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Quantitative human health risk assessment along the lifecycle of nano-scale copper-based wood preservatives

Danail Hristozov^{a,b}, Lisa Pizzol^{a,b}, Gianpietro Basei^a, Alex Zabeo^{a,b}, Aiga Mackevica^c, Steffen Foss Hansen^c, Ilse Gosens^d, Flemming R. Cassee^{d,e} , Wim de Jong^d, Antti Joonas Koivisto^f, Nicole Neubauer^g, Araceli Sanchez Jimenez^{g,h}, Elena Semenzin^a, Vrishali Subramanian^a, Wouter Fransmanⁱ, Keld Alstrup Jensen^f, Wendel Wohlleben^{f,g}, Vicki Stone^j and Antonio Marcomini^a

^aDepartment of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice, Italy; ^bGreendecision Srl, Venice, Italy; ^cDepartment of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark; ^dNational Institute for Public Health and the Environment, Bilthoven, Netherlands; ^eInstitute of Risk Assessment Studies, Utrecht University, Netherlands; ^fNational Research Centre for the Working Environment, Copenhagen, Denmark; ^gBASF SE, Ludwigshafen, Germany; ^hInstitute of Occupational Medicine, Edinburgh, UK; ⁱNetherlands Organisation for Applied Scientific Research TNO, Zeist, Netherlands; ^jSchool of Life Sciences, Nanosafety Research Group, Heriot-Watt University, Edinburgh, UK

ABSTRACT

The use of nano-scale copper oxide (CuO) and basic copper carbonate (Cu₂(OH)₂CO₃) in both ionic and micronized wood preservatives has raised concerns about the potential of these substances to cause adverse human health effects. To address these concerns, we performed quantitative (probabilistic) human health risk assessment (HHRA) along the lifecycles of these formulations used in antibacterial and antifungal wood coatings and impregnations by means of the EU FP7 SUN project's Decision Support System (SUNDS, www.sunds.gd). The results from the risk analysis revealed inhalation risks from CuO in exposure scenarios involving workers handling dry powders and performing sanding operations as well as potential ingestion risks for children exposed to nano Cu₂(OH)₂CO₃ in a scenario involving hand-to-mouth transfer of the substance released from impregnated wood. There are, however, substantial uncertainties in these results, so some of the identified risks may stem from the safety margin of extrapolation to fill data gaps and might be resolved by additional testing. Our stochastic approach successfully communicated the contribution of different sources of uncertainty in the risk assessment. The main source of uncertainty was the extrapolation from short to long-term exposure, which was necessary due to the lack of (sub)chronic *in vivo* studies with CuO and Cu₂(OH)₂CO₃. Considerable uncertainties also stemmed from the use of default inter- and intra-species extrapolation factors.

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1. Introduction

Preservation treatment is essential for increasing the service life of timber by imparting it with fungicidal and insecticidal properties. Copper-based preservatives have been widely used to treat softwood intended for commercial use due to their high performance and relatively low mammalian toxicity (Lebow and Foster 2005; Freeman and McIntyre 2008).

In response to the identified health risks from the chromated copper arsenate (CCA), chemical formulations without arsenic and chromium using ionic copper as the primary insecticide and fungicide were developed in the late 1980s.

Some key examples include the alkaline copper quaternary (ACQ), copper azole, and copper xylygen. Since then ionic copper formulations have become the dominant treatment for outdoor residential applications such as decking, gardening, fencing, and playground equipment in Europe. However, while they were effective in timber preservation, increased leaching of copper ions into the surrounding environment resulted in the degradation and structural failure of metal fasteners.

Micronized copper has been promoted as an alternative to ionic copper that can address these corrosion and treatment life issues (Freeman and McIntyre 2008). It has limited market penetration in

the EU due to a lack of regulatory approval, but over 75% of the residential lumber produced in the USA is nowadays treated with micronized copper (Freeman and McIntyre 2013) produced by mechanical grinding of compounds such as basic copper carbonate ($\text{Cu}_2(\text{OH})_2\text{CO}_3$) or copper oxide (CuO) with dispersing agents in a carrier solution (Freeman and McIntyre 2008). The size of the resulting particles ranges from 1 to 25,000 nm, with typically 90% of the particles below the size of 1000 nm (Freeman and McIntyre 2008). Leaching is significantly controlled in micronized wood treatments as compared to ionic treatments, and less than 5% of it is in particulate form (Platten et al. 2014). While a proportion of micronized copper formulations are nano-sized (Freeman and McIntyre 2008), the potential additional advantage offered by copper formulations within the nano-size range are evident (Clausen 2007; Evans et al. 2008; Kartal et al. 2009). Clausen (2007) argues that dispersion stability coupled with controlled particle size in nano-sized wood preservative formulations may greatly improve the penetration of the preservative in the wood, the treatability of refractory wood species, and the stability of finishes and coatings for above ground applications. Accordingly, nanoparticles of CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ have been increasingly considered for use in micronized wood treatment formulations (Clausen 2007; Evans et al. 2008; Kartal et al. 2009).

The increased use of nano-scale CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ as timber preservatives has raised concerns about the potential of these substances to cause undesirable human health effects. In spite of the fact that ionic copper formulations are currently thoroughly reviewed in Europe for their human and environmental risks under the Biocidal Products regulation (Regulation (EU) No. 528/2012), there are only few studies that attempted to assess their risks (US EPA 2003; Civardi et al. 2015). Therefore, we performed a quantitative human health risk assessment (HHRA) of nano-scale CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ along the lifecycles of antimicrobial and antifungal coatings and impregnations. This is the first quantitative estimation of the risks from these products from lifecycle perspective.

We applied the HHRA framework for chemicals as it has been considered by the European Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) applicable to nanomaterials (SCENIHR

2009). This approach consists of hazard identification, dose–response assessment, exposure assessment and risk characterization steps (Van Leeuwen and Vermeire 2007). We applied it as a probabilistic methodology designed to quantitatively estimate and communicate the uncertainties in each of these steps in order to demonstrate how they influence the final results (Pang et al. 2017; Tsang et al. 2017). Then we implemented this methodology as a software module in the web-based EU FP7 SUN project's Decision Support System (SUNDS, www.sunds.gd), which enabled it to estimate occupational, consumer and public health risks from manufactured nanomaterials along the lifecycles of nano-enabled products and to communicate the associated uncertainties.

This paper demonstrates the SUNDS HHRA module with dose–response data from *in vivo* experiments specifically designed to measure the subacute effects following inhalation and oral uptake of nanoscale CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$. The dose–response relationships were compared to external human exposure concentrations estimated for 13 relevant exposure scenarios (ES), which were formulated based on release data and contextual information generated for our case study antimicrobial and antifungal CuO -based acrylic coating and a $\text{Cu}_2(\text{OH})_2\text{CO}_3$ -containing impregnation applications.

2. Methods

2.1 Case study products

2.1.1 CuO used in an antimicrobial/antifungal wood protective coating

CuO pristine nanoparticles were obtained as a black powder from the company PlasmaChem GmbH, Berlin, Germany. They were synthesized by thermal decomposition of an inorganic precursor in solid phase. The synthesized and dry-milled $\text{Cu}_2(\text{OH})_2\text{CO}_3$ precursor was decomposed at approximately 350°C for several hours. The derived crystalline powder had a TEM particle size of 15–20 nm, a Brunauer–Emmett–Teller (BET) specific surface area of $47\text{ m}^2/\text{g}$ and a bulk material density of $6.3\text{ g}/\text{cm}^3$ according to the supplier. To check consistency with these data and complement them, we performed detailed physicochemical characterization of size (distribution), shape, crystallite phases, dispersability, agglomeration/aggregation, stability, surface area

and chemistry, chemical composition, and impurities. The adopted methods and the obtained results are described in detail in the Supporting Information (Table SI_1). They are not detailed in this section because the focus of this paper is on the risk characterization calculations, which are based on measured hazard and exposure and are, therefore, not directly dependent on the physicochemical properties.

The CuO nanopowder was dispersed in a solution by mixing according to an established BASF protocol (Tiarks et al. 2003). Specifically, we added it to a high-gloss acrylic wood coating, where the anticipated antimicrobial activity of the CuO would provide the additional functionalities of sealing the wood and serving decorative purposes. The wood-coating liquid was then applied either by spraying or brushing onto the surface of blocks of pine wood with dimensions of $2.5 \times 2.5 \times 1$ cm ($n = 70$). Some of the blocks were coated entirely with a CuO-free (TiO₂) coating to serve as a negative control. The rest of them were coated on one side with the TiO₂/CuO coating on a chemically inert substrate (Teflon or Poly Ethylene) and dried for a week in preparation for release experiments intended to generate data for formulating ES (cf. 2.2.2). The coatings were thoroughly characterised, and the results are reported in Pantano et al. (2018) and in the Supporting Information (Tables SI_2 and SI_3).

2.1.2 Cu₂(OH)₂CO₃ used in an antimicrobial/antifungal wood protective impregnation

Dispersed Cu₂(OH)₂CO₃ nanoparticles were obtained from PlasmaChem GmbH, Berlin, Germany. In the process of formulating an impregnation solution, the basic copper carbonate was wet milled until it reached nano-sized grade. The Cu₂(OH)₂CO₃ was combined with water, stabilizers, and co-biocides to make the stock solution. Small wood blocks were then immersed/soaked in this impregnation dispersion. This was adequate for research purposes, but on industrial scale pressure impregnation is typically carried out in steel cylinders or retorts. The wood is loaded on special tram cars and moved into the retort, which is then closed, evacuated and subsequently filled with preservative solution. Then pressure forces the preservative into the wood until the desired amount is absorbed.

The results of the performed detailed physicochemical characterization of the micronized Cu₂(OH)₂CO₃ suspension and the impregnated wood are reported in Pantano et al. (2018) and are summarized in the Supplemental Information (Tables SI_2 and SI_3).

2.2 Risk assessment by means of SUNDS

SUNDS is a web-based software system that has been designed to estimate occupational, consumer, public health, and environmental risks from nanomaterials in real industrial products along their lifecycles. In situations where the risks are not controlled SUNDS proposes suitable Risk Management Measures, including information about their costs versus the benefits of the technologies. The SUNDS framework was previously described by Subramanian et al. (2016). The computational risk assessment approach illustrated in this paper is part of the SUNDS Tier 2 and is described in more detail in Pizzol et al. This probabilistic HHRA module is designed to quantitatively estimate and communicate the uncertainties in each step of the risk analysis. The system, which is schematically depicted in Figure 1, can simultaneously assess risks in different lifecycle stages, targets, activities, and routes of exposure based on *in vivo* toxicity data and ES.

For each ES, based on a combination of the exposure assessment (estimation of external concentration) and hazard assessment (estimation of human effect threshold dose), the system produces a discrete value or a probability distribution of risk and the associated uncertainty. To do this, SUNDS uses exposure measurements, or if such are not available exposure can be estimated by means of models (e.g. NanoSafer, Ingestion Exposure Tool) that are either integrated in the system or externally interact with it through web services. To assess a human effect threshold SUNDS can use *in vivo* raw data to perform dose–response analysis by means of a dedicated model and then to correct the obtained Point of Departure (PoD) (i.e. Benchmark Dose, BMD) and extrapolate it to a human dose (HD) by means of the APROBA tool, which is integrated in the system. In some cases, the PoD (e.g. BMD or No-observed Adverse Effect Level, NOAEL) is available from the published literature and therefore can be directly imported in the system. This is what we did in this risk assessment, where the

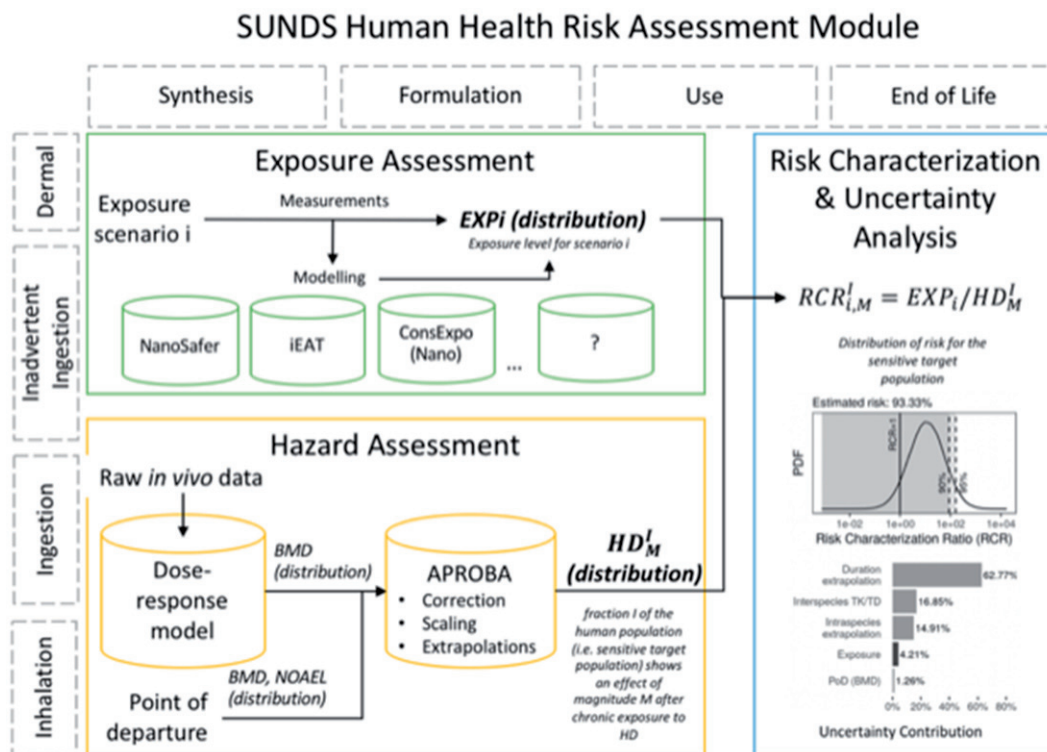


Figure 1. Structure, models, inputs and outputs of the human health risk assessment module of the SUN Decision Support System (SUNDS).

dose–response analysis involved PoD estimated in other studies, which were only corrected and extrapolated to HD in SUNDS by means of APROBA.

Sections 2.2.1–2.2.3 describe how the SUNDS HHRA module was applied for exposure and hazard assessment of the case-studies presented in Section 2.1 in order to assess occupational and consumer risks along their lifecycles and to communicate the associated uncertainties.

2.2.1 Exposure assessment

2.2.1.1 Formulation of exposure scenarios.

To gather the knowledge and expertise needed to formulate realistic workplace and consumer ES we performed a literature review, organized a dedicated workshop and obtained additional contextual information from the chemical company BASF.

Specifically, published literature from 2000 to 2016 was searched for relevant release and exposure assessment studies. To do this we queried the Web of Science database with combinations of the following keywords: nano, copper oxide, copper carbonate, micronized copper, CuO, CuCO₃, Cu₂(OH)₂CO₃, paint, impregnation,

exposure assessment, release, emission, exposure, workplace, consumer, use. The literature search resulted in a small number of documents, which were carefully analyzed. In addition, mapping of release hot spots along the lifecycles of the investigated products was performed as part of the SUN project (Steinfeldt 2017). We used these results as a basis to design the exposure assessment expert workshop.

The workshop took place on 22 January 2016 in Venice and was attended by 22 academic and industrial experts in human exposure assessment and copper-based timber preservatives from EU, US and Russia (cf. Supplemental Information, Table SI_4). The discussions resulted in generic ES, which were then further elaborated with information obtained from the literature and from the industrial company BASF. The formulated ES, listed in Table 2, covered the entire lifecycles (i.e. synthesis, formulation, use, end-of-life) of the investigated products. In cases when estimations of exposure were not available in the literature, such were derived in the SUN project by means of the experimental and modeling methods described in Section 2.2.2.

2.2.1.2 Estimation of exposure. The following experimental and modeling activities were performed with our case study products (cf. Section 2.1) in order to derive exposure estimations for each ES for performing risk assessment by means of SUNDS. The numbering of the different ES corresponds to Table 2.

ES 1 and 6 involving laboratory production, handling, and packing of nanoscale CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ powders. CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoscale powders are produced using sol gel synthesis. The sol gel synthesis and packing were performed in a fume hood where the bags were canned, and the cans were subsequently moved to a storage room. Occupational exposure measurements were performed, which resulted in breathing zone and far field respirable mass concentrations below the minimum detection limits of 161 and $26 \mu\text{g m}^{-3}$, respectively (Fonseca et al. 2018). The surface wipe samples analyzed with a Scanning Electron Microscope (SEM) did not reveal any CuO particles (Fonseca et al. 2018). Therefore, based on these results, we concluded that the exposure levels for ES 1 and 6 are negligible.

ES 2 and 7 involving pouring nanoscale CuO powders in the wood coating stock solutions. The nanoscale CuO pouring to the liquid matrix was not measured. The exposure levels were estimated by means of a one-box model (Hewett and Ganser 2017). Laboratory scale powder mixing was assumed to be performed without using any emission controls (i.e. worst-case scenario). The parameters used for modeling of manufacturing 100 L CuO preservative are the following: dustiness index = 104 mg/kg (moderate); mass flow = 1 kg/min (careful pouring); handling energy = 1 (equivalent release as in dustiness test); local emission controls = 1 (no control); pouring amount = 2.5 kg (poured from 1 kg bags, 9 min between pourings); room volume = 20 m^3 (small room); ventilation rate = 2 h^{-1} . The results of the modeling are reported in Table 2.

ES 3 involving application of CuO wood coating to the substrate. Because the Cu-based acrylic formulation is highly viscous, it is applied to the substrate by a brush. Release of respirable (PM4.5) droplets of this solution is assumed to be insignificant during brush painting (ECHA 2016), so the inhalation exposure is assumed negligible. Workers performing the brushing are supposed to wear protective

gloves to prevent direct skin exposure. If the gloves are worn correctly at all times, the skin exposure is insignificant. Hand-to-mouth exposure is also assumed to be negligible unless the worker touches mouth with contaminated gloves.

ES 4 involving scraping, sanding, and sawing wood treated with CuO preservative. The old CuO wood preservative coating is typically removed before surface treatment. Because the dry coating is viscoelastic (elasticity modulus of the matrix is 10^{-7} Pa) the coating is likely removed by scraping, which produces an insignificant amount of respirable particles: the smallest 10% size fraction of particles were $20 \mu\text{m}$ in size (Nowack et al. 2016).

Emission rates were estimated based on sanding and drilling release experiments, which were used to represent also sawing operations and are described in the Supporting Information (cf. Section SI2). The exposure levels were estimated based on these data by means of a single and two box (Hewett and Ganser 2017) models. The parameters used for modeling of sanding are the following: emission rate = $20 \mu\text{g}/\text{sec}$ where 2% is CuO_2 (sanding disc diameter 150 mm, grit size 80, rotation speed 1550 rpm, and contact force 17 N); local emission controls = 1 (no control); room volume = 100 m^3 (outdoor); FF ventilation rate = 10 h^{-1} (still air), near-field volume = 8 m^3 after Cherrie (1999); near-field air flow = $10 \text{ m}^3/\text{min}$. This resulted to a near-field (NF) concentration of $93 \mu\text{g}/\text{m}^3$ during continuous process. The results of the modeling are reported in Table 2.

ES 5 involving transfer to consumers' skin from surfaces by rubbing. Consumers are assumed to be handling painted wood with their hands without wearing protective gloves, which can lead to direct skin exposure and subsequent inadvertent ingestion by touching the area around the mouth. Hand exposure was assessed by conducting dermal transfer tests in the SUN project by means of the surface wiping method based on the NIOSH guideline Elements on Wipes: Method 9102 (NIOSH 2003). The experimental set-up and the obtained results are partly described in Mackevica et al. (2016) and are outlined in the Supporting Information (cf. Section SI2).

Perioral exposure was estimated using a modified version of the Ingestion Exposure Tool (iEAT) (Gorman et al. 2012), assuming that a person

touches a wood surface painted with CuO that has released CuO particles as a consequence of wearing and touches inadvertently the area around the mouth with subsequent ingestion by licking. The transfer efficiency of nanomaterials from finger tips to the perioral area was estimated experimentally. A worst case was assessed, where all the copper released from the wood is transferred to the finger tips. Each surface to hand event was presented as a hypothetical scenario where someone (with low or high hand moisture) touches the wood and then touches the perioral area. We assumed the finger area of contact was 1 cm^2 and the perioral area of contact also 1 cm^2 .

ES 11 involving children exposed directly to the $\text{Cu}_2(\text{OH})_2\text{CO}_3$ impregnated wood by skin transfer of copper to the mouth and related ingestion. The most likely place for children to come into contact with copper-based impregnated wood is a playground, where its skin can be exposed to copper with subsequent transfer to the mouth and related ingestion. Estimations of children exposure have been provided by Platten et al. (2014), where the wood surface area a child would come into contact with during a typical visit to a playground has been estimated along with potential transfer, ingested concentration per playground visit, and number of visits per week.

ES 13 involving leaching during contact with water and related potential human exposure. General population can come in contact with nano-scale CuO or $\text{Cu}_2(\text{OH})_2\text{CO}_3$ released by the wood during contact with water. To estimate the amount and form (particle or ion) of released copper, leaching experiments were performed in the SUN project according to the European standard EN 84 (ISO 1997), which describes an accelerated aging test of pine specimens treated with wood preservative formulations for simulating exposure to water (ISO 1997). The investigated material was the acrylic coating containing 1.5% CuO and 42.5% TiO_2 (pigment grade, non-nano) which was applied on pine wood (dimension: $2.6 \times 2.7 \times 1.1\text{ cm}$). The result from applying the test showed that the released copper was solely in ionic form (Pantano et al. 2018).

In the case of nano $\text{Cu}_2(\text{OH})_2\text{CO}_3$, Platten et al. (2014) reported results from leaching tests indicating that mostly ionic copper ($> \sim 95\%$) was released

from the treated wood and that the particulate copper that was released is attached to cellulose and is therefore not free in the leaching waters.

Based on these results, the human exposure to nanoparticles leaching during contact of CuO coatings and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ impregnations with water was considered negligible.

2.2.1.3 Derivation of exposure distributions. The above exposure levels were used to generate an exposure distribution (EXP_i) for each scenario i by means of SUNDS. When only deterministic values were available, normal or lognormal distributions were used to describe the probabilistic distribution of exposure as recommended by the US Environmental Protection Agency (US EPA 2001). Such distributions were created around the available deterministic values by fitting a one order of magnitude ($\pm 50\%$) wide confidence interval around the mean exposure estimate. The reason for this is that the exposure levels were estimated based on measurements or models, which introduce uncertainties in the EXP_i . Indeed, measurements are obtained by instruments, which present known errors, but many other aspects (e.g. preparing the samples, positioning of the instrument) add more uncertainties, often larger than the instrument errors. Moreover, the application of the one box and two box exposure models (Ganser and Hewett 2017) also introduced uncertainties associated with certain assumptions.

2.2.2 Hazard assessment

2.2.2.1 Hazard identification. To identify the hazards of CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoparticles, a literature review was performed, which showed that relevant *in vivo* inhalation or oral studies that considered multiple exposure doses (and were therefore suitable for dose-response assessment) did not exist (Gosens et al. 2016). Therefore, we designed and performed short-term inhalation and short-term oral studies in order to derive subacute data that according to the REACH Guidance on Chemical Safety Assessment (ECHA 2008) and the Guidance on Biocides Legislation (ECHA 2017) can be extrapolated for use in long-term HHRA. The used pristine nanomaterials and dispersions were the same described in 2.1. The study designs are

only shortly outlined in this section as they are described in detailed in Gosens et al (2016) and de Jong et al.

Short-term inhalation exposure. After an acclimatisation period, rats (8 weeks old, HsdCpb: WU) were exposed nose-only to a single generated exposure concentration of CuO nanoparticles or to clean air as a control for 5 consecutive days. By exposing the animals for various durations (18 min, 36 min, 90 min, 3 h, and 6 h), different dose levels were obtained. A 6 h concentration equivalent was derived by multiplying the duration of exposure by the exposure concentration (designated as dose $C \times T$) and scaling it to the highest exposure duration of 6 h to 13.2 mg/m^3 (for animals dedicated for toxicological examination) or 11.6 mg/m^3 (for animals dedicated for organ burden analysis). Repeated exposures to CuO nanoparticles via inhalation resulted in a linear increase in the determined lung burden, justifying the applied $C \times T$ concept.

Short-term oral exposure. Male rats (RjHan: WI, bred Specific Pathogen Free, barrier maintained during experiment) of 8–9 weeks old were obtained from Janvier Labs (Le Genest-Saint-Isle, Saint Berthevin, France). The CuO nanoparticle dispersions were orally administered by gavage using the following exposure doses: vehicle control, 1, 2, 4, 8, 16, and 32 mg/kg body weight (b.w.) and a pilot study with 64 mg/kg b.w. The doses were chosen based on information in the literature of soluble non-nano CuSO_4 , which indicated a No-observed Adverse Effect Level (NOAEL) of 16.3 mg/kg (Hébert 1993). The dose was administered as 0.1 ml per 20 g (1 ml per 200 g). In an additional study, one group of animals ($n = 4$) was exposed to a high dose of 512 mg/kg b.w. For the $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoparticles the administered doses were vehicle control, 4, 8, 16, 32, 64, and 128 mg/kg b.w. The animals were treated on 5 consecutive days (days 1–5) and autopsy was performed 24 h after the last oral administration (day 6). In addition, a recovery period of 3 weeks was included in the experiments to evaluate recuperation and possible persistence of the nanomaterials in the body. Autopsy of the recovery groups was performed on day 26, after three weeks of recovery.

2.2.2.2 Dose–response assessment. The dose–response assessment of the raw inhalation data was

not performed by means of SUNDS because it was done by Gosens et al. (2016) using the PROAST model. PROAST estimates a benchmark dose (BMD), which corresponds to a pre-defined benchmark response (BMR). The uncertainty of the BMD is reflected by providing a 90% confidence interval with an upper (BMDU) and lower (BMDL) limit.

The dose–response assessment of the ingestion data was also not performed by means of SUNDS, but by de Jong et al, who derived a Lowest Observed Adverse Effect Level (LOAEL) for decrease of total body weight, which was then divided by an uncertainty factor (UF) of 3 to calculate a NOAEL.

The BMD and NOAEL values derived from the two studies were imported in SUNDS, and used by the system as PoD, which were “corrected” to account for exposure duration differences between the animal experiments and the ES. In addition, allometric scaling was performed in the case of oral studies to consider physiological differences between the experimental animals and humans. These “corrected” probability distributions were then extrapolated to human effect threshold distributions by applying appropriate inter- and intra-species uncertainty factors (UF) (ECHA 2008).

The correction, allometric scaling, and extrapolations were performed by means of APROBA, which is a Microsoft Excel tool developed by the World Health Organisation’s International Programme on Chemical Safety (IPCS-WHO) and is programmed in SUNDS. It is able to perform approximate probabilistic (as well as deterministic) analysis of human dose extrapolation starting from animal dose–response results. The result of the probabilistic hazard assessment is a human effect threshold, called human dose HD_M^I at which a fraction I of the human population shows an effect of magnitude M after chronic exposure, with a specific confidence interval (e.g. 90%). This fraction I represents the sensitive target population, which is the portion of population that is more vulnerable to effects of exposure to the substance due to e.g. age or poor health status. APROBA contains default algorithms and values for performing correction and allometric scaling based on input information (cf. Section 3.2.2 and Table 4) (IPCS-WHO 2014). It also uses default extrapolation factors, which were proposed by the IPCS-WHO and are reported in Table 1.

Table 1. Generic uncertainty (extrapolation) factors (UF) for different aspects of the dose-response assessment assuming lognormal uncertainty distributions.

Aspect of hazard characterization	Lognormal P50	Lognormal P95/P50	Lognormal (P05, P95)	Comments
PoD uncertainty for NOAEL ^a : AF _{PoD-NOAEL}				
Continuous endpoint, chronic/subchronic study	1/3	4.7	(0.07, 1.6)	Ratio of NOAEL to BMD ₀₅ (5% relative change)
Continuous endpoint, developmental study	1/3	7.0	(0.05, 2.3)	Ratio of NOAEL to BMD ₀₅ (5% relative change)
Deterministic quantal endpoint	2/9	5	(0.04, 1.1)	Ratio of NOAEL to ED ₅₀ (50% response)
Stochastic quantal endpoint	2/3	4.7	(0.14, 3.2)	Ratio of NOAEL to BMD ₁₀ (10% extra risk)
Exposure duration: AF _{Dur}				
Subchronic → Chronic	2	4	(1/2, 8)	–
Subacute → Chronic	5	8	(5/8, 40)	–
Interspecies body size adjustment: AF _{Inter-BS}				
Oral	$\left(\frac{bw_{human}}{bw_{test\ species}}\right)^{0.3}$	$\left(\frac{bw_{human}}{bw_{test\ species}}\right)^{0.04}$	$\left(\frac{bw_{human}}{bw_{test\ species}}\right)^{(0.26, 0.34)}$	Use case-specific body weights
Inhalation	1/RDDR or 1/RGDR	2	(0.5, 2)/RDDR or (0.5, 2)/RGDR	Use case-specific RDDR (particle) or RGDR ^b (gas)
Interspecies TK/TD differences: AF _{Inter-TK/TD}				
Oral	1	3	(1/3, 3)	Given lack of alternative, can also be used for inhalation
Intraspecies differences for incidence I: AF _{Intra-I}				
I = 5%	5.0	2.8	(1.8, 14)	Log(GSD _H) P50 = 0.32 and P95/P50 = 2.2
I = 1%	9.7	4.3	(2.2, 42)	
I = 0.1%	20.4	7.0	(2.9, 143)	

Source: IPCS-WHO guidance document on evaluation and communication of uncertainty in hazard characterization (IPCS-WHO 2014).

BMD_x: benchmark dose for x% benchmark response; bw: body weight; ED₅₀: median effective dose; GSD_H: geometric standard deviation for interindividual variability in the human equipotent dose distribution; NOAEL: no-observed-adverse-effect level; P05: 5th percentile; P50: 50th percentile; P95: 95th percentile; PoD: point of departure; RDDR: regional deposited dose ratio; RGDR: regional gas dose ratio; TK/TD: toxicokinetic/toxicodynamic.

^aWhen using a NOAEL as the PoD, the uncertainty includes both the fact that the NOAEL is an approximation for the BMDL as well as the uncertainty in the underlying BMD (a ratio of 3 is assumed between the median estimate of the BMD and the BMDL).

^bFor gases, the RGDR is often assumed to be 1.

2.2.3 Risk characterization and uncertainty analysis

Risk was calculated by means of SUNDS based on the Risk Characterization Ratio ($RCR_{i,M}^I$) approach, which takes into account uncertainty and variability related to the incidence goal sensitive population. $RCR_{i,M}^I = EXP_i / HD_M^I$, where EXP_i represents an exposure level for scenario i . The $RCR_{i,M}^I$ distribution is classified as “non-acceptable” when it is above 1 for more than 10% of the sensitive population. The variability related to the rest of population is not taken into account in HD_M^I because when the sensitive population is at risk we assume that also the general population is at risk. The exposure situation “needs further consideration” when the $RCR_{i,M}^I$ is above 1 in 5–10%, and the risk is “acceptable” when it is above 1 for less than 5% of the sensitive population. These risk acceptability classes were defined based on the literature, which suggests that (in the case of probabilistic risk assessment) the risk can be acceptable if the 90th percentile of the population is safe, but more conservative values (i.e. the 95th percentile or the 99th percentile) can also be selected (US EPA 2001).

RCR distributions were generated for each of the ES by sampling the HD_M^I and EXP_i distributions in over 10 000 Monte Carlo simulations. The probability distribution of the RCR is affected by (1) the assumptions/considerations applied in the probabilistic hazard assessment and (2) the uncertainties associated with the exposure estimations. In the first case, selecting a specific population incidence goal (e.g. 5%) in the dose–response assessment implies that the resulting probabilistic distribution of the RCR protects 95% of the population, thus the RCR probability distribution represents the variability and uncertainty around the 95% of the assessed population.

The contribution of different sources to the overall uncertainty in the RCR was estimated for each ES by means of Monte Carlo. In each of the 10 000 simulations RCR was numerically estimated by randomly sampling 10 000 elements from the distributions of the PoD, exposure and UF. The contribution of each of these factors to the uncertainty in the risk estimate was quantified by assessing the level of correlation between the factor and the resulting RCR by means of the squared Spearman's rank correlation coefficient.

3. Results

3.1 Exposure assessment

Thirteen ES were formulated that covered the entire lifecycles (i.e. synthesis, formulation, use, end-of-life) of our case-study products: CuO-based acrylic coating and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ -containing impregnation (Table 2).

The exposure assessment of the CuO-based coating demonstrated that release of nanoparticles is possible at each lifecycle stage and can lead to both worker and consumer exposure in different formulations: as nanoscale powder, as liquid paint, or as a cured surface coating on wood. The handling of dry powders led to some significant exposure potential in the formulation lifecycle stage. If paint spraying is avoided, inhalation exposure to paint is assumed negligible during its application, but dermal and oral exposure could be relevant for both workers and consumers either via accidental dermal deposition when treating (painting) the wood or via hand-to-mouth (i.e. inadvertent oral) exposure. However, according to the latest studies dermal exposure is insignificant (Platten et al. 2016). Moreover, the dermal transfer testing of the painted wooden blocks (cf. Section 2.2.2) showed that there was nearly no release of nanoscale CuO from the paint matrix during surface wiping tests (Mackevica et al., 2016). However, after sanding of the paint surface, the observed CuO release was magnitudes higher. Accordingly, inhalation and inadvertent oral exposures were assessed in the case of occupational and consumer use during sanding activities and the results were used to represent also sawing operations.

The analysis of the literature on $\text{Cu}_2(\text{OH})_2\text{CO}_3$ -impregnated wood showed that the release of copper nanoparticles is typically negligible. The US EPA report provided an estimate of exposure for the concerning ES11 that involves children exposed directly to the treated wood by skin contact, transfer of copper to the mouth, and subsequent ingestion (Platten et al. 2014). Moreover, two other common exposure pathways were identified and assessed: leaching during contact with water and transfer during physical contact (cf. Table 2).

We used SUNDS to generate EXP_i probability distributions for each ES based on the estimated exposure levels, which demonstrated significant exposure potential for scenarios 2, 4, and 11

(Table 3). To account for unknown uncertainties due to measurement and modeling errors we established a one order of magnitude wide confidence interval around the deterministic inhalation exposure estimates for ES2 and ES4 (0.026 mg/m^3 and 0.36 mg/m^3 , respectively) and fitted the corresponding normal distributions. In ES11, starting from an exposure of 1.11 mg/d derived by averaging three visits to the playground over a week (Platten et al. 2014), we built a normal distribution representing uncertainty in the number of weekly visits characterized by the 5th percentile at $1.11/3 \text{ mg/d}$ and the 95th percentile at 1.11 mg/d . This bell-shaped curve was then divided by a uniform mixture of normal distributions representing the variability of weights of children (girls) aged from 8 to 36 months (mean: 10.95 kg , SD: 2.18 , $\text{CI}_{5\%}$: 7.6 kg , $\text{CI}_{95\%}$: 14.68 kg).

3.2 Hazard assessment

3.2.1 Hazard identification

The detailed results from the short-term inhalation exposure are available in Gosens et al. (2016), while the results from the short-term oral exposure are reported in de Jong et al (submitted). Therefore, only the main findings of relevance for the dose–response analysis (cf. Section 3.2.2) are outlined below.

Short-term inhalation exposure. Twenty-four hours after a 5-d exposure to CuO pristine nanoparticles, dose-dependent lung inflammation and cytotoxicity were observed as well as histological alterations of the nose epithelium. Lung histopathological examinations indicated alveolitis, bronchiolitis, vacuolation of the respiratory epithelium, and emphysema in the lung starting at a 6 h-concentration equivalent of 2.4 mg/m^3 .

After a recovery period of 22 d, limited lung inflammation was still observed leaving a small but significant elevation of macrophages in the airspace (at the highest dose of 13.2 mg/m^3). This inflammation was not accompanied by pathological changes or elevated biochemical markers of fibrosis. The histological alterations of the olfactory epithelium in the nose restored completely after 22 d. No histopathological changes were detected in the brain, olfactory bulb, spleen, kidney and liver. In conclusion, a 5-d, 6-h/d exposure equivalent to an aerosol of agglomerated CuO nanoparticles resulted

Table 2. Description of the exposure scenarios assessed for nanoscale CuO used in wood coating paints and nanoscale Cu₂(OH)₂CO₃ used in timber preserving impregnations.

Exposure scenario (ES)	LC stage	Target	Exposure route	Exposure level (EXP)	Additional information	Source
ES1: Laboratory scale CuO powder production, handling and packing	SYN	Worker	Inhalation	Negligible	Breathing zone and far field respirable mass concentrations below the minimum detection limits of 161 and 26 µg m ⁻³ , respectively, assuming background concentration level is 0 µg m ⁻³ .	Fonseca et al. (2018)
ES2: Pouring CuO nanoscale powder in the wood coating matrix	FOR	Worker	Dermal	Negligible	Surface contamination was not detected, and dermal and perioral exposure are negligible ^a .	SUN project deliverable 5.4
ES3: Applying CuO wood coating to the substrate	USE	Worker Worker	Inhalation Dermal	NF 26 µg/m ³ and FF 10 µg/m ³ Negligible Negligible	In case the fume cupboard was active the concentration was <0.2 µg/m ³ . If the fume cupboard would be switched off and pouring would be performed in a room the concentration would be in the NF 26 µg/m ³ and FF 10 µg/m ³ i.e. 130 times higher than with the fume cupboard. Surface contamination: dermal and perioral exposure are negligible ^a Since CuO wood preservative is highly viscous (viscosity N/A) it is applied by brush to the substrate. Release of respirable (PM _{4.5}) CuO wood preservative droplets is assumed to be negligible during brush painting. Thus, the inhalation exposure is negligible	ECHA (2016)
ES4: Sanding, cutting, drilling and sawing wood treated with CuO preservative	USE	Worker Worker, Consumer	Dermal Inhalation	Negligible 93 µg/m ³	Surface contamination: dermal and perioral exposure are negligible ^a Modeled NF CuO ₂ concentration during continuous outdoor sanding.	SUN project deliverable 5.4
ES5: Consumers transfer to skin from surfaces by rubbing	EOL	Worker Worker Consumer	Inhalation Dermal Dermal	Negligible Negligible Negligible	The percentage of treated wood in the waste is very low, thus reducing the emission of CuO The wiping test performed in the SUN project indicated insignificant transfer to the skin	Heggelund et al. (2016) Mackevica et al. (2016)
ES6: Cu ₂ (OH) ₂ CO ₃ powder production, handling and packing	SYN	Worker	Inhalation	Negligible	In this study, we assume that the occupational exposure levels during Cu ₂ (OH) ₂ CO ₃ production, handling and packaging are, like for CuO, below the detection limits, which were 161 µg m ⁻³ , 70, and 200 µg m ⁻³ , respectively, when assuming background concentration level is 0 µg m ⁻³ .	Fonseca et al. (2018)
ES7: Milling of Cu ₂ (OH) ₂ CO ₃ slurry for the impregnation stock solution	FOR	Worker Worker	Inhalation Dermal	Negligible Negligible	Surface contamination: dermal and perioral exposure are negligible. According to the latest skin penetration tests performed in SUN, dermal exposure is not relevant since the penetration rate is negligible for nanomaterials We assume that for the formulation phase, no inhalation exposure will occur due to negligible emissions to the air (SUN deliverable 2.3)	SUN project deliverable 2.3
ES8: Workers impregnating wood in an industrial setting	USE	Worker	Inhalation	Negligible	Surface contamination: dermal exposure is negligible since emissions are negligible	US EPA (1995)
ES9: Workers constructing garden fences, decking, cladding, playgrounds, vegetable gardens using the treated wood	USE	Worker Worker Worker	Dermal Inhalation Dermal	Negligible Negligible Negligible	For the vacuum pressure treatment process in industrial scenario, the emissions to air are limited. So, no relevant exposure scenarios are assessed Surface contamination: dermal and perioral exposure are negligible ^a For waterborne preservatives, very low emissions to air Surface contamination: dermal and perioral exposure are negligible. According to the latest skin penetration tests performed in SUN, dermal exposure is not relevant since the penetration rate is negligible for nanomaterials	US EPA (1995)
ES10: Consumer transfer to skin from surfaces by rubbing	USE	Consumer Consumer	Inhalation Dermal	Negligible Negligible	Inhalation exposure is assumed negligible Surface contamination: dermal and perioral exposure are negligible. According to the latest skin penetration tests performed in SUN, dermal exposure is not relevant since the penetration rate is negligible for nanomaterials	Mackevica et al. (2016)

(continued)

Table 2. Continued

Exposure scenario (ES)	LC stage	Target	Exposure route	Exposure level (EXP) mg/kg/d	Additional information	Source
ES11: Children exposed directly to the treated wood by skin contact, transfer of copper to the month and related ingestion	USE	Consumer	Oral	0.07 (SD 0.03)	Assuming an average weekly exposure of 1.11 mg/day, corresponding to three visits to the playground and dividing by the distribution of weights of children aged 8-36 months.	Platten et al. (2014)
ES12: Sanding, cutting, drilling and sawing wood treated with Cu ₂ (OH) ₂ CO ₃ preservative	EOL	Worker	Inhalation	Negligible	The percentage of treated wood in the waste is very low, thus reducing the emission of Cu ₂ (OH) ₂ CO ₃ . Dermal deposition was considered negligible as the workers wear gloves	Heggelund et al. (2016)
ES13: Leaching during contact with water and related potential human exposure (appl. to both CuO and Cu ₂ (OH) ₂ CO ₃)	USE	Consumer	Dermal	Negligible	Leaching experiments performed in SUN showed that the released copper was solely in ionic form. Platten et al. (2014) showed that mostly ionic copper (> ~95%) is released from the wood treated with Cu ₂ (OH) ₂ CO ₃ and that the particulate copper that was released is attached to cellulose and is therefore not free in the solution	Pantano et al. (2018) and Platten et al. (2014)
	EOL	Public	Oral	Negligible	The percentage of treated wood in the landfilled waste is very low. Release from landfills is negligible in general	Heggelund et al. (2016)

SYN: synthesis; FOR: formulation; EOL: end of life.

^aWorkers are assumed to wear protective gloves (e.g. nitrile) which prevent direct skin exposure. Thus, the skin exposure is assumed to be insignificant. Perioral exposure is also assumed to be insignificant unless a worker puts dirty glove in her/his mouth.

in a dose-dependent toxicity in rats, which almost completely resolved during a 3-week post-exposure period. The data for all endpoints measured were compared via the BMD calculated by PROAST. This allowed a ranking of the relative sensitivity of each endpoint to the inhaled CuO nanoparticles with biochemical markers and inflammatory cell number in the bronchoalveolar lavage fluid providing to be the most sensitive indicators for lung toxicity (Gosens et al. 2016).

Short-term oral exposure. *Copper oxide:* In the dose-response study with a maximum dose of 64 mg/kg, no signs of toxicity were noted. After treatment of 5 consecutive days, there was no difference in body weight between day 1 (start of treatment) and day 6 (24 h after end of treatment). In the additional group of animals treated with 512 mg/kg some indications for toxicity were observed based on changes in the body weight. Moreover, the results of the clinical chemistry showed that at day 6 alterations in the level of alkaline phosphatase and aspartate aminotransferase enzymes indicated the presence of liver toxicity. At the dose of 64 mg/kg, lactate dehydrogenase levels were also increased indicating cell and organ damage. Animals treated with 512 mg/kg showed similar alterations in clinical chemistry (low level of alkaline phosphatase, high level of aspartate aminotransferase, and high level of lactate dehydrogenase), and histopathological alterations in the liver (e.g. inflammation, hepatocellular hypertrophy, hepatocellular necrosis) thus supporting the data of the dose-response study. Therefore, the dose 512 mg/kg was taken as the LOAEL.

Copper carbonate: For Cu₂(OH)₂CO₃ nanoparticles, a dose-response study was performed with the highest dose being 128 mg/kg b.w. Repeated (five times) oral administration of the highest dose induced severe toxic responses in the treated animals as indicated by the behavior of the animals, frequent washing and piloerection. Based on these observations, the animals scheduled for prolonged observation (autopsy after a recovery period at day 26 after treatment) were autopsied prematurely at days 6 and 7, respectively, 24 and 48 hours after the last (day 5) treatment.

For animals treated with a dose up to 64 mg/kg b.w. both at days 6 and 26 after treatment, both body and organ weights did not show a difference

Table 3. Summary of exposure distributions (EXP_i) for each scenario i.

	ES1	ES2 inhalation	ES2 dermal	ES3	ES4 inhalation (consumer and worker)	ES4 peroral (consumer and worker)	ES4 end of life	ES5
5%	Negligible	1.30E-02	Negligible	Negligible	1.60E-01	2.35E-06	Negligible	Negligible
95%		3.90E-02			4.80E-01	9.87E-06		
50% (Median)		2.60E-02			3.20E-01	6.11E-06		
Mean		2.60E-02			3.20E-01	6.11E-06		
Mode		2.60E-02			3.20E-01	6.11E-06		
SD		7.90E-03			9.73E-02	2.29E-06		
	ES6	ES7	ES8	ES9	ES10	ES11	ES12	ES13
5%	Negligible	Negligible	Negligible	Negligible	Negligible	3.23E-02	Negligible	Negligible
95%						1.18E-01		
50% (Median)						6.80E-02		
Mean						7.06E-02		
Mode						6.30E-02		
SD						2.63E-02		

with the vehicle treated control animals. These results were consistent with the results from the hematological and clinical chemistry analyses. However, for the animals treated with 128 mg/kg b.w. at day 6 a decrease in body weight and weight of heart, liver, spleen, thymus was observed whereas adrenal weights were increased, the latter probably indicating a stress response due to the toxicity of the Cu₂(OH)₂CO₃ nanoparticles. In addition, several clinical chemistry parameters in the blood were affected (e.g. white blood cell increase, red blood cell decrease, and increases in ALT, AST, and LDH). Histopathological lesions were observed in various organs, notably the liver (hepatocellular vacuolation, hypertrophy, and necrosis, and single cell necrosis) (de Jong et al.).

3.2.2 Dose–response analysis

Deriving the PoD. The inhalation study argued that changes in the total number of inflammatory cells in the BAL can be considered a critical endpoint for inhalation risk assessment and proposed a BMR of 100% based on previous studies (Gosens et al. 2016). This BMR was used to calculate a BMDL of 0.16 mg/m³ and a BMDU of 0.29 mg/m³ by means of PROAST (Gosens et al. 2016). This BMD lognormal distribution was used as the PoD for risk assessment.

As far as CuO ingestion toxicity is concerned, based on the short-term oral exposure, a LOAEL for decrease of total body weight corresponding to 512 mg/kg was estimated (de Jong et al.). We divided this value by an UF of 3 to calculate a NOAEL of 170.67 mg/kg. The short-term oral study of the

Cu₂(OH)₂CO₃ derived a LOAEL of 128 mg/kg, which we similarly divided by an UF of 3 to estimate a NOAEL of 42.67 mg/kg b.w. These NOAEL values were corrected by means of APROBA (when needed) for differences in human and experimental exposure conditions and in respiratory volumes between experimental animals (at rest) and humans (light activity) and then used as PoD for risk assessment.

Selecting the uncertainty factors. The selected UF for CuO are for interspecies scaling, interspecies toxicokinetics and toxicodynamics, intraspecies differences, and differences in duration of exposure for both ingestion and inhalation. The selected UF for Cu₂(OH)₂CO₃ are interspecies scaling, interspecies toxicokinetics and toxicodynamics, intraspecies differences, and differences in duration of exposure for the ingestion pathway. The probabilistic distributions of these factors are the default values suggested by APROBA and reported in Table 1.

Deriving the distributions of HD. The PoD were used as inputs to APROBA, which was applied with the above inter- and intraspecies scaling and uncertainty factors as shown in Table 4 to derive lognormal distributions of long-term HDs for local and systemic effects due to both inhalation and ingestion of CuO and only ingestion of Cu₂(OH)₂CO₃. The results are reported in Table 5.

3.3 Risk characterization and uncertainty analysis

Figure 2 and Table 6 display the risks along the lifecycles of the investigated products and the associated sources of uncertainty estimated by means of

Table 4. APROBA input data and output results.

	Notes	Unit of measure	CuO inhalation	CuO ingestion	Cu ₂ (OH) ₂ CO ₃ ingestion
Inputs to APROBA					
Data type			CuO inhalation Continuous	CuO ingestion Continuous	Cu ₂ (OH) ₂ CO ₃ ingestion Continuous
Target BMR		%	100	5	5
PoD type			BMDL	NOAEL	NOAEL
PoD unit of measure			mg/m ³	mg/kg bw/d	mg/kg bw/d
PoD value			0.16	170.67	42.67
BMDU (in case of using the BMD approach)			0.29	–	–
Reference to support the PoD selection			Gosens et al. (2016)	de Jong et al	de Jong et al
Factor used to correct PoD to consider differences in human and experimental exposure conditions	Workers are assumed to be exposed 8 hours per day. This correction factor applies in case of inhalation studies		0.375	n.a.	n.a.
Exposure conditions		h/d	3	n.a.	n.a.
Factor used to correct PoD for differences in respiratory volumes between experimental animals (at rest) and humans (light activity)	This correction factor applies in case of inhalation studies		0.67	n.a.	n.a.
Corrected PoD value (BMDL)			0.040	–	–
Corrected BMDU (in case of using the BMD approach)	PoD (BMDL) and BMDU values corrected multiplying original values by the correction factors, in case of inhalation studies		.073	–	–
Data route			Inhalation Subacute	Oral Subacute	Oral Subacute
Study type			Rat	Rat	Rat
Test species			0.332	0.228	0.366
Species weight (average)		kg	70	70	10
Human weight		kg	5%	5%	1%
Population Incidence Goal (I)		%	95%	95%	95%
Probabilistic Coverage Goal		%	100	100	100
Overall deterministic UF		n.a.			
Outputs from APROBA					
NOAEL to BMD (LCL)	Uncertainty in transforming a NOAEL to BMD	n.a.	1	0.07	0.07
NOAEL to BMD (UCL)	This aspect addresses the interspecies adjustment to take into account differences in body size (e.g. allometric scaling).	n.a.	1	1.57	1.57
Interspecies scaling (LCL)	This aspect addresses remaining interspecies TK and TD (toxicokinetics and toxicodynamic differences) differences after accounting for body size differences.	n.a.	1	4.43	2.36
Interspecies scaling (UCL)	This aspect addresses uncertainty in using a less-than-chronic study (as specified in "Study type" previously) to estimate a chronic PoD.	n.a.	1	7.01	3.08
Interspecies TK/TD (LCL)	This aspect addresses the uncertainty in the amount of human variability in sensitivity. It depends directly on the "population incidence goal" entered previously	n.a.	0.333	0.333	0.333
Interspecies TK/TD (LCL)	HD distribution (lognormally distributed)	n.a.	3	3	3
Duration Extrapolation (LCL)	Unit of measure	n.a.	0.625	0.625	0.625
Duration Extrapolation (UCL)		n.a.	40	40	40
Intraspecies (LCL)		n.a.	1.77	1.77	2.24
Intraspecies (UCL)		n.a.	14.02	14.02	41.88
Results					
			Long term HD local effects mg/m ³	Long term HD systemic effects mg/kg body weight per day	Long term HD systemic effects mg/kg body weight per day
LCL (P05)			1.63-04	7.85E-02	0.041
UCL (P95)			2.88E-02	4.55E + 01	23.5

SUNDS. Three out of the 13 occupational and consumer ES resulted in RCR distributions ≥ 1 (i.e. risk present). The formulation stage ES2 had a high probability of risk compared with the other scen-

Table 5. Long-term HD log-normal probability distributions statistics for CuO (ingestion and inhalation routes) and for $\text{Cu}_2(\text{OH})_2\text{CO}_3$ (ingestion route).

	CuO inhalation	CuO ingestion	$\text{Cu}_2(\text{OH})_2\text{CO}_3$ ingestion
5%	1.63E-04	1.81E-01	4.09E-02
95%	2.88E-02	7.44E+01	2.35E+01
50% (median)	2.17E-03	3.67E+00	9.80E-01
Mean	7.48E-03	1.96E+01	6.33E+00
GM	2.17E-03	3.67E+00	9.80E-01
SD factor	4.82E+00	6.23E+00	6.90E+00

arios in the formulation lifecycle stage, with nearly 93.33% of the Monte-Carlo simulation results being ≥ 1 (i.e. 6.67% of the RCR resulted in no risk to the exposed sensitive population). Nearly 95.79% of the variation in this result was caused by uncertainty in the UFs, mainly the factor used for extrapolation from subacute to chronic effects (62.77%). In the use-stage ES4, a worst-case exposure estimation of 0.32 mg/m^3 determined a non-acceptable inhalation risk for 99.87% of the sensitive population of both workers and consumers. About 95.8% of the uncertainty in this result was again due to the UFs as the

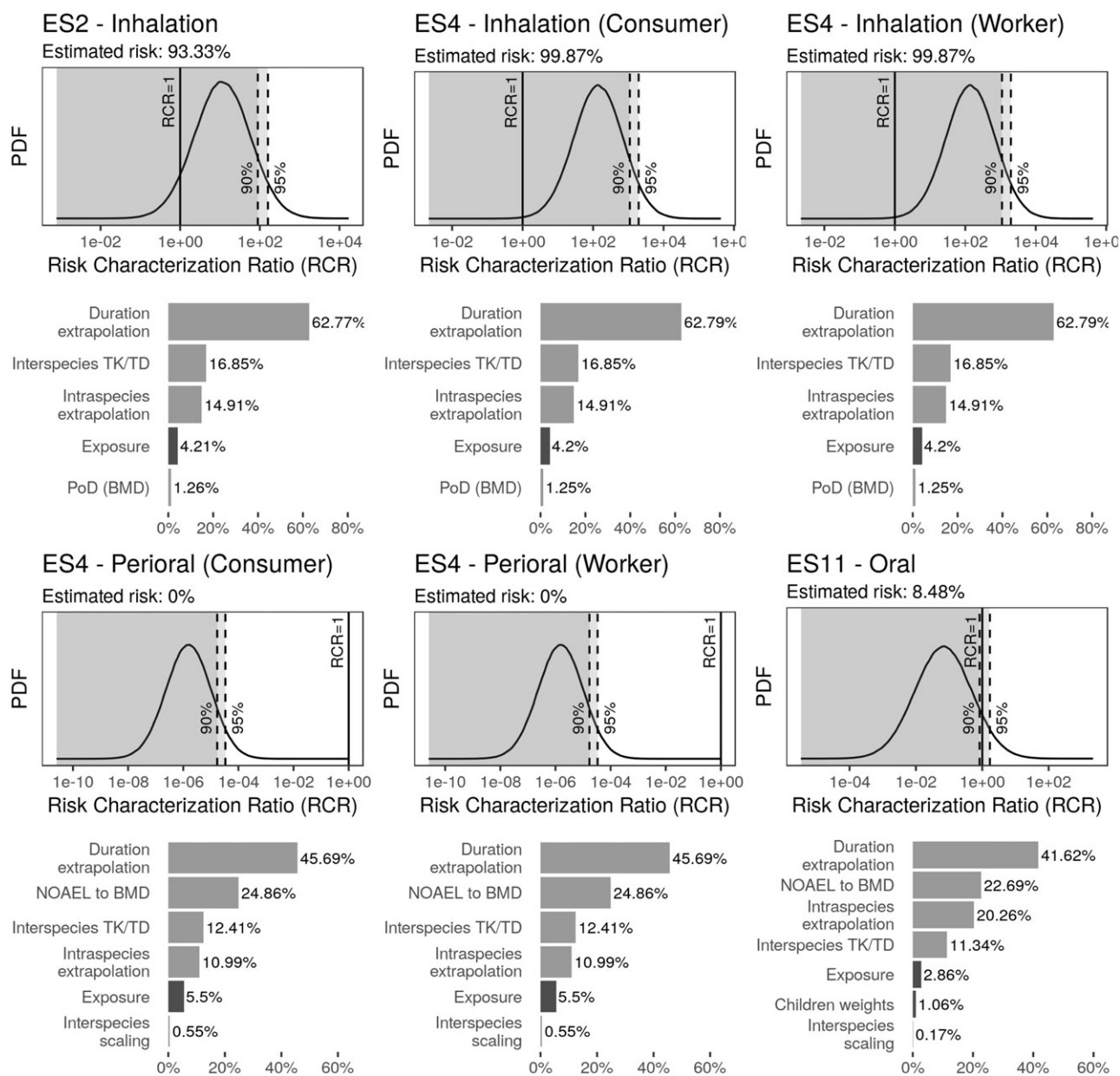


Figure 2. Risks along the lifecycles of the CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ -based products for all concerning exposure scenarios (ES). Contributions of the different sources of uncertainty to the total uncertainty, derived from over 10 000 Monte Carlo simulations, are highlighted.

Table 6. Risk characterization ratio (RCR) distributions of risk for all assessed exposure scenarios (ES). These statistics are the result from over 10 000 Monte Carlo simulations.

	ES1	ES2 inhalation	ES2 dermal	ES3	ES4 inhalation (consumer and worker)	ES4 perioral (consumer and worker)	ES4 end of life	ES5
5%	No risk	7.90E-01	No risk	No risk	9.74E+00	6.79E-08	No risk	No risk
95%	(negligible exposure)	1.60E+02	(negligible exposure)	(negligible exposure)	1.97E+03	3.37E-05	(negligible exposure)	(negligible exposure)
50%		1.12E+01			1.38E+02	1.51E-06		
Mean		4.13E+01			5.09E+02	8.96E-06		
GM		1.12E+01			1.38E+02	1.51E-06		
SD factor		5.02E+00			5.02E+00	6.60E+00		
Risk (Prob. RCR >1)		93.33%			99.87%	0.00%		
	ES6	ES7	ES8	ES9	ES10	ES11	ES12	ES13
5%	No risk	No risk	No risk	No risk	No risk	2.59E-03	No risk	No risk
95%	(negligible exposure)	(negligible exposure)	(negligible exposure)	(negligible exposure)	(negligible exposure)	1.70E+00	(negligible exposure)	(negligible exposure)
50%						6.63E-02		
Mean						4.63E-01		
GM						6.63E-02		
SD factor						7.18E+00		
Risk (Prob. RCR >1)						8.48%		

main underlying source was the extrapolation from subacute data to chronic effects. The perioral intake in ES4, instead, resulted in a safe scenario even for the most sensitive population. In contrast, the ES11 involving children exposed to the $\text{Cu}_2(\text{OH})_2\text{CO}_3$ through inadvertent ingestion is non-acceptable for 8.48% of the sensitive population. Similar to the other concerning scenarios 94.08% of the uncertainty in this result was caused by the UFs, but this time the contribution of the underlying sources was different: extrapolation from subacute to chronic effects = 41.62%; extrapolation from NOAEL to BMD = 22.69%; intraspecies extrapolation = 20.26%; interspecies toxicokinetics/dynamics = 11.34%; allometric scaling = 0.17%. The remaining 3.92% were from variation in exposure factors (i.e. exposure of the substance to sensitive children accounted for 2.86% of the uncertainty, while children weights contributed for 1.06%). The full characterization of the distributions used to perform the Uncertainty assessment is presented in the Supporting Information (Section S3), together with more detailed results for ES2 (Inhalation route of exposure), ES4 (Inhalation and Perioral routes of exposure) and ES11 (Oral route of exposure).

4. Discussion

This is the first quantitative HHRA of nanoscale CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ used for antimicrobial and antifungal treatment of wood. In contrast to the more classical deterministic approach our

probabilistic methodology was able to discriminate and communicate the different sources of uncertainty in the risk analysis to better inform the generation of additional data and/or the adoption of adequate risk management measures.

Specifically, it was possible to assess the uncertainty in the dose-response data by means of parametric bootstrapping. This enabled us to discover the largest source of uncertainty in the assessment, which was due to the extrapolation of the BMD derived from subacute animal experiments to long-term human dose. Therefore, in order to increase the confidence in our results it is important to repeat the analysis once subchronic or chronic *in vivo* inhalation and ingestion data become available.

Other considerable sources of uncertainty were the inter- and intraspecies UF. These default values were defined for regular chemicals based on historical precedence and if we assume that the CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoforms act according to different mechanisms of toxicity, then these factors may turn out to be inaccurate. In order to reduce this type of uncertainty, it may be necessary to establish nano-specific UF based on extensive analysis of the available physicochemical and toxicity data for nanomaterials.

The results from the dose-response analysis largely depend on the BMR. There is a lack of consensus among toxicologists regarding what effect size may demarcate adverse from non-adverse and there is an agreement that the BMR

may differ significantly among endpoints. Therefore, some authors suggested the evaluation of an uncertainty distribution for the BMR (Van Der Voet and Slob 2007), but we did not do this in our study. Instead, we used predefined values, which helped to communicate which BMR corresponds to which BMD distribution, but prevented us from considering this important parameter in the uncertainty analysis.

Other uncertainty arises from the fact that from the short-term exposure studies it is difficult to predict if (sub)chronic endpoints like sustained inflammation or fibrosis will be affected at longer exposures. In the short-term inhalation study, we found that lung inflammation was not completely resolved after day 22 but did not lead to fibrosis, while copper levels in the lung returned to baseline levels (Gosens et al. 2016). To reduce this kind of uncertainty, it is necessary to plan longer-term experiments considering a broader range of endpoints.

The exposure assessment of the dry nanoscale powders in this study was determined for worst-case scenarios, as risk management measures (e.g. emission controls, efficiency of local exhaust ventilation) that may reduce their airborne concentrations were not considered. Therefore, the impact of possible overestimations of exposures from powder handling in the workplace may have been significant in determining the high estimated risks associated with these scenarios. Therefore, these risks could be easily managed by applying appropriate risk management measures (e.g. engineering controls, personal protective equipment).

The potential risks of ES11 that involve children ingesting CuO or $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoparticles after hand to mouth transfer would be more difficult to control. In this case, the potentially most effective measures to be considered involve safety by design measures to reduce the release potential and/or the hazard of the material as well as consumer labeling and safety instructions.

Other sources of uncertainty in the exposure assessment may result from the fact that only external doses were considered in this study, while due to insufficient data the uptake and the translocation of the substances in the organism were not considered. Particle size distributions strongly influence the deposition pattern of nanoparticles in the lungs

and their dissolution kinetics in cases of soluble particles such as CuO or $\text{Cu}_2(\text{OH})_2\text{CO}_3$ (Gosens et al. 2016).

There are also considerable uncertainties in the measured external exposure concentrations in the air as they may quickly decline due to aggregation, agglomeration or surface deposition (Schneider and Jensen 2009). This means that nanoscale fractions measured close to the emission source may be eliminated by the time the particles are deposited in the lungs. Some specific sources of uncertainty that were not explicitly defined in this study include for example the time length of each work activity, the time-length of pauses between work activities. Exact values of these parameters will not always be known but can have a significant influence on nano-specific transformation processes such as aggregation, and thus on the overall inhalation exposure to nanoparticles.

In the lungs or the intestine the particles might completely dissolve, which would mean that only ions are uptaken in the systemic circulation and are translocated to the secondary organs. These phenomena could differ between species and the effects observed in animals could follow different mechanisms of toxicity as compared to the effects in humans. In order to reduce these uncertainties it is essential to perform kinetic studies and to appropriately measure or model the dissolution as well as the absorption, distribution, metabolization and excretion (ADME) kinetics of the investigated substances. The results from the kinetic studies that we performed in the SUN project showed that after short-term inhalation of CuO pristine nanoparticles, no other organs besides the nose and lung were affected based on histological analysis and organ weights. This could be explained by the lack of any increase in Cu levels compared with background levels in the liver, blood, brain, bone marrow, heart, kidney, and spleen at the applied exposure levels. After oral administration of CuO nanoparticles at day 6 increased levels of Cu was noted mainly in liver and lung starting at a dose of 32 mg/kg b.w. After oral administration of $\text{Cu}_2(\text{OH})_2\text{CO}_3$ nanoparticles increased Cu levels were observed in liver, lung, kidney, spleen, thymus, mesenteric lymph nodes, and to a lesser extent in testes and brain. This clearly shows that the two investigated materials have very different ADME profiles, but as long as

we do not understand their dissolution kinetics we can only guess what are the underlying reasons for this.

In general, nanomaterials (incl. CuO and Cu₂(OH)₂CO₃) are offered in many different grades that are optimized in terms of physicochemical properties for integration into specific applications. This study is a case-specific risk assessment where the nanomaterials used in the exposure and the hazard studies are similar. This is, however, often not the case and in order to avoid excessive case-by-case testing we should search for possibilities to group them based on similar physico-chemical, release, exposure, bio-kinetic, or toxicological information in order to facilitate read-across, which could reduce testing costs and the use of experimental animals.

5. Conclusions

Our assessment demonstrated unacceptable inhalation risks of CuO for worst-case ES involving handling of dry powders and sanding operations. In addition, we identified potentially unacceptable ingestion risks for the sensitive population of children exposed to Cu₂(OH)₂CO₃ nanoparticles by hand to mouth contact with impregnated wood. It should be noted, however, that there are significant uncertainties in these results, which should be resolved by additional testing. Therefore, the conclusion “unacceptable risk” may stem from the safety margin of extrapolations to fill data gaps and is, therefore, not a proof of actual risks.

Our analysis demonstrated that the main source of uncertainty is the extrapolation from subacute to long-term exposure, which was necessary due to the lack of (sub)chronic *in vivo* studies with CuO and Cu₂(OH)₂CO₃. Considerable uncertainties also stemmed from the use of default inter- and intraspecies UF for chemicals. The proposed approach is currently suited only for case-by-case risk assessments, but will also involve grouping and read-across for more efficient risk assessments in the future.

Disclosure statement

The authors report no conflicts of interest.

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ORCID

Flemming R. Cassee  <http://orcid.org/0000-0001-9958-8630>

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