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Research Paper

Occupational risk of nano-biomaterials: Assessment of nano-enabled magnetite contrast agent using the BIORIMA Decision Support System

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ABSTRACT

The assessment of the safety of nano-biomedical products for patients is an essential prerequisite for their market authorization. However, it is also required to ensure the safety of the workers who may be unintentionally exposed to the nano-biomaterials (NBMs) in these medical applications during their synthesis, formulation into products and end-of-life processing and also of the medical professionals (e.g., nurses, doctors, dentists) using the products for treating patients. There is only a handful of workplace risk assessments focussing on NBMs used in medical applications. Our goal is to contribute to increasing the knowledge in this area by assessing the occupational risks of magnetite (Fe_3O_4) nanoparticles coated with PLGA-b-PEG-COOH used as contrast agent in magnetic resonance imaging (MRI) by applying the software-based Decision Support System (DSS) which was developed in the EU H2020 project BIORIMA.

The occupational risk assessment was performed according to regulatory requirements and using state-of-the-art models for hazard and exposure assessment, which are part of the DSS. Exposure scenarios for each life cycle stage were developed using data from literature, inputs from partnering industries and results of a questionnaire distributed to healthcare professionals, i.e., physicians, nurses, technicians working with contrast agents for MRI. Exposure concentrations were obtained either from predictive exposure models or monitoring campaigns designed specifically for this study. Derived No-Effect Levels (DNELs) were calculated by means of the APROBA tool starting from in vivo hazard data from literature. The exposure estimates/measurements and the DNELs were used to perform probabilistic risk characterisation for the formulated exposure scenarios, including uncertainty analysis. The obtained results revealed negligible risks for workers along the life cycle of magnetite NBMs used as contrast agent for the diagnosis of tumour cells in all exposure scenarios except in one when risk is considered acceptable after the adoption of specific risk management measures. The study also demonstrated the

Acronyms: ATMP, Advanced Therapy Medicinal Product; BAL, Bronchoalveolar Lavage; BMD, Benchmark Dose; CES, Contributing Exposure Scenario; CSA, Chemical Safety Assessment; dART, Dermal Advanced REACH Tool; DNEL, Derived No-Effect Level; DSS, Decision Support System; EC, European Commission; ECEL, Exposure Control Efficacy Library; ECHA, European Chemicals Agency; EFs, Extrapolation Factors; ES, Exposure Scenario; EV, Exposure Value; EUON, European Union Observatory for Nanomaterials; FDA, Food and Drug Administration; FESEM, Field Emission Scanning Electron Microscope; GM, Geometric Mean; HD, Hazard Dose; LCL, lower confidence limit; LOAEL, Lowest Observed Adverse Effect Level; MD, Medical Device; MRI, Magnetic Resonance Imaging; NBM, nano-biomaterial; NOAEL, No-Observable Adverse Effect Level; NP, nanoparticle; NM, nanomaterial; OEL, Occupational Exposure Limit; PEG, Polyethylene glycol; PLGA, Poly (lactic-co-Glycolic Acid); PLGA-*b*-PEG-COOH, Poly (lactic-co-Glycolic Acid) (PLGA)-*block*- Polyethylene glycol (PEG)-carboxylic acid; PoD, Point of Departure; RCR, Risk Characterisation Ratio; REACH, Regulation, Evaluation, Authorization and Restriction of Chemicals; RMM, Risk Management Measure; SME, Small-Medium Enterprise; SPION, superparamagnetic iron oxide nanoparticle; SUN, Sustainable Nanotechnologies; SUNDS, SUN Decision Support System; TARMM, Technological Alternatives and Risk Management Measures; TEM, Transmission Electron Microscope; UCL, Upper confidence Limit; WHO/IPCS, World Health Organization International Programme on Chemical Safety Workgroup.

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added value of using the BIORIMA DSS for quantification and communication of occupational risks of nano-biomedical applications and the associated uncertainties.

1. Introduction

The convergence between nanotechnology and biotechnology has offered great improvements in several biomedical applications through the use of nano-biomaterials (NBMs) in diagnostic, therapeutic and regenerative medicine (Wang et al., 2018). Indeed, as NBMs exhibit distinctive mechanical, electrical, and optical properties compared to other microscopic structures, they have been used for drug delivery, bioimaging, as biosensors, contrast agents or as important components in medical implants (Pelaz et al., 2017; Sitharaman, 2011).

Along with the increasing need to effectively evaluate the safety for patients intentionally exposed to the nano-enabled biomedical applications for treatment purposes, there are still substantial gaps in the understanding of the occupational risks resulting from unintentional exposure to nanomaterials (NMs) that may be released from these products (Brouwer et al., 2016; Leso et al., 2019; Nasser and Lynch, 2019). This includes risks not only for workers exposed to NBMs during synthesis, production and end-of-life treatment, but also the risks to the healtcare professionals (e.g., doctors, dentists, nurses, assistants), who may be exposed to the materials during their application to the patients (Giubilato et al., 2020).

The established regulatory risk assessment paradigm for chemicals can be applied to assess the risks of NMs (Dekkers et al., 2016; European Chemicals Agency (ECHA), 2016a, 2016b, 2016c; European Chemicals Agency (ECHA), 2014a, 2014b, 2014c; Grieger et al., 2019; SCENIHR, 2009a; Hristozov et al., 2016, Hristozov et al., 2012;Organisation for Economic Co-operation and Development (OECD), 2012) and, considering that the NBMs used in the medical sector are a special category of NMs, nano-specific approaches can be applied also to the risk assessment of these materials. The adaptation of such approaches for physico-chemical characterisation, hazard and exposure assessment has been the main aim of the EU H2020 BIORIMA project, which developed a framework for risk assessment and management of NBMs used in medical devices (MD) and advanced therapy medicinal products (ATMP) (Giubilato et al., 2020). This framework complements the preclinical benefit-risk analysis of these technologies with a complete assessment of their risks for workers (including healthcare professionals) and the environment.

Since the NBMs are first of all chemical substances, their human health and environmental safety is regulated by the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, which requires a Chemical Safety Assessment (CSA) for each substance produced or imported in quantities above 10 t per year (EC 1907/2006). The CSA involves hazard assessment, exposure assessment and risk characterisation, but requires specific considerations when applied to NMs, such as considering simultaneously several physico-chemical properties in addition to chemical composition (e.g., size distribution, shape, number concentration, surface properties) (Organisation for Economic Co-operation and Development (OECD), 2019).

While some examples of occupational risk assessment of NMs used in industrial products can be found in literature (Hristozov et al., 2018; Pizzol et al., 2018; Silva et al., 2015), the occupational risks of NBMs used in medical applications have been far less investigated, especially for medical professionals, with few exceptions such as the assessment of the potential exposure to NPs of dentists (Van Landuyt et al., 2014; Van Landuyt et al., 2012). Therefore, it is necessary not only to perform more studies in this regard, but also to develop tools that can facilitate the occupational risk assessment for nano-enabled biomedical products (Leso et al., 2019; Murashov, 2009; Murashov and Howard, 2015).

To address this need, in the EU H2020 BIORIMA project we developed a Decision Support System (DSS) to support stakeholders from industry (especially SMEs), consultancy and regulation in occupational risk assessment and management of NBMs applied in medical applications, more specifically MDs and ATMPs (https://biorimadss.greendecision.eu/). The use of this web-based system can facilitate the assessment of risks for product manufacturers, healthcare workers as well as end-of-life processing and waste disposal personnel through the application of up-to-date exposure and hazard assessment tools.

The objective of this paper is to demonstrate the approach for conducting occupational risk assessment for NBMs that we adopted in the BIORIMA project. To this end, we applied the BIORIMA DSS to a real case study: magnetite (Fe_3O_4) NPs coated with Poly (lactic-*co*-Glycolic Acid) (PLGA)-*block*- Polyethylene glycol (PEG)-carboxylic acid (PLGA-*b*-PEG-COOH) used as contrast agent for the diagnosis of solid tumours in Magnetic Resonance Imaging (MRI).

2. Materials and methods

2.1. Case study material

The investigated case study is a dispersion of magnetite (Fe_3O_4) NPs coated with PLGA-*b*-PEG-COOH and its physico-chemical characteristics are reported in Table 1. Details on physico-chemical characterisation are reported in Supplementary information (SI1), where the work performed by Song et al., 2008 was used as a reference to obtain effective density value.

Due to magnetic properties, biocompatibility and biodegradability, these superparamagnetic iron oxide nanoparticles (SPIONs) have been used in several types of application in oncological medicine (Ansari et al., 2018; Soetaert et al., 2020) and their size permits to enhance contrast in MRI, while the biocompatible coating of PEG and PLGA improves tumour targeting and increases the circulation time (Cole et al., 2011; Kim et al., 2019). Moreover, under an alternating magnetic field, studies revealed that magnetite NPs can be used for localised hyperthermia at the tumour site by transforming magnetic field into heat (Chatterjee et al., 2011).

The investigated magnetite NPs coated with PLGA-*b*-PEG-COOH have been designed and produced by Colorobbia Consulting industry (Vinci, Florence, Italy) and are currently under the pre-clinical investigation for the market authorisation process. Specifically, the application

Table 1
Physicochemical characteristics of magnetite NPs coated with PLGA-*b*-PEG-COOH.

Parameter	Technique	Results
Particles size distribution (nm)	TEM	23.5 ± 6
Shape	TEM	Monodispersed and spherical particles
Hydrodynamic diameter (nm)	DLS	51 ± 1
Z potential (mV)	ELS	-53 ± 2.1
Effective density (g/cm^3)	Volumetric centrifugation	1.12

of magnetite NPs as contrast agent in MRI for the identification of solid tumour is considered. In the current study, no material transformations along the life cycle of the product are investigated as they are not likely and/or significant in the assessed exposure scenarios.

2.2. BIORIMA Decision Support System

The BIORIMA Decision Support System (DSS) is an adaptation of the SUNDS system (Subramanian et al., 2016) designed to estimate occupational and environmental risks of NBMs used in MD and ATMP along their life cycle. SUNDS was designed with the aim of supporting the assessment and management of environmental and human health risks of nanomaterials used in industrial applications and consumer products along their entire life cycle. The system can be used at two levels of complexity. At the first level, the NanoSCAN tool (developed within the LICARA project) can check supplier risks, competing products, market opportunities or perform risk/benefit analysis and is targeted at SMEs for regulatory safety assessments and product innovation decisions, reducing R&D&I costs. The second level (Risk Assessment and Risk Control) performs quantitative (deterministic or probabilistic) risk assessment of nanomaterials along the lifecycle of nano-enable products and, if needed, supports the selection of appropriate risk control measures; it is intended mainly for application by industry. As for human health risks, as detailed in the works by Pizzol et al., 2018 and Hristozov et al., 2018 on nano-pigments used in automotive plastics and nanoscale copper-based wood preservatives, respectively, SUNDS allows users to assess risks for workers, consumers, and the general population. The BIORIMA DSS has, instead, been specifically designed with the aim of supporting NBMs manufacturers, regulatory bodies and standardization authorities in assessing environmental and occupational risks associated with the unintentional exposure to NBMs used in biomedical applications, necessary to complement the risk-benefits analysis of these products for the patients to whom the products are intentionally administered/applied. The BIORIMA DSS, therefore, focuses on the quantitative assessment of risks for workers and the environment considering the peculiarities of biomedical applications in terms of release and exposure scenarios. In cases of risks that are not adequately controlled, the system proposes to the end-user suitable risk management measures (e.g., engineering controls, Personal Protective Equipment), including information about their efficacy.

Specifically, the system is divided into two modules: Risk Assessment, which is subdivided in Occupational and Ecological Risk Assessment, and Risk Control. In the Occupational Risk Assessment section, which is demonstrated in this paper, the user can input deterministic or probabilistic exposure values obtained, for example, from a monitoring campaign, or calculate them by applying occupational exposure models (i.e., a 2-box model for inhalation, iEAT for ingestion exposure). For the hazard assessment, deterministic or probabilistic Derived No-Effect Level (DNEL) values can be directly inserted as input or derived from raw toxicity data by applying dose-response and intra/inter-species extrapolation models (i.e., PROAST, APROBA).

The resulting estimation of human health risk is always quantitative through the identification of the Risk Characterisation Ratio (RCR) which is the ratio between the measured/estimated exposure value and the DNEL. The RCR can be either deterministic (risk acceptable when RCR <1, not acceptable ≥ 1) or probabilistic using the following classes: 1) acceptable (when the threshold of one is higher than the 95th percentile of the RCR distribution), 2) needs further consideration (threshold of one between the 90th and 95th percentile) and 3) not acceptable (threshold of one below the 90th percentile). If the resulting risks are unacceptable, Risk Management Measures (RMMs) and their corresponding efficacy values specific for each route of exposure can be selected from the Exposure Control Efficacy Library (ECEL) database, which is connected with the BIORIMA DSS.

2.3. Occupational risk assessment

The approach for occupational risk assessment of NBMs adopted in the DSS is based on the REACH CSA (European Chemicals Agency (ECHA), 2016a), which was already applied to NMs in a number of studies such as Hristozov et al., 2018 and Pizzol et al., 2018. The CSA approach implemented in the DSS includes three steps: (1) hazard assessment, (2) exposure assessment, and (3) risk characterisation, including uncertainty analysis.

2.3.1. Hazard assessment

This step consists of hazard identification and dose-response assessment. The hazard identification involves the gathering and evaluation of the available information on the adverse health effects of the substance. The main issue to address is whether the existing evidence suggests a potential risk for the human health. To identify the relevant hazard information for magnetite NPs, a literature review focused on the following human health endpoints required in the REACH CSA guidance was performed: acute toxicity, irritation and corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity (European Chemicals Agency (ECHA), 2017a, 2017b).

The dose-response assessment characterises the relationship between the dose of the substance administered during animal studies and the observed in vivo effects by means of statistical modelling. The final goal is to estimate an acceptable human exposure level such as the Derived No-effect Level (DNEL), which is defined by REACH (Annex I, 1.0.1) as the level of exposure above which humans should not be exposed (European Chemicals Agency (ECHA), 2012a). The DNEL can then be compared to measured or estimated exposure levels to calculate risks in specific exposure scenarios. The starting point for estimating DNEL is the Point of Departure (PoD), or in other words the highest safe dose based on which adverse effects are not likely to occur in the test animals. The PoD can be a No Observed Adverse Effect Level (NOAEL) or the lower confidence limit of the Benchmark Dose (BMD).

Two tools are included in the BIORIMA DSS to support dose-response assessment and extrapolation of the PoD to DNEL: PROAST and APROBA. When toxicological information from in vivo testing is available, a BMD can be estimated by using PROAST (c), a software package developed by the Netherland's National Institute for Public Health and the Environment (RIVM) for the statistical analysis of dose-response data. This model has been adopted in toxicological studies as it provides probabilistic distributions of BMD (Gosens et al., 2016; Gosens et al., 2015; Gosens et al., 2014). The BMD is estimated from the complete dose-response dataset by fitting dose-response models. Statistical uncertainties in the data are taken into account in the confidence interval around the BMD, whose lower limit (denoted as BMDL) is the PoD that is used as a starting point for deriving the DNEL by applying inter- and intra-species extrapolation factors (EFs). This extrapolation is performed by using APROBA, which was developed by the World Health Organization International Programme on Chemical Safety Workgroup (WHO/IPCS). APROBA performs probabilistic (as well as deterministic) analysis of human dose extrapolation starting from animal dose-response results (e.g. NOAEL, BMDL) considering EFs distributions and based on the European Chemicals Agency (ECHA) guidelines (European Chemicals Agency (ECHA), 2012b).

2.3.2. Exposure assessment

The occupational exposure assessment is the process of characterizing, estimating, measuring, and modelling the magnitude, frequency, and duration of contact with a substance (including NM) as well as the number and characteristics of workers exposed considering the different route of exposure (Vallero, 2014). Inhalation, dermal contact and ingestion are the main routes of exposure to be addressed for nanoforms under REACH regulation (European Comission (EC), 2018). Inhalation is considered the primary route by which NPs in the form of free, unbound, airborne particles will enter the bodies of workers and, once

inhaled, NPs will deposit in different regions of the respiratory tract, depending upon their particle size (ISO/TR 12885:2008 (International Organization for Standardization (ISO), 2008). As there is insufficient information on the penetration of NPs through the skin, (Europe and Food Safety Authority (EFSA), 2017; World Health Organization (WHO), 2006) and local effects that NPs could create on the skin, dermal exposure also needs to be assessed by deposition from the air, by the direct contact with the substance or with contaminated surfaces (i.e., lab objects, clothing) (European Chemicals Agency (ECHA), 2016a) in each life cycle stages. Ingestion exposure typically occurs when substances are accidentally transferred from contaminated hands to the peri-oral region (European Chemicals Agency (ECHA), 2016a, 2016b, 2016c).

2.3.2.1. Identification of exposure scenarios. For each stage of the life cycle of the NM under assessment, specific exposure scenarios (ESs) should be identified and described considering i) information on the NM, ii) the process and activities performed by workers, iii) the presence of any RMMs and iv) the estimates of exposure that can be quantified under the described conditions (Read et al., 2014). For each ES, a number of Contributing Exposure Scenarios (CES) can be identified as described in ECHA guidance documents (European Chemicals Agency (ECHA), 2016a, European Chemicals Agency (ECHA), 2014b), which refer to specific activities where release of NMs may take place.

Estimation of exposure for each CES can be performed through direct monitoring as well as using exposure models. Site-specific measurements of known quality are often preferred over model estimates and are also needed to validate and improve models (Pizzol et al., 2018). However, for most exposure scenarios, such measurements are hardly available (European Chemicals Agency (ECHA), 2012a, 2012b), which requires the use of models to estimate exposure values.

In this work, the development of CESs along the life cycle of the investigated nano-enabled contrast agent was based on the recommendations of Read et al., 2014. Information on work cycle, substances, workplace conditions, targets and risk control measures were collected from the literature as well as from a developed questionnaire for healthcare personnel coupled with interviews to the contacted workers.

Due to confidentiality restrictions on industrial production, detailed information about the synthesis of magnetite NPs cannot be disclosed and the synthesis stage has been excluded from the assessment. For this reason, the occupational risk assessment was conducted for the following life cycle stages: product manufacturing, use and end-of-life. CESs of the product manufacturing were identified through several interviews with the nano-enabled product manufacturers. For the use stage, information was collected based on a questionnaire (SI2) following the recommendations described in Read et al., 2014 and listed in the table in SI6. This questionnaire was filled in by three medical radiologists, three radiology technicians and three nurses of the University Hospital of Padova (Italy) in order to define activities performed by healthcare staff during the administration of contrast agent as well as the use of specific risk management measures. CESs for the end-of-life were identified considering all the possible types of disposal of medical devices and from information obtained from semi-structured interviews to workers at the Department of prevention and public hygiene at the University Hospital of Padova, to healthcare waste disposal workers, and workers of the incinerators of Verona and Padova (Italy).

2.3.2.2. Monitoring campaign. A monitoring campaign was performed at Colorobbia Consulting industry with the aim of quantifying the release of magnetite NPs during the activities performed by workers in the product manufacturing stage. The tiered approach suggested by NIOSH and updated by OECD, 2015 for the evaluation of nanoparticles at workplaces was considered (Organisation for Economic Co-operation and Development (OECD), 2015).

At Tier 1, information related to the workplace, specific characteristics of magnetite NPs and workplace activities were collected during a scoping visit at Colorobbia Consulting industry in 2019. At this stage, the identification of possible emission sources during the product manufacturing activities as well as the use of specific risk management measures were addressed. In general, activities such as weighing and mixing of suspension of magnetite NPs are performed by workers which can cause airborne NMs release (Ding et al., 2017). For this reason, a basic exposure assessment was performed using for online measurements an optical particle sizer (OPS) (TSI, Model 3330) and the Aerasense NanoTracer (Oxility, Eindhoven, The Netherlands) connected with a Tygon tube (length 1 m) settled at 30 cm from the mouth of the worker to collect data near the breathing zone of the worker. Two high flow peristaltic pumps (Casella, model APEX) containing a polycarbonate HEPA filter were fixed on the lab coat of the worker settled at 30 cm from the mouth to collect particles in air. Filters were then observed by scanning electronic microscopy analysis using a Field Emission Scanning Electron Microscope, FESEM (Carl Zeiss Sigma NTS, Germany) for off-line measurements. Elemental analysis was performed by image analysis using FESEM coupled to an energy dispersive X-ray micro-analyser (EDS, mod. INCA). More information can be found in SI3.

As during the monitoring campaign the investigated NPs may have a similar size range derived from other industrial processes (Demou et al., 2008), in this study measurements of background were performed by monitoring workers' activities performed under the typical working conditions but without using the magnetite NPs. Moreover, as it is not clear whether a concentration of the investigated NPs can be considered 'significantly high' during an activity compared to the corresponding concentration without nano-activity, the practical approach proposed by Brouwer et al., 2013 was followed. Accordingly, three main parameters were defined for analysing time series measurements: i) the ratio of the average concentrations as determined by on-line instruments between nano-activity and non-nano-activity higher than 2, ii) the presence of nanoparticles on the SEM grids by EDX analysis, iii) the absence of other activities generating the investigated NPs. The evaluation criteria between nano-activity (A) and non-nano activity (B) can be defined as:

ratio A/B $\geq$ 2: likely;

ratio A/B $>$ 1.05 and $<$ 2: possibly/not excluded;

ratio A/B $<$ 1.05: not likely.

Information related to other activities performed at Colorobbia Consulting during the monitoring campaign was collected to exclude possible release of iron in other compartments of the industry.

Transformation of the obtained values from particle concentration to mass concentration were performed by following the equation described in SI4.

2.3.2.3. Exposure models. When exposure measurements are not available, predictive models can be used to perform occupational exposure assessment for NMs. Indeed, as workplace measurements of NMs are relatively complex in healthcare sector and in waste disposal, exposure models may be required to provide estimates of exposure especially in those ESs when a direct monitoring is difficult to perform.

For this reason, in the context of BIORIMA project, a 2-box model was implemented for the quantification of the inhalation exposure for workers unintentionally exposed to NBMs along the life cycle of a MD or an ATMP containing NBMs, based on Ganser and Hewett, 2017. The inhalation model has been coded in Python and it is included with a graphical interface within the BIORIMA DSS. This model can simulate the particle behaviour in a well-mixed room predicting the near-field (NF, close to the emission source) and far-field (FF, inside the room but distant from the emission source) concentrations. This model considers some inputs parameters regarding the physico-chemical characteristics of the NM (i.e., particle size, density and fraction of pristine NM), characteristics of the activity performed by workers (i.e., mass of material used, task duration, generation rate, number of repetitions and the type of activity) and room conditions (i.e., room volume and number

of air changes per hour). The first step is to calculate the total emission of NM during the activity. The activity release rate allows to calculate the total emission rate to the air (in mg/min and for the worst case) for different activities that could lead to a NM release in any of the life cycle stages (e.g., synthesis of a NM, handling or transferring, use or end-of-life). Then, different equations are applied to calculate the steady state concentration (in mg/m^3) assuming a constant emission rate, and the transient concentration that leads to a generation curve followed by a decay curve. The inhalation model provides as final exposure output the NF and FF concentration over time, which are then reported in the BIORIMA DSS as the average concentration during the work shift (mg/m^3). The most conservative value between NF and FF concentrations is then used to calculate the final risk by dividing it by the DNEL for inhalation exposure, using a precautionary approach.

Considering a possible exposure of healthcare workers to NBMs from hand to mouth contact (Murashov and Howard, 2015), the predictive model iEAT developed by Gorman et al., 2012 has been selected to estimate inadvertent ingestion exposure in the workplace, following the approach proposed by Pizzol et al., 2018 and Hristozov et al., 2018 (assuming that a person touches a surface contaminated with the investigated NMs and then touches inadvertently the area around the mouth with subsequent ingestion by licking). The iEAT model is included in the BIORIMA DSS. This model identified four compartments (i.e., the source, air, surface contaminant layer, oral cavity), nine processes of mass transport between the compartments (i.e., emission, deposition, resuspension or evaporation, transfer, removal, redistribution, decontamination, penetration and/or permeation, swallowing) and uses a database with more than 500 empirically measured transfer efficiencies in order to calculate the Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL) of the ingestion dose in mg/kg bw/d (these values serve then in the DSS to calculate a normal distribution of the ingestion dose, used in the probabilistic risk assessment).

Due to the lack of dermal exposure models in literature for NMs, REACH equations for dermal exposure were used, based on the work performed in Goede et al., 2018; McNally et al., 2019 where the mechanistic Dermal Advanced REACH Tool (dART) is presented. This model has been developed to quantify dermal exposure for low-volatile liquid and in this work, its application in a dispersion of NPs is demonstrated. dART tool is based on three main equations where dermal exposure is calculated by a sum of i) the deposition of the investigated substance from the air to the hands, ii) the direct emissions and/or direct contact with the liquid, and iii) the transfer from contaminates surfaces. After the application of each equation, the final output is a deterministic estimation of hand exposure in mg/cm^2/d using the standardized value of hand surface found in European Chemicals Agency (ECHA) (2017b).

2.3.3. Risk characterisation and uncertainty analysis

Risks can be assessed in either deterministic or probabilistic terms and are considered acceptable when: i) exposure is below prescribed no-effect threshold (e.g., occupational exposure limit - OEL), or ii) ESs have a negligible exposure, or iii) risk characterisation ratio (RCR) is lower than 1.

If exposure cannot be excluded, the RCR value is calculated based on Eq. 1:

$$RCR = \frac{EV}{HD} \quad (1)$$

where EV is the exposure value or the probabilistic distribution of exposure determined for a specific ES, and HD is the hazard dose represented by the DNEL that can be both a probabilistic distribution or a deterministic value.

The units of the exposure and the units used for deriving the DNEL must be the same (European Chemicals Agency (ECHA), 2014c). For systemic effects, the units of DNELs are mg/m^3 for inhalation, and mg/kg*bw or mg/kg*bw/day for oral and dermal exposure. For local effects, the unit of DNELs is mg/m^3 for inhalation, while for dermal exposure it is mg/cm^2 skin, mg/person/day (e.g., calculated based on the deposited amount per cm^2 times the actually exposed body area), or a measure of concentration (% or ppm) (European Chemicals Agency (ECHA), 2012a, 2012b).

Once the risk is estimated, its acceptability can be classified according to an approach based on confidence intervals. Specifically, in case the risk is presented deterministically, two classes are identified: acceptable (RCR < 1) and not acceptable (RCR > 1). As probabilistic risk distributions typically follow a right-skewed lognormal distribution, the risk is acceptable if the 90th percentile of the exposed target is safe, but conservative values can also be selected (i.e., the 95th percentile or the 99th percentile) (Pizzol et al., 2018).

To support risk communication of the obtained results, uncertainties need to be clearly assessed. In BIORIMA DSS, uncertainty contribution to RCR by each involved factor is estimated by means of the Monte Carlo approach with 10,000 trials. At each trial, the RCR is numerically estimated by randomly sampling elements from the BMD/NOAEL distribution, exposure values, and from EF distributions used to derive the DNEL. The contribution to uncertainty of each factor is quantified by assessing the level of correlation between the factor and the resulting RCR by means of squared Spearman's rank correlation coefficient (Helton and Davis, 2003). Uncertainties related to the dose-response data has been performed by means of parametric bootstrapping. The contribution of each EF is selected as the arithmetic mean of each resulting curve and appropriate figures are developed for communication purposes.

2.4. Risk management measures

If the resulting risks are unacceptable, the adoption of RMMs can be selected based on route of exposure as well as its efficacy of protection of NMs.

Considerations on specific requirements during the preparation of drug containing NMs and its administration can be found in European Agency for Safety and Health at Work, 2013. For example, for inhalation exposure the use of HEPA filters, respiratory cartridges and masks with fibrous filtering materials are considered effective against airborne NMs (e.g., half- or full-face masks with P3/FFP3 or P2/FFP2 filters), while for dermal exposure, the adoption of two pair of gloves is considered effective for the protection from NMs and the use of protective clothing made with cotton fabrics should be avoided.

In the BIORIMA DSS, specific RMMs can be selected from an inventory of Technological Alternatives and Risk Management Measures (TARMM) from the ECEL database (Fransman et al., 2008), which permits to select the best RMM considering not only its efficacy value, but also its cost of implementation as well as its average life duration.

3. Results

3.1. Hazard assessment

Relevant information on the toxicity of the magnetite for the inhalation, ingestion and dermal contact exposure routes was found in the European Union Observatory for Nanomaterials (EUON) website (https://echa.europa.eu/registration-dossier/-/registered-dossier/15989/7/6/1) and extracted from the respective REACH registration dossier. The magnetite NPs considered in the dossier can be used as a reference for hazard assessment of the dispersion of magnetite NPs coated with PLGA-*b*-PEG-COOH as these polymers have been approved by the Food and Drug Administration (FDA) as biocompatible because they can be degraded into non-toxic lactic acid and, accordingly, these polymers should not be considered in the risk assessment (Liang et al., 2019).

For inhalation exposure, a sub-chronic study performed by Pauluhn, 2012 was selected. In this study, rats were exposed to powder of

Table 2
Inputs and outputs of the APROBA tool for each route of exposure.

Inputs		Route of exposure		
		Inhalation	Dermal	Ingestion
Type of PoD		Continuous	Continuous	Continuous
Magnitude of Effect		0.05	0.05	0.05
PoD		NOAEL	NOAEL	NOAEL
Value of PoD		3.525	75	2000
Study type		Subchronic	Subacute	Chronic
Test species		Rats	Rats	Rats
Species weight (kg)		0.35	0.2	0.2
Human weights (kg)		70	70	70
Population Incidence Goal		0.05	0.05	0.05
Probabilistic Extrapolation Factors	Uncertainty in NOAEL as surrogate of BMD	LCL: 0.07	LCL: 0.07	LCL: 0.07
		UCL: 1.57	UCL: 1.57	UCL: 1.57
	Interspecies scaling	LCL: 1	LCL: 4.59	LCL: 4.59
		UCL: 1	UCL: 7.33	UCL: 7.33
	Remaining interspecies toxicokinetic/toxicodynamic differences	LCL: 0.33	LCL: 0.33	LCL: 0.33
		UCL:3	UCL: 3	UCL: 3
	Uncertainty in exposure duration	LCL: 0.5	LCL: 0.63	LCL: 1
		UCL: 8	UCL: 40	UCL: 1
	Uncertainty for intraspecies variability	LCL: 1.77	LCL: 1.77	LCL: 1.77
		UCL: 14	UCL: 14	UCL: 14
Output		Inhalation (mg/m^3)	Dermal (mg/kg/d)	Ingestion (mg/kg bw/d)
DNEL		LCL: 0.08	LCL: 0.08	LCL: 23.6
		UCL: 13.8	UCL: 31.6	UCL: 1830

PoD: Point of Departure, LCL: Lower Confidence Level, UCL: Upper Confidence Level.

magnetite (Fe_3O_4) for 6 h per day, 5 days per week, for 13 weeks at target concentrations of 0 (dry air), 10, 15 and 50 mg/m^3 (20 rats per sex per group) which correspond to a near field exposure determined by gravimetric analysis of 0, 4.7 ± 0.6, 16.6 ± 3.0 and 52.1 ± 6.4 mg/m^3 respectively. The NOAEL was determined after the identification of significative pulmonary effects through five different endpoints: histopathology, changes in bronchoalveolar lavage (BAL) protein, increase in total cell counts in BAL, and increase of absolute and relative neutrophilic granulocytes in BAL. Based on these endpoints, the NOAEL value of 4.7 mg/m^3 was proposed by the author for sub-chronic inhalation. This value was firstly corrected to derive a Point of Departure (PoD) which considers the effective exposure of the target (European Chemicals Agency (ECHA), 2012b). Indeed, the exposure duration in animal testing is 6 h/d and need to be modified to reflect the 8 h/d of workers exposure. To achieve this, ECHA suggests to apply a correction factor of 0.75 to obtain the corrected NOAEL for 8 h/d (European Chemicals Agency (ECHA), 2012b) obtaining the corrected NOAEL of 3.5 mg/m^3. Then, this value has been used as PoD to calculate the corresponding DNEL using APROBA in the BIORIMA DSS.

In order to estimate the oral and dermal toxicity, the study by Remya et al., 2016 was selected. In this study, the authors performed prolonged and repeated administrations for oral exposure (low dose of 500 mg/kg bw; medium dose – 1000 mg/kg bw, and High dose – 2000 mg/kg bw) in rats of a mean weight of 0.2 g following the OECD 453 guideline for 90 days (Organisation for Economic Co-operation and Development (OECD), 2018). Results showed an increase in glutathione reductase activity in high dose treated animals, which suggests that the system is combating some oxidative stress but in a controlled manner. Indeed, there is no significative difference in the antioxidant parameters of the treated animals compared to the control. The value of 2000 mg/kg bw was used to estimate the corresponding DNEL value.

As for dermal toxicity, Remya et al., 2016 performed sub-acute studies by exposing three rats to different concentrations of NPs (Low-25 mg/kg, Medium-50 mg/kg, and High–100 mg/kg) 6 h daily for 28 days. Results revealed no observable signs of tissue damage in kidney, liver or spleen, and no noticeable change in the haematological and biochemical parameters of treated animals. The authors affirmed that no skin sensitization or irritation can be observed. For this reason, the corresponding DNEL value was calculated using a NOAEL value of 100 mg/kg as point of departure, firstly multiplying the NOAEL for 0.75 obtaining the corrected NOAEL value of 75 mg/kg for 8 h/d (European Chemicals Agency (ECHA), 2012b).

The NOAEL values extracted from the above studies were used to derive DNELs for each exposure route, by applying APROBA, using the interspecies and intraspecies scaling and extrapolation factors reported in Table 2 and performed over 10,000 Monte Carlo simulations to derive lognormal distributions of $DNEL_{long\text{-}term}$ for local and systemic effects due to inhalation, ingestion and dermal exposure to magnetite.

DNEL values in mg/kg/d were obtained (LCL: 0.08, UCL: 31.6) using APROBA tool. However, as hand dermal exposure is measured in $mg/cm^2/d$, DNEL values were modified following ECHA, 2012b document by using the standardized body weight for workers (70 kg) and total body surface (16,600 cm^2) defined in ECHA, 2017b, obtaining the final hand dermal DNEL values of LCL: 0.003, UCL: 1.33 $mg/cm^2/d$.

3.2. Exposure assessment

The description of all the CESs, the assessed exposure routes and related exposure estimations identified along the life cycle of the case-study NBM is reported in Table 3 and in details in SI5.

Specifically, as reported in Table 3, for inhalation exposure, results obtained from the questionnaire for healthcare personnel showed a negligible exposure during CES6 and CES7 (additional specifications in SI6). Exposure during product manufacturing for CES1, CES2, CES3 and CES5 was also evaluated as negligible based on the results of the monitoring campaign. Indeed, although the ratio of the average concentrations determined between nano-activity and non-nano-activity (as measured by the NanoTracer) was a value >2 in CES1 and CES5, morphological analysis and chemical analysis of the filter during nano and non-nano activities revealed the absence of iron and the only presence of ceramics in both the filters (SI7). For this reason, it is possible to exclude a release of magnetite NPs during activities performed in CES1, CES2, CES3, CES5.

For CES4, the inhalation model was applied (see input data in SI8), and the concentration of magnetite NPs estimated in the Near Field and Far Field is reported in Table 3.

Considering the end-of-life stage (CES8), once the investigated material is injected to the patient using a syringe, it is classified as highly hazardous health-care waste containing sharps contaminated with blood and need to be treated in incinerator (World Health Organisation

Table 3
Life cycle stages and Contributing Exposure Scenarios (CES) assessed for the magnetite NPs used as contrast agent, the source of information for each route of exposure and the final exposure estimation in mg/m^3 for inhalation, mg/cm^2/d for dermal and in mg/kg bw/d for ingestion exposure.

Life cycle stages	Contributing Exposure scenario	Exposure route	Estimation method	Exposure estimation
Product manufacturing	CES1: Weighting, solution preparation and mixing	Inhalation	Monitoring campaign	NE
		Dermal	dART equations	1.47E-3
		Ingestion	iEAT model	LCL: 1.87E-04
				UCL: 2.92E-03
	CES2: Formation of coated NPs in a mixing chamber	Inhalation	Monitoring campaign	NE
		Dermal	dART equations	NE
		Ingestion	iEAT model	NE
	CES3: Dialysis and concentration	Inhalation	Monitoring campaign	NE
		Dermal	dART equations	NE
		Ingestion	iEAT model	NE
	CES4: Filtration and packaging in glass bottles	Inhalation	Inhalation model	NF: 6.15E-06
				FF: 2.46E-06
		Dermal	dART equations	3.19E-3
		Ingestion	iEAT model	LCL: 2E-04
				UCL: 3.08E-03
	CES5: Cleaning and maintenance	Inhalation	Monitoring campaign	NE
		Dermal	dART equations	2.46E-3
		Ingestion	iEAT model	LCL: 2E-04
				UCL: 3.08E-03
Use	CES6: Injection administration	Inhalation	Questionnaire	NE
		Dermal	dART equations	0.80E-06
		Ingestion	iEAT model	LCL: 2E-04
				UCL: 3.08E-03
	CES7: Cleaning and waste disposal	Inhalation	Questionnaire	NE
		Dermal	dARTequations	7.97E-3
		Ingestion	iEAT model	LCL: 2E-04
				UCL: 3.08E-03
End-of-life	CES8: Incineration	Inhalation	Interviews	NE
		Dermal	dART equations	0
		Ingestion	iEAT model	LCL: 6.71E-03
				UCL: 9.34E-02

NE: negligible exposure, LCL: Lower Confidence Limit, UCL: Upper Confidence Limit, NF: Near Field, FF: Far Field.

(WHO), 2014).

The understanding of NMs behaviour during solid waste incineration is still at an early stage (Organisation for Economic Co-operation and Development (OECD), 2016). Combustion temperature and melting/boiling points, chemical composition, size and oxidation state of the nanomaterial are significant determinants of the fate of the NM during incineration. Inorganic NPs that escape destruction during the incineration process will mostly end up in the bottom ash (United Nations Environment Programme (UNEP), 2018). As for the exposure of workers employed at incineration facilities, in general occupational exposure levels to airborne dust can be considered negligible during routine operations of an incineration plant, while significant exposure to airborne dust could occur during cleaning and maintenance operations where workers are handling air pollution residues (fly ash) or bottom ash created during the combustion process (Institute of Occupational Medicine (IOM), 2012). This also applies to nanomaterials: because of their large surface-to-volume ratios, NPs tend to adhere to surfaces in the furnace chamber, boilers, heat exchanger tubes and the wet scrubber (Walser et al., 2012), and then removed with compressed air which can disperse residual NPs. Therefore, as concluded by Walser et al., 2012, attention should be paid during maintenance operations, when exposure to NPs trapped in the system may increase. However, incinerator maintenance activities are not routine operations and the quantity of magnetite NPs currently used as contrast agent is very limited. Thus, in the absence of additional literature data or predictive models and given the fact that an ad-hoc monitoring was not feasible, in this work we assumed that a negligible inhalation exposure in CES 8 was a reasonable conclusion. These assumptions have been also confirmed by the two directors of incinerators consulted for this specific case-study, as they stated that the use of appropriate emission control technologies at the incinerator prevent a release of contaminated air in the workplace.

Dermal exposure for each CES was calculated using the equations defined in dART tool and reported in SI9. Results showed the lowest value of exposure during the administration of the contrast agent (CES6) due to the semi-automatic process of injection, while the highest value is obtained in CES7, when healthcare personnel clean contaminated surfaces (Table 3). A negligible exposure was assessed for CES2 and CES3 because these activities are performed in a closed reactor.

Oral intake through the hand-to-mouth exposure was quantified using the iEAT model (see input data reported in SI10) while exposure estimates are reported in Table 3. Results revealed no differences in the oral exposure during activities performed by workers along the entire life cycle, except for workers at the incinerator who may be more exposed to contaminated objects containing NPs compared to all the other CESs. Indeed, all the activities during product manufacturing and in the use stage are performed in a 'clean workplace where surfaces are regularly decontaminated' (as defined by the iEAT). Therefore, the lowest value of surface contamination (represented by the hand loading parameter in SI10) was assigned to these CESs, while, since the incinerator can be considered as a 'clean industrial environment' as well as a 'dirty industrial environment with visible contamination', higher values of surface contamination were selected as input in CES8.

3.3. Risk characterisation and uncertainty analysis

The results of the hazard and exposure assessment are integrated during the risk characterisation to obtain RCR probability distributions. For all the CESs where an exposure cannot be excluded, the RCR probability distributions as a result from over 10,000 Monte Carlo simulations are reported in Figs. 1–3 for inhalation, dermal and ingestion respectively. In Table 4 the mean values of RCR for each CES are summarized.

As can be seen from Table 4, a negligible inhalation exposure is obtained for all CESs except for CES4, where an acceptable risk is obtained as the RCR is less than 1 for >95% of the sensitive population (Fig. 1a). The uncertainty associated to the risk estimations can be

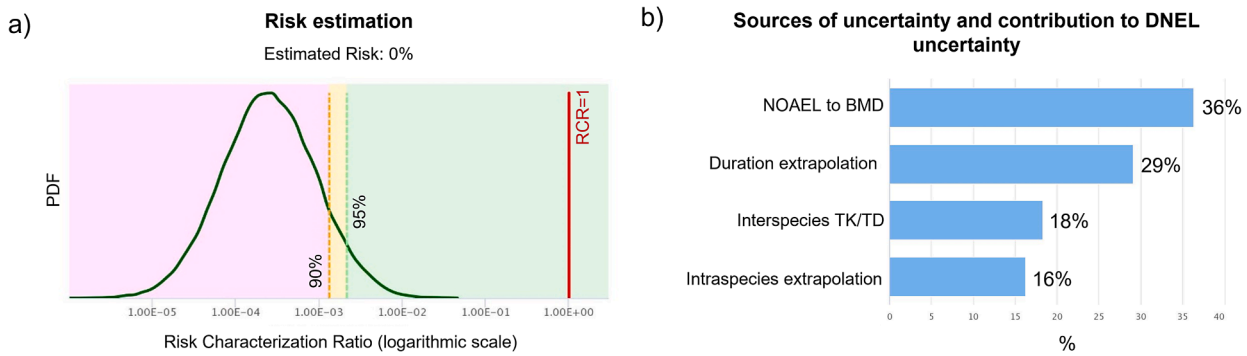

Fig. 1. a) Risk Characterisation Ratio distribution of CES4 for inhalation exposure and b) contributions of the different sources of uncertainties to the total uncertainty related to the derivation of the DNEL for inhalation. PDF: probability distribution function, TK: toxicokinetic, TD: toxicodynamic.

assessed considering the probabilistic distributions used for the derivation of the long-term human dose for inhalation. Indeed, as the deterministic value of the near field has been used to derive exposure estimation, no uncertainties are obtained for exposure assessment. Uncertainties related to the derivation of the DNEL can be ascribed to the choice of using a NOAEL instead of the BMD as Point of Departure for a 36%, the duration extrapolation from sub chronic to chronic for a 29%, while 18% and 26% are associated to inter- and intraspecies extrapolation factors, respectively (Fig. 1b).

As can been seen from Fig. 2, all the reported CESs have an acceptable risk for dermal exposure as the RCR is below1 for >95% of the sensitive population (Fig. 2a-d), except for dermal exposure in CES7 (Fig. 2e) where the RCR is 0.63 ± 3. For this reason, the application of proper RMMs to control the risk is needed and a possible choice is to consider the use of Personal Protective Equipment. In this regard, a pair of nitrile gloves was selected from the ECEL library with an efficacy of 97% for NMs. After recalculating the RCR with the new scenario with PPE, an acceptable risk value of 0.02 ± 0.1 is obtained (Fig. 2f).

As for inhalation exposure, risk uncertainties are related only to the derivation of the DNEL for dermal exposure (Fig. 2g) which can be ascribed to the extrapolation factors used to derive DNEL from a subacute NOAEL for a 48%, the choice of using a NOAEL instead of the BMD as Point of Departure for a 26%, while 13% and 12% are associated to inter- and intraspecies extrapolation factors, respectively.

As for oral exposure, an acceptable risk is obtained for all the CESs since the RCR is always below 1, where Fig. 3a represents risk distribution for CESs 1–7 and Fig. 3b for CES8.

Once the iEAT model is used, the BIORIMA DSS permits to identify the uncertainties related to the use of this model in the risk evaluation. Indeed, uncertainty associated to the use of iEAT model in the risk evaluation can be defined as a 25% in CES 1–7 (Fig. 3c) and for a 19% in CES 8 (Fig. 3d).

Uncertainties related to the derivation of the DNEL can be ascribed to the choice of using a NOAEL instead of the BMD as Point of Departure for a 51%, while 25% and 23% are attributed to inter- and intraspecies extrapolation factors, respectively (Fig. 3e).

4. Discussion and conclusion

In this study, a probabilistic occupational risk assessment approach for NBMs has been applied to a nano-enabled biomedical product: i.e., a dispersion of magnetite NPs coated with PLGA-*b*-PEG-COOH used as contrast agent in MRI applications.

The strength of the proposed probabilistic approach (in comparison to the more conventional deterministic ones) is its ability to clearly communicate sources of uncertainty in the quantitative estimates of hazard and exposure.

In case qualitative information is obtained and used in the assessment (for example, when exposure is characterized through questionnaires or interviews, for data-scarce scenarios), the BIORIMA DSS cannot incorporate and evaluate the associated contribution to the overall uncertainty. This current limitation of the DSS could be tackled in future development of the tool, for example implementing Value-of-Information approaches (Zabeo et al., 2019) that could be used to quantify how targeted collection/generation of additional information may achieve optimal (cost-efficient) reduction of uncertainty in the risk assessment results.

In this work, important sources of uncertainty can be ascribed to hazard assessment, namely i) the choice of the PoD to derive a DNEL (e.g., NOAEL, BMD), ii) the type of toxicological tests performed (e.g., acute, sub-acute, sub-chronic or chronic tests) and iii) the inter and intra species variability.

In details, important considerations are needed for the choice of the PoD. Indeed, as the NOAEL value is dependent on experimental study design (e.g., selection of dose levels, the range between doses), while the BMD is derived from the complete dataset of dose-response data, ECHA guidance suggested that BMD is preferred over NOAEL for the derivation of the DNEL (European Chemicals Agency (ECHA), 2012a). Indeed, advantages of using a BMD instead a NOAEL is that i) a BMD is derived using all experimental data and reflects the dose-response pattern to a greater degree, ii) the BMD is independent of predefined dose levels and spacing of dose levels, iii) the BMD makes more reasonable use of sample size, with better designs resulting in higher Benchmark doses (European Chemicals Agency (ECHA), 2012a).

As no NOAEL values for ingestion and for dermal exposure of magnetite NPs are currently available in the literature, concentrations which represent the highest tested concentrations that do not cause long term effects are considered as NOAEL using a conservative approach. From the obtained results, the choice of using a NOAEL value as an estimate of the BMD is the largest source of uncertainty in the derivation of the $DNEL_{ingestion}$ and $DNEL_{inhalation}$, while it is the second largest source of uncertainty in $DNEL_{dermal}$ derivation.

When a PoD from a chronic study is available, then using this value to derive a DNEL should be preferred as this would require no use of assessment factors to extrapolate for the duration of the study (e.g. from sub-acute or sub-chronic to chronic) (European Chemicals Agency (ECHA), 2012a). In our assessment, due to the lack of relevant chronic data, we derived a chronic PoD starting from a sub-acute study using a probabilistic EF with a confidence interval equal to 0.62 and 40. This extrapolation is a major source of uncertainty for the $DNEL_{dermal}$ that we used in our risk assessment. To derive $DNEL_{inhalation}$, a sub-chronic PoD was used (EF: LCL: 0.5, UCL:8), while no extrapolation factors were needed for the ingestion route of exposure where a suitable chronic study was available and therefore was used to derive the $DNEL_{ingestion}$. In conclusion, to increase the confidence in the evaluation of toxicological effects of magnetite NPs for dermal exposure, it is important to repeat the assessment once sub-chronic or chronic data become available, identifying not only local but also systemic effects. However, as the

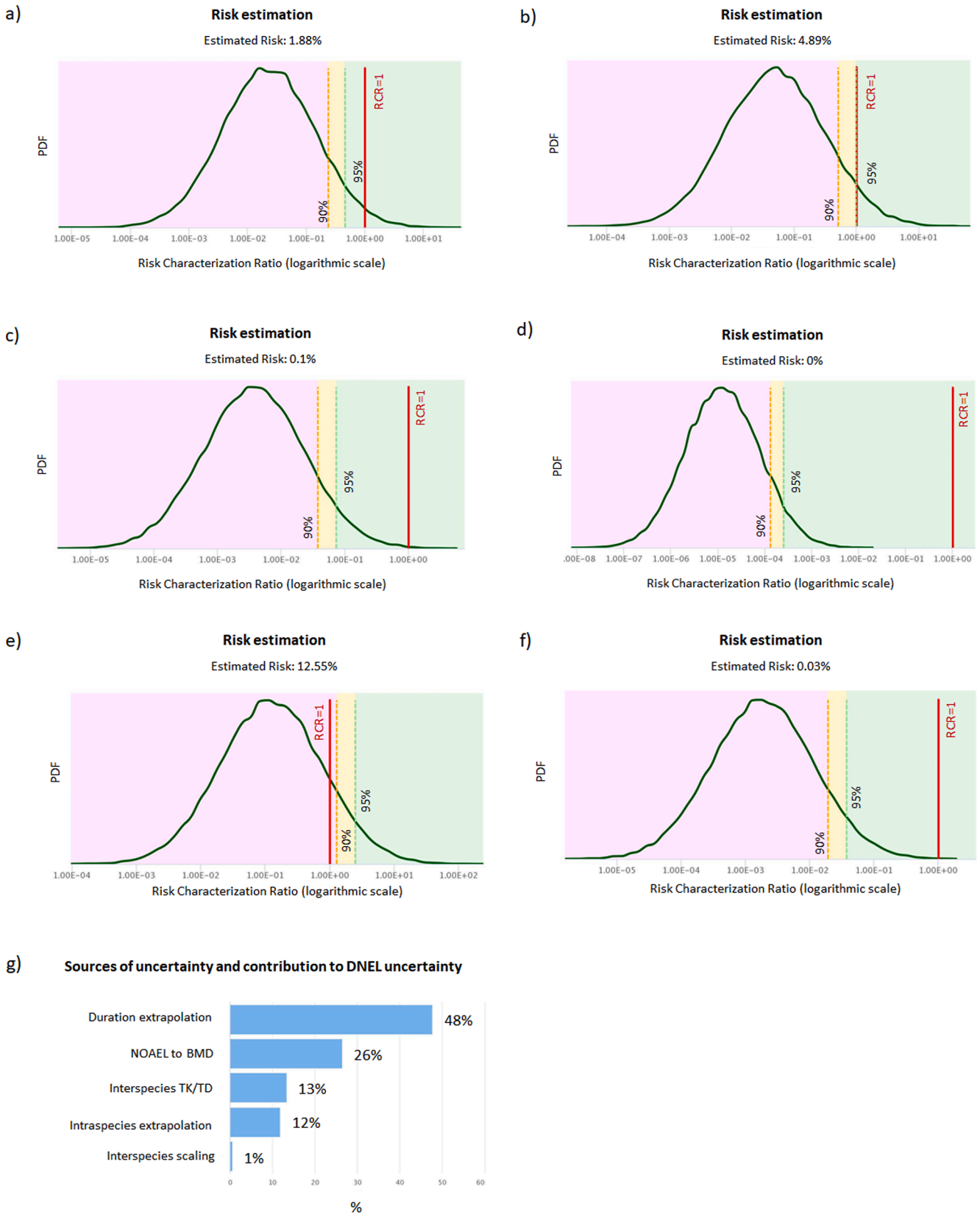

Fig. 2. RCR distribution for dermal exposure in a) CES 1, b) CES4, c) CES5, d) CES6, e) CES 7, f) CES 7 adding proper RMMs and g) contributions of the different sources of uncertainties to the total uncertainty related to the derivation of the DNEL for dermal exposure.

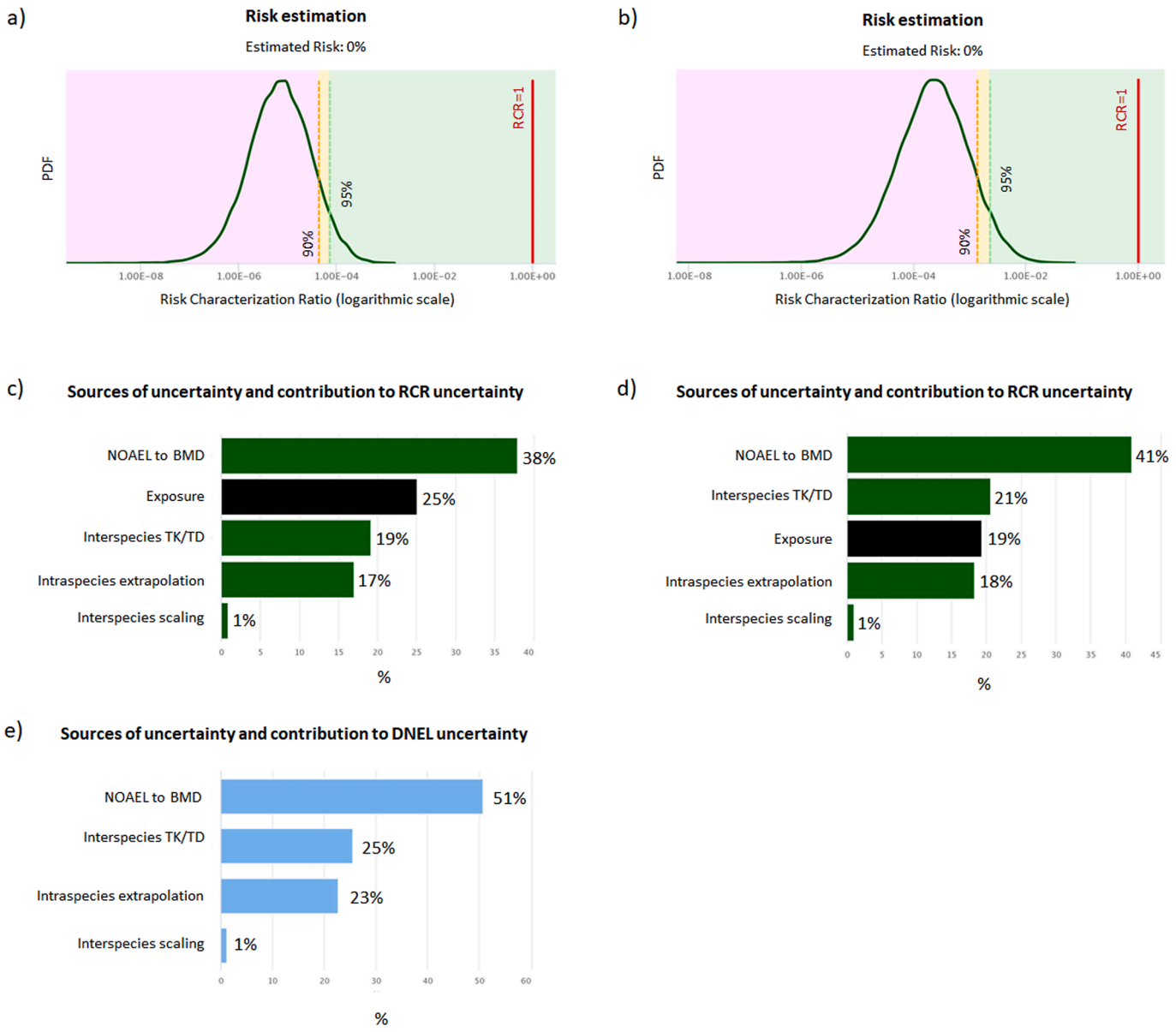

Fig. 3. RCR distribution for ingestion exposure in a) CES 1-CES7 and b) CES8, c) uncertainty related to the derivation of RCR in CES1-CES7 and d) in CES8, e) contributions of the different sources of uncertainties to the total uncertainty related to the derivation of the DNEL for ingestion exposure.

dispersion of magnetite NPs coated with PLGA-*b*-PEG-COOH can be classified as non-soluble in water, its formulation is expected not to lead to a significative dissolution of the NPs once it reaches the workers' skin and if the skin of workers is expected not to be seriously damaged, dermal adsorption of magnetite NPs coated with PLGA-*b*-PEG-COOH can be considered very low, taking into account the classification provided in the ECHA guideline for dermal adsorption of NPs (European Chemicals Agency (ECHA), 2020).

The sources of uncertainty related to intra- and inter-species variability (i.e., the differences between animals and humans and between humans) were also considered in the study but using the default EF values proposed for chemical substances, which may not be fully adequate for nanomaterials. To reduce the uncertainty related to intra- and inter-species extrapolations, it is worth investing future efforts into deriving nano-specific EFs by applying in silico tools to the large body of toxicity data already available in the literature.

Uncertainties of this assessment can also be attributed to the use of toxicological data of a substance similar to the investigated material instead of the substance itself. Indeed, this work uses toxicity data not derived from ad-hoc studies on magnetite NPs coated with PLGA-*b*-PEG-COOH, but data for iron oxide (not in nano form) for inhalation toxicity and iron oxide NPs coated with dextran for dermal and oral toxicity respectively, which may cause an increase of the uncertainty on the final risk evaluation. However, as ECETOC (ECETOC, 2013) concluded that local toxicity of the poorly soluble particles of low toxicity (PSPs) is independent of the particle size (i.e., micro or nano-sized material) and is threshold related, and since magnetite NPs can be classified as PSPs (Pauluhn, 2012), local toxicity at different concentrations investigated by Pauluhn, 2012 was considered as starting point to define a DNEL$_{inhalation}$ even if it is not in the nano-range (the average diameter of the tested particles was 981 nm). In order to estimate the oral and dermal toxicity, the study by Remya et al., 2016 was selected as the investigated material (dextran stabilized iron oxide NPs) may be considered similar to the magnetite NPs investigated in this paper, as both are in nano-form and coated with a polymer used for medical applications.

Considering exposure assessment, the quantification of dermal exposure was determined based on dART equations for low volatile liquids (instead of applying a nano-specific exposure modelling

Table 4
Risk values for Contributing Exposure Scenario for each route of exposure.

Life cycle stages	Contributing Exposure scenario	Exposure route	Risk value	
			Mean	SD
Product manufacturing	CES1: Weighting, solution preparation and mixing	Inhalation	0	
		Dermal	3.4E-01	8E-04
		Ingestion	1.85E-05	4.47E-05
	CES2: Formation of coated NPs in a mixing chamber	Inhalation	0	
		Dermal	0	
		Ingestion	0	
	CES3: Dialysis and concentration	Inhalation	0	
		Dermal	0	
		Ingestion	0	
	CES4: Filtration and packaging in glass bottles	Inhalation	1E-02	2E-02
		Dermal	7.3E-01	2E-03
		Ingestion	1.95E-05	4.43E-05
	CES5: Cleaning and maintenance	Inhalation	0	
		Dermal	5.0E-02	1E-04
		Ingestion	1.95E-05	4.43E-05
Use	CES6: Injection administration	Inhalation	0	
		Dermal	4.9E-05	2.17E-04
		Ingestion	1.95E-05	4.43E-05
	CES7: Cleaning and waste disposal	Inhalation	0	
		Dermal	6.3E-01	3.0E+0
		Dermal after RMMs	2.0E-02	1.0E-01
		Ingestion	1.95E-05	4.43E-05
End-Of-Life	CES8: Incineration	Inhalation	0	
		Dermal	0	
		Ingestion	5.94E-04	1.48E-03

SD: Standard Deviation.

approach) and this choice could lead to an approximative estimation of hand exposure to magnetite NPs, which probably causes an overestimation of dermal exposure in CES7. However, the use of specific RMMs resulted effective in the reduction of the final risk value. Indeed, after the application of a pair of nitrile gloves, the resulting risk is considered acceptable. Therefore, the applications of a dermal exposure model specific for NPs is advisable and will help risk assessors in obtaining a more realistic dermal exposure estimation.

The use of the iEAT model demonstrates its applicability to estimate ingestion exposure of magnetite NPs from hand-to-mouth contact. However, the iEAT model uses a reduced set of parameters to characterize ESs, and this does not allow to differentiate CESs with different characteristics. For this reason, no significant differences were obtained for the selected CESs.

Results obtained from the monitoring campaign in product manufacturing stage demonstrates the importance of following the OECD tiered approach when planning occupational exposure monitoring. As shown in the current study, the use of particle counters without the characterisation of the particles sampled could lead to an incorrect interpretation of results, causing an overestimation of the effective workers exposure of the investigated NP. Indeed, results revealed that the combination of online and offline measurements permits to distinguish between background particles and a (no) release of magnetite NPs. When monitoring campaigns were not possible to perform, the 2-box model was used to quantify inhalation exposure, revealing its ability to quantify near field and far field exposure of NBMs.

The final risk evaluation permits to conclude that risks for workers who use or manage magnetite NPs coated with PLGA-*b*-PEG-COOH used as contrast agent in MRI along its entire life cycle are not significant.

However, as specific tasks performed by healthcare personnel are currently not represented by models showed in this work, further improvements need to be done in order to quantify exposure of healthcare personnel in the use stage. The same observation applies to workers involved in waste management during end-of-life processes. This would require performing extensive research of all the activities performed by healthcare personnel who are managing NBMs, or workers involved in the disposal of waste incorporating NBMs, as well as to perform occupational monitoring campaigns to obtain experimental data. Moreover, given the diversity and the high number of tasks performed by the different categories of healthcare personnel and the continuous changing of type of applications of nano-enabled biomedical products, the development of specific ESs or CESs would require their continuous evaluation, improvement, and verification. It is worth highlighting that due to the increasing interest in NBMs and their medical applications, the development of occupational risk assessment of NBMs will be an essential task for their market authorization, investigating not only the safety of patients but also workers who may be potentially exposed to these nano-enabled biomedical products.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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