicological testing. Sonication is often used to produce well-dispersed ENM suspensions as an alternative to, or in combination with, dispersing agents.

According to Handy et al. (2012), there are no standard sonication protocols in terms of time, temperature, sonication power, volume of solution sonicated, type (batch vs. probe), and properties (micro- vs. macro- probe) of sonication devices. However, recommendations have been published, for example, by the Duke University's Center for the Environmental Implications of Nanotechnology (CEINT) and the National Institute of Standards and Technology (NIST) (Taurozzi et al., 2012). Whatever option is selected, the

possible effect of sonication on samples must be verified; hence, treatment of samples before (eco)toxicological testing (duration, stir speed, sonication power, etc.) should be fully documented.

IV.5 Final remarks

Overall, the integration of a life cycle perspective in the hazard and exposure assessment of biocidal active substances and ENMs in NEPs/NEAs has confirmed new sources of exposure for the selected life cycle stages and compartments. For future studies on:

(i) the environmental hazard assessment of biocidal active substances ➲ need to study the impact of metabolites in the aquatic compartments and to complete the existing information on the toxicity associated to STP microorganisms for both parent compounds and metabolites;

(ii) the release of particles during machining operations of (nano)composites ➲ need to establish a rating system based on quantified measurements of airborne particles for specific materials and degradation processes preferably as a function of total removed mass;

(iii) the release of particles during consumer use of nano-additivated printing inks ➲ need to collect proper data for risk assessment associated to long-term exposure to low concentrations of particles emitted during the printing process;

(iv) the development of CFs for (the released forms of) ENMs ➲ need to develop straightforward approaches resulting in the integration of consolidated models with nano-specific provisions.

IV.6 References

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V. CONCLUSIONS AND THESIS

CONCLUSIONS

I. Biocidal products represent a source of environmental exposure to highly toxic active substances during their use and end of life stages. In general, such substances can easily reach aquatic compartments and can be transformed in metabolites, degradation and transformation products that are generally less toxic than parent compounds towards trophic levels representative of the fresh/marine water and sewage treatment plant (STP) compartments, however, 22–36% presented the same toxicity and ~6% were more toxic.

II. There is no toxicological information for around 50% of the metabolites of biocidal products for fish, invertebrates and algae. There is scarce toxicity data for STP microorganisms with information available only for 37% of the biocidal substances and 4% of metabolites.

III. Machining of nanocomposites in post-manufacturing operations represents a source of exposure for ENMs used as fillers to increase the performance of plastics (e.g. equivalent tensile strength with lower density). Airborne nanosized particles are released in different scenarios simulating mechanical processes both by industrial and consumer stakeholders at the indoor compartment using polypropylene samples with different types of fillers (wollastonite, montmorillonite, talc –at micrometric scale-) and neat polypropylene. However, no unique trend in the airborne particle emissions for the samples of interest across the whole range of simulated scenarios takes place.

IV. There is a need to standardize the release assessment of ENMs from nanocomposites in machining operations. Data provided as a function of the concentration of released airborne particles per total quantity of mass removed is suggested as a suitable metric to compare the emissions associated to the different scenarios.

V. The consumer use at the household compartment of polyethylene glycol cadmium telluride quantum dots (PEG-CdTe QDs) added to printing inks to provide new or enhanced properties (e.g. anti-counterfeiting) represents a potential source of exposure to PEG-CdTe QDs.

VI. CdTe-QDs emitted during inkjet printing might cause potential health impacts as revealed by an *in vitro* basal acute toxicity screening. Aerosol of PEG-CdTe QDs ink delivered to the cells under the Air Liquid Interface (ALI) inhalation simulating conditions at a concentration of 54.7 ng/ml (37.1 ng/ml Cd and 17.6 ng/ml Te), resulted in decreased cell viability by 25.6% (±15.7%) when compared with the clean air condition. A 9.0% (±11.9%) decrease of cell viability was also observed for atomized solvent ink.

VII. Characterization factors (CFs) for toxicity-related impact categories (i) Human toxicity non-cancer effects and (ii) Freshwater ecotoxicity to be accounted for in Life Cycle Impact Assessment (LCIA) were derived for CdTe QDs. The combination of USEtox® and SimpleBox4Nano which represent the UNEP-SETAC consensus model for human health and freshwater toxicity impact categories and a fate model calculating size dependent fate factors for ENMs, respectively, was not achieved to derive CFs for pristine and released forms of ENMs due to a lack of correspondence in the mass balance rate constants and in the common constants that define the environmental compartments by the two models.

THESIS

The implementation of a life cycle oriented perspective when assessing the risks associated to both conventional and emerging pollutants leads to the identification of novel risks that differ from those derived from the manufacturing stages. In the case of ENMs, the simulation of the different processes that NEP/NEAs undergo throughout their life cycle demonstrates the release and associated exposure and hazard towards human health and/or the environment of the released ENMs, which needs to be investigated and subsequently accounted for in Life Cycle Assessment (LCA) in order to improve the overall sustainability of these emerging pollutants.

VI. ANNEX

ANNEX

In addition to the papers that form the main body of this Ph.D. thesis, the following publications dealing with life cycle approaches in the risk assessment of both conventional and emerging pollutants were also developed during the Ph.D. study (in chronological order of publication):

- **Blázquez, M.**; Andreu-Sánchez, O.; Ballesteros, A.; Fernández-Cruz, M.L.; Fito, C.; Gómez-Ganau, S.; Gozalbes, R.; Hernández-Moreno, D.; de Julián-Ortiz, J.V.; Lombardo, A.; Marzo, M.; Ranero, I.; Ruiz-Costa, N. and Benfenati, E. (2021) Computational tools for the assessment and substitution of biocidal active substances of ecotoxicological concern: the LIFE-COMBASE project [CHAPTER; In Chemometrics and Chemoinformatics in Aquatic Toxicology. Roy, K. (ed). 1st ed.] [Wiley-Blackwell, DOI: https://onlinelibrary.wiley.com/doi/book/10.1002/9781119681397].
- Hernández-Moreno, D.; **Blázquez, M.**; Navas, J.M.; Fernández-Cruz, M.L. (2021). Fish cell lines as good screening tools to predict the acute toxicity in fish of biocidal active substances and their relevant environmental metabolites. Aquatic Toxicology 242; 106020. (DOI: https://doi.org/10.1016/j.aquatox.2021.106020).
- Starost, K.; Frijns, E.; Van Laer, J.; Faisal, N.; Egizabal, A.; Elizetxea, C.; **Blázquez, M.**; Nelissen, I. and Njuguna, J. (2021) A study on the nanoparticle emissions into environment during mechanical drilling of polyester, polypropylene and epoxy nanocomposite materials. [CHAPTER 5; In Health and Environmental Safety of Nanomaterials. Njuguna, J.; Pielichowski, K.; Zhu, H. (eds). 2nd ed.] [Elsevier Ltd., DOI: https://doi.org/10.1016/B978-0-12-820505-1.00011-0].
- **Blázquez, M.**; Andreu-Sánchez, O.; Fernández-Cruz, M.L.; Ranero, I. and Benfenati, E. (2020) Comparing *in vivo* data and *in silico* predictions for acute effects assessment of biocidal active substances and metabolites for aquatic organisms. *Ecotoxicology and Environmental Safety* 205. DOI: https://doi.org/10.1016/j.ecoenv.2020.111291.
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- Starost, K.; Frijns, E.; Van Laer, J.; Faisal, N.; Egizabal, A.; Elizetxea, C.; Nelissen, I.; **Blázquez, M.** and Njuguna, J. (2017) The effect of nanosilica (SiO_2) and nanoalumina (Al_2O_3) reinforced polyester nanocomposites on aerosol nanoparticle emissions into the environment during automated drilling. *Aerosol Science & Technology* 51 (9); PP: 1035-1046. DOI: https://doi.org/10.1080/02786826.2017.1330535.

The main objective of the present thesis has consisted in implementing a **life cycle oriented perspective** when assessing the risks associated to synthetic chemicals including **biocides** and **engineered nanomaterials** (ENMs) as examples of conventional and emerging contaminants, respectively.

In the case of **biocidal products**, in general, once used, biocidal active substances can easily reach aquatic compartments where they are transformed in **metabolites, degradation and transformation products**. However, the hazard of such substances towards trophic levels representative of the freshwater compartment is **less known** than that of the parent compounds.

For **EMNs**, the different processes that nanotechnology enabled products and/or applications (NEPs/NEAs) undergo throughout their life cycle need to be **simulated in a controlled environment** for the assessment of their potential **release and associated exposure** to their released forms.

We have developed two **nano-release assessment studies** selecting (i) a NEA in solid state, generally aimed to be used at industrial scale at the indoor compartment (polypropylene based nanocomposite samples integrating different types of (nano)fillers) and (ii) a NEP in liquid state, aimed to be used by consumers at the indoor compartment (nano-additivated inkjet printing ink).

We have demonstrated that under a holistic, life cycle oriented perspective that includes use and end of life stages, **novel risks** differing from those derived from the manufacturing stage occur.

Finally, we have evaluated the possibility to derive **new characterization factors** for human respiratory effects and ecotoxicity of one of the case studies for ENMs in order to account for the specific impacts of these emerging pollutants in the life cycle assessment framework according to ISO 14040.