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

Hristozov, Danail [1,2]; Pizzol, Lisa [1,2]; Zabeo, Alex [1,2]; Basei, Gianpietro [1]; Mackevica, Aiga [3]; Hansen, Steffen Foss [3]; Gosens, Ilse [4]; Cassee, Flemming R. [4,10]; de Jong, Wim [4]; Koivisto, Joonas [5]; Neubauer, Nicole [6]; Sanchez Jimenez, Araceli [7]; Semenzin, Elena [1]; Subramanian, Vrishali [1]; Fransman, Wouter [8]; Jensen, Keld Alstrup [5]; Wohlleben, Wendel [6]; Stone, Vicki [9]; Marcomini, Antonio [1]

1. Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice, Italy
2. Greendecision Srl., Venice, Italy
3. Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark
4. National Institute for Public Health and the Environment, Bilthoven, Netherlands.
5. National Research Centre for the Working Environment, Copenhagen, Denmark.
6. BASF SE, Ludwigshafen, Germany
7. Institute of Occupational Medicine, Edinburgh, United Kingdom
8. Netherlands Organisation for applied scientific research TNO, Zeist, Netherlands
9. Heriot-Watt University, School of Life Sciences, Nanosafety Research Group, Edinburgh, United Kingdom
10. Institute of Risk Assessment Studies, Utrecht University, Netherlands.

Abstract

The use of nano-scale copper oxide (CuO) and basic copper carbonate ($Cu_2(OH)_2CO_3$) in both ionic and micronized wood preservatives has raised concerns about the potential of these substances to cause adverse humans health effects. To address these concerns, we performed quantitative (probabilistic) human health risk assessment (HHRA) along the lifecycles of these chemicals used in antifungal wood coatings and impregnations. 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 $Cu_2(OH)_2CO_3$ in a scenario involving the hand-to-mouth transfer of 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 may be resolved by additional testing.

The adopted stochastic approach was preferred to deterministic analyses in the sense that it can communicate the contribution of each source of uncertainty and therefore can help in developing strategies to reduce it. Our analysis demonstrated that the main source of uncertainty is the extrapolation from short to long term exposure, which was necessary due to the lack of (sub)chronic *in vivo* studies with CuO and $Cu_2(OH)_2CO_3$. Considerable uncertainties also stemmed from the use of default inter- and intra-species extrapolation factors. The proposed approach is currently unable to assess the uncertainties resulting from using data from studies involving different nanoforms of the same substance, which makes it only suitable to apply on a case-by-case basis.

Keywords: Probabilistic human health risk assessment, Engineered nanomaterials, Copper oxide; Copper carbonate; Occupational and consumer exposure scenarios, Benchmark dose, SUN Decision Support System

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 (Freeman and McIntyre 2008, Lebow and Foster 2005).

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 80s. Some key examples include the alkaline copper quaternary (ACQ), copper azole, and copper xyligen. 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 of metal fasteners and subsequent structural failure.

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 ($Cu_2(OH)_2CO_3$) or copper oxide (CuO) with

62 dispersing agents in a carrier solution (Freeman and McIntyre 2008). The size of the resulting particles
63 ranges from 1 to 25000 nm, with typically 90% of the particles below the size of 1000 nm (Freeman and
64 McIntyre 2008). Leaching is significantly controlled in micronized wood treatments as compared to ionic
65 wood treatments, and less than 5% of it was in particulate form (Platten et al. 2014). While a proportion
66 of micronized copper formulations are nano-sized (Freeman and McIntyre 2008), the potential
67 additional advantage offered by copper formulations within the nano-size range are even more
68 substantially being considered (Clausen 2007, Evans et al. 2008, Kartal et al. 2009). Clausen (2007)
69 argues that dispersion stability coupled with controlled particle size in nano-sized wood preservative
70 formulations may greatly improve preservative penetration, treatability of refractory wood species and
71 stability of finishes and coatings for above ground applications. Accordingly, nanoparticles of CuO and
72 $Cu_2(OH)_2CO_3$ have been increasingly considered for micronized wood treatment formulations (Clausen
73 2007, Evans et al. 2008, Kartal et al. 2009).

74 The increased use of nano-scale CuO and $Cu_2(OH)_2CO_3$ as timber preservatives has raised concerns about
75 the potential of these substances to cause undesirable human health effects. In spite of the fact that
76 ionic copper formulations are currently thoroughly reviewed in Europe for their human and
77 environmental risks under the Biocidal Products regulation (Regulation (EU) No 528/2012), there are
78 only few studies that attempted to assess their risks (US EPA 2003, Civardi et al. 2015). Therefore, we
79 performed a quantitative human health risk assessment (HHRA) of nano-scale CuO and $Cu_2(OH)_2CO_3$
80 along the lifecycles of antimicrobial/antifungal coatings and impregnations. This is the first quantitative
81 estimation of the risks from these products from lifecycle perspective.

82 We applied the HHRA framework for regular chemicals as it has been considered by the European
83 Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) applicable to
84 nanomaterials (SCENIHR 2009). This approach consists of hazard identification, dose-response
85 assessment, exposure assessment and risk characterization steps (Van Leeuwen and Vermeire 2007).
86 We applied it as a probabilistic methodology designed to quantitatively estimate and communicate the
87 uncertainties in each of these steps in order to demonstrate how they influence the final results (Tsang
88 et al. 2017, Pang et al. 2017). Then we implemented this methodology as a software module in the web-
89 based EU FP7 SUN project's Decision Support System (SUNDS), which enabled it to estimate
90 occupational, consumer and public health risks from manufactured nanomaterials along the lifecycles of
91 nano-enabled products.

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 $Cu_2(OH)_2CO_3$. 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 on a CuO-based acrylic coating and a $Cu_2(OH)_2CO_3$-containing impregnation.

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 $Cu_2(OH)_2CO_3$ 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 m^2/g and a bulk material density of 6.3 g/cm^3 according to the supplier. To check consistency with these data and complement them, we performed detailed physicochemical characterisation 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 characterisation 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 x 2.5 x 1 cm (n=70). Some of the blocks were coated entirely with a CuO-free (TiO_2) coating to serve as a negative control. The rest of them were coated on one side with the TiO_2/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

121 ES (cf. 2.2.2). The coatings were thoroughly characterised, and the results are reported in Pantano et al.
122 (2018) and in the Supporting Information (Tables SI_2 and SI_3).

123 ### *2.1.2 $Cu_2(OH)_2CO_3$ used in an antimicrobial/antifungal wood protective impregnation*

124 Dispersed $Cu_2(OH)_2CO_3$ nanoparticles were obtained from PlasmaChem GmbH, Berlin, Germany. In the
125 process of formulating an impregnation solution, the basic copper carbonate was wet milled until it
126 reached nano-sized particles. The $Cu_2(OH)_2CO_3$ was then combined with water, stabilisers and co-
127 biocides to make the stock solution. Small wood blocks were then immersed/soaked in this
128 impregnation dispersion. This was adequate for research purposes, but on industrial scale pressure
129 impregnation is typically carried out in steel cylinders or retorts. The wood is loaded on special tram cars
130 and moved into the retort, which is then closed, evacuated and subsequently filled with preservative
131 solution. Then pressure forces the preservative into the wood until the desired amount is absorbed.

132 The results of the performed detailed physicochemical characterisation of the micronised $Cu_2(OH)_2CO_3$
133 suspention and the impregnated wood are reported in Pantano et al. (2018) and are summarised in the
134 Suplemental Information (Tables SI_2 and SI_3).

135 ## *2.2 Risk assessment by means of SUNDS*

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

146 For each ES, based on a combination of the exposure assessment (estimation of external concentration)
147 and hazard assessment (estimation of human effect threshold dose) the system produces a discrete
148 value or a probability distribution of risk and the associated uncertainty. To do this, SUNDS uses
149 exposure measurements, or if such are not available exposure can be estimated by means of models

150 (e.g. NanoSafer, Ingestion Exposure Tool) that are either integrated in the system or interact with it
151 externally. To assess a human effect threshold SUNDS can use *in vivo* raw data to perform dose-
152 response analysis by means of a dedicated model and then to correct the obtained Point of Departure
153 (PoD) (i.e. Benchmark Dose (BMD)) and extrapolate it to a human dose (HD) by means of the APROBA
154 tool, which is integrated in the system. In some cases, the PoD (e.g. BMD or No-observed Adverse Effect
155 Level (NOAEL)) is available from the published literature and therefore can be directly imported in the
156 system instead of analysing raw data. This is the case of this risk assessment, where the dose-response
157 analysis involved PoD estimated in other studies, which were only corrected and extrapolated to HD by
158 means of SUNDS/APROBA as it is described in 2.2.2.

159 *Insert Figure 1 here*

160 The following sections 2.2.1-2.2.3 describe how the SUNDS HHRA module was applied for exposure and
161 hazard assessment of the case-studies presented in 2.1 to assess occupational and consumer risks along
162 their lifecycles and to communicate the associated uncertainties.

163 ### *2.2.1 Exposure assessment*

164 #### *2.2.1.1 Formulation of exposure scenarios*

165 To gather the knowledge and expertise needed to formulate realistic workplace and consumer ES we
166 performed a literature review, organised a dedicated workshop and obtained additional contextual
167 information from the industrial companies BASF and Koppers Inc.

168 Specifically, published literature from 2000 to 2016 was searched for relevant release and exposure
169 assessment studies. To do this we queried the Web of Science database with combinations of the
170 following keywords: nano, copper oxide, copper carbonate, micronized copper, CuO, $CuCO_3$,
171 $Cu_2(OH)_2CO_3$, paint, impregnation, exposure assessment, release, emission, exposure, workplace,
172 consumer, use. The literature search resulted in a small number of documents, which were carefully
173 analysed. In addition, mapping of release hot spots along the lifecycles of the investigated products was
174 performed as part of the SUN project (Steinfeldt 2017). We used these results as a basis to design the
175 exposure assessment expert workshop.

176 The workshop took place on 22 January 2016 in Venice and was attended by 22 academic and industrial
177 experts in human exposure assessment and copper-based timber preservatives from EU, US and Russia.
178 The discussions resulted in generic ES, which were then further elaborated with information obtained

179 from the literature and from the industrial companies BASF and Koppers Inc. The formulated ES covered
180 the entire lifecycles (i.e. synthesis, formulation, use, end-of-life) of the investigated products and are
181 listed in Table 2. In cases when estimations of exposure were not available in the literature, such were
182 derived in the SUN project by means of the experimental and modelling methods described in 2.2.2.

183 ***2.2.1.2 Estimation of exposure***

184 The following experimental and modelling activities were performed with our case study products (cf.
185 2.1) in order to derive exposure estimations for each of the formulated ES for performing risk
186 assessment by means of SUNDS. The numbering of the different ES corresponds to Table 2.

187 ***ES 1 and 6 involving laboratory production, handling and packing of nanoscale CuO and $Cu_2(OH)_2CO_3$***
188 ***powders***

189 CuO and $Cu_2(OH)_2CO_3$ nanoscale powders are produced using sol gel synthesis. The sol gel synthesis and
190 packing were performed in a fume hood where the bags were canned, and the cans were subsequently
191 moved to a storage room. Occupational exposure measurements were performed, which resulted in
192 breathing zone and far field respirable mass concentrations below the minimum detection limits of 161
193 and 26 µg m^{-3}, respectively (Fonseca et al.). The surface wipe samples analysed with a Scanning Electron
194 Microscope (SEM) did not reveal any CuO particles (Fonseca et al.). Therefore, based on these results we
195 concluded that the exposure levels for ES 1 and 6 are negligible.

196 ***ES 2 and 7 involving pouring nanoscale CuO powders in the wood coating stock solutions***

197 The nanoscale CuO pouring to the liquid matrix was not measured. The exposure levels were estimated
198 by means of a one-box model (Hewett and Ganser 2017). Laboratory scale powder mixing was assumed
199 to be performed without using any emission controls (i.e. worst-case scenario). The parameters used for
200 modelling of manufacturing 100 L CuO preservative are the following: Dustiness index = 104 mg/kg
201 (moderate); mass flow = 1 kg/min (careful pouring); handling energy = 1 (equivalent release as in
202 dustiness test); local emission controls = 1 (no control); pouring amount = 2.5 kg (poured from 1 kg bags,
203 9 minutes between pourings); room volume = 20 m^3 (small room); ventilation rate = 2 h^{-1}. The results of
204 the modelling are reported in Table 2.
205

206 ***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 µm 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 modelling of sanding are the following: Emission rate = 20 µg/sec where 2 % is CuO_2 (sanding disc dimeter 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)[20]; near-field air flow = 10 m^3/min. This resulted to a near-field (NF) concentration of 93 µg/m^3 during continuous process. The results of the modelling 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 described in detail in Mackevica et al. (submitted) 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 were 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 $Cu_2(OH)_2CO_3$ impregnated wood by skin transfer of copper to the month 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 $Cu_2(OH)_2CO_3$ 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 x 2.7 x 1.1 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 $Cu_2(OH)_2CO_3$, Platten et al. (2014) reported results from leaching tests indicating that mostly ionic copper (>~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.

263 Based on these results, the human exposure to nanoparticles leaching during contact of CuO coatings
264 and $Cu_2(OH)_2CO_3$ impregnations with water was considered negligible.

265 ***2.2.1.3 Derivation of exposure distributions***

266 The above exposure levels were used to generate an exposure distribution (EXP_i) for each scenario *i* by
267 means of SUNDS. When only deterministic values were available, normal or lognormal distributions
268 were used to describe the probabilistic distribution of exposure as recommended by the US
269 Environmental Protection Agency (US EPA 2001). Such distributions were created around the available
270 deterministic values by fitting a one order of magnitude (+/-50%) wide confidence interval around the
271 mean exposure estimate. The reason for this is that the exposure levels were estimated based on
272 measurements or models, which introduce uncertainties in the EXP_i. Indeed, measurements are
273 obtained by instruments, which present known errors, but many other aspects (e.g. preparing the
274 samples, positioning of the instrument) add more uncertainties (often larger than the instrument
275 errors). Moreover, the application of the one box and two box exposure models (Ganser and Hewett
276 2017) also introduced uncertainties associated with certain assumptions.

277 ***2.2.2 Hazard assessment***

278 ***2.2.2.1 Hazard identification***

279 To identify the hazards of CuO and $Cu_2(OH)_2CO_3$ nanoparticles, a literature review was peformed, which
280 showed that dedicated *in vivo* inhalation or oral studies that considered multiple exposure doses (and
281 were therefore suitable for dose-response assessment) did not exist (Gosens et al. 2016). Therefore, we
282 designed and performed short-term inhalation and short-term oral studies in order to derive subacute
283 data that according to the REACH Guidance on Chemical Safety Assessment (ECHA 2008) and the
284 Guidance on Biocides Legislation (ECHA 2017) can be extrapolated for use in long-term HHRA. The used
285 pristine nanomaterials and dispersions were the same described in 2.1. The study designs are only
286 shortly outlined in this section as they are described in detailed in Gosens et al (2016) and De Jong et al
287 (submitted).

288 **Short-term inhalation exposure**

289 After an acclimatization period, rats (8 weeks old, HsdCpb: WU) were exposed nose-only to a single
290 generated exposure concentration of CuO nanomarticles or to clean air as a control for 5 consecutive
291 days. By exposing the animals for various durations (18 min, 36 min, 90 min, 3 h, and 6 h), different dose

292 levels were obtained. A 6 h concentration equivalent was derived by multiplying the duration of
293 exposure by the exposure concentration (designated as dose C x T) and scaling it to the highest exposure
294 duration of 6 h to 13.2 mg/m^3 (for animals dedicated for toxicological examination) or 11.6 mg/m^3 (for
295 animals dedicated for organ burden analysis). Repeated exposures to CuO nanoparticles via inhalation
296 resulted in a linear increase in the determined lung burden, justifying the applied C x T concept.

297 **Short -term oral exposure**

298 Male rats (RjHan: WI, bred Specific Pathogen Free, barrier maintained during experiment) of 8-9 weeks
299 old were obtained from Janvier Labs (Le Genest-Saint-Isle, Saint Berthevin, France). The CuO nanopaticle
300 dispersions were orally administered by gavage using the following exposure doses: vehicle control, 1, 2,
301 4, 8, 16, 32, mg/kg body weight (b.w.) and a pilot study with 64 mg/kg b.w. The doses were chosen
302 based on information in the literature of soluble non-nano $CuSO_4$, which indicated a No-observed
303 Adverse Effect Level (NOAEL) of 16.3 mg/kg (Hébert 1993). The dose was administered as 0.1 ml per 20
304 g (1 ml per 200 g). In an additional study one group of animals (n=4) was exposed to a high dose of 512
305 mg/kg b.w. For the $Cu_2(OH)_2CO_3$ nanoparticles the administered doses were, vehicle control, 4, 8, 16, 32,
306 64, and 128 mg/kg b.w. The animals were treated on five consecutive days (days 1-5) and autopsy was
307 performed 24 hours after the last oral administration (day 6). In addition, a recovery period of 3 weeks
308 was included in the experiments to evaluate recuperation and possible persistence of the nanomaterials
309 in the body. Autopsy of the recovery groups was performed on day 26, after three weeks of recovery.

310 ***2.2.2.2 Dose-response assessment***

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

315 The dose-response assessment of the ingestion data was also not performed by means of SUNDS, but by
316 De Jong et al (submitted), who derived a Lowest Observed Adverse Effect Level (LOAEL) for decrease of
317 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 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 extrapolation factors (EF) (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. 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.

Insert Table 1 here

2.2.3 Risk characterization & 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 populaiton. $RCR_{i,M}^I = EXP_i/HD_M^I$, where $EXPi$ 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% to 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

347 acceptable if the 90th percentile of the population is safe, but more conservative values (i.e. the 95th
348 percentile or the 99th percentile) can also be selected (USEPA 2001; USEPA 2014a; USEPA 2014b).

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

356 The contribution of different sources to the overall uncertainty in the RCR was estimated for each ES by
357 means of Monte Carlo. In each of the 10 000 simulations RCR was numerically estimated by randomly
358 sampling 10 000 elements from the distributions of the PoD, exposure and UF. The contribution of each
359 of these factors to the uncertainty in the risk estimate was quantified by assessing the level of
360 correlation between the factor and the resulting RCR by means of the squared Spearman's rank
361 correlation coefficient [8].

362 # 3. Results

363 ## *3.1 Exposure assessment*

364 13 ES were formulated that covered the entire lifecycles (i.e. synthesis, formulation, use, end-of-life) of
365 our case-study products: CuO-based coating paint and $Cu_2(OH)_2CO_3$-containing impregnation (Table 2).

366 The exposure assessment of the CuO-based coating demonstrated that release of nanoparticles is
367 possible at each lifecycle stage and can lead to both worker and consumer exposure in different
368 formulations: as nanopowder, as liquid paint, or as a cured surface coating on wood. The handling of dry
369 powders led to some significant exposure potential in the formulation lifecycle stage. If paint spraying is
370 avoided, inhalation exposure to paint is assumed negligible during its application, but dermal and oral
371 exposure could be relevant for both workers and consumers either via accidental dermal deposition
372 when treating (painting) the wood or via hand-to-mouth (i.e. inadvertent oral) exposure. However,
373 according to the latest studies dermal exposure is insignificant (Platten et al. 2016). Moreover, the
374 dermal transfer testing of the painted wooden blocks (cf. 2.2.2) showed that there was nearly no release
375 of nanoscale CuO from the paint matrix during surface wiping tests (Mackevica et al.). However, after

376 sanding of the paint surface, the observed CuO release was magnitudes higher. Accordingly, inhalation
377 and inadvertent oral exposures were assessed in the case of occupational and consumer use during
378 sanding activities and the results were used to represent also sawing operations.

379 The analysis of the literature on $Cu_2(OH)_2CO_3$-impregnated wood showed that the release of copper
380 nanoparticles is typically negligible. The US EPA report provided an estimate of exposure for the
381 concerning ES11 that involves children exposed directly to the treated wood by skin contact, transfer of
382 copper to the month and subsequent ingestion (Platten et al. 2014). Moreover, two other common
383 exposure pathways were identified and assessed: leaching during contact with water and transfer during
384 physical contact (cf. Table 2).

385 *Insert Table 2 here*

386 We used SUNDS to generate EXP_i probability distributions for each ES based on the estimated exposure
387 levels, which demonstrated significant exposure potential for scenarios 2, 4 and 11 (Table 3). To account
388 for unknown uncertainties due to measurement and modelling errors we established a one order of
389 magnitude wide confidence interval around the deterministic inhalation exposure estimates for ES2 and
390 ES4 (0.026 mg/m^3 and 0.36 mg/m^3, respectively) and fitted the corresponding normal distributions. In
391 ES11, starting from an exposure of 1.11 mg/day derived by averaging three visits to the playground over
392 a week (Platten et al. 2014), we built a normal distribution representing uncertainty in the number of
393 weekly visits characterized by the 5th percentile at 1.11/3 mg/day and the 95th percentile at 1.11
394 mg/day. This bell-shaped curve was then divided by a uniform mixture of normal distributions
395 representing the variability of weights of children (girls) aged from 8 to 36 months (mean: 10.95 kg, SD:
396 2.18, $CI_{5\%}$: 7.6 kg, $CI_{95\%}$: 14.68 kg).

397 *Insert Table 3 here*

398 ## *3.2 Hazard Assessment*

399 ### *3.2.1 Hazard identification*

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

403 ***Short-term inhalation exposure***

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

408 After a recovery period of 22 days, limited lung inflammation was still observed leaving a small but
409 significant elevation of macrophages in the airspace (at the highest dose of 13.2 mg/m^3. This
410 inflammation was not accompanied by pathological changes or elevated biochemical markers of fibrosis.
411 The histological alterations of the olfactory epithelium in the nose restored completely after 22 days. No
412 histopathological changes were detected in the brain, olfactory bulb, spleen, kidney and liver. In
413 conclusion, a 5-day, 6-hour/day exposure equivalent to an aerosol of agglomerated CuO nanoparticles
414 resulted in a dose-dependent toxicity in rats, which almost completely resolved during a 3-week post-
415 exposure period. The data for all endpoints measured were compared via the BMD calculated by
416 PROAST. This allowed a ranking of the relative sensitivity of each endpoint to the inhaled CuO
417 nanoparticles with biochemical markers and inflammatory cell number in the bronchoalveolar lavage
418 fluid providing to be the most sensitive indicators for lung toxicity (Gosens et al. 2016) .

419 ***Short-term oral exposure***

420 Copper oxide: In the dose response study with a maximum dose of 64 mg/kg, no signs of toxicity were
421 noted. After treatment of 5 consecutive days there was no difference in body weight between day 1
422 (start of treatment) and day 6 (24 hours after end of treatment). In the addional group of animals
423 treated with 512 mg/kg some indications for toxicity were observed based on changes in the body
424 weight. Moreover, the results of the clinical chemistry showed that at day 6 alterations in the level of
425 alkaline phosphatase and aspartate aminotransferase enzymes indicated the presence of liver toxicity.
426 At the dose of 64 mg/kg lactate dehydrogenase levels were also increased indicating cell and organ
427 damage. Animals treated with 512 mg/kg showed similar alterations in clinical chemistry (low level of
428 alkaline phosphatase, high level of aspartate aminotransferase, and high level of lactate
429 dehydrogenase), and histopathological alterations in the liver (e.g. inflammation, hepatocellular

430 hypertrophy, hepatocellular necrosis) thus supporting the data of the dose response study. Therefore,
431 the dose 512 mg/kg was taken as the LOAEL.

432 Copper carbonate: For $Cu_2(OH)_2CO_3$ nanoparticles a dose response study was performed with the
433 highest dose being 128 mg/kg b.w. Repeated (5 times) oral administration of the highest dose induced
434 severe toxic responses in the treated animals as indicated by the behaviour of the animals, frequent
435 washing and piloerection. Based on these observations the animals scheduled for prolonged observation
436 (autopsy after a recovery period at day 26 after treatment) were autopsied prematurely at days 6 and 7,
437 respectively 24 and 48 hours after the last (day 5) treatment.

438 For animals treated with a dose up to 64 mg/kg b.w. both at day 6 and day 26 after treatment both body
439 and organ weights did not show a difference with the vehicle treated control animals. These results
440 were consistent with the results from the haematological and clinical chemistry analyses. However, for
441 the animals treated with 128 mg/kg b.w. at day 6 a decrease in body weight and weight of heart, liver,
442 spleen, thymus was obeserved whereas adrenal weights were increased, the latter probably indicating a
443 stress response due to the toxicity of the $Cu_2(OH)_2CO_3$ nanoparticles. In addition, several clinical
444 chemistry parmeters in the blood were affected (e.g. white blood cell increase, red blood cell decrease,
445 and increases in ALT, AST, and LDH) Histopathological lesions were observed in various organs, notably
446 the liver (hepatocellular vacuolation, hypertrophy, and necrosis, and single cell necrosis) (de Jong et al.).

447 ### *3.2.2 Dose-response Analysis*

448 ***Deriving the PoD***

449 The inhalation study argued that changes in the total number of inflammatory cells in the BAL can be
450 considered a critical endpoint for inhalation risk assessment and proposed a BMR of 100% based on
451 previous studies (Gosens et al. 2016). This BMR was used to calculate a BMDL of 0.16 mg/m^3 and a
452 BMDU of 0.29 mg/m^3 by means of PROAST (Gosens et al. 2016) . This BMD lognormal distribution was
453 used as the PoD for risk assessment.

454 As far as CuO ingestion toxicity is concerned, based on the short-term oral exposure De Jong et al
455 (submitted) a LOAEL for decrease of total body weight corresponding to 512 mg/kg was estimated. We
456 divided this value by an UF of 3 to calculate a NOAEL of 170.67 mg/kg. The short term oral study of the
457 $Cu_2(OH)_2CO_3$ derived a LOAEL of 128 mg/kg, which we similarly divided by an UF of 3 to estimate a

458 NOAEL of 42,67 mg/kg b.w. These NOAEL values were corrected by means of APROBA (when needed)
459 for differences in human and experimental exposure conditions and in respiratory volumes between
460 experimental animals (at rest) and humans (light activity) and then used as PoD for risk assessment.

461 ***Selecting the Uncertainty Factors***

462 The seleted UF for CuO are for interspecies scaling, interspecies toxicokinetics and toxicodynamics,
463 intraspecies differences and differences in duration of exposure for both ingestion and inhalation. The
464 seleted UF for $Cu_2(OH)_2CO_3$ are interspecies scaling, interspecies toxicokinetics and toxicodynamics,
465 intraspecies differences and differences in duration of exposure for the ingestion pathway. The
466 probabilistic distributions of these factors are the default values suggested by APROBA and reported in
467 Table 1.

468 ***Deriving the distributions of HD***

469 The PoD were used as inputs to APROBA, which was applied with the above inter- and intra-species
470 scalling and and uncertainty factors as shown in Table 4 to derive lognormal distributions of long-term
471 HDs for local and systemic effects due to both inhalation and ingestion of CuO and only ingestion of
472 $Cu_2(OH)_2CO_3$. The results are reported in Table 5.

473 *Insert Tables 4 and 5 here*

474 ## *3.3 Risk characterization & Uncertainty analysis*

475 Figure 2 and Table 6 display the risks along the lifecycles of the investigated products and the associated
476 sources of uncertainty estimated by means of SUNDS. 3 out of the 13 occupational and consumer ES
477 resulted in RCR distributions ≥ 1 (i.e. risk present). The formulation stage ES2 had a high probability of
478 risk compared to the other scenarios in the formulation lifecycle stage, with nearly 93.33% of the
479 Monte-Carlo simulation results being ≥ 1 (i.e. 6.67% of the RCR resulted in no risk to the exposed
480 sensitive population). Nearly 95.79% of the variation in this result were caused by uncertainty in the
481 UFs, mainly the factor used for extrapolation from subacute to chronic effects (62.77%). In the use-stage
482 ES4, a worst-case exposure estimation of 0.32 mg/m^3 determined a non-acceptable inhalation risk for
483 99.87% of the sensitive population of both workers and consumers. 95.8% of the uncertainty in this
484 result was again due to the UFs as the main underlying source was the extrapolation from subacute data

485 to chronic effects. The perioral intake in ES4, instead, resulted in a safe scenario even for the most
486 sensitive population. In contrast, the ES11 involving children exposed to the $Cu_2(OH)_2CO_3$ through
487 inadvertent ingestion is non-acceptable for 8.48% of the population. Similarly to the other concerning
488 scenarios 94.08% of the uncertainty in this result was caused by the UFs, but this time the contribution
489 of the underlying sources was different: extrapolation from subacute to chronic effects = 41.62%;
490 extrapolation from NOAEL to BMD = 22.69%; intraspecies extrapolation = 20.26%; interspecies
491 toxicokinetics/dynamics = 11.34%; allometric scaling = 0.17%. The remaining 3.92% were from variation
492 in exposure factors (i.e. exposure of the substance to sensitive children accounted for 2.86% of the
493 uncertainty, while children weights contributed for 1.06%). The full characterization of the distributions
494 used to perform the Uncertainty assessment is presented in the Supporting Information (Section S3),
495 together with the complete results for ES2 (Inhalation rout of exposure), ES4 (Inhalation and Perioral
496 routes of exposure) and ES11 (Oral route of exposure).

497

498 *Insert Figure 2 and Table 6 here*

499 4. Discussion

500 This is the first quantitative HHRA of nanoscale CuO and $Cu_2(OH)_2CO_3$ used for antimicrobial and
501 antifungal treatment of wood. In contrast to the more classical deterministic approach our probabilistic
502 methodology was able to discriminate and communicate the different sources of uncertainty in the risk
503 analysis (Figure 1) to better inform the generation of additional data and/or the adoption of adequate
504 risk management measures.

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

510 Other considerable sources of uncertainty were the inter- and intraspecies EF. These default values were
511 defined for regular chemicals based on historical precedence and if we assume that the CuO and
512 $Cu_2(OH)_2CO_3$ nanoforms act according to different mechanisms of toxicity, then these factors may turn

513 out to be inacurate. In order to reduce this type of uncertainty it may be necessary to establish nano-
514 specific EF based on extensive analysis of the available physicochemical and toxicity data for
515 nanomaterials. This requires the development of data management and curation capabilities to check
516 the quality of data prior to their analysis.

517 The results from the dose-response analysis largely depend on the BMR. There is a lack of consensus
518 among toxicologists regarding what effect size may demarcate adverse from non-adverse and there is
519 an agreement that the BMR may differ significantly among endpoints. Therefore, some authors
520 suggested the evaluation of an uncertainty distribution for the BMR (Van Der Voet and Slob 2007), but
521 we did not do this in our study. Instead, we used predefined values, which helped to communicate
522 which BMR corresponds to which BMD distribution, but prevented us from considering this important
523 parameter in the uncertainty analysis.

524 Other uncertainty arises from the fact that from the short-term exposure studies it is difficult to predict
525 that no (sub)chronic endpoints like sustained inflammation or fibrosis will be affected at longer
526 exposures. In the short-term inhalation study, we found that lung inflammation was not completely
527 resolved after 22 days but did not lead to fibrosis, while copper levels in the lung returned to baseline
528 levels (Gosens et al. 2016).

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

536 The potential risks of ES11 that involves children ingesting CuO or $Cu_2(OH)_2CO_3$ nanoparticles by skin
537 contact, transfer of copper to the month and related ingestion would be more difficult to control. In this
538 case, the potentially most effective measures to be considered involve safety by design measures to
539 reduce the release potential and/or the hazard of the material as well as consumer labelling and safety
540 instructions.

541 Other sources of uncertainty in the exposure assessment may result from the fact that only external
542 doses were considered in this study, while due to insufficient data the uptake and the translocation of
543 the substances in the organism were not considered. Particle size distributions strongly influence the
544 deposition pattern of nanoparticles in the lungs and their dissolution kinetics in cases of soluble particles
545 such as CuO or $Cu_2(OH)_2CO_3$ (Gosens et al. 2016).

546 There are also considerable uncertainties in the measured external exposure concentrations in the air as
547 they may quickly decline due to aggregation, agglomeration or surface deposition (Schneider and Jensen
548 2009). This means that nanoscale fractions measured close to the emission source may be eliminated
549 by the time the particles are deposited in the lungs. Some specific sources of uncertainty that were not
550 explicitly defined in this study include for example the time length of each work activity, the time-length
551 of pauses between work activities. Exact values of these parameters will not always be known but can
552 have a significant influence on nano-specific transformation processes such as aggregation and thus the
553 inhalation exposure to nanoparticles.

554 In the lungs or the intestine the particles might completely dissolve, which would mean that only ions
555 are uptaken in the systemic circulation and are translocated to the secondary organs. These phenomena
556 could differ between species and the effects observed in animals could follow different mechanisms of
557 toxicity as compared to the actual effects in humans. In order to reduce these uncertainties it is
558 essential to perform kinetic studies and to appropriately measure or model the dissolution as well as the
559 absorption, distribution, metabolisation and excretion (ADME) kinetics of the investigated substances.
560 The results from the kinetic studies that we performed in the SUN project showed that after short-term
561 inhalation of CuO pristine nanoaprticles, no other organs besides the nose and lung were affected based
562 on histological analysis and organ weights. This could be explained by the lack of any increase in Cu
563 levels compared to background levels in the liver, blood, brain, bone marrow, heart, kidney, and spleen
564 at the applied exposure levels. After oral adminstration of CuO nanoparticls at day 6 increased levels of
565 Cu was noted mainly in liver and lung starting at a dose of 32 mg/kg b.w. After oral adnistration of
566 $Cu_2(OH)_2CO_3$ nanoparticles increased Cu levels were observed in liver, lung, kidney, spleen, thymus,
567 mesenteric lymp nodes, and to a lesser extent in testes and brain. This clearly shows that the two
568 investigated materials have very different ADME profiles, but as long as we do not understand their
569 dissolution kinetics we can only guess what are the underlying reasons for this.

570 In general nanomaterials (incl. CuO and $Cu_2(OH)_2CO_3$) are offered in many different grades that are
571 optimized in terms of physicochemical properties for integration into specific applications. This study is a
572 case-specific risk assessment where the nanomaterials used in the exposure and the hazard studies are
573 similar. This is however often not the case in order to avoid excessive case-by-case testing we should
574 search for posibilities to group them based on physico-chemical, release, exposure, bio-kinetic or
575 toxicological information in order to facilitate read-across, which could reduce testing costs and the use
576 of experimental animals.

577 ## 5. Conclusions

578 Our assessment demonstrated unacceptable inhalation risks of CuO for worst-case ES involving handling
579 of dry powders and sanding operations. In addtion, we identified potentially unacceptable ingestion
580 risks for the sensitive populaion of childred exposued to $Cu_2(OH)_2CO_3$ nanoparticles by hand to mouth
581 contact with impregnated wood. It should be noted, however, that there are significant uncertainties in
582 these results, which should be resolved by additional testing. Therefore, the conclusion "unacceptable
583 risk" may stem from the safety margin of extrapolations to fill data gaps and is therefore not a proof of
584 actual risks.

585 Our analysis demonstrated that the main source of uncertainty is the extrapolation from subacute to
586 long-term exposure, which was necessary due to the lack of (sub)chronic *in vivo* studies with CuO and
587 $Cu_2(OH)_2CO_3$. Considerable uncertainties also stemmed from the use of default inter- and intra-species
588 UF for chemicals. The proposed approach is currently suited only for case-by-case risk assessments, but
589 will be extended to enable also grouping and read-across for more efficient analysis.

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594 ## Declaration of interest

595 The authors report no conflicts of interest.

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Tables

Table 1. Generic Uncertainty (Extrapolation) Factors (UF) for different aspects of the dose-response assessment assuming lognormal uncertainty distributions. Source: IPCS-WHO guidance document on evaluation and communication of uncertainty in hazard characterisation (IPCS-WHO 2014).

Aspect of hazard characterization	*Lognormal P50*	*Lognormal P95/P50*	*Lognormal (P05, P95)*	*Comments*
PoD uncertainty for NOAEL[a]: $AF_{PoD\text{-}NOAEL}$				
Continuous end-point, chronic/subchronic study	1/3	4.7	(0.07, 1.6)	Ratio of NOAEL to BMD_{05} (5% relative change)
Continuous end-point, developmental study	1/3	7.0	(0.05, 2.3)	Ratio of NOAEL to BMD_{05} (5% relative change)
Deterministic quantal end-point	2/9	5	(0.04, 1.1)	Ratio of NOAEL to ED_{50} (50% response)
Stochastic quantal end-point	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: $AF_{Inter\text{-}BS}$				
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: $AF_{Inter\text{-}TK/TD}$				
Oral	1	3	(1/3, 3)	Given lack of alternative, can also be used for inhalation
Intraspecies differences for incidence *I*: $AF_{Intra\text{-}I}$				
I = 5%	3.4	2.8	(1.8, 14)	$Log(GSD_H)$ P50 = 0.32
I = 1%	5.7	4.3	(2.2, 42)	and P95/P50 = 2.2
I = 0.1%	10	7.0	(2.9, 143)	

BMD_x: benchmark dose for *x*% benchmark response; bw: body weight; ED_{50}: 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

[a] When 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).

[b] For gases, the RGDR is often assumed to be 1.

747 **Table 2.** Description of the exposure scenarios assessed for nanoscale CuO used in wood coating paints and nanoscale $Cu_2(OH)_2CO_3$ used in timber preserving impregnations.

Exposure scenario (ES)	LC stage	Target	Exposure route	Exposure level (EXP_i)	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.)
		Worker	Dermal	negligible	Surface contamination was not detected, and dermal and perioral exposure are negligible*	
ES2: Pouring CuO nanoscale powder in the wood coating matrix	FOR	Worker	Inhalation	NF 26 µg/m^3 and FF 10 µg/m^3	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.	SUN project deliverable 5.4
		Worker	Dermal	negligible	Surface contamination: dermal and perioral exposure are negligible*	
ES3: Applying CuO wood coating to the substrate	USE	Worker	Inhalation	negligible	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)
		Worker	Dermal	negligible	Surface contamination: dermal and perioral exposure are negligible*	
ES4: Sanding, cutting, drilling and sawing wood treated with CuO preservative	USE	Worker, Consumer	Inhalation	93 µg/m^3	Modelled NF CuO_2 concentration during continuous outdoor sanding.	SUN project deliverable 5.4
		Worker, Consumer	Dermal, Perioral	Dermal: negligible Perioral: 6,11E-06 (SD 2.29E-06) mg/kg/day	Surface contamination: dermal exposure is negligible, while perioral exposure has been assessed for consumers based on the IEAT model and considering an average of 6,3 hand-to-mouth (oral or perioral) contacts per day.	SUN project deliverable 5.4, (Gorman Ng et al. 2016)
	EOL	Worker	Inhalation	negligible	The percentage of treated wood in the waste is very low, thus reducing the emission of CuO.	(Heggelund et al. 2016)
		Worker	Dermal	negligible		
ES5: Consumers transfer to skin from surfaces by rubbing	USE	Consumer	Dermal	negligible	The wiping test performed in the SUN project indicated insignificant transfer to the skin.	(Mackevica et al. In preparation)
ES6: $Cu_2(OH)_2CO_3$ powder production, handling and packing	SYN	Worker	Inhalation	negligible	In this study, we assume that the occupational exposure levels during $Cu_2(OH)_2CO_3$ 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.)
		Worker	Dermal	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	
ES7: Milling of $Cu_2(OH)_2CO_3$ slurry for the impregnation stock solution	FOR	Worker	Inhalation	negligible	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
		Worker	Dermal	negligible	Surface contamination: dermal exposure is negligible since emissions are negligible	
ES8: Workers impregnating wood in an industrial setting	USE	Worker	Inhalation	negligible	For the vacuum pressure treatment process in industrial scenario, the emissions to air are limited. So, no relevant exposure scenarios are assessed	(US EPA 1995)
		Worker	Dermal	negligible	Surface contamination: dermal and perioral exposure are negligible*	
ES9: Workers constructing garden fences, decking, cladding, playgrounds, vegetable gardens using the treated wood	USE	Worker	Inhalation	negligible	For waterborne preservatives, very low emissions to air	(US EPA 1995)
		Worker	Dermal	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	
ES10: Consumer transfer to skin from surfaces by rubbing	USE	Consumer	Inhalation	negligible	Inhalation exposure is assumed negligible	(Mackevica et al. In preparation)
		Consumer	Dermal	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	
ES11: Children exposed directly to the treated wood by skin contact,	USE	Consumer	Oral	0.07 (SD 0.03) mg/kg/d	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)

transfer of copper to the month and related ingestion						
ES12: Sanding, cutting, drilling and sawing wood treated with $Cu_2(OH)_2CO_3$ preservative	EOL	Worker	Inhalation	negligible	The percentage of treated wood in the waste is very low, thus reducing the emission of $Cu_2(OH)_2CO_3$.	(Heggelund et al. 2016)
		Worker	Dermal	negligible	Dermal deposition was considered negligible as the workers wear gloves.	
ES13: Leaching during contact with water and related potential human exposure (appl. to both CuO and $Cu_2(OH)_2CO_3$)	USE	Consumer	Oral	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 $Cu_2(OH)_2CO_3$ and that the particulate copper that was released is attached to cellulose and is therefore not free in the solution.	(Pantano et al. 2018),(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)
* Workers 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. Legend: SYN = Synthesis; FOR = Formulation; EOL = End of life						

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

	ES1	ES2 Inhalation	ES2 Dermal	ES3	ES4 Inhalation (Consumer and Worker)	ES4 Perioral (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		

769 **Table 4.** APROBA input data and output results.

	Notes	Unit of measure	CuO Inhalation	CuO Ingestion	$Cu_2(OH)_2CO_3$ Ingestion
Inputs to APROBA					
			CuO Inhalation	**CuO Ingestion**	**$Cu_2(OH)_2CO_3$ Ingestion**
Data type			Continuous	Continuous	Continuous
Target BMR		%	100	5	5
PoD type			BMDL	NOAEL	NOAEL
PoD unit of measure			mg/m3	mg/kg bw/day	mg/kg bw/day
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 (submitted)	De Jong et al (submitted)
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/day	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)	PoD (BMDL) and BMDU values corrected multiplying original values by the correction factors, in case of inhalation studies.		0,040	---	---
Corrected BMDU (in case of using the BMD approach)			0,073	---	---
Data route			Inhalation	Oral	Oral
Study type			Subacute	Subacute	Subacute
Test species			Rat	Rat	Rat
Species weight (average)		kg	0,332	0,228	0,366
Human weight		kg	70	70	10
Population Incidence Goal (I)		%	5%	5%	1%
Probabilistic Coverage Goal		%	95%	95%	95%
Overall deterministic UF		n.a.	100	100	100
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)		n.a.	1	1,57	1,57
Interspecies scaling (LCL)	This aspect addresses the interspecies adjustment to take into account differences in body size (e.g. allometric scaling).	n.a.	1	4,43	2,36
Interspecies scaling (UCL)		n.a.	1	7,01	3,08
Interspecies TK/TD (LCL)	This aspect addresses remaining interspecies TK and TD (toxicokinetics and toxicodynamic differences) differences after accounting for body size differences.	n.a.	0,333	0,333	0,333
Interspecies TK/TD (LCL)		n.a.	3	3	3
Duration Extrapolation (LCL)	This aspect addresses uncertainty in using a less-than-chronic study (as specified in "Study type" previously) to	n.a.	0,625	0,625	0,625
Duration Extrapolation		n.a.	40	40	40

(UCL)	estimate a chronic PoD.				
Intraspecies (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.	1,77	1,77	2,24
Intraspecies (UCL)		n.a.	14,02	14,02	41,88
Results	HD distribution (lognormally distributed)		long term HD local effects	long term HD systemic effects	long term HD systemic effects
	Unit of measure		mg/m3	mg/kg body weight per day	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

Table 5. Long-term HD log-normal probability distributions statistics for CuO (ingestion and inhalation routes) and for $Cu_2(OH)_2CO_3$ (ingestion route).

	CuO Inhalation	**CuO Ingestion**	**$Cu_2(OH)_2CO_3$ 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

Table 6. Risk Characterisation 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 (Negligible exposure)	7,90E-01	No Risk (Negligible exposure)	No Risk (Negligible exposure)	9,74E+00	6,79E-08	No Risk (Negligible exposure)	No Risk (Negligible exposure)
95%		1,60E+02			1,97E+03	3,37E-05		
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 (Negligible exposure)	No Risk (Negligible exposure)	No Risk (Negligible exposure)	No Risk (Negligible exposure)	No Risk (Negligible exposure)	2,59E-03	No Risk (Negligible exposure)	No Risk (Negligible exposure)
95%						1,70E+00		
50%						6,63E-02		
Mean						4,63E-01		
GM						6,63E-02		
SD factor						7,18E+00		
Risk (Prob. RCR > 1)						8,48%		

Figures

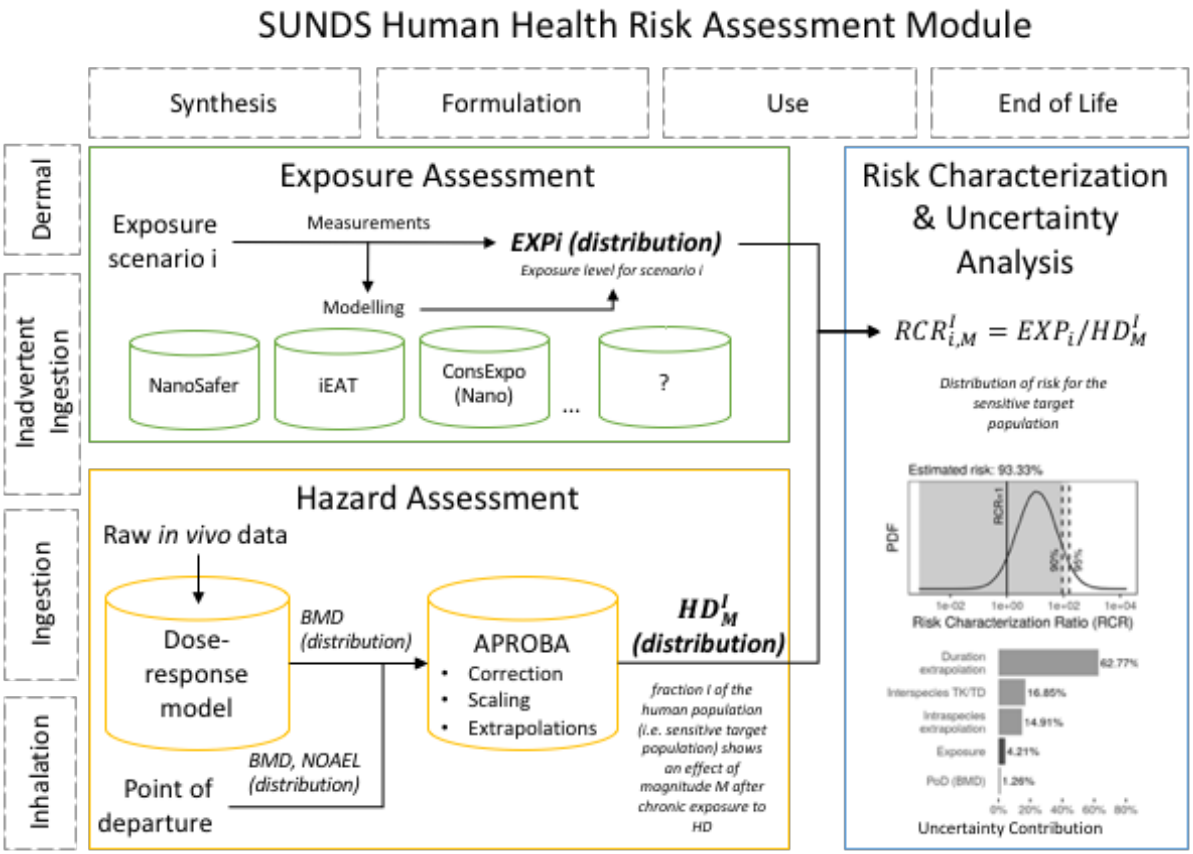

Figure 1. Structure, models, inputs and outputs of the human health risk assessment module of the SUN Decision Support System (SUNDS).

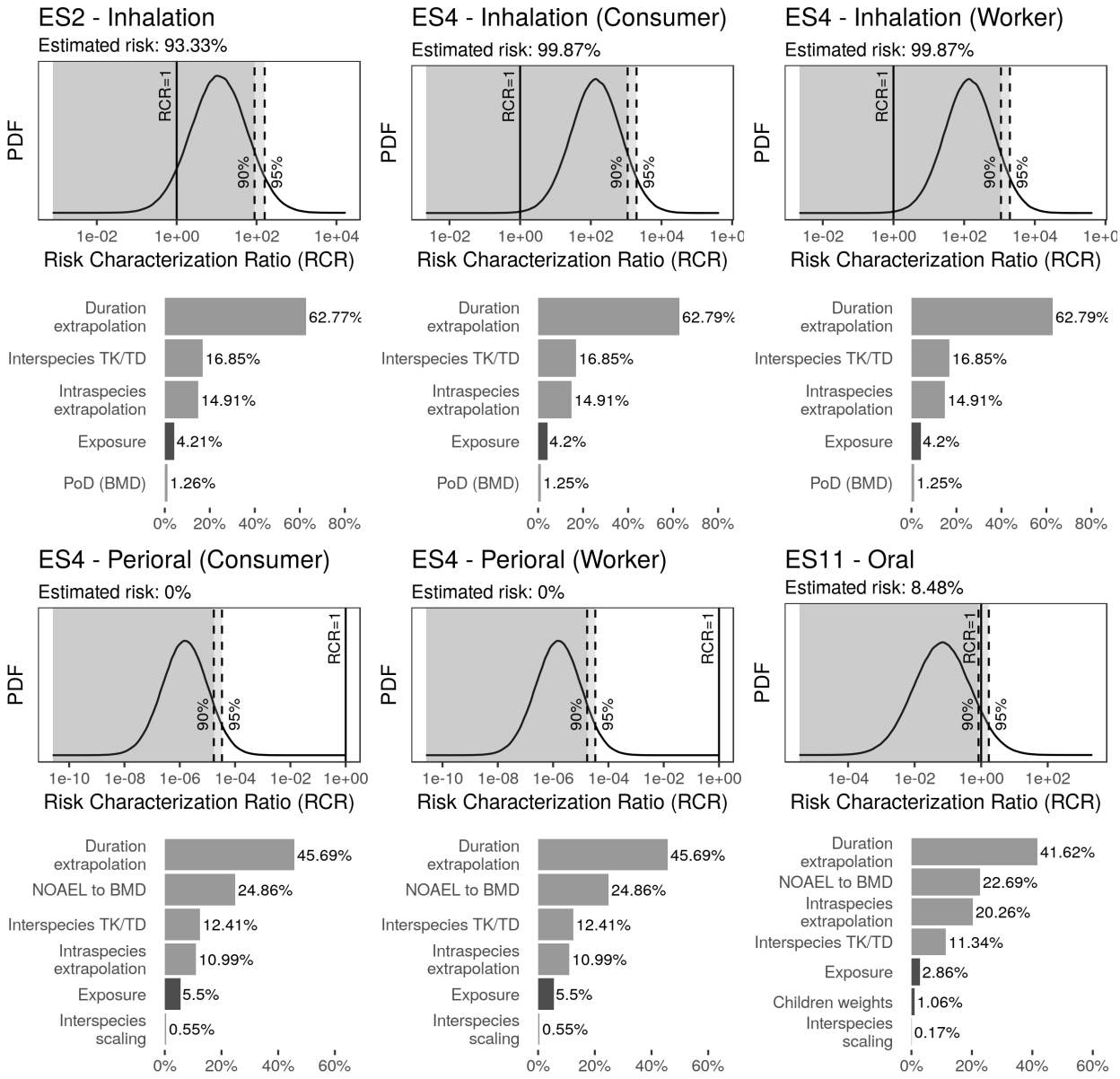

Figure 2. Risks along the lifecycles of the CuO and $Cu_2(OH)_2CO_3$ based products for all concerning exposure scenarios (ES). Contributions of the different sources of uncertainty to the total uncertainty, derived from over 10 000 Monte Carlo simulations, are highlighted.