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Helsinki, 09 July 2019

Substance name: Zinc oxide (ZnO)
EC number: 215-222-5
CAS number: 1314-13-2
Date of latest submission(s) considered[1]: 10 July 2018
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of Zinc oxide (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

a. Requested Information

Based on Article 46(1) of the REACH Regulation (Regulation (EC) No 1907/2006), you are requested to submit the following information on the forms of the registered substance as specified in Appendix 1:

1. Subchronic Inhalation Toxicity: 90-Day Study (OECD test guideline (TG) 413[3]) combined with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 421) in rat (nose-only) with i) extended histopathology of lung, liver, brain, olfactory bulb and heart (appropriate ZnO particle determination in these organs), and ii) neurotoxicity and developmental (neuro)toxicity evaluation, including detailed clinical observations addressing potential neurobehavioural effects. Test materials and specific conditions are described in detail in Appendix 1.

2. In Vivo Mammalian Alkaline Comet Assay (OECD TG 489) in rat, inhalation route (nose-only), including site-of-contact genotoxicity evaluation in lung epithelial cells and nasal mucosa cells, as well as systemic genotoxicity evaluation in liver in addition to bone marrow. Test materials and specific conditions are described in detail in Appendix 1.

3. Information on transformation, dissolution and dispersion stability of manufactured and imported nanoforms of zinc oxide that are covered by the registration dossier(s) submitted for zinc oxide

    a. Screening Test (24 h) from OECD Guidance Document on

[1] This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

[3] OECD TG 413, latest adopted version updated in 2018.

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Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media ENV/JM/MONO(2001)9, OECD Series on Testing and Assessment Number 29; 2001 as further specified in Appendix 1.

b. OECD TG 318 on dispersion stability of nanomaterials in simulated environmental media; Information on ecotoxicity further specified in Appendix 1.

4. Freshwater Algae and Cyanobacteria, Growth Inhibition Test (test method: OECD TG 201, 2006, EU method C.3) with the algae species *Desmodesmus subspicatus* on different nanoforms of zinc oxide (based on the results from request 3.) that are covered by the registration dossier(s) submitted for zinc oxide; as further specified in Appendix 1.

5. Long-term toxicity on invertebrates (*Daphnia sp.*) (test method: *Daphnia magna* reproduction test, EU C.20/OECD TG 211): *Daphnia magna* Reproduction Test, EU C20 on different nanoforms of zinc oxide (based on the results from request 3.) that are covered by the registration dossier(s) submitted for zinc oxide; as further specified in Appendix 1.

6. Information on uses and operational conditions of zinc oxide in nanoforms; Information on supported use conditions and characteristics of the nanoforms of zinc oxide in their use as component for the production of paints and coatings and their use as component for the production of polymer-matrices, plastics, thermoplastics and related preparations and as a component for the production of rubber, resins and related preparations and for the identified uses considered relevant for consumer exposure by the evaluating Member State Competent Authority (evaluating MSCA), as specified in Appendix 1.

You have to provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **09 February 2022**. In addition, full study reports shall be submitted for all studies under requests 1. – 5.

Table 1: Summary of the testing strategy

Test requested	**Conditions when to perform test**	**Estimate of time required***
1. Subchronic Inhalation Toxicity: 90-Day Study (OECD TG 413, latest adopted version updated in 2018) combined with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 421) in rat (nose-only) with additional examinations specified in	Unconditionally	28 months (including preparatory work and pre-testing) (3+28=31 months)

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Appendix 1		
2. In Vivo Mammalian Alkaline Comet Assay (OECD TG 489), in rat, inhalation route (nose-only), including site-of-contact genotoxicity evaluation in lung epithelial cells and nasal mucosa cells, and systemic genotoxicity evaluation in liver in addition to bone marrow.	Unconditionally. Time for preparatory work and pre-testing for an adequate selection of test material(s) is only added to request 1, as the required testing and preparation work for requests 1 and 2 are identical and only need to be performed once.	18 months (3+18=21 months)
3a.+3b. Information on transformation, dissolution and dispersion stability	Unconditionally	12 months (3+12=15 months)
4. Freshwater Algae and Cyanobacteria, Growth Inhibition Test (test method: OECD TG 201, 2006, EU method C.3)	Unconditionally, selection of test material based on results from 3a and 3b	9 months (3+12+9=21 months)
5. Long-term toxicity on invertebrates (Daphnia sp.) (test method: Daphnia magna reproduction test, EU C.20/OECD TG 211)	Unconditionally, selection of test material based on results from 3a and 3b.	9 months (3+12+9=21 months)

*The normal time that performance of an individual study takes. In brackets is explained the breakdown of time needed for the sequential testing in order to deliver all the information requested by the ultimate deadline.

The reasons of this decision and any further test specifications are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential

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and not included in the public version of this decision.

b. Who performs the testing?

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

c. Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[4] by Christel Schilliger-Musset, Director of Hazard Assessment

[4] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

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Appendix 1: Reasons

The scope of this substance evaluation is limited to the properties of and information on forms of Zinc oxide containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm (In accordance with the Commission Recommendation of 18 October 2011 on the definition of nanomaterial[5]).

For this purpose, 'particle' means a minute piece of matter with defined physical boundaries; 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components and 'aggregate' means a particle comprising of strongly bound or fused particles.

The term 'nanoform', when used in this decision, shall relate to a nanoform that can be specified based on differences in the parameters, like particle number size distribution, surface functionalisation or treatment, shape, aspect ratio and other morphological characterisation, information on assembly structure or surface area (specific surface area by volume and/or specific surface area by mass).

The registrants that can demonstrate not to manufacture or import such forms are not requested to provide the information. In the absence of explicit and suitable information in all available individual registration dossiers, ECHA is not in a position to determine whether and which individual registration dossier actually covers any specific nanoform of the substance.

Based on the evaluation of all relevant information submitted on zinc oxide and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State Competent Authority (evaluating MSCA) to complete the evaluation of whether the substance constitutes a risk to human health and the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify the concern for human health and the environment.

The registered substance was evaluated under Regulation (EEC) No. 793/93, however nanoforms were not specifically considered there.

Zinc oxide is manufactured and/or imported in the European Economic Area in 100 000 - 1 000 000 tonnes per year. This substance has a harmonised classification in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) as Aquatic Acute 1 and Aquatic Chronic 1 (H 400, H 410).

[5] OJ L275,20.10.2011, p.38

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Zinc oxide is manufactured and imported as nanoforms and non-nanoforms. The amount of the nanoforms is unclear but likely in the range of 1000-10000 t/a (Sun et al., 2014). There is evidence from the information in the registration dossiers that also forms of zinc oxide not considered as nanoforms in the registration dossiers may potentially fulfil the definition of a nanoform. This is further substantiated by data from literature indicating that some production processes typically result in nanoforms (Mahmud et al., 2006).

Uses, exposure and potential risk

According to information in the registration dossier zinc oxide in nanoforms is used as a component or an additive for various purposes including the manufacture of electronic components, production of clear coatings, polymer-matrices, plastics, thermoplastics and related preparations, and cosmetic emollients used for sunscreen, skin care and pharmaceuticals preparations. In the course of the substance evaluation process additional uses have been identified. According to information provided by registrants acknowledging the registration of zinc oxide in nanoforms products containing zinc oxide in nanoforms are used at industrial sites and by professionals and consumers. Significant exposure to consumers and environment cannot be excluded.

Based on information in the registration dossier and information from the published literature, as detailed below in the sections for human health and environment, there is a concern that zinc oxide in nanoforms may cause specific target organ toxicity from repeated exposure (STOT-RE), may be a germ cell mutagen, may cause adverse effects on reproduction and may be toxic to aquatic organisms.

Based on this exposure and hazard information, there is a potential risk for consumers and the environment.

As the available information is not sufficient to conclude on potential target organs, and genotoxic and reproductive toxicity properties as well as on effects for aquatic organisms, further information is needed, as explained below.

Use in cosmetics and animal testing

It is noted that zinc oxide in nanoforms is also used in cosmetics and personal care products according to the Cosmetics Regulation (EC) No 1223/2009.

As noted above, there are also uses under REACH. ECHA's factsheet on the interface between REACH and Cosmetics Regulations[6], developed jointly with the European Commission, provides that registrants of substances that use the substance also for non-cosmetic uses (i.e. mixed-use substances) are permitted to perform animal testing, as a last resort, for all endpoints requiring vertebrate testing.

Zinc oxide in nanoforms may be exclusively used in cosmetics and personal care

[6] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf/2fbcf6bf-cc78-4a2c-83fa-43ca87cfb314

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products according to the Cosmetics Regulation by some of the Registrants. The stages of manufacturing of the Substance and/or formulation of cosmetic products are taking place in the EEA and there is no indication that they are carried out under strictly controlled conditions. As potential worker exposure may exist, testing for human health concerns endpoints is necessary to assess the risks from exposure to workers and therefore in order to fulfil the relevant REACH requirements. In addition, animal testing for environmental endpoints is not restricted according to the Cosmetics Regulation (EC) No 1223/2009. This is in accordance with the above factsheet.

Human health

General considerations on the "ion-only" hypothesis

In your registration dossier you propose a category approach for i) Zn-salts, ii) microsized (bulk) ZnO, iii) coated ZnO in nanoform (nano ZnO) and iv) uncoated nano ZnO, based on the assumption of toxicological equivalency of these zinc compounds/forms of ZnO, as you propose that their toxicity is generally only driven by released zinc cations (Zn^{2+}) (in the following called 'ion-only hypothesis'). However, no further evidence for this assumption was provided except for water solubility data and results of bio-elution studies on some ZnO nanoforms (see below). Although ECHA agrees that Zn^{2+} release is an important driver of ZnO toxicity, ECHA considers this explanation without further toxicological evidence as inadequate to conclude that ZnO in nanoform (uncoated and coated) is toxicologically equivalent to rapidly dissolving zinc salts and microsized ZnO.

In contrast to zinc salts, zinc oxide is not readily soluble under neutral pH conditions (water solubility < 0.1 mg/L) (SCCS, 2012a). However, the more the pH deviates from physiologically buffered pH conditions, the higher the ZnO dissolution rate becomes (Bian et al., 2011; Li et al., 2013). In more extreme pH environments, such as in lysosomal compartment, ZnO dissolves rapidly and completely. Accordingly, in addition to the toxic potential of the Zn ion, and depending on the specific route of ZnO exposure, particle-related effects such as (local) inflammatory foreign body responses, adsorption, persistence, carry-over effects, translocation, cellular uptake, have to be considered when assessing the toxic potential of ZnO. Thus, even if the zinc cation is the ultimate toxic agent, it is unclear when and where it is released to become effective (e.g. extracellular or intracellular).

You have provided data on results of bio-elution studies conducted with a few of the registered coated and uncoated ZnO nanoforms. For all of the tested nanoforms, a "rapid and mostly complete dissolution" was reported at weakly acidic conditions in artificial lysosomal fluids, while very low dissolution was observed at neutral conditions in artificial alveolar fluids. From these results, you concluded that "no significant differences in Zn^{2+} elution is observed between coated, non-coated and micro-scaled ZnO" and that "read across between various forms of ZnO (micro-scale, nano (coated or uncoated)) is fully supported".

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ECHA does not share this conclusion. The reproducibility between laboratories is unsatisfactory (Henderson et al., 2014). Existing data show that there are marked differences in (water)solubility of the same ZnO nanoform due to small differences in test media composition or other test conditions (e.g. static vs. non-static) (Eixenberger et al., 2017; SCCS, 2012a). Further literature data demonstrate that results of in vitro dissolution studies with ZnO in nanoform using artificial body fluids do not necessarily reflect in vivo conditions (Cho et al., 2013; Mu et al., 2014; Paek et al., 2013; Xiaoli et al., 2017). In fact there are studies indicating that different physico-chemical characteristics of ZnO in nanoforms might impact dissolution, cellular uptake and toxicity (Hsiao and Huang, 2011; Kim et al., 2014; Mu et al., 2014; Paek et al., 2013; Xu et al., 2010). Differences in bioavailability and occurrence/ appearance of Zn^{2+} ions in specific target tissues/organs between micro- and nano-sized ZnO, and between different nanoforms of ZnO can be expected (e.g. Konduru et al., 2014). The variable findings suggest that several physicochemical characteristics such as particle size, presence and type of surface modification, surface charge and/or agglomeration might influence toxicokinetics and toxicity of ZnO in nanoform. Moreover, literature data on toxicity indicate that various nanoforms of ZnO cause effects that cannot be explained alone by Zn^{2+} ion release.

As there is evidence that nanoforms of ZnO may impact dissolution, cellular uptake and toxicity differently compared to bulk ZnO, the toxicity described in the current dossier(s) may be underestimated for uses of ZnO in nanoforms.

As exposure to ZnO in nanoforms cannot be excluded due to wide dispersive uses, there is a potential risk for human health that is specific to ZnO in nanoforms.

Hence, this substance evaluation is limited to ZnO nanoforms of similar dimensional specifications as those nanoforms in the registration dossiers.

Requests

1 Subchronic Inhalation Toxicity: 90-Day Study (OECD TG 413, latest adopted version updated in 2018) combined with the Reproduction/Developmental Toxicity Screening Test (OECD TG 421) specified additional examinations

The concern(s) identified

The substance evaluation raised specific concerns with regard to (systemic) repeated dose inhalation toxicity, neurotoxicity, reproductive toxicity (fertility), as well as regarding developmental (neuro)toxicity, which all shall be addressed using requested study design.

Repeated dose inhalation toxicity

The registration dossier cites a subchronic (90 day) inhalation toxicity study in rats according to OECD TG 413 as key study, which tested the respirable dry-generated aerosols of the coated ZnO nanoform [redacted] (triethoxycaprylylsilane-coated nano

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ZnO) at 0.3, 1.5 and 4.5 mg/m³ and a microsized ZnO pigment at 4.5 mg/m³ ([redacted], 2013). The study design emphasized effects in the lung and included bronchoalveolar lavage (BAL), lung cell proliferation and systemic Zn estimation in different tissues. Data of two associated short-term inhalation studies of 5 days ([redacted], 2009) and 14 days ([redacted], 2011) of exposure are also presented, which ECHA primarily regards as range-finding studies and supporting information in terms of site-of-contact toxicity. The 14 day study also included uncoated nano-sized ZnO ([redacted]) at one single concentration (8 mg/m³). A subacute 28 day study was also presented ([redacted], 2014), which tested microscaled ZnO at four concentrations (0.5, 1.5, 3.0 and 4.5 mg/m³). A subchronic inhalation study with ZnO in bulk form was not provided.

The short-term inhalation studies reported dose-dependent, but slight and partially reversible inflammatory effects in the respiratory tract, which occurred already at lower exposure concentrations. Accordingly, the highest exposure group in the subchronic toxicity study was adjusted to 4.5 mg/m³. The subchronic inhalation study determined a NOAEL of 1.5 mg/m³, based on slight effects restricted to the respiratory tract. Local effects at 4.5 mg/m³ [redacted], as well as in the micro ZnO treatment included affected bronchoalveolar lavage fluid (BALF) parameters, bronchioloalveolar hyperplasia, as well as alveolar granulocyte and interstitial mononuclear cell infiltration in the lungs and decreased cell proliferation rates. (Multi)focal epithelial hyaline (eosinophilic) droplets in the nasal cavity and hyperplasia of the olfactory epithelium were detected as well. Increased absolute and relative lung weights were only observed in micro ZnO treatment.

No systemic toxicity was reported in any of these inhalation studies. The 14 day inhalation study did not reveal systemic toxicity either at up to 8 mg/m³ for the tested nanoforms (coated and uncoated). However, the duration of this study is too short to be predictive for subchronic and chronic effects. In contrast, significant increases in the absolute and relative amount of segmented neutrophils and decreases in blood lymphocytes (relative) were observed at 4.5 mg/m³ [redacted] (coated nano ZnO) after 90 days of inhalation, which was not seen in case of the concurrent micro ZnO treatment at the same concentration. The authors considered these haematological findings as incidental without further explanation. Furthermore, increases in relative and absolute left testis weights, albeit statistically significant in all ZnO exposure groups one day post-exposure, were considered to be incidental by the study authors, as no obvious dose-response relationship could be observed. Nevertheless, similarly after 14 days of exposure to the coated [redacted] at ≥ 0.5 mg/m³ or the uncoated [redacted] at 8 mg/m³ marked but statistically not significant increases in absolute and relative (left and right) testes weights were observed. ECHA considers that these effects require further clarification.

In the subchronic study a "practically complete dissolution of retained ZnO" was assumed after 90 days of inhalation and it was stated that "no relevant amounts of increased nanoscaled ZnO were detected in any body compartment demonstrating the rapid elimination". However, in the provided short-term studies, 5- and 14 days of exposure led to an increase in Zn content in the liver ([redacted] treatment, only after

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5 days of exposure: 133 % compared to control), kidney ([redacted] and micro ZnO treatment: up to 139 %) and brain ([redacted] and micro ZnO treatment: up to 160 %), measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Moreover, one day post subchronic exposure of rats to the coated [redacted] (at ≥ 1.5 mg/m^3) but not to micro ZnO, significantly decreased Zn levels were observed in kidneys, indicating that nano ZnO inhalation exposure might impact Zn homeostasis. The study authors disregarded this effect without further explanation. In lungs of rats treated with the uncoated [redacted] as well as the coated [redacted], nanoscaled structures resembling nanoparticles were found within the cytoplasm of (predominantly) alveolar macrophages in the 14 day exposure study, indicating incomplete dissolution of nano ZnO in the lung. These ingested structures could not be verified as ZnO particles, as the surrounding Zn content impaired a conclusive analytical analysis, but particulate nano structures were not detected in the parallel micro ZnO treatment.

ECHA concludes that the selected dose levels in subchronic inhalation toxicity testing of ZnO, which were triggered by slight pulmonary inflammation in dose finding studies, were insufficient to assess systemic toxicity. A justification for exclusive testing of these rather low exposure concentrations was not provided in the dossier. An acute nose-only inhalation toxicity study cited in the dossier tested uncoated ZnO nanomaterial at 1790 mg/m^3 without showing mortality. This test concentration was reported to be the highest possible concentration to produce respiratory particles ([redacted], 1997). Thus, limitations for testing higher concentrations than 4.5 mg/m^3 in the subchronic repeated dose toxicity test are not obvious.

Taken together, the subchronic inhalation study is considered insufficient to inform on systemic toxicity and on STOT-RE classification of ZnO in nanoform.

ECHA acknowledges that some information on repeated inhalation of different (nano)forms of ZnO is available, in particular with regard to respiratory toxicity. However, a clear and robust comparative testing concept is not recognisable. In fact, where comparative data is available, this indicates that_different (nano)forms of ZnO accumulate differently in the lung and in secondary organs and induce different toxic effects with distinct recovery profiles. This challenges your statement that: "The effects after exposure to [redacted] and the reference substances (uncoated nanoscaled ZnO: [redacted] and a microscaled ZnO), were comparable", either because of incompleteness of comparative testing or due to differences already seen in the available study results. Your conclusion that "quality and dose dependency of effects are independent of ZnO particle size and coating of the surface" is not justified.

Furthermore, only one nanoform was tested in the 90 day study, namely the coated [redacted], and an available 28 day subacute inhalation toxicity study tested microsized ZnO only. Toxicity data with respect to uncoated nano ZnO was only provided from a 14 day range-finding study, where the uncoated [redacted] was tested at one concentration only concurrently with the coated [redacted]. Data from this study indicates that the two tested ZnO nanoforms, as well as micro ZnO behave differently with regard to biokinetics and toxicity. Therefore it is unclear, if the specific coated nanoform tested in

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the subchronic 90 day study is the most relevant nano ZnO with regard to regulatory relevant repeated dose testing and whether quality and dose dependency of effects are in fact independent of ZnO particle size and coating of the surface, as you state in the dossier.

Test animals were exposed to dry-generated particle dusts, which usually agglomerate rapidly (Ahn et al., 2017). A mass median aerodynamic diameter (MMAD) < 3.0 µm (geometric standard deviation (GSD) ca. 1.5) was determined for the 90 day study, indicating a high proportion of respiratory particles. However, the toxicological equivalency of weakly and strongly agglomerating ZnO nanoparticles was not demonstrated. In fact, liquid preparations are commercially available with size-customized ZnO nanoparticle dispersal, covering a broad spectrum of agglomerate sizes from the low nanometre range to several micrometres. Hence, a risk arising from exposure to nebulised ultrafine ZnO dispersions that differ from that of dry aerosol exposure might exist, and this needs to be clarified as specified in section 'Considerations on the test method and testing strategy'.

Several experimental repeated dose inhalation studies were identified in the literature, which indicate further health concern by inhaled nano ZnO, in particular with regard to systemic toxicity of uncoated nanoforms. These studies were not considered in the registration dossier. Importantly, in addition to pulmonary inflammation, several studies reported systemic distribution and/or severe effects in other organs depending on administration method, in particular in brain, heart and liver.

Besides reversible local effects in BALF of mice, Adamcakova-Dodd et al. (2014) measured significantly elevated Zn levels in heart immediately after subchronic (13 weeks, whole body) but not after subacute (2 weeks, whole body) inhalation exposure to nebulized, well characterised uncoated nano ZnO (average particle size: 26 nm; aerosol size distribution < 50 nm) at 3.5 mg/m^3 (measured by ICP-MS). In another study exposing male SD rats to uncoated nano ZnO (50 nm) for 2 weeks via inhalation (whole-body; at 1.1 and 4.9 mg/m^3), similarly BAL parameters were affected, and the degree of adverse local effects decreased after 30 days without exposure (Chuang et al., 2014). In addition, mild to moderate inflammatory cell infiltration in **cardiac tissue** was observed one day after the 14 day exposure period, leading to focal fibrosis 7 days and myocardium degeneration and necrosis of cardiac tissue 30 days post-exposure. In this study, increases in white blood cell, lymphocyte and granulocyte counts were also detected in the peripheral blood of treated rats at different time points after exposure. In a further study, intranasal spraying of uncoated nano ZnO (20 nm; dry-powder spray; 2.5 mg/kg bw) on 3 consecutive days not only induced severe lung lesions in rats, which were still visible 12 and 36 h post-exposure without mitigation, but also resulted in significant **liver** damage, including inflammation, interstitial hyperaemia, fatty degeneration around the central vein and expanded hepatocyte necrosis (Wang et al., 2010). Regarding effects in the **brain**, inhalation of 12 – 14 nm uncoated nano ZnO for three days (whole body; daily increasing concentrations: 6.8, 11.4 and 22.3 mg/m^3) resulted in the detection of numerous electron-dense, nanoscaled black spots in the cerebrum of male rats, which were assumed to be ZnO nanoparticles (Kao et al., 2012).

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Synaptosome samples from olfactory bulbs of those exposed rats showed significantly higher Zn levels than those from controls. A direct olfactory bulb-brain translocation pathway for ZnO nanoparticles in rats was hypothesized, as it was previously proposed for several other nanomaterials as well (Oberdörster et al., 2004).

Supporting evidence for particle-driven toxicity is coming from a number of in vivo experiments testing ZnO in nanoform via other routes than inhalation, reporting distinct (adverse) systemic effects by exposure to different nanoforms of ZnO or nano and bulk ZnO (Kim et al., 2014; Li et al., 2012; Liu et al., 2017; Park et al., 2016; Park et al., 2014a; Park et al., 2014b; Pasupuleti et al., 2012; Shrivastava et al., 2014; Wang et al., 2007; Wang et al., 2017). The mode of action, however, was not clearly demonstrated in these studies.

The additional studies, though exploratory in nature and with technical deficits, nevertheless indicate a target organ spectrum of toxicity for nano ZnO, which has not been reported for bulk ZnO. The studies also challenge the implicit assumption that coated nano ZnO, which has been tested most extensively in repeated dose inhalation toxicity studies (as [redacted]) is representative for all ZnO nanoforms and being the most toxic ZnO nanoform, respectively.

Thus, an extended protocol of an OECD TG 413 study is required, including appropriate determination of nano ZnO in the target organs liver, cardiac muscle and brain as well as a detailed histopathology of these organs in a comparative testing approach to clarify the concerns raised by weight of evidence analysis of the available data. Considering that even little variability of study parameters may greatly affect the outcome of test results, ECHA deems parallel testing or at least testing under constant conditions as necessary.

Neurotoxicity

The findings by Kao et al. (2012), who detected nanostructures in the cerebrum of rats exposed to uncoated nano ZnO via inhalation for three days, raise a concern regarding neurotoxicity of ZnO in nanoform after repeated inhalation. Scars were detected particularly in endosomes of brain tissue. Moreover, synaptosome samples from olfactory bulbs of exposed rats showed significantly higher Zn levels than those from control rats, suggesting a direct olfactory bulb-brain translocation pathway for ZnO nanoparticles in rats, as it was concluded for other nanomaterials as well (Oberdörster et al., 2004). Other studies further supported the identified concern. In a guideline conform repeated inhalation toxicity study ([redacted], 2011) significant increases in Zn levels in brain 14 days after 2 weeks of nose-only exposure to the uncoated [redacted], the coated [redacted] and micro ZnO treatment were detected, with highest Zn levels at the lowest test concentration. In addition, after 5 days of exposure to [redacted], the relative brain weight of treated animals was significantly increased ([redacted], 2009). Unfortunately, only in the 14 day exposure study specific organs and tissues were screened for nano ZnO in particulate form and brain tissue was not among them.

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90 days of exposure to micro ZnO but not [redacted] led to increases in relative brain weight 29 days post-exposure ([redacted], 2013).

A number of additional repeated dose studies presented in the scientific literature further substantiate a neurotoxicity concern, reporting behavioural alterations (Han et al., 2011; Liu et al., 2017), effects on brain weight (Ko et al., 2015; Wang et al., 2016), as well as nanoparticles in brain neurons of exposed animals (Shrivastava et al., 2014).

Taken together, these findings indicate that exposure to nano ZnO may lead to abnormalities in the central nervous system and/or in nerves. ECHA, thus, considers that further information clarifying the potential of nano ZnO to induce neurotoxicity is necessary.

Reproductive toxicity (fertility)

In your registration dossiers, only reproductive toxicity studies with rapidly dissolving zinc salts are presented. None of the studies provided in the dossier tested ZnO, neither in bulk nor in nanoform. A read-across approach to zinc salts was justified by the assumption that after intake the biological activity of the zinc compounds are determined exclusively by the zinc cation ("ion-only hypothesis"). However, evidence for the feasibility of this approach with regard to reproductive toxicity is missing. This is particularly true for uncoated and coated nanoforms of ZnO, respectively.

The registration dossier cites a subchronic (90 day) inhalation toxicity study in rats according to OECD TG 413, which tested the respirable dry-generated aerosols of the coated [redacted] at 0.3, 1.5 and 4.5 mg/m^3 and a microsized ZnO pigment at 4.5 mg/m^3 ([redacted], 2013). In this study, increases in relative and absolute left epididymis and testis weights were reported, which however – albeit the reported statistical significance in all ZnO exposure groups 1 day post-exposure – were considered to be incidental by the study authors, as no obvious dose-response relationship could be observed. Similar (but statistically not significant) increases in absolute and relative (left and right) epididymis and testes weights were observed as well after 14 days of exposure to the coated [redacted] at ≥ 0.5 mg/m^3 or the uncoated [redacted] at 8 mg/m^3. ECHA considers that these reproductive effects require further investigation.

Supporting evidence for reproductive toxicity (fertility) of ZnO in nanoform was identified in several of in vivo experiments investigating effects of exposure to nano ZnO by other routes than inhalation. These studies reported adverse reproductive impacts of ZnO nanoforms, comprising impaired spermatogenesis and testes lesions (Han et al., 2016; Hussein et al., 2016; Moridian et al., 2015; Talebi et al., 2013), increases in uterus and epididymis weight (Jo et al., 2013; Park et al., 2014a; Park et al., 2014b) and affected testosterone levels (Moridian et al., 2015; Talebi et al., 2013). In addition, several in vitro studies identified in the scientific literature reported adverse reproductive effects of nano ZnO (Han et al., 2016; Liu et al., 2016; Zhao et al., 2016; Zhao et al., 2015).

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Developmental (neuro)toxicity

With regard to developmental toxicity, only one study has been provided which tested the prenatal developmental toxicity of a single coated ZnO nanoform by low-dose inhalation exposure. For the second species no nano specific information was provided, but rather information on a study in rabbits with a proposed read across substance: zinc sulphate. A specific read across justification was not provided, except for the statement that after intake, the biological activities of the zinc compounds are determined exclusively by the zinc cation (i.e. "ion-only hypothesis"), which however lacks evidence and justification.

Triethoxycaprylylsilane-coated nano ZnO ([redacted]) was used to expose rats in an OECD TG 414 prenatal developmental toxicity study by inhalation of a dry aerosol [redacted], 2013). Up to the highest administered dose (7.5 mg/m^3) no embryotoxicity was observed. Regarding maternal toxicity, increase in lung weight and moderate alveolar lipoproteinosis has been observed. It is noted that the same material tested via inhalation at comparable conditions (max. 8 mg/m^3 for 14 days) on non-pregnant rats did not reveal those effects. The representativeness of [redacted] for ZnO and more specifically for ZnO in nanoform has not been justified for this endpoint. Furthermore, a justification for the inhalation route and the relatively low dosing regimen is not provided. It is also noted that available inhalation studies tested an aerosol of [redacted] that was generated by dispersing a dry powder, the agglomeration behaviour of which might substantially differ from that of ultrafine dispersions that are commercially available.

Supporting evidence for particle-driven developmental toxicity is coming from a number of in vivo experiments testing ZnO in nanoform via other routes than inhalation, reporting diverse adverse effects on offspring development, such as skeletal and visceral alterations (Hong et al., 2014a; Hong et al., 2014b), increased foetal resorptions and reduced pup survival (Jo et al., 2013). In mouse embryo culture experiments, treatment by nano ZnO resulted in zinc absorption and morphological changes of treated embryos (Jung et al., 2015).

In addition, evidence for developmental neurotoxicity of ZnO in nanoform was found in the literature. In these studies significantly increased zinc concentrations in the brain of offspring (Xiaoli et al., 2017) were reported, as well as disruption of the monoaminergic system (Okada et al., 2013), and behavioural alterations (Alimohammadi et al., 2018; Han et al., 2011), including learning and memory deficits (Xiaoli et al., 2017).

ECHA concludes that the provided data is insufficient to clarify the potential health concern and that the additional data further raise a concern with respect to developmental (neuro)toxicity. Further regulatory robust testing, however, is considered not proportionate at the moment. ECHA concludes that additional histopathological, neuro-behavioural and neuropathological examinations of offspring (according to OECD TG 426) shall be added to the requested combined Subchronic Inhalation Toxicity: 90-Day Study (OECD TG 413) with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 421), to be

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able to clarify the concern regarding developmental (neuro)toxicity of nano ZnO.

Why new information is needed

Available data in the registration dossiers on repeated dose inhalation toxicity are indicative of specific health concerns of inhaled ZnO nanoparticles. In addition, a number of other studies on inhaled nano ZnO from the scientific literature support and extend this concern. The identified health concerns encompass both, local (respiratory tract) and systemic toxicity; the latter in particular with regard to neuronal and cardiovascular damage was not described for inhaled zinc oxide or zinc salts in the registration dossiers.

It is generally accepted that for nanomaterials the inhalation route is of particular health concern, primarily due to the likelihood of human exposure via this route, and to direct and indirect inflammatory effects in the respiratory tract, to interference with and/or uptake by lung cells, as well as to extrapulmonary transport of nanoparticles. ZnO in nanoform is assumed to be a nanomaterial of low biopersistence. However, convincing in vivo evidence for rapid and complete dissolution following inhalation is missing, partially due to analytical challenges to demonstrate the presence of ZnO particles. In contrast to zinc salts, the water solubility of ZnO is low under neutral pH conditions and the production of stable, ultrafine ZnO nanoparticle dispersions is an asset in commercial applications. The provided inhalation studies, stressing occupational dust formation, disregarded dispersibility and stabilisation issues of potential consumer uses, such as wet aerosol spraying.

In this context, the inadequacy of the provided data becomes apparent. Most inhalation studies tested a specific coated nanoform of ZnO ([redacted]), though its eligibility as representative test material has not been justified. You have only provided very limited data on uncoated nano ZnO in the registration dossiers, although uncoated nano ZnO has the highest production volume of all registered ZnO nanoforms and several studies indicated differences in toxic profile and potency, respectively, compared to coated nano ZnO yielding severe adverse effects (e.g. Chuang et al., 2014; [redacted], 2013; [redacted], 2011; Jain et al., 2013; Kao et al., 2012).

The registration dossier further does not contain sufficient data regarding reproductive toxicity of ZnO in nanoform, while data from the scientific literature indicate severe health effects of ZnO in nanoform, including adverse effects on sexual function and fertility.

You also provided only limited data regarding developmental (neuro)toxicity. Data from the published literature, however, indicate severe developmental health concerns after repeated exposure of parental animals with nano ZnO, including developmental neurotoxicity.

Hence, ECHA concludes that based on the limited information on nano ZnO you submitted and taking additional experimental data from the open literature into account, the available information raises a concern regarding repeated dose toxicity and

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neurotoxicity of nano ZnO with regard to the inhalation route. Moreover, nano ZnO may be of risk to human health with respect to reproductive toxicity (fertility) and developmental (neuro)toxicity. ECHA considers that clarification of these multiple concerns is vital and that further information demonstrating the equivalency of different ZnO nanoforms, as well as bulk ZnO and other_zinc compounds is necessary in order to evaluate the read-across you presented.

Transmission electron microscopy (TEM) analysis of commercial zinc oxide nanomaterial preparations reveal a broad spectrum of particle distribution, ranging from highly agglomerated particles in dry test aerosols to customized ultrafine liquid dispersions of poorly agglomerating nanoparticles. There is potential exposure to highly and poorly agglomerated ZnO nanoparticles via occupational exposure and as a result of professional and commercial uses (e.g. spray application). ECHA, hence, considers it necessary to test not only respirable dry aerosols but also nebulised wet aerosols of ultrafine dispersions under size-controlled conditions.

Finally, the DNEL derivation for ZnO in nanoform for the inhalation route in the registration dossiers is considered inappropriate, as it is not derived in accordance with the Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.15: Consumer exposure assessment.

What is the possible regulatory outcome

The results of the requested study will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the nanoforms of the Substance should be classified for specific target organ toxicity as defined in the CLP Regulation (EC) No 1272/2008. The evaluating MSCA will also assess whether the Substance should be proposed for identification as a substance of very high concern (SVHC) under Article 57 of REACH, which would lead to stricter risk management measures than those currently in place.

Additionally the information from the study can be used for an adequate risk assessment and establishment of DNELs in particular for controlling the potential risks to consumers and the general population from nano ZnO.

Considerations on the test method and testing strategy

Testing strategy

The requested information requirements related to human health make use of the combination of testing for repeated inhalation toxicity (OECD TG 413) and reproductive toxicity according to OECD TG 421 via the inhalation route with additional specifications, including neurotoxicity and developmental (neuro)toxicity evaluation, as indicated in detail below. This strategy strives to generate comprehensive and regulatory valid information but considers the adequacy and proportionality of information requests.

To obtain information on coating, size, and dissolution dependence, testing must

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compare two nanoforms of ZnO (a coated and an uncoated form) and also include microsized ZnO as well as a zinc salt as 'size control' and 'solubility control', respectively. The latter is required to inform on the "ion-only hypothesis", whereas the former informs on the nano-specificity of effects. For ECHA this is also a reasonable compromise in gaining maximum comparable information, especially with regard to the toxicological representativeness of the tested nanoforms on the one hand and consideration of adequacy and proportionality of information requests on the other hand.

Test materials

The testing strategy requires the testing of two nanoforms of ZnO (coated and uncoated) plus a microsized ZnO and a rapidly dissolving Zn salt as control substances (four test items in total). This strategy is deemed proportionate to inform on the impact of relevant factors such as size, solubility, and surface modification. Alternatively, all registered nanoforms may be tested separately.

The selection of the four test items is at your discretion. However, the following boundary criteria shall be followed:

a. Two nanoforms of ZnO, one coated and one uncoated form, shall be tested at three concentrations, preferably in parallel and equimolar for the two nanoforms.

b. Concomitantly, microsized ZnO and a rapidly dissolving zinc salt (of similar solubility as $ZnCl_2$), shall be tested at one concentration, referring to the top dose in a. to provide information on size and dissolution dependence of effects ('size control' and 'solubility control', respectively).

c. The nanoforms and the microsized ZnO selected from the forms covered by the joint registration shall meet the following minimum criteria:

I. Substance identity:

Identical starting material of the coated and the uncoated ZnO nanoform;

II. Particle size distribution:

i) Nanoforms:
> 50 % of the particles in the number size distribution with a diameter < 100 nm

ii) Micro-sized ZnO:
> 80 % of the particles in the number size distribution with a diameter > 1 µm

Size distribution curves of the nano- and the non-nanoform shall not overlap. A considerably large size difference between the nano- and the

non-nanoform is essential in order to conclude on the significance of particle size.

III. Morphology of the nanoform:

Near spherical to slight rod shape;

IV. Surface chemistry of the nanoform:

Stable coating, corresponding to one of the coatings described in the registration dossier - preferentially of positive surface charge.

A robust justification for the selection of the test materials shall be provided.

d. For the purpose of selecting the test materials and interpreting the results of the test, the nanomaterials require comprehensive physico-chemical characterisation beforehand according to the Guidance on information requirements and chemical safety assessment, Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals (Version 1.0; May 2017) and Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance (Version 2.0; May 2017), as they are expected to play a decisive role in the toxicology of nano ZnO:

- The chemical composition/Impurities. Method: Energy dispersive X-ray analysis (EDX), taking note of the OECD Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C) and including a detailed description of the methodology;

- The granulometry, which shall include primary particle size and shape, aggregate/agglomerate size and primary particle size distribution (number-based). For this purpose a combination of at least two methods, an image-based one (e.g. TEM) and a statistical one, shall be used.

- The crystalline size and structure. Method: X-ray diffraction (XRD), including a detailed description of the methodology;

- The specific surface area (by volume). Method: for powders BET (ISO 9277:2010), for suspensions calculation based on theoretical model;

- The surface treating agent(s), including chemical identity (IUPAC name and numerical identifiers (CAS and EC), type of reaction with the ZnO surface, relative coverage of the ZnO surface (as this information is part of the substance identity, the information should be added in IUCLID sections 1.2 and 1.4);

- The dustiness. Method: rotating drum method (prEN 15051-2);

- The zeta potential and the point of zero charge. Method: Laser-Doppler electrophoresis or electrophoretic light scattering to be performed at fixed low salt concentration and at fixed particle concentration, taking note of the OECD Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C).

e. When choosing test media, great attention shall be paid to particle stability. In particular, buffer systems leading to enhanced dissolution of ZnO shall be avoided (e.g. see Eixenberger et al. (2017)).

Specific test conditions

a. Assessment of impact of agglomeration and degree of dispersal: Commercial nano ZnO encompasses a broad spectrum of agglomeration, from almost single particle dispersions (e.g. in various liquid media/formulations) to highly clustered materials (in particular when generated as dry aerosol). This spectrum shall be adequately considered in the test conditions. Therefore, preliminary comparative toxicity studies using different size fractions of nano ZnO shall be performed to evaluate whether the parameter agglomeration can be neglected in the main study. For pre-testing the toxicity of fine and ultrafine dispersed nanomaterials appropriate exposure methods should be considered. This can be done in vitro or in vivo as considered appropriate. If agglomeration proves to be an influencing factor with respect to toxicity, the main study needs to be performed with at least two respirable size fractions, a strongly and a weakly agglomerating fraction.

b. Continuous monitoring of the particle/agglomerate size distribution and measurements of actual test concentrations within the atmosphere using appropriate methods is required.

c. To enhance the statistical power of the test, the number of parental animals shall be increased in order to provide at least 15 pregnant females per group.

d. At least three test concentrations shall be used per nanoform. The highest test concentration shall reveal clear systemic toxicity but not severe suffering or death. Otherwise a limit concentration of ≥ 0.2 mg/L/6h/d shall be used (this corresponds to a 10-times lower concentration than the maximum concentration as set in GD 39 and OECD TG 413).

e. Males/sires of the F0 generation shall be continuously treated until sacrifice of pups used for neuro-behavioural and neuropathological evaluation at PND 22. Dams shall be continuously treated until (and including) day 19 of gestation and dosing shall be re-initiated as soon as possible and no later than PND 4. The total exposure duration for both sexes of the F0 generation shall be at least 90 days, according to paragraph 17 of OECD TG 413.

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f. Each test and control group shall contain a sufficient number of animals to enable the required testing strategy (analyses according to OECD TGs 413 and TG 421, as well as the additional evaluations requested under i–j and l-o) and adequate statistical analysis.

g. According to paragraph 50 of OECD TG 413, you shall perform the mandatory bronchoalveolar lavage fluid (BALF) analysis, including analysis of lactate dehydrogenase (LDH), total protein or albumin, as well as cell counts and differentials for alveolar macrophages, lymphocytes, neutrophils, and eosinophils.

h. Histopathology shall be carried out according to paragraph 57 of OECD TG 413 in all control groups (including size and solubility controls) and at least at the highest test concentration (n = 10/sex/group). However, it is strongly recommended that histopathological analyses are performed at the lower test concentrations as well. If there are findings at the highest dose level, the lower doses are to be investigated in any case.

i. At the end of the exposure and after termination, the presence of (nano)particulate structures shall be assessed in lung, heart, brain, olfactory bulb and liver using appropriate methodology (n ≥ 5/sex/group). If (nano)particles were detected, their chemical composition shall be analysed using appropriate methodology to be able to differentiate between zinc oxide, other zinc compounds and elemental zinc (n ≥ 3/group).

j. Detailed clinical observations and ophthalmological examinations according to paragraphs 32 – 34 and 38 of OECD TG 424 (Neurotoxicity Study in Rodents) shall be performed in F0 animals at all test concentrations and in controls (n ≥ 10/sex/group) to address potential neurotoxic effects.

k. If an adjustment of litter sizes is considered necessary, adjustment shall be performed according to OECD TG 421, paragraph 33. Nevertheless, each test and control group shall contain sufficient numbers of offspring to enable the required testing strategy (analyses according to OECD TG 421, paragraphs 39 - 49, as well as the additional evaluations requested under l-n) and adequate statistical analysis.

l. During treatment and observation periods, more detailed clinical observations of the offspring shall be conducted according to OECD TG 426, paragraphs 28 – 33 (n ≥ 1/sex/litter/group), and in 1 male and 1 female per litter pre-weaning testing of behavioural ontogeny, including motor activity according to paragraphs 34 and 35 of OECD TG 426 shall be performed, as well.

m. At least 5 pups/sex/group from different litters shall be sacrificed at postnatal day (PND) 22 according to OECD TG 426 and neuropathological analyses of brain (according to OECD TG 426, paragraphs 39 – 45), as well as full histopathological analysis of the other organs shall be conducted. Gross necropsy of pups shall

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encompass visual inspection for obvious skeletal and tissue alterations.

n. The presence of (nano)particulate structures shall be assessed in brain and liver of at least 5 pups/sex/group from different litters after sacrifice at PND 22 (all treatments and controls) using appropriate methodology. If (nano)particles were detected, their chemical composition shall be analysed using appropriate methodology to be able to differentiate between zinc oxide, other zinc compounds and elemental zinc (n ≥ 3/group).

o. A satellite (reversibility) animal group for each control and test material (n ≥ 5 animals/sex/group), using the highest test concentration, shall be added to the main extended repeated dose toxicity study to observe reversibility, persistence, or delayed occurrence of toxicity in F0 animals during a post-exposure period. The post-exposure period shall be at least 45 days. In addition to BALF analysis, organ histopathology shall be performed.

Consideration of alternative approaches

The request for the subchronic inhalation toxicity study is suitable and necessary to obtain information that will allow clarification whether there is a potential risk for systemic toxicity after repeated inhalation of nano ZnO, as indicated by scientific literature data. The OECD TG for subchronic inhalation toxicity (OECD TG 413) has been adapted to nanomaterial testing only recently (June 2018). There is no alternative for using vertebrate animals to provide information on the concerns identified.

In addition, there are currently no validated non-vertebrate or alternative tests to address the reproductive toxicity concerns identified, apart from individual instead of combined testing. There is no alternative for using vertebrate animals to provide information on the concerns identified.

Consideration of your comments to this study request

- *Roughly summarised, you are of the opinion that the three provided repeated dose inhalation studies with one coated ZnO nanoform ([redacted]) are sufficient for DNEL derivations and risk assessment of all registered ZnO nanoforms and that all other information (from the scientific literature) should be discarded due to its unreliability.*

  The evaluating MSCA is of the opinion that

  1) the provided 5 and 14 day studies are rated as range-finding studies, too short to be actually predictive for subchronic and chronic effects (this does not even comply to a sub-acute OECD TG 412 study),

  2) uncoated nano ZnO was tested at only one concentration and only in the 14 day study, limiting predictions with regard to the comparability of

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biokinetics behaviour and toxicity of the tested coated and uncoated ZnO nanoforms,

3) the toxicological representativeness of the tested nanoform in the OECD TG 413 key study for all registered ZnO nanoforms is not sufficiently justified. Data from the 14 day repeated dose-testing do not sufficiently demonstrate that the coated [redacted] is indeed more toxic than the uncoated [redacted] (let alone other ZnO nanoforms), as most of the weak local effects were observed after both, exposure to the uncoated [redacted] and the coated [redacted] and no information on dose-dependency of the uncoated nano ZnO treatment is available. In addition, in a publication from one co-registrant, it is suggested that the uncoated nanoform [redacted] might in fact be more toxic than the coated counterpart: "Presumably, the uncoated ZnO [redacted] [redacted] was slightly more toxic [in rat precision-cut lung slices] than its coated counterpart, since ZnO [redacted] [redacted] did not induce significant effects at all test substance concentrations." (Landsiedel et al., 2014; Sauer et al., 2014),

4) no data was provided demonstrating that the observed effects were (solely) based on Zn^{2+}-ion release, as you state in your ion-only hypothesis. Strikingly, in contrast to this hypothesis, in the respective 14 day repeated dose study ([redacted], 2011), nanoscaled structures resembling nanoparticles were found within the cytoplasm of (predominantly) alveolar macrophages in lungs of rats of the uncoated [redacted] as well as the coated [redacted] treatments, indicating incomplete dissolution of nano ZnO in the lung,

5) no adequate justification was provided justifying why 4.5 mg/m^3 was chosen as the highest dose in the provided OECD TG 413 study. It is deemed too low (triggered by slight pulmonary inflammation in a shorter dose finding study) to observe systemic toxicity. The OECD TG 413 states, that the "maximum concentration tested should consider: 1) the maximum attainable concentration, 2) the need to maintain an adequate oxygen supply, and/or 3) animal welfare considerations.[...] For particles aerosol testing > 2 mg/L should only be attempted if a respirable particle size can be maintained/achieved...",

6) testing in the OECD TG 413 design further did not demonstrate the toxicological equivalency of weakly and strongly agglomerating ZnO nanoparticles. The provided data, on the contrary, indicates that e.g. lung burden and organ weights might be affected distinctly depending on the degree of agglomeration,

7) results of all three provided repeated inhalation studies revealed differences and inconsistency in deposition behaviour and toxicity between the different ZnO (nano)forms tested,

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8) further data from the scientific literature (discussed above) also suggest that several physico-chemical characteristics may influence toxicokinetics and, thus, toxicity of ZnO in nanoform. Literature information also demonstrated that inhalation exposure to uncoated and/or smaller nano ZnO (at similarly low or even lower concentrations than tested in the provided OECD TG 413 study) results in local effects but also in adverse systemic effects.

Altogether, the provided limited data is considered insufficient for adequate hazard assessment of all registered ZnO nanoforms.

Inclusion of relevant scientific literature is a common procedure to identify and/or support concerns. Thus, it was part of the Weight of Evidence approach (WoE) applied to support the identified concerns and underline the consequential necessity for clarification of the concerns by regulatory more robust information. In WoE of the provided and additional data from the scientific literature, concerns arise with respect to repeated dose inhalation toxicity of ZnO in nanoform, which need clarification by testing of different ZnO nanoforms under comparative conditions.

- *You highly recommend to split the combined study design (TG 413 + 489) into separate studies on the grounds that technical challenges which are already present in a complex inhalation study might adversely affect the genotoxicity outcomes of the Comet assay.*

  It is partially acknowledged that the combined parallel testing approach (OECD TGs 413 and 489) may have practical limitations. Accordingly, the information request has been amended to request individual testing for i) repeated dose inhalation toxicity (OECD TG 413) (combined with the Reproduction/ Developmental Toxicity Screening Test, OECD TG 421) and ii) in vivo genotoxicity (OECD TG 489).

Consideration of the Proposals for Amendment (PfA)

One PfA proposed to provide details regarding which BAL parameters should be investigated in the requested study. It was further asked whether a reference to the testing schemes listed in the Annex of OECD TG 413 (option A and option B) might be considered helpful.

Information was added to the paragraph 'Specific test conditions', specifying that the mandatory BALF analysis reported in paragraph 50 of OECD TG 413 is considered sufficient.

The selection of test scheme A or B largely depends on the outcome of the obligatory range-finding study. However, as ZnO is sort of intermediate material with regard to its dissolution behaviour, the more stringent and informative test scheme B (in particular in terms of post exposure observations) should be followed throughout as far as possible for the sake of comparability.

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Nevertheless, as stated in paragraph 'Specific test conditions' (f) above, it is at the discretion of the Registrants to ensure that each test and control group contains a sufficient number of animals to enable the required testing strategy, including the additional requested investigations, and adequate statistical analysis. As with the other specific analyses requested, the decision now specifies that the satellite group for analysis of reversibility of effects is to be performed with ≥ 5 animals/sex/group according to the Annex of OECD TG 413.

In your comments to this PfA you indicated that you agree with the PfA and support the conclusion that the mandatory BALF analyses are to be performed according to OECD TG 413. You further agreed with the PfA indicating that it might be useful to add a reference to the testing schemes listed in the Annex of OECD TG 413 (option A and option B). It is noted that the choice of the testing scheme depends on the outcome of the dose-range-finding study.

Another PfA suggested to perform the Reproduction/Developmental Toxicity Screening test (OECD TG 421) with the Subchronic Inhalation Toxicity Study (OECD TG 413), as the dissolution of ZnO nanoparticles in the gastric fluid was suggested to be much higher than in the phagolysosomal and alveolar fluid and consequently a higher systemic exposure of ZnO nanoparticles via the inhalation route might be expected.

It is agreed that further assessment of inhalation exposure is urgently needed, including investigation of potential site-of-contact and specific target organ toxicity, as well as neurological responses. As significant exposure of humans to nano ZnO via the inhalation route can be expected, further assessment of inhalation exposure is also necessary for determining fertility effects, as well as developmental (neuro)toxicity of ZnO in nanoform.

The request has therefore been modified to omit the request for oral testing in the combined testing approach (OECD TG 422) and to request a Subchronic Inhalation Toxicity: 90-day Study (OECD TG 413) combined with the Reproduction/Developmental Toxicity Screening test (OECD TG 421), in agreement with the received PfAs. This study request is also in line with your comments to the initial version of the draft decision, in which you proposed a similar study design as now requested under 1.

Conclusion

Based on the targeted substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the requested studies using the registered substance subject to this decision as specified above:

Subchronic Inhalation Toxicity: 90-Day Study (OECD TG 413, latest adopted version updated in 2018) combined with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 421) in rat (nose-only) with i) extended histopathology of lung, liver, brain, olfactory bulb and heart (appropriate ZnO particle determination in these organs), and ii) neurotoxicity and developmental (neuro)toxicity evaluation, including detailed

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clinical observations addressing potential neurobehavioural effects. The test shall be carried out according to the conditions stated above. The test materials shall be selected according to the section "Test materials".

2 In Vivo Mammalian Alkaline Comet Assay (OECD TG 489)

The concern(s) identified

The substance evaluation raised specific concerns with regard to (site-of-contact) genotoxicity after inhalation exposure.

Genotoxicity in vitro

You provided a number of in vitro and in vivo studies, differentiating the (ZnO) "nanomaterial" and "zinc oxide" as test materials, while "nanomaterial" was treated as a single entity. You concluded absence of a gene mutation potential in vitro based on a number of negative bacterial assays and one mammalian cell gene mutation test. ECHA considers negative outcomes of nanomaterial testing in bacterial mutation assays of low informational value (Guidance on information requirements and chemical safety assessment, Appendix R7-1 for nanomaterials applicable to Chapter R7a: Endpoint specific guidance, May 2017). A submitted mammalian cell gene mutation test according to OECD TG 476, investigating the impact of the coated [redacted], the uncoated [redacted] and microscaled ZnO at various concentrations, showed a marked increase in mutation frequency but was rated ambiguous by you because of the high level of cytotoxicity in mouse lymphoma cells ([redacted], 2011). ECHA notes that the levels of cytotoxicity observed in this study were acceptable according to the corresponding OECD TG (476). The study by [redacted] (2011) therefore is deemed positive. In an in vitro Mammalian Chromosome Aberration OECD TG 473 guideline compliant study [redacted] ([redacted], 2010) the above mentioned three ZnO (nano)forms did not induce significantly increased chromosome aberrations in hamster embryo lung fibroblasts (V79). Interestingly, V79 proved more resistant towards Zn-induced cytotoxicity (up to 50 µg/ml [redacted] was tested in contrast to a maximum dose of 10 µg/ml applied to lymphoma cells in the OECD TG 476 test). The uncoated [redacted] and micro-scaled ZnO were tested only at one concentration each in this experiment.

Other in vitro genotoxicity studies you cited yielded conflicting results. Following OECD TG 473, Hikiba et al. (2005) observed a dose-dependent increase in structural chromosome aberrations in Syrian hamster embryo cells after treatment with high purity ZnO used in dental practice. In another dental study, ZnO induced chromosome aberration in primary dental pulp cells. Interestingly, concomitantly tested zinc chloride failed to induce structural chromosome aberrations (Someya et al., 2008). However, the representativeness of the tested dental ZnO in these two studies for ZnO nanomaterial is unclear, also due to poor characterisation of the test material (Hikiba et al., 2005; Someya et al., 2008). An earlier study, Suzuki (1987), has been cited as supporting

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evidence. In this study dental ZnO was tested in Syrian hamster embryonic cells by a variety of in vitro genotoxicity assays. Unfortunately, material characterisation, such as particle size and the form of the zinc oxide in preparation, is lacking in this study as well. The results of the performed sister chromatid exchange (SCE) test was rated ambiguous by you. ECHA notes that the SCE assays are not part of the standard battery of tests to investigate chromosome aberration. In an in vitro chromosome aberration test, similar to OECD TG 473, Gümüs et al. (2014) reported structural chromosome aberrations in primary peripheral blood lymphocytes, treated with uncoated nano ZnO (particle diameter: 45 nm; fragment size: 450 nm), which lacked clear dose-dependence. However, concomitantly measured micronucleus frequency in the lymphocytes increased significantly and dose-dependently without excessive cytotoxicity. Corradi et al. (2012) observed increased micronuclei in A549 human lung epithelial carcinoma cells exposed to uncoated nano ZnO ([redacted]) but only at highly cytotoxic concentrations. You further assessed the clastogenic potential on the basis of data obtained from several independent studies using the alkaline single gel electrophoresis (comet) assay, testing different coated and uncoated ZnO nanoforms on a variety of mammalian cell types. Three of the six studies you presented were positive for coated and uncoated ZnO nanoforms, respectively (human nasal mucosa cells: Hackenberg et al. (2011a); Hackenberg et al. (2011b); human hepatoblastoma cells: Kermanizadeh et al. (2012a); Kermanizadeh et al. (2012b)), two testing uncoated nano ZnO only were rated ambiguous by you (Alarifi et al., 2013; Lin et al., 2009) and one was negative for coated but positive for uncoated nano ZnO (Kermanizadeh et al., 2013). Altogether the provided information on clastogenicity is deemed inconclusive.

Additional in vitro evidence from the published literature (not considered in the registration dossier) support a suspected DNA damaging activity of ZnO in nanoform. Most data stems from positive micronucleus and comet assays, performed on a variety of ZnO nanomaterials in different cell types. The majority of these studies indicate oxidative DNA damage (Gerloff et al., 2009; Ghosh et al., 2016; Sliwinska et al., 2015; Yin et al., 2010; Yin et al., 2015). However, there are also studies which reported DNA damage in the absence of ROS generation (Demir et al., 2014b; Heim et al., 2015; Kononenko et al., 2017). Response differences correlated with primary particle size in several but not all of the studies, thus no clear conclusion on the impact of particle size on the genotoxic potential of nano ZnO can be drawn. More recent studies tested the commercial uncoated ZnO nanoforms [redacted] and [redacted] positively in the comet assay and in the micronucleus test, respectively (El Yamani et al., 2016; Roszak et al., 2016). Two alternative assays confirmed the genotoxic potential of ZnO: Nano-sized crystalline ZnO significantly induced DNA double strand breaks in the γH2A·X immunofluorescence assay suggesting clastogenic activity (Heim et al., 2015). Evidence was also provided for gene mutation by two nanoforms of ZnO of different primary particle size by results of CD59 gene loci mutation assay which used hamster-human hybrid cells of a specific karyotype (Wang et al., 2015). These and other studies showed differences in the genotoxic potential between ZnO and other zinc compounds (such as $ZnCl_2$ or zincite) as well. Moreover, several of these studies examined the uptake of ions vs. particles. It has to be stressed that none of the experimental studies was in itself sufficiently convincing

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in favouring or rejecting ion- or particle-borne genotoxicity as the predominant mechanism. This is partially due to the technical difficulties in determining the dissolution rate of the ZnO in the assay system, the measurement of extra- and intracellular zinc levels as well as discrimination between the uptake/fate of cationic zinc, other zinc compounds and/or ZnO nanoparticles.

Nanoparticle coatings of commercial ZnO nanoparticles may serve different desirable functions such as improved dispersibility and stabilisation. The registration dossier did not systematically investigate how the chemistry and the stability of the coating would affect the outcome of a genotoxicity assay. Besides manufactured coating adsorption of molecules from the incubation medium such as bovine serum albumin was shown to protect cells in vitro from clastogenic activity (Roszak et al., 2016). On the other hand, co-exposure of ZnO nanoparticles with 10 % foetal bovine serum increased the frequency of micronuclei.

Other factors that might affect the genotoxic potential of ZnO are photogenotoxicity, oxidative stress, "opsonisation" and aging. There are several studies that reported an impact of light on genotoxicity testing of ZnO (Demir et al., 2014a; Dufour et al., 2006; Gopalan et al., 2009; Jones et al., 2013; Pal et al., 2016) and that handling samples without controlling changing illumination conditions (as is true in the majority of existing studies) may alter the test results.

The crucial role of oxidative stress in ZnO-induced toxicity has recently been reviewed (Feng et al., 2017; Kwon et al., 2014). Oxidative stress is induced by increase of ROS production and decrease of antioxidants such as glutathione (GSH). In terms of genotoxicity, these conditions foster oxidative DNA damage. In fact, a number of the cited clastogenic studies (comet assays and micronucleus test in particular) concomitantly demonstrated an intracellular increase of ROS and/or depletion of GSH induced by nano ZnO but also lipid peroxidation and induction of endonuclease-sensitive sites, the latter a more direct indicator of oxidative DNA damage. However, there are also reports that could not find an association between DNA damage and increased intracellular ROS, indicating that other factors may be relevant in nano ZnO-induced genotoxicity. Several studies added the antioxidant drug N-acetyl-L-cysteine (NAC) to the assay system thereby enhancing GSH levels and thus proving oxidative stress-mediated genotoxicity. However, studies which co-exposed NAC with ZnO such as Pati et al. (2016) in a recent combined in vitro and in vivo study, should be treated with caution as cysteine is known to bind Zn^{2+} with high affinity, forming complexes (Pace and Weerapana, 2014). It should be considered that ZnO is less prone to form ROS via Fenton reactions, unless it is doped with iron or another ion with variable oxidation state.

ECHA concludes that the evidence on genotoxicity/mutagenicity in vitro for nano ZnO is inconsistent. An (oxidative) DNA-damaging potential is likely, indicated by a number of positive results from micronucleus and comet assays, but requires further elucidation. ECHA notes the disparate outcomes of individual studies, which could – at least in part – be due to deficiencies in the testing of nanomaterials in in vitro genotoxicity assays, as standardisation of

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test methods for nanomaterial (NM) is still missing. In fact, important issues, such as possible impact from agglomeration/sedimentation, cellular uptake, adsorption and media depletion, have not been resolved.

The specific challenges of in vitro testing of nanomaterials has been demonstrated recently by the high inter-laboratory variability of genotoxicity test results obtained for nano ZnO and other nanomaterials within the European joint project Nanogenotox (2013). Most strikingly, a recent scientific publication reported that common cell culture media buffer systems such as HEPES induces the rapid dissolution of ZnO nanoparticles, conversion to zinc phosphate/carbonate precipitates and increased cytotoxicity (Eixenberger et al., 2017).

Genotoxicity in vivo

Based on a limited number of controversial in vivo tests you concluded the absence of a relevant genotoxic potential of nano ZnO:

Li et al. (2012) were unable to detect a higher frequency of micronucleated polychromatic erythrocytes (PCE) or a variation in the ratio of PCE to total erythrocytes 24, 48, or 72 h after oral exposure of male ICR mice, treated with either uncoated ZnO nanoparticles or microparticles at three different concentrations (1250, 2500 and 5000 mg/kg bw). Intraperitoneal injection of 15, 30 or 60 mg/kg bw of the registered coated material [redacted] into male NMRI mice did not induce a higher frequency of micronuclei in bone marrow cells 24 or 48 hours (only highest dose) later (in contrast to appropriate positive controls). A slight inhibition of erythropoiesis (PCE/ NCE ratio) at 60 mg/kg was interpreted as an indication that the test substance actually reached the bone marrow ([redacted], 2009a; Landsiedel et al., 2010). It is noted that intraperitoneal administration is not a physiological exposure route. Unfortunately, when investigating the genotoxicity of different (nano)forms of commercial coated ZnO ([redacted] and [redacted]) and TiO_2 nanomaterials in various tests, Landsiedel et al. (2010) did not include any ZnO nanoforms in the comet assay on alveolar lavage cells from the lung of rats after a 5-day nose-only inhalation exposure.

However, [redacted] (2011) tested the coated [redacted] (0.5, 2, and 8 mg/m^3), the uncoated [redacted] and microscaled ZnO (8 mg/m^3 each) in a mammalian erythrocyte micronucleus test (according to OECD TG 474) and a hOGG1 modified comet assay in BAL cells after 14 days of nose-only inhalation exposure of Wistar rats. With regard to the micronucleus test, there was no evidence of a significantly enhanced mean frequency of micronucleated erythrocytes due to [redacted] or microscaled ZnO exposure in males or females, as compared to the vehicle control groups (clean air) at any dose level. The reduced PCE/NCE ratio in the positive control (cyclophosphamide) and in females exposed to the highest [redacted] or [redacted] dose was interpreted as indication that the bone marrow was reached. The result of the comet assay was rated as ambiguous by the authors. BAL cells were analysed 1 and 14 days after the end of the inhalation exposure, respectively, in the absence or presence of the repair endonuclease hOGG1 to detect oxidative DNA damage. After 24 hours, no clear DNA damage was

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observed but after 14 days of recovery, all three (nano)forms demonstrated a genotoxic potential including oxidative DNA damage, which was statistically significant at the highest dose tested for the coated [redacted] only but showing a tendency of dose-dependency. However, no oxidative DNA damage was detected in lung epithelial cells using immunohistochemical methods. The robustness of the comet assay was affected by the limited viability of the isolated BAL cells. The authors concluded that the data on in vitro genotoxicity was partially supported by evidence for local genotoxic effects, however questioned whether BAL cells are a suitable surrogate for lung epithelial cells. The latter were not tested in this study.

Additional in vivo studies not cited in the registration dossier provided inconclusive evidence. A comet assay following intratracheal instillation detected DNA damage in BAL cells immediately after exposure but possibly interfering with cytotoxicity (Jacobsen et al., 2015). Three oral studies reported conflicting outcomes. One study provided oxidative DNA damage in liver tissue (Sharma et al., 2012), another one did not find any genotoxicity in liver or stomach after oral gavage for four different ZnO nanoforms, differing in size and surface charge, neither in the comet assay nor in the micronucleus test (Kwon et al., 2014). A third study, which administered ZnO nanorods in drinking water, was also negative but missed inclusion of positive controls (Bollu et al., 2016). Finally, in contrast to the coated [redacted], uncoated 80 nm ZnO nanoparticles dose-dependently induced (systemic) chromosome aberration and micronuclei in bone marrow and peripheral erythrocytes, respectively, following intraperitoneal injection (Ghosh et al., 2016). However, a comet assay was negative in these cells (corroborating in vitro findings) and ambiguous in liver cells.

ECHA concludes that the available in vivo data raise a human health concern with regard to genotoxicity of nano ZnO. In particular, the observed genotoxic effects by nano ZnO in the lung following inhalation exposure require further testing with potential target cell populations. Impact of the coating should also be studied. In addition, the bioavailability of ZnO at extrapulmonary target organs requires detailed analysis.

Why new information is needed

ECHA considers that the database on genotoxicity you provided is both inconclusive and incomplete, and does not resolve the concern for human health. Further experimental evidence, mostly from comet assays and micronucleus tests indicating an (oxidative) DNA damaging potential of ZnO in nanoform, has been found in the scientific literature, though of inconsistent robustness. ECHA acknowledges that there are substantial methodological challenges associated with in vitro genotoxicity testing of nanomaterials and ZnO in nanoform in particular. ECHA considers that your data do not demonstrate that the nanoparticulate nature is irrelevant and that extracellular, rapidly released zinc cations are the exclusive determining factor i.e. that your "ion-only hypothesis" is justified.

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In fact, there is a lot of uncertainty in terms of the prevailing mechanism of nano ZnO genotoxicity. Broadly, two hypotheses on the mechanism of genotoxicity for zinc oxide in nanoform are currently discussed, a "particle" one and an "ion" one (Feng et al., 2017; Kwon et al., 2014; Roszak et al., 2016; Vandebriel and De Jong, 2012). The "ion-only hypothesis" assumes that the genotoxic effects are caused exclusively by Zn^{2+} released from ZnO extracellularly and taken by cells, where it may interfere with intracellular signalling and metabolism (indirect mechanism) or by entering the nucleus interfering with DNA directly. Thus, the genotoxicity is highly correlated to the dissolution rate of ZnO particles in the extracellular milieu and their surface area. The smaller the particles, the higher the proportion of released Zn ions. This would result in a quantitative difference in dissolved zinc from bulk material and is highly dependent on the pH of the medium, the effective uptake of Zn ions and the absence of extracellular adsorption and/or complexation and dilution. The "particle" hypothesis favours the uptake of the nanoparticles by endocytosis or diffusion and its compartmentalization inside the cell before dissolution and zinc ion formation. This "Trojan horse" effect would be particularly effective in compartments of extreme pH, especially at acidic conditions such as in lysosomes. This mechanism would result not only in quantitative but also in qualitative differences between nano- and bulk particles, as nanoforms would be more easily internalised (perhaps facilitated by different types of coating), which give rise to a high concentration of intracellular Zn^{2+}. In addition, other particle-borne properties (e.g. contaminants, coatings) may contribute to genotoxicity. The different mechanisms would also have an impact on the target tissue in case of in vivo testing, as the "ion-only hypothesis" assumes systemic availability and toxicity, whereas the "particle hypothesis" would rather be restricted to genotoxicity at the site-of contact.

The available in vivo data mirrors this uncertainty. You concluded on the absence of a mutagenic potential of nano ZnO because systemic genotoxicity (bone marrow, peripheral blood cells) was not observed ([redacted], 2009b; [redacted], 2011; Landsiedel et al., 2010). On the other hand, you questioned the relevance of target cells of a positive comet assay results demonstrating (oxidative) DNA lesion in BAL cells after inhalation exposure to nano ZnO ([redacted], 2011). It is also noted that ECHA considers the dosing of the latter study as insufficient to exclude systemic effects.

ECHA concludes that the available data raise a concern regarding human health, specifically with respect to mutagenicity of nano ZnO. More specific in vivo information is required, taking into account both local and systemic effects. The impact of coating, zinc salts, and oxidative damage should be specifically addressed concomitantly.

What is the possible regulatory outcome

The results of the requested study will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the nanoforms of the Substance should be classified for mutagenicity as defined in the CLP Regulation (EC) No 1272/2008. The evaluating MSCA will also assess whether the Substance should be proposed for identification as a Substance of Very High Concern (SVHC) under Article 57

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of REACH, which would lead to stricter risk management measures than those currently in place.

Additionally the information from the study can be used for an adequate risk assessment and establishment of DNELs in particular for controlling the potential risks to consumers and the general population from nano ZnO.

Considerations on the test method and testing strategy

Testing strategy

To obtain information on coating, size and dissolution dependence, testing should compare two nanoforms of ZnO (a coated and an uncoated form) and also include microsized ZnO as well as a zinc salt as 'size control' and 'solubility control', respectively. The latter is required to inform on the "ion-only hypothesis", whereas the former informs on the nano-specificity of effects. For ECHA this is also a reasonable compromise in gaining maximum comparable information, especially with regard to the toxicological representativeness of the tested nanoforms on the one hand, and consideration of adequacy and proportionality of information requests on the other hand.

Test materials

See request 1.

Specific test conditions

a. Assessment of the impact of agglomeration and degree of dispersal: Commercial nano ZnO encompasses a broad spectrum of agglomeration, from almost single particle dispersions (e.g. in various liquid media/formulations) to highly clustered materials (in particular when generated as dry aerosol). This spectrum shall be adequately considered in the test conditions. Therefore, preliminary comparative toxicity studies using different size fractions of nano ZnO shall be performed to evaluate whether the parameter agglomeration can be neglected in the main study. For pre-testing the toxicity of fine and ultrafine dispersed nanomaterials appropriate exposure methods should be considered. This can be done in vitro or in vivo as considered appropriate. If agglomeration proves to be an influencing factor with respect to toxicity, the main study needs to be performed with at least two respirable size fractions, a strongly and a weakly agglomerating fraction.

b. Continuous monitoring of the particle/agglomerate size distribution and measurements of actual test concentrations within the atmosphere using appropriate methods is required.

c. An appropriate positive control shall be administered via inhalation in the requested study on hand. If administering the positive control, a known mutagen inducing DNA strand breaks (e.g. see table 1 of OECD TG 489), via inhalation

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proved to be difficult due to practical and safety reasons, the positive control may be administered by a different route, e.g. by intratracheal instillation.

d. Local genotoxicity shall be investigated in lung (lung epithelial cell suspensions) and in nasal mucosa cells. In addition to bone marrow, liver parenchyma shall be investigated as systemic target tissue. Sampling for genotoxicity investigations shall be done no later than 4 hours after the last exposure treatment. Thus, careful consideration should be given to the tissue sampling for comet analysis alongside the requirements of tissue sampling for other types of toxicological and toxicokinetics assessments, respectively.

e. Gonads shall be stored adequately for potential subsequent investigation of indication of germ cell mutagenicity mutagenicity - (% tail DNA in comet assay).

Consideration of alternative approaches

The request for the in vivo comet assay is suitable and necessary to obtain information that will allow clarification whether there is an actual risk for mutagenicity after repeated inhalation of nano ZnO. Genotoxicity testing in vivo according to OECD TG 489 is required as positive results after inhalation exposure need to be clarified on relevant target tissues. The deficits in standardisation of current protocols for in vitro testing of nanomaterials have been pointed out. Therefore, there is no alternative for using vertebrate animals to provide information on the concerns identified.

Consideration of the Proposals for Amendment

One PfA stressed that there might not be many laboratories that can perform the analysis on the nasal tissue and that analysis of nasal tissue is not properly validated.

The analysis of nasal tissue within the in vivo comet assay is not properly validated, yet. However, literature data indicate that the comet assay is suitable for the analysis of nasal tissue (e.g. del Castillo et al., 2002; Glück and Gebbers, 2000; Heydens et al. 1999; Jeffrey et al., 2006; Klein et al., 1999).
As this is an additional parameter added to the already validated analyses to be performed within the requested OECD TG 489, the outcome of the specific nasal tissue analysis is deemed additionally advantageous and specifically valuable to be able to evaluate the site-of-contact genotoxicity of nano ZnO.

You agreed to the PfAs that genotoxicity testing of nasal tissue lacks standardisation. ECHA, however, adheres to the requested study design, as nasal tissue has been identified as target tissue of concern. References have been added regarding this type of investigation (within an OECD TG 489 study).

Another PfA indicated that there are various pitfalls that need to be considered when combining OECD TG 413 and OECD TG 489, and that both studies shall only be

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combined as long as this will not impair the validity of and the results from each individual study.

In accordance with the proposals for amendment received, separate testing of OECD TG 413 and OECD TG 489 is now considered inevitable, as the repeated dose inhalation toxicity study is now requested to be combined with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 421). Thus, a combined testing of OECD TGs 413 and 489 is no longer feasible.

A third PfA proposed to specify the route of administration of the positive control, as it is important that the same route should be used for test and control substances when measuring site-of-contact effects. This, however, might prove difficult due to practical reasons. Hence, besides inhalation, also intratracheal instillation was suggested as route of administration for the positive control.

It is agreed on that the positive control ideally should be administered via the inhalation route, but it is also acknowledged that this might prove difficult due to practical and safety reasons. Hence the routes of administration, which shall be considered in the present case, are now specified in section 'Specific test conditions'.

Conclusion

Based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you shall carry out the following study using the registered substance subject to this decision as specified above:

In Vivo Mammalian Alkaline Comet Assay (OECD TG 489) in rat, inhalation route (nose-only), including site-of-contact genotoxicity evaluation in lung epithelial cells and nasal mucosa cells, as well as systemic genotoxicity evaluation in liver in addition to bone marrow. The test shall be carried out according to the conditions stated above. The test materials shall be selected according to the section "Test materials". See references (e.g. del Castillo et al., 2002; Glück and Gebbers, 2000) for further details on analysing nasal tissue in a comet assay.

Consideration of your additional comments with regard to HH testing

- *In the decision testing of respirable dry aerosol and nebulised wet aerosols of ultrafine dispersions under size-controlled conditions is required before the actual inhalation tests start. You indicate that for you it is unclear what pre-testing is really required.*

  The request aims to demonstrate that agglomeration does not confound toxicity testing. The testing of respirable dry aerosol as well as nebulised wet aerosols refers to reported differences in the degree of dispersal of ZnO nanoparticles in dry state or in various liquid media/formulations. Therefore, preliminary comparative toxicity studies using different size fractions of nano ZnO (small agglomerates, ultrafine dispersed vs. larger agglomerates, fine dispersed) shall

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be performed to evaluate whether the parameter agglomeration can be neglected in the main toxicity study or whether several size-fractions have to be tested. Pre-testing can be done in vitro or in vivo as considered appropriate. For specifics of pre-testing please refer to the amended chapter "Specific test conditions" under request 1 and 2.

- *You consider it crucial that the evaluating MSCA chooses or at least provides advice on the nanoform(s) to be tested.*

  The selection of representative nanoforms fulfilling the specific requirements set out in section "test materials" should be at your discretion, as you know best the specifications of your materials. The evaluating MSCA, however, is willing to provide support in the selection of test materials by reviewing a list with several specified candidate test material(s) you selected and justified before the human health (HH) testing actually starts.

- *You propose a step-wise testing approach with dissolution studies of all ZnO nanoforms as a first step to further substantiate your "ion-only" hypothesis. Depending on the results of these studies (and if additional data is deemed necessary by you), you propose to perform an in vitro/vivo TK/ADME study to compare nano ZnO forms regarding possible repeated dose effects. You further expect that consequential in vivo oral testing will not be necessary, as you anticipate that in strongly acidic solutions all ZnO regardless of the form will readily and completely solubilise, indicating that all Zn(O) forms are comparable with respect to toxicity.*

  The toxicity of ZnO in nanoform in the organism cannot be adequately predicted based solely on in vitro bio-dissolution testing. Comparative in vivo data corroborating any correlation between in vitro dissolution rate (or behaviour) and in vivo toxicity of nano ZnO is missing in the dossier. On the contrary, where comparative data is available, this rather indicates that there are actual differences in distribution, toxic potential and target organs between different ZnO (nano)forms, as described in detail above. It is further noted that according to ECHAs "Read-Across Assessment Framework (RAAF)" (ECHA, 2017d), in vitro studies are insufficient as standalone information for read-across justification.

  Therefore, the fate and toxicity of ZnO in nanoform cannot be sufficiently explained solely based on in vitro dissolution data. Without relevant comparative in vivo data, the read-across between (nano)forms of ZnO, i.e. the "ion-only" hypothesis provided by you is insufficiently justified. In vivo testing is rather deemed necessary to get information on target tissues depending on the route of exposure of ZnO in nanoform and on particle-related uptake, as well as intracellular effects.

The general value of categorised approaches for preliminary particle characterisation is acknowledged. However, ultimately determination of applicability requires validation of

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the data in vivo. ECHA is, hence, not convinced regarding the step-wise testing approach you proposed, but rather adheres to the requested study design. However, ECHA agrees on the proposal to perform toxicokinetic analyses in vivo. To comply with the 3R principle, it is suggested to integrate toxicokinetics analyses into the requested repeated dose toxicity studies. Furthermore, the decision considerably reduces the test materials to two nanoforms (representing different categories: coated and non-coated), a "size control" and a "solubility control", to be able to gain sufficient information with respect to comparability of toxicokinetics and toxicity of ZnO in nanoform by still taking the 3R principle into account.

- *You are of the opinion that the need for data on accumulation of nano ZnO in several organs as well as specification of the nano ZnO form is not justified related to the human health concern, as accumulation is not considered as an indicator for adverse effects by you.*

Regarding the requested data on the accumulation of nano ZnO in organs, this aspect does not refer to simple Zn/ZnO measurements (e.g. by ICP-MS), but rather refers to the detection and identification of particulate matter in the respective organs. As results from the provided studies, as well as from several publications found in the scientific literature indicate that exposure to nano ZnO (orally and via inhalation, for details see above) might lead to nanoparticle accumulation in organs causing adverse effects, the assessment of accumulation of nanoparticles and their identification in several specific organs to be crucial as a first tier.

- *You state that for human health requests some specific test conditions, e.g. neurotoxicity testing and measurement of potential neuron or synapse damage and inclusion of satellite (reversibility) animal groups will not impact the existing DNEL derivations and are therefore considered not necessary.*

The need for clarification of the concern that nano ZnO can in fact induce neurotoxicity in adult and developing animals as essential, as this concern was raised based on several findings from publications found in the scientific literature. Moreover and contrary to your statement, additional testing, including testing for e.g. neurotoxicity, might very well alter the DNELs derived in the CSR.

This specifically concerns the derived long-term DNELs for systemic effects (oral and inhalation) provided in the dossier, which are currently based on a study, that has not tested ZnO in any (nano)form, but rather tested another zinc compound. A specific justification for this read-across is not provided in the dossier, except for reference to the "ion-only" hypothesis, which is rejected by ECHA and the evaluating MSCA. Results of studies actually testing ZnO in nanoform in a validated repeated dose study design, hence, might affect the derived oral and inhalation DNELs for systemic effects drastically, as literature information indicates. Furthermore, this also concerns the provided derived inhalation DNELs for long-term local effects, which are currently based on a low-dose repeated inhalation study with a single coated ZnO nanoform. As evidence and justification for the toxicological representativeness of this nanoform is missing in the

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dossier, this information is considered insufficient for DNEL derivation. The lack of comparative data limits the significance of the observed results to the tested coated ZnO nanoform, as sufficient evidence indicating the possibility of an adequate read-across to other, ZnO (nano)forms is lacking. In fact, additional studies from the scientific literature (cited and described in detail above) suggest that inhalation exposure to other ZnO nanoforms at similarly low or even lower concentrations than tested in the study used for DNEL derivation, can result in adverse local but also adverse systemic effects.

Thus, these concerns need to be addressed appropriately. Nevertheless, parallel neurotoxicity testing within the standard protocols can be technically and logistically demanding and changed the DD accordingly to further account for proportionality and animal welfare.

Regarding the inclusion of satellite (reversibility) animal groups for each tested material in the repeated dose toxicity tests, information from the scientific literature suggest, that repeated inhalation and oral exposure, respectively, to ZnO in nanoform can yield severe systemic effects, which - in some cases - were reported to persist or even progress over time. Thus, the implementation of satellite groups evaluating reversibility/persistence/progression of effects after repeated oral dosing/repeated inhalation with nano ZnO as justified, as they might very well affect DNEL derivation.

- *You disagree with several claims made in the draft decision, because based on publications which are "exploratory in nature and with obvious technical deficits" it is not appropriate to question the results of valid guideline studies and request further animal testing.*

  Regarding the guideline and GLP-conform data on nano ZnO presented in the dossier, the results of these studies are not questioned. However, there are some limitations regarding selected test concentrations, test material selection and comparability to other nanoforms, as stated in detail in request 1.

  All in all, the evaluating MSCA performed a thorough WoE analysis for each evaluated endpoint based on all information provided by you, as well as further information retrieved from the scientific literature. Consideration of such literature, even if rather exploratory in nature, is a common procedure to identify supporting information to build up lines of evidences. Hence, further information from the scientific literature contributed to the WoE applied to support the raised concerns and to underline the necessity for clarification of the concerns by regulatory more robust information. Shortcomings of these publications are addressed in this decision. The overall outcome plausibly supported a concern. For publications in which shortcomings were noted or which raised questions with respect to scientific validity/credibility the evaluating MSCA cross-checked the authors' expertise and publication record.

  For a number of human health endpoints such as oral repeated dose toxicity and fertility, respectively, you did not provide any information with respect to nano

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ZnO, which additionally justifies consideration of explorative data from the scientific literature. Moreover, this information is relevant for comparison, as frequently you provided information only for one specific nanoform, without justifying that this nanoform is toxicologically representative for other ZnO nanoforms. In some cases, scientific results contradict the presented ones, which thus further points towards the need for clarification. Thus, the toxicological information provided in the technical dossier is insufficient and additional health concerns have been identified which need clarification by robust studies.

- *At least one registrant of nano ZnO claims that the imported or manufactured ZnO nanoforms are solely used for cosmetic and pharmaceuticals preparations. It was further highlighted that Regulation (EC) No 1223/2009 on Cosmetic Products explicitly prohibits performing animal tests with cosmetic ingredients after March 2013 and if animal testing is performed after that date, a marketing ban of the respective ingredient can be imposed, wherefore the respective registrants are not willing to participate in the performance of such animal tests.*

  Based on a Proposal for Amendment additional text on uses and exposure was added at the beginning of Appendix 1 which also addresses the Registrant's concern on testing of substances exclusively used for cosmetic uses.

Consideration of other Proposals for Amendment with respect to human health testing

One PfA proposed to rewrite the sections "What is the regulatory outcome" of each human health study request.

The evaluating MSCA agreed with the proposed text and changed these paragraphs accordingly.

You indicated that you do not agree with this PfA, as the new text states that "the information from the [requested] study can be used for an adequate risk assessment and establishment of DNELs in particular for controlling the potential risks to consumers and the general population from nano ZnO". You are of the opinion that the available 90 day study is sufficient for DNEL derivation and risk assessment of nano ZnO. However, as stated in detail in section 'Consideration of your comments to this study request' under request 1, ECHA does not consider the available data sufficient for DNEL derivation of all registered nanoforms of ZnO. Moreover, as there is an additional concern for reproductive toxicity of nano ZnO, the requested (combined) study (OECD TGs 413 and 421 is deemed necessary for an adequate risk assessment of ZnO in nanoforms and will contribute to the clarification of the concerns raised.

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Environment

The concern identified

Your registration dossiers list a number of ecotoxicity studies featuring data exclusively collected for uncoated nanoforms of ZnO. Studies on acute and chronic effects from literature are also discussed in the registration dossiers. Test organisms studied include fish, aquatic invertebrates (fresh and salt water), algae (fresh and saltwater), aquatic and terrestrial plants, sediment organisms, microorganisms, as well as soil macroorganisms. Thus, the dossiers include a large collection of ecotoxicity data of uncoated nanoforms of zinc oxide. However, the relation of the nanoforms investigated in these studies to the nanoforms registered remains unclear. Only few studies included in your registration dossiers specifically address one of the nanoforms defined in the dossier. Most of the tested ZnO nanoforms covered by literature target primary particles sizes around 30 nm and feature no coating, thus, they only resemble some of the registered ZnO nanoforms (i.e. "Zinc oxide nano").

Your conclusions on most of the ecotoxicity endpoints aim to support the hypothesis that nanoforms of zinc oxide are less or equally toxic than the microsized and/or dissolved form inducing Zn^{2+} cations (hypothesis that zinc ions represent the worst-case). For this aim, mainly studies were selected as key studies which feature a comparison of the ecotoxic effects of a nanoform of zinc oxide and its ionic counterpart. And indeed, some studies indicate that nanoscale zinc oxide is less or equally toxic than the tested ionic counterparts.

However, there are several studies concluding that zinc oxide in nanoform seems to be more toxic than the ionic form (Li et al., 2017; Manzo et al., 2013; Santo et al., 2014; Xiong et al., 2011; Yu et al., 2011; Yung et al., 2015; Zhu et al., 2009) or the microsized form (Manzo et al., 2013). This effect may be due to a higher toxic potency, an additional mode of action or an altered way of exposure.

As environmental exposure to zinc oxide in nanoforms cannot be excluded due to wide dispersive uses, there is a further concern for toxicity to aquatic organisms that is specific to the zinc oxide in nanoforms as explained below. This potential risk needs to be clarified.

Aquatic plants and Algae

In your registration dossiers on ZnO many studies on effects of nanoscale ZnO on algae are available. Three of the key studies in the registration dossiers feature effect values on salt water algae, one on fresh water algae. However, none of the key studies was performed using one of the registered ZnO nanoforms as a test substance.

Effect concentrations were calculated either from nominal concentrations or concentrations not specified to be nominal or measured. This has to be considered as considerable limitation as based on agglomeration and dissolution processes strong

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variations from the nominal concentrations and thus altered effect concentrations are assumed. Effect values are in the same range for micro-sized and nano ZnO and the respective zinc salt with the exception of one study with the saltwater algae *D. tertiolecta* (Manzo et al., 2013). For this species a 10-fold increase in the NOEC (4 days, growth inhibition) was found for micro-sized ZnO compared to nano ZnO (0.8 mg/L for micro-sized, 0.08 mg/L for nano, expressed as elemental Zn). Manzo et al. (2013) conclude that nano-sized ZnO is more toxic than its micro-sized counterpart. As toxicity of the Zn salt was even higher, Manzo et al. (2013) concluded that based on a constraint of diffusion for the nano- and micro-sized ZnO the bioavailability might be altered and that mainly specific physico-chemical properties determine the difference in toxicity of micro-sized and nanoform, so such behaviour cannot be strictly related to the toxic action of zinc metal ions.

Differences in toxicity of different ZnO forms were also found in studies with marine diatoms (Li et al., 2017; Yung et al., 2015) for which lower effect concentrations for the nanoforms were found compared to the zinc salt. Thus, the relative toxicities of nano and salt forms of ZnO were different in the study by Manzo et al. (2013) compared to those by Li et al. (2017) and Yung et al. (2015). Although it is not known which factors contribute to the relative toxicities, the results clearly show that deducing toxicity data from other metal oxide species or zinc salts might misinterpret the toxic action and manifestation.

These studies (Manzo et al., 2013; Li et al., 2017; Yung et al., 2015) were not performed by using one of the registered zinc oxide nanoforms. Nevertheless, these studies provide evidence for nanospecific effects beyond ion toxicity. Hence, they raise a concern on the proper assessment of a nanospecific toxicity to aquatic organisms which needs to be clarified for the registered zinc oxide nanoforms.

Aquatic invertebrates

In your registration dossiers one study addresses long term toxicity of nano ZnO on aquatic invertebrates. In this study one of the registered ZnO nanoforms ([redacted]) has been tested (Adam et al., 2014). It is a comprehensive study according to OECD TG 211 and includes a comparison of the tested nanoform to a respective Zn salt ($ZnCl_2$). Effect concentrations were calculated based on measured concentrations and expressed as elemental Zn. The results showed similar chronic effects on reproduction for nanoscale ZnO and $ZnCl_2$ (NOEC (nano): 58 µg/L Zn and NOEC ($ZnCl_2$): 40 µg/L Zn). In parallel, dissolution kinetics of the ZnO nanoform in different test concentrations in the test media were investigated, showing a fast dissolution of the ZnO nanoform between 69% (average minimum) to 100% within a few hours upon spiking. Due to this, the authors conclude that toxicity of nano zinc oxide is mainly mediated by the dissolved zinc ion. Even though the study is well conducted, it remains unclear how the results and observations on long term toxicity and dissolution kinetics relate to the unknown toxicity of the other registered nanoforms with deviating sizes, morphologies and surface functionalities which may alter dissolution, agglomeration and thus bioavailability.

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Three additional studies on long term toxicity for daphnia of ZnO nanoforms can be found in your registration dossier. The studies are well conducted; two of them considered nanoforms also registered in the dossier. However, one of these studies (Adam et al., 2015) cannot be considered as a chronic test in terms of growth and reproduction. It rather presents a 10 day exposure test with a 10 day recovery period on juveniles and as such cannot be considered for chronic effects on daphnia for which the endpoint reproduction is central for interpretation.

The other study (Lopes et al., 2014) investigated feeding inhibition upon 4 days which cannot be considered as chronic data either. Nevertheless, reproduction of a registered ZnO nanoform was investigated and effects based on nominal concentrations were found to be in a similar range for all zinc exposures. However, as it is generally acknowledged that transformation processes like the dissolution kinetics, agglomeration and sedimentation will mainly influence toxicity and bioavailability of ZnO nanoforms, the question remains how this specific registered nanoform investigated in this study can be used to represent the toxicity of all other registered nanoforms of zinc oxide and be considered as representative of a worst case.

This is highlighted by the third study (Bacchetta et al., 2017) using a nanoform of ZnO different from those in the registration. The author concluded that based on their data Zn^{2+} ions play a key role but ZnO nanoparticles are able to cause specific toxic effects based on their capacity to release Zn^{2+} ions after being internalised in the cells as indicated in a 21 day study in daphnia. This is supported by Santo et al. (2014) who showed that acute toxic effects of zinc oxide nanoparticles are independent from the dissolved Zn^{2+} available in the media in a 48 hour study in daphnia.

These findings raise further concerns for nanospecific toxicity to aquatic organisms especially if the dissolution of the various zinc oxide nanoforms is slow or incomplete.

Conclusion

As described above for aquatic plants and algae and aquatic invertebrates, available data raise a concern for nano-specific toxicity to aquatic organisms to be clarified.

It is known that transformation, dissolution and dispersion stability, which are determined by environmental conditions as well as physico-chemical properties of the individual nanoform (like size, morphology and surface functionality), influence exposure and subsequently effects to environmental organisms (Levard et al., 2012; Misra et al., 2012; Misra et al., 2014; Starnes et al., 2015). The toxic potential of the registered nanoforms may deviate from the tested nanoforms (as published in the literature) as they may differ from each other in terms of dissolution and dispersion stability.

Based on the strong dependence of bioavailability and thus toxicity on dissolution and dispersion stability of the nanoforms in environmental settings, which is also acknowledged by you (e.g. in the endpoint summary on bioaccumulation); ECHA also believes that ecotoxicity of the registered nanoforms of ZnO cannot be predicted by data

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from ionic zinc forms *per se* (hypothesis that zinc ions represent the worst-case), nor be comprehensively represented by ecotoxicological data on ZnO nanoforms covered by literature data.

Why new information is needed

As mentioned above, there are studies available on the toxicity of nanoscale zinc oxides which show lower effect values than the ionic counterpart (also reviewed in (Notter et al., 2014)). ECHA concludes that ecotoxicity data for the nanoforms cannot be deduced from ionic species alone without any risk of underestimation. Therefore there is a need for individual hazard assessment of every nanoform, as long as valid arguments for conservative grouping are not in place.

As aquatic organisms are supposed to be sensitive towards environmental pollutants, including nanomaterials, and due to the special function of aquatic toxicity data for classification, there is a need to reliably clarify your conclusion regarding the use of ecotoxicological data of the ionic form for the nanoform with regard to aquatic organisms and to provide evidence that the selected literature studies used for verifying the conclusion cover the properties of the various registered ZnO nanoforms.

Therefore, further data are needed, to

a) verify or refute your hypothesis that nanoscale zinc oxide features a reduced or equal ecotoxicity than the ionic form and thus data on the ionic form are conservative and protective enough to assess the hazard of nanoforms and

b) justify that the tested forms (from literature) are representative for all nanoforms covered in the registration dossiers.

Requests

3. **Information on transformation, dissolution and dispersion stability of the manufactured and imported nanoforms of zinc oxide that are covered by the registration dossier(s) submitted for zinc oxide**
   a. **Screening Test (24 hours) from OECD Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media, , OECD Series on Testing and Assessment Number 29, ENV/JM/MONO(2001)9; 2001 (OECD, 2001)**

   b. **OECD TG 318 on dispersion stability of nanomaterials in simulated environmental media (OECD, 2017)**

One purpose of this request is to enable you to select nanoforms that you shall test under requests 4 and 5.

Dissolution, transformation and dispersion stability are fundamental parameters that influences fate during testing and knowledge on these parameters are needed as a first

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step in preparation of further test strategies and to interpret test results (OECD, 2014; Reihlen et al., 2018; Steinhäuser et al., 2017). Beside the influence of test media and chemical composition, properties of the nanoforms, like size, shape and surface coating influence dissolution, transformation as well as dispersion stability (ECHA, 2017b; Hartmann et al., 2014). In addition, dissolution, transformation and dispersion stability influence each other. Furthermore, it is highlighted that dissolution, transformation and dispersion stability are (beside other parameters) very important to build hypotheses for grouping and read across for environmental hazard assessment (ECHA, 2017a; RIVM, 2015).

The test results of requests 3a and 3b will subsequently allow to decide on the relevance of the data already provided in your registration dossiers and to group the nanoforms with respect to their behaviour under environmentally relevant conditions. Representative nanoforms shall then be selected from these groups and be tested under request 4 and 5. Taken together the information from requests 3, 4 and 5 will then allow the evaluating MSCA to conclude on the environmental behaviour and hazard of all nanoforms covered in the registration and to decide on further risk management measures for individual groups of nanoforms.

The information under 3.a. and 3.b. shall be provided for each ZnO nanoform registered. The extended dispersion stability test (part of request 3.b.) does not need to be performed for those nanoforms which show low dispersion stability or high dispersion stability, respectively, under all conditions of the screening test. The methods applied for sampling and analytics shall ensure that the remaining nanomaterials will be separated from the aqueous phase. Information on method and energy input for agitation shall be reported.

What are possible further risk management measures

The requested information will be used by the evaluating MSCA for the risk assessment of zinc oxide in nanoform. Based on the results of the requests additional risk management measures may be proposed. For example, the requested data on specific nanoforms (or groups of nanoforms) could lead to different M factors and thus different environmental classification of mixtures containing ZnO nanoforms, compared to the current situation. The current harmonised classification for ZnO for aquatic hazards is Aquatic Acute 1 and Aquatic Chronic 1 but does not include M factors. Thus, the new test results could indicate that the criteria for a higher M factor than currently used are met for acute and/or chronic hazard for certain nanoforms of ZnO. As there is currently no harmonised M factor, M factors for ZnO nanoforms must be set by the manufacturers, importers and downstream users in accordance with CLP Article 10(4). A higher M factor could lead to the consequence that a larger proportion of mixtures containing ZnO nanoforms would be classified as hazardous to the aquatic environment as well as to a more stringent classification of those mixtures containing ZnO nanoforms which are currently classified as hazardous to the aquatic environment. This would enhance risk management measures and would then also have an impact with regard to other legislations which make direct reference to the classification and labelling according to

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the CLP Regulation. Also, a harmonised classification for specific ZnO nanoforms including an M factor could be considered.

Consideration of your comments on request 3

For performing the 24 hour dissolution screening test you proposed in your comments to use a pH value of 7.6 as this is a pH value at which there is an equilibrium with air for the ecotoxicity testing. This proposal seems plausible as it is in the range of pH conditions noted for the ecotoxicity test conditions.

You also asked for advice on experimental aspects for fractionating dissolved and particulate fraction resulting from the dissolution test. According to literature ultracentrifugation in combination with ultrafiltration has successfully been used. To realise preferable small cut-off diameters filtration membranes with low kDa values are applied. Thus, publications such as e.g. (Li et al., 2011), (Miller et al., 2010) or (Merdzan et al., 2014) used filtration membranes with cut off values of 3kDa. Reference for appropriate analytic methods can be found at e.g. (Misra et al., 2012), (Merdzan et al., 2014) or (Ma et al., 2012). When conducting centrifugal ultrafiltration appropriate timescale and acceleration for the material under investigation needs to be defined. Care should be taken about the influence of separation time on further dissolution and should be disclosed within the calculation of dissolved fraction. Furthermore, it is advantageous to use membrane materials that show low absorption of released ions.

Consideration of your comments on the testing strategy

In your comments you question the sense of performing the study requested under 3.b. by applying OECD TG 318 and considered this information rather a research issue than relevant for the overall testing strategy of the environment related requests. Alternatively, you proposed to determine the dispersion stability within the 24 hour dissolution screening test.

The aim of the studies requested under 3.a. and 3.b. is explained above. The investigation of dispersion stability within the dissolution test is not expedient. The dissolution screening test according to GD 29 takes place under permanent agitation where particles and agglomerates are not able to settle. In consequence only the agglomeration size can be determined and it is currently questionable how reliable and robust that approach would be and which analytic techniques would deliver proper values: e.g. electron microscopic pictures can be afflicted by preparation artefacts and can be statistically weak depending on the number of provided pictures, DLS measurements overvalue bigger agglomerates and do not deliver transparent information on particle/agglomerate sizes. In contrast TG 318 provides a mass concentration of the stable fraction in the supernatant (OECD, 2017). With OECD TG 318 a standardised test protocol is available, an additional advantage compared to your proposal.

Furthermore, ECHA does not agree to your assessment regarding the importance of this information:

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- The regulatory relevance of this endpoints for environmental risk assessment of nanoforms is well known and internationally accepted,

- With TG 318 a standardised test method exists which can be used to reliably and reproducibly address this endpoint,

- even though in general deviations from standard test protocols could be accepted for reliable assessment of data on request 3.a. and 3.b., it is inevitable to apply standardised test methods, i.e. to employ OECD TG 318 to address request 3.b. instead of collecting data by using protocols not intended for that endpoint. It is anticipated for that request to strengthen the significance of the data. Conversely, it is unclear if the protocol of GD 29 would provide meaningful results for dispersion stability.

Therefore, the request to conduct the study on dispersion stability using the OECD TG 318 is maintained.

4. **Freshwater Algae and Cyanobacteria, Growth Inhibition Test (test method: OECD TG 201, 2006, EU method C.3)**

5. **Long-term toxicity on invertebrates (*Daphnia sp.*) (test method: *Daphnia magna* reproduction test, EU C.20/OECD TG 211);: *Daphnia magna* Reproduction Test, EU C20**

Test materials:

The information requested under 4. and 5. shall be provided for the following ZnO nanoforms based on the results from requests 3.a. and 3.b.:

a. For the nanoform with the highest, lowest and a mean dissolved Zn^{2+} concentration based on the results from request 3.a. If all nanoforms show very similar (≤±10%) dissolved Zn^{2+} concentration only one representative nanoform shall be selected based on this step. If the Zn^{2+} concentration in the mean case is very similar (≤±10%) to the highest/lowest dissolved Zn^{2+} concentration only tests with the nanoforms of the highest and lowest dissolved Zn^{2+} concentration shall be performed; and

b. For one representative nanoform from the group of nanoforms with low dispersion stability based on the screening test, high dispersion stability based on the screening test and with condition-depending dispersion stabilities based on the full test, respectively (as defined in OECD TG 318 in paragraph 57.) based on the results from request 3.b. If no nanoform belongs to one of these groups no tests on ecotoxicity for that group is needed. It is possible that the selected nanoforms

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from request 3.b. are identical with the selected nanoforms based on request 3.a) (so the overall number of tests to be conducted varies between 1 - 6).

Consideration of your comments on test materials

You highlighted in your comments that the draft decision states that the overall number of tests to be conducted under requests 4 and 5 varies between 1 and 5. In response, a maximum of 3 nanoforms based on request 3.a. and a maximum of 3 nanoforms based on request 3.b. are to be tested. The draft decision was modified accordingly. However, the probability of overlap of nanoforms to be tested based on the results of requests 3.a and 3.b is assumed to be high.

You asked for the evaluating MSCA's advice on the selection of nanoforms of ZnO to be tested under request 4 and 5. It is up to you to choose the appropriate nanoforms to be tested based on the results from requests 3.a. and 3.b. The selection of representative nanoforms specified under section "test materials" is at the discretion of the registrants, as they know best the specifications of their materials. The evaluating MSCA, however, is willing to support you in the selection of test materials by discussing the results of requests 3.a. and 3.b. when they are available.

Specific test conditions:

a. The following adaptations shall be made in the test media to be used for request 4 and 5, respectively: to minimise complexation of zinc ions, the amount of $Na_2EDTA{\cdot}2(H_2O)$ shall be minimised (by balancing the molar concentrations of iron and $Na_2EDTA{\cdot}2(H_2O)$). The pH shall be the pH at which the medium equilibrates with air (generally around 7.6) and the temperature shall be 20 °C.

b. For conducting these studies, the OECD Guidance on Sample Preparation and Dosimetry (ENV/JM/MONO(2012)40 (OECD, 2012), in particular sections III, IV, and V-A through V-C) and ECHA Guidance on information requirements and CSA – Appendix on nanomaterials applicable to Chapter R7a and R7b Endpoint specific guidance (ECHA, 2017b; ECHA, 2017c) shall be consulted. Methods of stock preparation and for application of test substance shall be fully reported. The composition of the test media shall be fully reported (including at least ionic strength, calcium concentration and hardness, pH, alkalinity, dissolved organic matter, and presence of dispersing agents). In addition, the following important conditions shall be taken into account:

    i. Throughout the study, the concentration of the test substance in the aquatic phase and the ratio between particulate and ionic zinc shall be monitored in samples from the test vessels, using analytical techniques that enable distinction between the concentrations of the nanoform of zinc oxide and ionic zinc. This monitoring data shall be reported. If relevant, information on stability of surface coatings during test performance shall be provided.

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ii. The study setup shall include a control with exposure to zinc chloride to enable distinction between the toxicity of the nanoform of zinc oxide and ionic zinc.

c. The nanoforms of zinc oxide that are tested shall be sufficiently characterised. Besides dispersion stability and dissolution rate this also includes primary particle size, surface area, surface charge and surface composition/surface chemistry. Guidance in this regard can be taken from the ProSafe Joint document (Steinhäuser et al., 2017). The information on characterisation shall be included in the full study reports.

Consideration of alternative approaches

The requests are suitable and necessary to obtain information that will allow clarifying whether there is a risk for the environment. More explicitly, when considering available alternatives it is the least onerous way to obtain information. Possible alternatives would be testing all nanoforms, additional parameters as well as requesting fish tests (possibly for all or on all selected nanoforms). Testing all nanoforms without considering results based on request 3.a. and 3.b. could potentially lead to creation of surplus information of little value. Testing of fish without considering the results from requests 3 to 5 could lead to potentially unnecessary vertebrate testing.

Testing fewer nanoforms is not effective and does not generate the similar and sufficient information for the registered nanoforms of zinc oxide.

Consideration of your comments on the testing strategy

You call for considering "the consistency of patterns" of ZnO ecotoxicity that you were presenting in your comments "rather than to focus on exemptions". Based on the remaining knowledge gaps on specific ecotoxicity of different nanoforms and unknown species sensitivity towards Zn species for nanoforms, ECHA doubts that there is enough evidence for a consistent pattern of ecotoxicity that also applies to (all) nanoforms. Therefore, ECHA is of the opinion that the requested test strategy for environment with all its elements (requests 3a+b, 4 and 5) features an appropriate, logical and strategical stepwise approach to verify the pattern hypothesized in the registration dossier for the registered nanoforms based on the cited literature.

Consideration of Proposals for Amendment (PfA)

A PfA suggested the deletion of requests 3 to 5 as well as the request for a long-term toxicity study in fish in order to focus on the human health concern first, and based on the argument that the provided justification for the testing did not clarify how it was proportionate in relation to the planned further risk management measures. In addition the PfA states that it is unclear which and how many nanoforms should be tested and the deletion of the requests would make the decision focused first on human health

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endpoints. You agreed to the PfA and its arguments. In addition you stated that the soluble form is causing the toxicity which is already accounted for in the risk characterisation and related risk management measures and no further environmental risk needs to be managed.

It is not agreed to completely delete or postpone the requests for environmental studies. For adequate risk management and to ensure a high level of protection for human health and the environment both concerns should be addressed in parallel. The consequence of deriving an M factor is one of the possible risk management measures. (Environmental) classification and labelling is seen as an important risk management measure and M-factors have an important impact on the classification of mixtures, as it has been clarified in the decision. Based on the description above it is concluded that ecotoxicity of the registered nanoforms of ZnO cannot be predicted by data from ionic zinc forms *per se*.

Nevertheless, based on another PfA the request for a long-term toxicity study on fish (FELS test) was removed from the draft decision and the deadline for testing was shortened accordingly.

The FELS test was initially requested in the draft decision as information on long term fish toxicity of nanoscale ZnO is very limited and also the studies listed in the registration dossier as well as the additional study cited in your previous comments are not adequate to represent long term toxicity of (registered) ZnO nanoforms on fish. In addition, several acute studies on fish toxicity showed specific toxic effects of nanoforms of ZnO beyond ionic toxicity (Xiong et al., 2011; Yu et al., 2011; Zhu et al., 2009). However, based on the currently missing information requested in requests 3 - 5 it is currently difficult to conclude on the most sensitive nanoform for fish testing. The possible alternative would be the testing of fish using all nanoforms identified or selected from requests 3 a and b. However, as this would lead to multiple vertebrate tests, it was decided to omit the request for the time being.

You, nevertheless, also stated that you are planning to perform the screening test of transformation/dissolution (request 3.a above in this decision) and the dispersion stability test (request 3.b above). For the dispersion stability test, you request whether the test, if kept in the decision, can be performed at pH 7.6, instead of the 3 pHs stipulated in the test guideline method.

According to the OECD TG 318 governing the dispersion stability test, the extended test does not need to be performed for those nanoforms which show low dispersion stability or high dispersion stability, respectively, under all conditions of the screening test.

The aim of OECD TG 318 is to describe the dispersion stability under environmentally relevant conditions taking into account the major drivers for this endpoint and their variabilities. Narrowing down the test conditions contrary to the TG would decrease the significance of its results. The aim of the request is to identify similarities and differences of the registered ZnO nanoforms and with that to group the nanoforms with respect to

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their behaviour under environmentally relevant conditions. Besides this, grouping based on the information on all pHs (including pH 4 and pH 9) is also relevant to extrapolate the results from the ecotoxicity studies to all (groups of) nanoforms covered by the registration.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the above mentioned studies using the registered substance subject to this decision as specified above.

Full study reports

For requests 1. – 5. full study reports shall be provided including detailed information about the conduction of the studies. There are many factors affecting the toxicity and physicochemical behaviour of nanomaterials, therefore full study reports are required to reliably interpret the results.

Consumer Exposure

6. Information on uses and operational conditions of zinc oxide in nanoforms;

The concern(s) identified

Zinc oxide in nanoform is registered for identified uses with high potential of exposure in the sense of a high possibility of consumer exposure, or a possibility of a relevant level of consumer exposure, or both. Considering the hazardous potential on human health and the potential of the identified uses for consumer exposure, the exposure assessment is of particular importance.

The evaluating MSCA has performed its own exposure assessment for consumers taking into account the available information on the identified uses. However, due to limitations of this it could fail to reflect the actual exposure situation.

Why new information is needed

Further information is required to ensure any conclusions on potentially required risk management measures that will be based on an exposure assessment reflecting the use conditions of the actual exposure situation.

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Your registration dossier does not contain a quantitative exposure assessment for zinc oxide in nanoforms. At present, it is not clear if the evaluating MSCA has captured all identified uses and gained knowledge on all registered nanoforms. It is also not clear if the actual maximum content of zinc oxide in nanoform has been used for exposure assessment. In addition, the consumer exposure assessment might be very conservative and could fail to reflect a realistic exposure situation due to:

- the underlying assumptions on the use of zinc oxide in nanoform and their impact on the applicable consumer exposure scenarios built next to maximum contents of zinc oxide in nanoform in the mixtures and articles, and on operational conditions, such as activities of consumers with the article, route, contact area, duration and frequency of their exposure to the zinc oxide in nanoform; and/or

- the methodology employed.

It should be noted that there is more than one option to refine the methodology. The information request is structured to ensure its appropriateness. Measured data on the release of zinc oxide in nanoform would be one way enabling to refine the exposure estimate and in consequence the risk assessment for conclusion on the additional concerns identified. It is to be noted that all hazardous properties and effects are to be considered, as they become evident. However, if respective data sets do not become available, refinements could further be achieved by employing other models (e.g. ConsExpo nano).

The present exposure assessment by the evaluating MSCA is based on a conservative low tier modelling. It employs a tool which has not been designed or validated for nanoforms of substances. From this it can be assumed that the real exposure will be lower than the outcome of the modelling. The exposure assessment takes into account scientific opinions by the European Food Safety Agency (EFSA) and the Scientific Committee on Consumer Safety (SCCS). It does not establish the consumer exposure resulting from the use of zinc oxide in nanoforms in sunscreen and other cosmetics. However, the consumer exposure level resulting from such uses will be taken into account if the need to establish their contribution to the consumer exposure level for risk characterisation becomes apparent later in the substance evaluation process.

Available information

Publicly available information on the uses of zinc oxide in nanoform (e.g. The Commission Staff Working Paper on Types and uses of nanomaterials including safety aspects (SWD (2012) 288 final) (EC, 2012)), and Public reports from the French Nanoregister (MTES, 2013; MTES, 2014; MTES, 2015; MTES, 2016) indicate a variety of possible uses for the production of mixtures and articles used by consumers. To differentiate between theoretically possible and registered uses, a clarification on the actual identified uses was sought via informal contact with relevant registrants.

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All active registrants of zinc oxide were contacted prior to the substance evaluation process to clarify which of them register the zinc oxide in nanoforms and to gain information on the identity of their nanoforms and uses. The response rate to this questionnaire was approximately 40%. In addition, the evaluating MSCA analysed the information given in the registration dossiers for indications of referring to nanoforms of zinc oxide. The lead registrant, who does not register zinc oxide in nanoforms and selected registrants which have acknowledged to register it in nanoforms were contacted for clarification and additional information on the identified uses.

In this context the registrants provided (confidential) information on the actual use, end product and its conditions of use by consumers, the end product´s content/concentration of zinc oxide in nanoform and the identity of the nanoform used for the production of the end product (mixture/article). Notably, the detail of this information was restricted to the extent of knowledge on downstream uses by the individual registrants.

This obtained information was used to conclude on the form of the end product used by consumers (like liquid, spray, powder), whether the nanoform occurs free or fixed, the shape of the nanomaterial in the product, the routes of exposure to address, whether a direct and intense contact occurs, the exposure event duration, the exposure frequency and other important parameters for the exposure evaluation of substances in nanoform and used for the exposure assessment performed by the evaluating MSCA.

It should be noted that the assessment of potential risks from particle related effects of the substance in nanoform requires consideration of information and parameters beyond those needed for assessment if the toxicity is considered to only relate to the water soluble Zn cations ("ion only hypothesis"), which is followed by the registrants.

For the scenario the evaluating MSCA took also into account additional information sources to deal with the knowledge gaps remaining after the informal contacts with the registrants.

Uses considered relevant for consumer exposure

Based on the information available to the evaluating MSCA, the uses a) as component for the production of paints and coatings and b) its use as component for the production of polymer-matrices, plastics, thermoplastics and related preparations next to the uses as a component for the production of rubber, resins and related preparations will lead to the end products most relevant for consumer exposure assessment.

The end products for consumers arising from them can be categorised into mixtures (e.g. paints, coatings, adhesives and sealants) and articles that have either been treated with the mixtures (e.g. a painted toy or a coated piece of fabric) or manufactured from the compounds/master batches arising from the use group "b)" mentioned above (e.g. rubber or plastic articles).

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Taking together the identified uses and use descriptions of all registrants that have acknowledged uses of zinc oxide in nanoform the level of knowledge on the actual type of end products used by consumers does not allow to conclude in detail on the product details and other parameters to take into account for exposure assessment.

The mixtures could occur in form of liquids, gels, sprays and powders. The nanoforms therein are considered to be contained in either a fluid, soft matrix, or a powdery matrix easily becoming airborne. The consumer behaviour for their use will generally cause a direct contact with the product.

Manufactured articles are considered likely to provide a more solid firm matrix for the nanoforms than mixtures or involve an attachment of the nanoforms onto their surface, requiring release by migration and/or mechanical stress induced abrasion processes during the use of the article for contact. Treated articles will generally involve some kind of matrix for the nanoforms (e.g. dried paint, cured adhesive, thin film coating), but their direct attachment onto its surface may also occur (e.g. UV blocking nanoforms grown onto cotton fibres or particulate spray residues).

Based upon these "release" considerations, articles are contemplated as (potentially) minor source of consumer exposure, than mixtures. Yet, daily handling and intense contact may occur.

In addition the level of information is insufficient to conclude on refined operational conditions (e.g. indoor/outdoor use, exposure event duration, exposure frequency and other main drivers of exposure).

In consideration of these findings the evaluating MSCA built its exposure scenarios as broad sentinel default scenarios intended to cover all potential types of end products covered by the registered use descriptions for the products and articles indicated above for which literature indicates availability on the market. The scenarios incorporate also assumptions on the maximum content of zinc oxide in nanoform required to consider. However, the evaluating MSCA requires confirmation that the conditions of use and categories taken into account for its exposure assessment are supported by the registrants and are in line with their communication in the supply chain up to the end product intended to be used by consumers.

Exposure from mixtures

The evaluating MSCA performed a low tier exposure assessment with ECETOC TRA Version 3. This modelling tool offers the required broad default scenarios and its algorithms have been considered as in principle applicable to substances in nanoform (Clark et al., 2010; Clark et al., 2012; Mackevica and Foss Hansen, 2016a; Mackevica and Foss Hansen, 2016b; Van Tongeren, 2011) when the results are interpreted with caution to the short-comings for each route.

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In view of the potential (sub-)categories to consider oral, dermal and inhalation exposure were taken into account for all mixtures. Furthermore, a frequent use (daily to weekly) of some products in the categories needs to be considered for the subsequent risk assessment of the identified uses.

On consumer level the identified use for the production of paints and coatings needs to consider the product categories 9a coatings and paints, thinners, paint removers, 9b fillers, putties, plasters, modelling clay and 9c finger paints.

The consideration of the inhalation exposure is necessary due to the existence of sprayable applications for this product category on the market and the overarching use description in the registrations covering such uses. The SCCS has clarified the product types included in the term "sprayable application" in the opinion SCCS/1539/14 (SCCS, 2015). In lack of detailed information on the use conditions of zinc oxide in nanoform (e.g. maximum content of zinc oxide in nanoform, and the type of paints/coatings), the exposure assessment was based on default scenarios in ECETOC TRA but with an iteration of the content to 10 %. The vapour pressure was set to 0 Pa. The content was chosen based on the results on product contents of surface coatings published by (Vorbau et al., 2009) [as cited in (Mackevica and Foss Hansen, 2016b)] and information received from registrants.

It should be noted, that this type of products may contain concentrations of up to 50% of zinc oxide.

Table 2 Results of Exposure Assessment with ECETOC TRA 3 by the evaluating MSCA for consumer use of paints & coatings

Category	Dermal, [mg/kg/d]	Oral, [mg/kg/d]	Inhalation, [mg/kg/d]	Inhalation, [mg/m^3]
PC 9a	***1,43E+01***	--	***9,44E+00/***	***1,25E+03/***
PC 9b	***2,54E+01***	***1,00E+01***	--	--
PC 9c	***2,54E+01***	***1,35E+01***	--	--

Table 3 Most conservative results of Exposure Assessment with ECETOC TRA 3 by the evaluating MSCA for consumer use of paints & coatings in consideration of all potential categories.

Use	Dermal, [mg/kg/d]	Oral, [mg/kg/d]	Inhalation, [mg/kg/d]	Inhalation, [mg/m^3]
Paints &	***2,54E+01***	***1,35E+01***	***9,44E+00/***	***1,25E+03/***

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Coatings				

On consumer level the identified use as component for the production of polymer-matrices, plastics, thermoplastics and related preparations as well as a component for the production of rubber, resins and related preparations has to consider the use of sealants / adhesives / mastics linked to mixtures of the category PC 1 (adhesives, sealants) and PC 9b. On professional level this type of use is also linked to PC 24 (lubricants, greases, release products). However, this was not included in the assessment.

In the absence of sufficiently detailed information on the use conditions of zinc oxide in nanoform (e.g. maximum content of zinc oxide in nanoform, and the general type of sealants / adhesives / mastics), the exposure assessment was based on default scenarios in ECETOC TRA but with an iteration of the content to 2,5 % for PC1, 10 % for PC 9b and 6% for PC 24. The vapour pressure was set to 0 Pa.

Table 4 Results of Exposure Assessment with ECETOC TRA 3 by the evaluating MSCA for consumer use of sealants / adhesives / mastics

Category	Dermal, [mg/kg/d]	Oral, [mg/kg/d]	Inhalation, [mg/kg/d]	Inhalation, [mg/m³]
PC 1	***3,57E+00***	--	***8,57E+00***	***9,38E+01***
PC 9b	***2,54E+01***	***1,00E+01***	--	--

Table 5 Most conservative results of Exposure Assessment with ECETOC TRA 3 by the evaluating MSCA for consumer use of sealants / adhesives / mastics in consideration of all potential categories.

Use	Dermal, [mg/kg/d]	Oral, [mg/kg/d]	Inhalation, [mg/kg/d]	Inhalation, [mg/m³]
Sealants / Adhesives / Mastics	***2,54E+01***	***1,00E+01***	***8,57E+00***	***9,38E+01***

The modelling demonstrates that the use of the considered mixtures by consumers will lead to an exposure of consumers via the oral, dermal and inhalation route.

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Unlike cosmetics and other personal care products, the product categories considered above are not intended for direct use on the skin and the exposed body areas to take into account are generally smaller (see e.g. scenarios for sun screen products, paints and surface impregnation products in a recently performed scientific project (Larsen et al., 2015) and ConsExpo Factsheets on DIY, Paints, Cosmetics and cleaning products (Bremmer et al., 2006; Bremmer and van Engelen, 2007; Prud'homme de Lodder et al., 2006; ter Burg et al., 2007). In view of this, it seems plausible that the dermal exposure from the above mentioned products remains below the exposure contributions of the cosmetics and personal care products.

In addition, the evaluating MSCA took notice of the scientific opinions on use of zinc oxide nanoforms by the European Commission's Scientific Committee on Consumer Safety (SCCS/1489/12 (SCCS, 2012b)), its Addendum to the Opinion SCCS/1489/12 on zinc oxide nanoforms (SCCS, 2014)), and its Opinion for clarification of the meaning of the term „sprayable applications/products" for the nanoforms of Carbon Black CI 77266, Titanium Oxide and Zinc Oxide; SCCS/1539/14 (SCCS, 2015).

The evaluating MSCA is aware that the scientific opinions by the SCCS only apply to the nanoparticles that have been part of the dossier for SCCS/1489/12 or are similar materials in accordance with SCCS/1518/13 and criteria mentioned therein.

The evaluating MSCA is aware that some of the nanoforms registered for the productions of mixtures and articles used by consumers were not part of the SCCS dossier. Furthermore, the SCCS consideration that nanoparticles with the given characteristics at concentrations up to 25% do not pose a risk for adverse effects to human health does not apply to applications that might lead to inhalation exposure to ZnO nanoparticles (such as sprayable products).

To be noted by the registrants: the evaluating MSCA considered your statements on main applications of zinc oxide in nanoforms leading to potential consumer exposure, the authorisation of zinc oxide in nanoforms for use in cosmetics and the relations of volumes of zinc oxide in nanoform as compared to volumes of bulk material used for the production of mixtures and articles used by consumers indicated in your registration dossier. Based on the above mentioned findings on i) routes contributing to the exposure resulting from the mixtures within your registered uses and ii) the routes and nanoforms to which the scientific opinions on safe use of zinc oxide in nanoforms in cosmetics apply, the evaluating MSCA does not regard your above indicated statements on zinc oxide in nanoforms and consumer exposure as sufficient to support an argumentation of safe use of zinc oxide in nanoforms for consumers. While no information is requested in this regard, you are recommended to present the argumentation supporting your conclusion on a safe use more clearly in your registration dossier, including the consideration of all routes.

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Exposure from articles

Assessment of consumer exposure to ZnO in nanoform needs to take into account additional contributions from the fraction released from articles by migration or mechanical forces.

Exposure modelling with ECETOC TRA assumes an instantaneous oral exposure and instantaneous complete transfer from the article contact layer to the skin for dermal exposure. This assumption is likely to overestimate the exposure resulting from release mechanisms.

Taking into account the knowledge about diffusional properties of nanoparticles in polymers and the solubility characteristics of the zinc oxide nanoparticles EFSA recently concluded, that while still being present in the plastic article, it will not migrate in nanoform from Low-density Polyethylene (LDPE) if used as a transparent ultraviolet light absorber in unplasticised polymers at up to 2% weight (EFSA, 2016). Their observations suggest the expected rate of migration will be low even if the experimental conditions are not entirely met, e.g. articles produced with addition of more than 2% content or plasticised plastics need to be considered for the articles under consideration.

Zinc oxide in nanoform used for textile functionalization can be located uniformly distributed within the material or coated onto its surface. For textiles functionalized with silver, Wagener et al. (2016) recently demonstrated that nanocomposites release a smaller amount of Ag in comparison to surface-coated textiles. Another mechanism for release to take into account is mechanical forces (e.g. abrasion or chewing). They could contribute to dermal, oral and inhalation exposure of consumers. For mechanical stress related release processes some measurement data is available indicating no release of free zinc oxide nanoparticles [(Göhler et al., 2010; Vorbau et al., 2009) as cited in (Mackevica and Foss Hansen, 2016b)]]. However, it remains unclear if these experiments are representative for the registered uses.

To be noted by the registrants: Based on the above mentioned considerations, no information on the release of zinc oxide in nanoform from articles is requested. However, you are strongly recommended to present supporting data for contributions of treated and manufactured articles to consumer exposure for all routes in your argumentation on safe use of zinc oxide in nanoforms in your registrations.

Information requested

In order to re-evaluate the current assumption on applicable exposure scenarios and to strengthen the exposure assessment, further information is required on

i) not yet captured identified uses and operational conditions, including the identity and properties of the respective nanoforms used, in the form of

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- a use description at least in the detail of section 2.2 of the Chemical Safety Report given in accordance with the ECHA Guidance on Information Requirements and Chemical Safety Assessment. Chapter *R.12*, and
- the maximum content (w/w %) of zinc oxide in nanoform to consider for them;

ii) the identified uses and the supported operational conditions of zinc oxide nanoforms as component for the production of paints and coatings and as component for the production of polymer-matrices, plastics, thermoplastics and related preparations, and as a component for the production of rubber, resins and related preparations, and consumer use of paints and coatings, and consumer use of sealants/adhesives/mastics, and service life of rubber articles, and service life of plastic articles, and service life of articles treated with the mixtures mentioned above in form of

- data on the routes of exposure to be considered in the consumer exposure assessment of the above mentioned identified uses and article service life stages, and

- supported operational conditions for the identified uses and article service life stages considered relevant for consumer exposure by the evaluating MSCA as specified above (maximum content (w/w %) of zinc oxide in nanoform, exposure duration, application duration, product amount used, exposure frequency, indoor or outdoor use) including information on the supported type of mixtures and articles produced allowing a robust more precise conclusion on the expected exposure, and

- data on the maximum content (w/w %) of the zinc oxide in nanoform on the level of industrial uses among the above mentioned identified uses.

If your registration does not comprise zinc oxide in nanoforms you can submit the requested information under points i) and ii) by informing ECHA, that you do not register zinc oxide in nanoforms.

If your registration comprises zinc oxide in nanoforms and you have been in contact with the evaluating MSCA acknowledging the registration of zinc oxide in nanoforms in the questionnaire and indicated your identified uses in the questionnaire, your uses are captured and you can submit the requested information under point i) by informing ECHA that your registration comprises no "not yet captured uses".

If your registration comprises zinc oxide in nanoforms and you have not acknowledged the registration of zinc oxide in nanoforms as response to the questionnaire and you have no other identified uses of zinc oxide in nanoforms than listed in the requested information under point ii), you may also submit the requested information under point i) by informing ECHA that your registration comprises no "not yet captured uses".

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In all other cases the information set out under point i) is to be submitted for all identified uses of zinc oxide in nanoform not mentioned in the requested information under point ii) together with the identity and properties of the respective nanoforms.

If your registration comprises zinc oxide in nanoform the information set out under point ii) is to be submitted for the indicated identified uses and article service life stages in your registration.

Consideration of your general comments on consumer uses

- *You indicated you will complete the list of uses and associated nano ZnO content to the limit of REACH recital 30 via the zinc industry consortium, providing data on (i) routes of exposure, (ii) operational conditions and (iii) maximum content of nano ZnO for life cycle of paint, rubber and plastics.*

ECHA appreciates your efforts but points out that the information should be collected not only from members of the consortium but the other registrants too, to represent the current situation best and that the collection of the information should best be approached by actively contacting all registrants to obtain the information. In addition,, depending on the identified uses of all registrants of zinc oxide in nanoform a complete documentation may need to go beyond the mentioned life cycle of paint, rubber and plastics.

Consideration of your comments on the information requirements for Consumer Exposure

- *Acknowledging that some uses might not have been covered due to missing information by members of the zinc industry consortium and other registrants and/or downstream users at the time of the analysis you propose several actions:*

  i) With regard to available information, to update the uses and exposure scenarios;

  ii) With regard to uses considered relevant for consumer exposure, to review the potential consumer exposure including a documentation in appropriate scenarios (added further to e-SDS for communication in supply chains) and consideration of available tools for exposure assessment (knowing that, presently, none is fully validated for nanomaterials).

  iii) Also with regard to uses considered relevant for consumer exposure to include an entry in the section of uses advised against described as "the use of zinc oxide in sprayable application products as described in SCCS/1489/12 on zinc oxide nanoforms (SCCS, 2014), and SCCS/1539/14 (SCCS, 2015), Opinion for clarification of the meaning of the term "sprayable applications/products"";

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iv) With regard to exposure from articles present supporting data for contributions of treated and manufactured articles to consumer exposure for all routes in its argumentation on safe use of zinc oxide in nanoforms.

In conclusion you inform you will perform the activities that are indicated within the general comment on consumer uses (see above).

In addition to the considerations of the general comment on consumer uses (see above) ECHA appreciates the proposed efforts to document the conditions of safe use throughout the supply chain. For the proposed inclusion of the use of zinc oxide in nanoforms in "sprayable applications/products" with reference to the mentioned descriptions by SCCS in the section of "uses advised against" it is noted, that SCCS opinions may cover different types of non-food consumer products. However, the scope of the SCCS opinions stated in the comment is the use of zinc oxide in some nanoforms in cosmetics while the registration dossier reports other uses beyond cosmetic uses and seems to include additional nanoforms. Should the aim of the proposal be, to exclude inhalation exposure by all uses of registered zinc oxide in nanoforms special care will need to be taken of the wording in order to ensure that the scope of the entry matches the intention.

Consideration of Proposals for amendment

A PfA suggests to delete the information request based on four observations:

- *The request appears wide and imprecise;*
- *The request may go beyond the control of the Registrants if it means that specific information in the supply chain beyond the manufacture, import and use of the Substance by the Registrants themselves is being requested;*
- *The request includes 2 recommendations to the Registrants which are not enforceable and deemed unnecessary;*
- *It is unclear, if the requested information would be necessary to conclude on the necessary regulatory actions. Here it is also pointed out, that "Authorities may not need to have very detailed exposure information in order to conclude on the need for regulatory risk management actions."*

In response, authorities "may not need to have very detailed exposure information in order to conclude on the need for regulatory risk management actions" in all cases. However, in this case the requested information has been tailored to meet the needs for a robust fit for purpose low tier exposure assessment. One subset [information request i)] is the information to ensure that the evaluating MSCA has considered in its exposure assessment for consumers all registered uses of zinc oxide in nanoform. This is because a majority of the active registrants of zinc oxide did not react to its questionnaire aiming at identifying the registrants of zinc oxide in nanoform.

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The other subset [information request ii)] is the information to ensure that the evaluating MSCA's assumptions on routes and operational conditions in the exposure assessment for consumers match those which are supported by the registrants. These assumptions are based on the information on registered uses of zinc oxide in nanoform provided by the contacted registrants and other sources. The request is necessary since there are registrants acknowledging the registration of zinc oxide in nanoforms who did not provide information for demonstration of safe use of zinc oxide in nanoforms throughout all life cycle stages in their dossiers. This lack of information includes stages considered relevant by the evaluating MSCA for consumer exposure assessment.

The requested information is restricted to information which the registrants will need to fulfil their future legal obligations under REACH to demonstrate safe use of their zinc oxide nanoforms throughout all life cycle stages. Submitting this information does not require specific information beyond the control of the registrants. It is the obligation of the registrant to supply this kind of information for their communication of safe use throughout the supply chain and it is the obligation of downstream users to check if their use is covered by the scenario they receive and act accordingly if it is not. The registrants do not have to rely on specific information in the supply chain to fulfil their obligation to communicate the safe use supported by them.

The complex information given within the information request has been structured to inform on

- the identified concerns,
- why new information is needed,
- the available information,
- uses considered relevant for consumer exposure,
- exposure from mixtures,
- exposure from articles, and
- the information requested.

The information request indicates two subsets of information required and additional specifications in the bullet points associated to them. Therefore, the request is not wide and imprecise.

With regard to the argumentation that the recommendations to the Registrants are not enforceable and deemed unnecessary the evaluating MSCA agrees, that such recommendations are not enforceable within substance evaluation. For this reason both notes are positioned outside the section on the "Information requested" and include a clear statement, that they form no basis of information requests.

In consideration of the above the request is maintained.

However, based on the concern of requesting to detailed information and a perceived uncertainty in another PfA of the request being sufficiently specific/detailed to enable registrants to know what is missing in their dossiers to enable a reliable risk assessment editorial amendments have been included to address those concerns.

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Consideration of your comments on the Proposals for amendment

Your support of the PfA to delete the information request is noted. However, for the reasons explained above, the information request was maintained.

What is the possible regulatory outcome

The information will be used to re-evaluate the current assumption on applicable exposure scenarios. This is necessary in order to take into account the actual uses and their operational conditions for the consumer exposure assessment and to perform a realistic exposure assessment for the nanoforms of ZnO. Together with the hazard data the exposure assessment will lead to a robust risk assessment and potentially to regulatory risk management measures, if considered necessary.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you shall provide the following:

Information on uses and operational conditions of zinc oxide in nanoforms; Information on supported use conditions and characteristics of the nanoforms of zinc oxide in their use as component for the production of paints and coatings and their use as component for the production of polymer-matrices, plastics, thermoplastics and related preparations and as a component for the production of rubber, resins and related preparations and for the identified uses considered relevant for consumer exposure by the evaluating MSCA.

Consideration of the time needed to perform the requested studies

The deadline takes into account the time (+ 3 months) that you may need to agree which of the registrant(s) need to perform the required tests. It has been set to allow for sequential testing or other sequential information gathering or information generation approaches as appropriate and also takes into account that a combination of the studies under requests 1 and 2 might not be feasible as indicated in your comments.

In addition you indicate that you need more time to perform all requested human health tests, as considerable pre-work including the required pre-tests is deemed necessary. ECHA notes that considerable pre-work including the performance of pre-tests is needed before main testing can start. ECHA considers the default 18 months for performing the OECD TG 413 (request 1), plus additional 4 months for preparatory work, as well as another 6 months for the performance of pre-test(s) as sufficient to perform the requested study. The time needed for testing has been adapted accordingly. As pre-testing and choice of test materials for request 1 and 2 are identical, and the fact that selected test materials can also be used for the oral study requested under request 3, the overall time frame does not need adaptation.

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to the potential hazards of ZnO in nanoform and its wide dispersive use, consumer use and exposure of the environment, Zinc oxide CAS No 1314-13-2 (EC No 215-222-5) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2017. The updated CoRAP was published on the ECHA website on 21 March 2017. The competent authority of Germany (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding

1. Human Health:
    a. Insufficient justification for the applied read across/category approach with respect to most HH endpoints
    b. Repeated dose toxicity (RDT): oral and inhalation route
    c. Neurotoxicity
    d. Genotoxicity of nano ZnO
    e. Reproductive toxicity (fertility)
    f. Developmental toxicity
2. Potential additional effects on environmental organisms and different bioavailability of the nanoforms of zinc oxide.

The evaluating MSCA considered that further information was required to clarify the above mentioned concerns. Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 16 March 2018.

ECHA notified you of the draft decision and invited you to provide comments.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took the comments from you, which were sent within the commenting period, into account and they are reflected in the reasons (Appendix 1). The request(s) and the deadline were amended.

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Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and amended the draft decision. An information request regarding long-term toxicity in fish (FELS test) has been removed. The proposals for amendment are reflected in the reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendments.

Your comments on the proposed amendments were taken into account by the Member State Committee.

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-64 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

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Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to otherwise fulfil the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on the composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will carry out the study on behalf of the other registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx?CaseNumber=SEV-215-222-5-1

   Further advice can be found at:
http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the registrants to perform the stud(y/ies) on behalf of all of them.