Environmental assessment of nanomaterial use in Denmark

Final report, 15 September 2015

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Ministry of Environment and Food
The Danish Environmental Protection Agency

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Preface

This report is the final report under the project "Nanomaterials – Occurrence and effects in the Danish Environment" ("NanoDEN"). The NanoDEN project was commissioned by the Danish EPA in December 2012 and is hereby completed. The project is one among a number of projects funded by the Danish EPA aiming to increase the knowledge and understanding regarding occurrence of engineered nanomaterials in Denmark and the possible risks posed by these to humans and the environment.

The NanoDEN project is part of the initiative of the Danish government and the Red-Green Alliance (a.k.a. Enhedslisten) called "Bedre styr på nanomaterialer" (Better control of nanomaterials) for 2012-2015 that focuses on the use of nanomaterials in products on the Danish market and their consequences for consumers and the environment.

The NanoDEN project has been carried out by a project team with participation of COWI A/S (lead partner) (overall project leader: Jesper Kjølholt), the Technical University of Denmark (DTU Environment) (project leader: Anders Baun) and the Swiss Nano Modelling Consortium (SNMC) (project leader: Fadri Gottschalk).

COWI is the lead partner for this report, which has been prepared by Jesper Kjølholt and Anna Brinch (COWI A/S), Fadri Gottschalk and Bernd Nowack (SNMC), and Hans-Christian Holten Lützhøft, Nanna B. Hartmann and Anders Baun (DTU Environment).

A Steering Committee with the following participants was established for the NanoDEN project:
- Flemming Ingerslev, Danish EPA (Chairman and project responsible)
- Katrine Bom, Danish EPA
- Jørgen Larsen, Danish EPA
- Jesper Kjølholt, COWI A/S (project manager)
- Anders Baun, DTU Environment
- Fadri Gottschalk/Bernd Nowack, SNMC.

Executive Summary

Background and Objective

This is the concluding report of the project "Nanomaterials – occurrence and effects in the Danish environment" (abbreviated NanoDEN), which is one among of a number of projects commissioned by the Danish Environmental Protection Agency as part of the Danish Government's initiative "Better Control of Nanomaterials" ("Bedre styr på nanomaterialer"). These projects have aimed to investigate and generate new knowledge on the presence of nanomaterials on the Danish market and assess the possible associated risks to consumers and the environment.

The overall objective of NanoDEN has been to discuss and assess whether engineered nanomaterials (ENMs)[1] released to and occurring in the Danish environment give reason for environmental concern. This is also the overarching question in the current report, which, additionally, has the more specific objective of performing a concrete environmental risk assessment of ten engineered nanomaterials selected in the project to represent ENMs of relevance to Denmark. The assessment is based on the results of the preceding sub-projects of NanoDEN that have addressed the following themes related to ENMs: environmental fate and behaviour, modelling of environmental exposure, and effects in the (aquatic) environment.[2]

- Titanium Dioxide (TiO_2), photostable
- Titanium Dioxide (TiO_2), photocatalytic
- Zinc Oxide (ZnO)
- Silver (Ag)
- Carbon Nanotubes (CNTs)
- Copper carbonate/Copper oxide ($CuCO_3$ / CuO)
- Nano Zero Valent Iron (nZVI)
- Cerium Dioxide (CeO_2)
- Quantum Dots (QDs)
- Carbon Black (CB)

Main findings and conclusions

The main question "do engineered nanomaterials (ENMs) released to and occurring in the Danish environment give reason for environmental concern" is addressed through a number of more specific sub-questions. The reason for this is that ENMs, just like traditional chemicals, are used in many different applications and have a wide range of individual properties. It is therefore impossible to give one single answer that can embrace this variety.

- *Do some individual ENMs, or groups of ENMs, constitute a risk of environmental effects now or within a not too distant future?*

With the current scientific knowledge, and current use patterns and volumes, none of the ENMs selected for this study appear to constitute a general environmental risk or to be of significant environmental concern (i.e. they do not at the same time show high toxicity to aquatic organisms and occur at significant levels in the environment). ENMs of potential concern are those that are either highly toxic (e.g. Ag, CuO, CNT) or have a high environmental exposure combined with more

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[1] In the context of NanoDEN, nanomaterials are defined as materials consisting of particles with one or more external dimensions between 1 and 100 nm.

[2] For full references to these reports, please consult the list of references. See: Hartmann *et al.* (2014), Gottschalk *et al.* (2015a and 2015b), and Lützhøft *et al.* (2015).

moderate toxicity (e.g. titanium dioxide and carbon black). To both of these groups applies that their use volumes shall increase significantly compared to today's levels before the potential risk will materialise. Concerning the remaining the selected ENMs it is assessed to be unlikely that any of them should constitute a general environmental risk (ZnO, nZVI, CeO_2 and QD). The possible risk that local effects could occur in special cases has not been evaluated in this study and due to scarcity of data it has not been possible to determine risk quotients for soil- and sediment-dwelling organisms or to assess bioaccumulation risks.

- *Are ENMs, as a group, covered sufficiently by the ENMs studied in this project?*

The ENMs selected for this project represent: i) partly materials that are currently used in some of the highest tonnages among nanomaterials, ii) partly materials that together represent a wide range of applications in relevant consumer products and industrial/professional processes, iii) partly materials of different physico-chemical character (metals, other inorganics, carbon-based ENMs), and iv) materials with a significant span regarding toxicity and behaviour in the environment.

Hence, we believe that the selection made is representative considering the purpose of the NanoDEN project, and the delimitations made in the project are not considered to compromise the possibility of discussing ENMs from an overall risk perspective in this report.

- *Are certain functionalities or types of uses of ENMs more critical than others from an environmental perspective?*

The critical functionalities and types of uses are in principle all those leading to direct releases to and exposure of one or more environmental compartments, i.e. without passing through e.g. sewage treatment plants, incineration plants or other waste treatment processes. Examples of such critical uses are outdoor paints and other coatings for building facades or other constructions, sun lotions, impregnation of wood for outdoor constructions (on land or in water), anti-fouling paints for ships, fuel additives, fillers for tyres, and artificial lawns (rubber filler made from used tyres). Some of these uses lead to aquatic exposure due to discharges of untreated runoff from roofs and paved surfaces (e.g. CeO_2 and CB) while others affect directly the soil environment (such as use of CuO for wood impregnation) or even the marine environment (ENMs in antifouling paints). It should be mentioned that the assessments are made only for the "pristine" form of the ENMs, i.e. the materials without coatings or other types of surface functionalization as typically used in commercial applications.

- *Is the Danish situation with regard to ENMs comparable to the general situation in the EU?*

In Denmark, there seems to be virtually no production of basic nanomaterials and manufacturing of nano-containing materials and products appears to be quite limited. Therefore, industrial point sources are probably of less importance in Denmark than in other countries in the EU with more extensive industrial production. The actual use of nano-products by consumers is overall assumed to be more or less in line with other European countries. The degree of re-cycling of waste incineration residuals (slags etc.) for outdoor constructions such as base-materials for roads is high in Denmark compared to many other EU countries. Such "open" types of re-cycling imply a potential risk of higher environmental exposure for some ENMs, in particular of (subsurface) soil and groundwater.

- *Is there, based on the above considerations, reasons for general concern with regard to nanomaterials in the Danish environment?*

Based on the data review and the assessments of exposure and effects of ENMs in the aquatic environment performed in this project, no causes for significant environmental concerns regarding nanomaterials in the Danish environment have been identified at the current level and character of use of these materials. However, a few ENMs should be kept under observation (or some assumptions regarding their use be clarified), either because they already today are released in significant quantities to the environment (TiO_2, CB) or because their environmental properties (in particular ecotoxicity) place them in a group of materials of potential concern (Ag, Cu, CNT). The remaining ENMs are either used only for very specific applications in small quantities or in applications that result in only very low releases to the environment.

Environmental risk assessment of selected ENMs

To provide a foundation for the overall environmental considerations presented above, a concrete environmental risk assessment of the selected ENMs was performed in line with the principles used in risk assessment of chemical substances in general. However, due to limitations in the available data on ecotoxicological effects, the assessment is limited to the freshwater compartment.

The results of the assessments are summarised in the table below in the form of risk quotients (= PEC/PNEC[3]) for effluents from sewage treatment plants (STPs) and fresh surface waters (regional scenario, after full distribution). The model calculations of the level of environmental exposure (PEC) were carried out on basis of probability distributions and, therefore, the table presents risk quotients both for the most probable exposure concentration modelled and the highest concentration within the 95 percent confidentiality interval of the total distribution range.

ENM	**Environmental compartment**	**Risk Quotient (PEC/PNEC)**	
		Most probable value	Max value (95% conf. int.)
TiO_2 (photostable)	STP effluent	0.7	5.1
	Surface water, fresh	0.0002	0.006
TiO_2 (photocatalytic)	STP effluent	0.09	0.79
	Surface water, fresh	1.5 E-05	3.9 E-04
ZnO	STP effluent	≈0	-#
	Surface water, fresh	1.8E-04	0.0052
Ag	STP effluent	0.042	4.88
	Surface water, fresh	0.001	0.004
CNT	STP effluent	3.57E-04	4.17E-03
	Surface water, fresh	1.19E-06	1.79E-05
CuO	STP effluent	3.8	12.1
	Surface water, fresh	0.0059	0.018
nZVI	STP effluent	-##	-##
	Surface water, fresh	-##	-##
CeO2	STP effluent	1.79E-03	1.15E-02
	Surface water, fresh	7.69E-07	1.92E-05
QD	STP effluent	-##	-##
	Surface water, fresh	-##	-##
CB	STP effluent	-*	-*
	Surface water, fresh	-**	-**

-: estimation of PEC/PNEC not possible

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[3] PEC = Predicted Environmental Concentration; PNEC = Predicted No-Effect Concentration. If PEC/PNEC is <1 the risk of biological effects is considered acceptably low.

* possibly >1; **possibly <<1

possibly <1; ## possibly <<1

It appears from the table that if the risk assessment is based on the most probable value for the predicted environmental concentration (PEC), only 1-2 nanomaterials (copper oxide and possibly carbon black) are estimated to show risk quotients higher than unity in STP effluents, i.e. there is a potential risk of biological effects close to the points of discharge into the aquatic environment for these two ENMs. If the highest effluent concentration in the 95 percent confidence interval is used, the risk quotient for silver and titanium dioxide also exceed unity but the highest calculated risk quotient (for copper oxide) is still only about 12. A risk reduction of this magnitude will normally be possible to obtain for STP effluents discharging into rivers or coastal marine waters as the value of unity does not have to be met in the effluent itself but only at the border of a mixing zone established around the discharge point. None of the average concentrations calculated for freshwater after full distribution will lead to risk quotients anywhere near the value of unity.

Please note that for nano-copper, the assessment is based on the assumption that all copper-based preservation of wood is done with nano-copper, which is not the situation today (but potentially possible in the future). For carbon black (CB) it is conservatively assumed that that CB-products consist exclusively of nano particles, and this is not necessarily the case in reality.

Data gaps and uncertainties

Due to the fact that "nano" is a very new scientific area, the above assessments and conclusions have been reached on basis of limited existing knowledge e.g. with regard to analytical and test methodologies, as well as availability of specific data on the individual nanomaterials. From a regulatory perspective and in relation to society's possibilities of managing and, if necessary, introducing environmental regulation of engineered nanomaterials, the following data and procedures are considered to be the most important to obtain/generate:

- Data that can define the actual environmental exposure to ENMs more precisely than now, e.g.:
  - more specific (Danish) data on the sources and releases from industrial facilities, as well as resulting from the use of nano-products by consumers;
  - data clarifying to what extent and for how long ENMs released to the environment exist as nanoparticles in the environment before they either dissolve or are no longer on nano form due to aggregation/agglomeration;
  - Better data on retainment of ENMs in STPs and waste incineration plants.

- Data that can verify the PECs predicted by modelling i.e. analytically measured data (which requires better analytical methods for ENMs to be developed);

- Data that can lead to more precise and reliable PNECs than today, i.e. more data (especially chronic) on individual ENMs as well as testing methods that are more suitable to ENMs than the current.

- Ecotoxicity data for organisms from other compartments than freshwater (e.g. soil, marine, and sediment-living organisms) since interpolation between environmental compartments is presently not feasible.

- Data that can validate that the use of current methods for deriving PNEC will lead to protective concentrations with regards to environmental effects of ENMs

Sammenfatning

Baggrund og formål
Dette er den afsluttende rapport i projektet "Nanomaterialer – forekomst og effekter i det danske miljø" (forkortet NanoDEN), som er et af en række projekter iværksat af Miljøstyrelsen som en del af udmøntningen af regeringens finanslovsaftale, kaldet "Bedre styr på nanomaterialer". Disse projekter har haft som hovedformålet at undersøge og generere viden om industrielt fremstillede nanomaterialer på det danske marked.

Hovedformålet med NanoDEN har været at undersøge og belyse om tilførslen til og forekomsten af industrielle nanomaterialer (ENM) i det danske miljø giver anledning til miljømæssig bekymring[4]. Dette er også det bagvedliggende hovedspørgsmål i nærværende rapport, der desuden har haft som mere specifikt delformål at foretage en konkret miljømæssig risikovurdering af nedennævnte nanomaterialer, der blev udvalgt i projektet til at repræsentere ENM af relevans for Danmark. Vurderingen er baseret på resultaterne af de forudgående delprojekter under nanoDEN, der har omhandlet hhv. miljømæssig opførsel og skæbne, modellering af miljøeksponering samt effekter i (vand)miljøet[5].

- Titandioxid, TiO_2 (hhv. fotostabilt og foto-katalytisk)
- Zinkoxid, ZnO
- Sølv, Ag
- Kulstofnanorør, CNT (carbon nano-tubes)
- Kobbercarbonat, $CuCO_3$ / kobberoxid, CuO
- Nanojern i oxidationstrin nul, nZVI (nano zero-valent iron)
- Ceriumdioxid, CeO_2
- Kvantepunkter, QD (quantum dots)
- Carbon black, CB

Projektets hovedresultater/-konklusioner
Hovedspørgsmålet "giver forekomsten af industrielle nanomaterialer i det danske miljø anledning til miljømæssig bekymring" er forsøgt besvaret ud fra en række mere konkrete delspørgsmål idet ENM, lige som traditionelle kemikalier, har så mange forskellige anvendelser og egenskaber, at der ikke kan gives ét, samlet svar, der kan dække dem alle.

- *Er der bestemte ENM, eller grupper af ENM, der i dag eller inden for en overskuelig fremtid udgør en miljømæssig risiko?*

Blandt de udvalgte ENM vurderes der ikke at være nogen materialer på nanoform, der ud fra den eksisterende viden og med de nuværende anvendelser og omfang af forbrug kan siges at udgøre en miljømæssig risiko i Danmark (dvs. de har ikke både høj giftighed for vandlevende organismer og stor forekomst i miljøet). Potentielt kritiske ENM skal findes blandt dem, der enten har en høj giftighed (sølv, kobberoxid, CNT) eller som med en mindre giftighed giver anledning til betydelig eksponering af miljøet (titandioxid og CB). For begge grupper gælder, at anvendelsen af dem skal stige betydeligt i forhold til dagens niveau for at risikoen bliver reel. For de resterende blandt de udvalgte ENM vurderes det som usandsynligt, at de skulle udgøre en generel risiko (ZnO, nZVI,

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[4] Nanomaterialer er i forbindelse med NanoDEN defineret som materialer fremstillet af partikler med en eller flere eksterne dimensioner på mellem 1 og 100 nm.
[5] For fulde referencer til disse rapporter, se rapportens referenceliste under Hartmann *et al.* (2014), Gottschalk *et al.* (2015a og 2015b) og Lützhøft *et al.* (2015).

CeO_2 og QD). Det har ligget uden for projektet at vurdere risikoen for, at der i særlige tilfælde kan forekomme lokale påvirkninger, og på grund af mangel på relevante data har der ikke kunnet beregnes risikokvotienter for jord- og sedimentlevende organismer eller konkluderes på risikoen for bioakkumulering.

- *Er ENM, som samlet gruppe, dækket tilstrækkeligt med de udvalgte ENM?*

De ENM, der er udvalgt til dette projekt, repræsenterer dels nanomaterialer, der er blandt dem der anvendes i de højeste tonnager, dels materialer med et bredt spektrum af forventede anvendelser i relevante forbrugerprodukter, industrielle processer og miljøteknisk remediering, og endelig har de en række forskellige fysisk-kemiske og miljømæssige karakteristika. Derfor anses de udvalgte ENM for at være repræsentative ift. det overordnede formål med NanoDEN-projektet og de fravalg, der er foretaget, vurderes ikke at være kritiske ift. at kunne diskutere de overordnede perspektiver.

- *Er der funktionaliteter eller typer af anvendelser af ENM, der er særligt kritiske ud fra et miljømæssigt perspektiv?*

De kritiske funktionaliteter og anvendelser er i princippet dem, der medfører direkte udslip til og eksponering af en eller flere dele af miljøet, dvs. uden først at passere gennem renseanlæg eller forbrændingsanlæg eller andre former for emissionsbegrænsende tekniske foranstaltninger. Eksempler på sådanne kritiske anvendelser er udendørs malinger eller andre udendørs facadebelægninger, imprægnering af træ til udendørs anvendelser, bundmalinger til skibe, additiver til bilmotorer og bildæk, kunstgræsbaner etc. Nogle af disse anvendelser medfører eksponering af ferskvandmiljøer som følge af udledninger af ubehandlet afstrømning fra tage og veje (f.eks. CeO_2 og CB), mens andre fører til eksponering af jordmiljøet (CuO) eller havmiljøet (skibsmalinger). Det skal også nævnes, at vurderingerne er foretaget på baggrund af den kemiske sammensætning af de udvalgte ENM. Vurderinger af betydningen af overfladefunktionalisering og coatings af kommercielt anvendte partikler er således ikke inkluderet.

- *Er situationen i Danmark mht. ENM sammenlignelig med situationen i andre europæiske lande?*

I Danmark er der ingen eller kun ganske ubetydelig basal produktion af ENM og formodentlig også meget begrænset fremstilling af materialer eller produkter indeholdende nanomaterialer. Derfor er industrielle punktkilder antagelig af mindre betydning end i et antal andre lande med højere grad af industriel produktion. I øvrigt vurderes anvendelsen af ENMs hos forbrugerne at være omtrent på linje med den i andre europæiske lande. I Danmark er der en ret høj grad af genanvendelse af slagger mv. fra affaldsforbrænding, der potentielt kan medføre en forøget miljøeksponering, primært af dybere jordlag og grundvand.

- *Er der, på baggrund af ovenstående, grund til overordnet miljømæssig bekymring pga. nanomaterialer i Danmark?*

På det foreliggende vidensgrundlag, baseret på vurderinger af virkninger af ENM i ferskvand, er der i nærværende projekt ikke identificeret årsager til væsentlig miljømæssig bekymring relateret til industrielle nanomaterialer i det danske miljø. Nogle få specifikke ENM bør dog holdes under observation (eller forholdene omkring dem afklares nærmere). Dette enten fordi de allerede i dag er beregnet at have ganske høje udslip til miljøet (TiO_2, CB) eller fordi de har miljøegenskaber (giftighed), der placerer dem i en gruppe af potentielt problematiske materialer (Ag, Cu, CNT). De fleste øvrige ENM anvendes enten til meget specielle formål i små mængder og/eller til formål, der ikke eller kun i meget ringe grad giver anledning til udslip til miljøet.

Miljømæssig risikovurdering af udvalgte ENM
Der er, som baggrund for de foranstående overordnede miljømæssige betragtninger foretaget en konkret miljømæssig risikovurdering af de udvalgte ENM efter de samme principper som ved miljømæssig risikovurdering af kemikalier. På grund af datamæssige begrænsninger mht. biologiske effekter er vurderingen dog begrænset til (fersk)vandsmiljøet.

Resultatet af de foretagne vurderinger er sammenfattet i nedenstående tabel udtrykt ved risikokvotienter (PEC/PNEC[6]) for hhv. udløb fra renseanlæg og ferskvandmiljøer (regional betragtning, efter opblanding). Modelberegningerne af eksponering i vandmiljøet er foretaget på baggrund af sandsynlighedsfordelinger og derfor er der i tabellen angivet risikokvotienter både for den modellerede mest sandsynlige koncentration og for den højeste koncentration inden for 95%-sandsynlighedsintervallet af teoretisk mulige værdier.

ENM	**Type af vandmiljø**	**Risikokvotient (PEC/PNEC)**	
		Mest sandsynlige værdi	Max. værdi (95% interval)
TiO_2 (fotostabilt)	Udløb fra renseanlæg	0.7	5.1
	Overfladevand, fersk	0.0002	0.006
TiO_2 (fotokatalytisk)	Udløb fra renseanlæg	0.09	0.79
	Overfladevand, fersk	1.5 E-05	3.9 E-04
ZnO	Udløb fra renseanlæg	≈0	- #
	Overfladevand, fersk	1.8E-04	0.0052
Ag	Udløb fra renseanlæg	0.042	4.88
	Overfladevand, fersk	0.001	0.004
CNT	Udløb fra renseanlæg	3.57E-04	4.17E-03
	Overfladevand, fersk	1.19E-06	1.79E-05
CuO	Udløb fra renseanlæg	3.8	12.1
	Overfladevand, fersk	0.0059	0.018
nZVI	Udløb fra renseanlæg	- ##	- ##
	Overfladevand, fersk	- ##	- ##
CeO_2	Udløb fra renseanlæg	1.79E-03	1.15E-02
	Overfladevand, fersk	7.69E-07	1.92E-05
QD	Udløb fra renseanlæg	- ##	- ##
	Overfladevand, fersk	- ##	- ##
CB	Udløb fra renseanlæg	- *	- *
	Overfladevand, fersk	- **	- **

-: PEC/PNEC ikke mulig at estimere.

* muligvis >1; **muligvis <<1

muligvis <1; ## muligvis <<1

Det fremgår af tabellen, at hvis man tager udgangspunkt i den mest sandsynlige værdi for miljøkoncentrationen (PEC) er der kun 1-2 materialer (kobber og muligvis CB), der har risikokvotienter større end 1 i udløbspunktet for udledninger fra renseanlæg (dvs. der foreligger en potentiel miljørisiko). Tages der udgangspunkt i max. koncentrationen for 95%-konfidensintervallet

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[6] PEC = Predicted Environmental Concentration (forventet miljømæssig koncentration);
PNEC = Predicted No-Effect Concentration (den forventede højestekoncentration uden biologiske effekter i miljøet).

kommer sølv og titandioxid også med, men den maksimale risikokvotient (for kobber) er dog stadig kun ca. 12. Et sådant niveau for nødvendig risikoreduktion vurderes som almindeligvis opnåelig i forbindelse med renseanlæg. Der er ingen af de beregnede gennemsnitskoncentrationer i overfladevand efter fuld fordeling i vandmiljøet, der vil føre til risikokvotienter blot i nærheden af 1.

Der skal her gøres opmærksom på, at for kobber er vurderingen baseret på en antagelse om, at al kobberbaseret træimprægnering foregår med nano-kobber, hvilket ikke er tilfældet i dag (men potentielt mulig i fremtiden), og for carbon black (CB) er det konservativt antaget, at hele den anvendte mængde er som nano, hvilket ikke nødvendigvis er tilfældet i virkeligheden.

Manglende viden og usikkerheder

Ovenstående vurderinger og konklusioner er truffet på baggrund af den eksisterende viden, en viden der på grund af, at nano er så nyt et miljøvidenskabeligt område, har en række begrænsninger både med hensyn til egnede undersøgelsesmetoder og med hensyn til konkrete, materialespecifikke data. I forhold til samfundets muligheder for at håndtere og eventuelt miljømæssigt regulere industrielle nanomaterialer er følgende data/oplysninger fundet at være de væsentligste at få adresseret:

- Data som kan fastlægge den miljømæssige eksponering for ENM mere præcist end i dag, f.eks.:
  - flere specifikke (danske) data om kilder og afgivelser fra industrielle virksomheder såvel som pga. anvendelse af nanoprodukter hos almindelige forbrugere;
  - data som kan klarlægge i hvilken grad og i hvor lang tid ENM, der er tilført miljøet, vedbliver at eksistere som nanopartikler førend de enten er gået i opløsning eller ikke længere er på nanoform pga. aggregering/agglomerering mv.
  - bedre data om tilbageholdelse af ENM i renseanlæg og forbrændingsanlæg.

- Data som kan verificere de modellerede PEC-værdier, dvs. analytisk-kemiske data (hvilket vil kræve udvikling af flere og mere egnede analysemetoder).

- Data som kan føre til fastsættelse af mere præcise og mere pålidelige PNEC end i dag, dvs. flere data (især kroniske) for de enkelte ENM såvel som testmetoder, der er mere egnede (skræddersyede) til ENM end de nuværende (som er udviklet til kemikalier).

- Økotoksikologiske data for organismer i andre miljøer end ferskvand, f.eks. det marine miljø, jord og sediment, da interpolation mellem de forskellige miljøer p.t. ikke er mulig.

- Data som kan validere, at anvendelsen af eksisterende metoder til fastlæggelse af PNEC for kemikalier også giver et tilstrækkeligt beskyttelsesniveau for ENM.

1. Introduction

Background and Objective

The Danish EPA has, as part of the Danish Government's initiative "Better Control of Nanomaterials" ("Bedre styr på nanomaterialer"), commissioned a number of projects aiming at investigating and generating new knowledge on the presence of nanomaterials in products on the Danish market and assess the possible associated risks to consumers and the environment. One of these projects is the current project on nanomaterials in the Danish environment (NanoDEN), which has the overall objective of discussing and assessing whether engineered nanomaterials (ENMs) released to and occurring in the Danish environment give reason for concern.

The current, final sub-project of NanoDEN (sub-project 6) being reported here will partly summarise (to some extent using identical phrases) and discuss the main results and findings of the previous sub-projects under the NanoDEN "umbrella" and partly present an environmental assessment of each of the ENMs selected for the study with the overall aim to address the fundamental question of the project, i.e. if engineered nanomaterials in the Danish environment give reason for concern.

Selection of nanomaterials

In the context of the NanoDEN project, ENMs are defined as manufactured materials with one or more external dimensions between 1 and 100 nm. In that respect ENMs is a part of a broader group of nanomaterials, which can result from natural, anthropogenic (incidental) and engineered (intentional) processes, however the NanoDEN project focuses on ENMs, as this is the most relevant group of nanomaterials from an up-stream regulatory point of view. The following nanomaterials were selected for this project as they together are considered to comprise the majority of currently widely used ENMs and also represent a range of typical functionalities and applications of ENMs:

- Titanium Dioxide (TiO_2) (rutile and anatase)
- Zinc Oxide (ZnO)
- Silver (Ag)
- Carbon Nanotubes (CNTs)
- Copper carbonate/Copper oxide ($CuCO_3$ / CuO)
- Nano Zero Valent Iron (nZVI)
- Cerium Dioxide (CeO_2)
- Quantum Dots (QDs)
- Carbon Black (CB)

In the NanoDEN project, $CuCO_3$ nanoparticles are included as case study particles for copper-containing nanoparticles. However, for the effects part of the risk assessment $CuCO_3$ has been replaced by CuO due to the fact that nano-CuO has been studied to some extent in ecotoxicity studies, whereas studies for nano-$CuCO_3$ are lacking.

Contents of the report and guidance to the reader
The report is structured as follows:

Chapter 2 gives an introduction to the approach and methodology that has been applied for the determination of sources, environmental exposure and effects, and for conducting the specific environmental assessment of the ten nanomaterials selected for this particular study (considered representative for the majority of current ENMs).

Chapter 3 contains the specific, individual environmental assessments of the selected ENMs and includes for each a short summary of the main findings from the previous NanoDEN sub-projects regarding ENM sources and releases, environmental behaviour and exposure, environmental effects and PNECs, ending up with a formal risk assessment basically using the same approach as being applied in REACH for chemicals. At the end of each material-specific section in the chapter, a summary of the main "risk relevant" environmental features of the ENM in question is provided and an overall summary of the determined aquatic risk quotients is included at the end of the chapter.

The subsequent **Chapter 4** provides a broader discussion of issues related to the main elements of an environmental assessment of nanomaterials, namely the sources and releases of the materials to the environment, the environmental exposure, the environmental effects and the approach to risk assessment of ENMs as well as number of cross-cutting issues.

A number of uncertainties and data gaps encountered in the project are discussed in **Chapter 5**, which address both issues related to assessment of nanomaterials in general and issues specifically related to the selected nanomaterials.

Chapter 6 aims to discuss and conclude, to the extent possible, on overall issues and possible general concerns related to nanomaterials in the Danish environment rather than focusing on the individual materials. However, the previous findings on the selected materials are used to support the overall conclusions and perspectives presented.

2. Approach and methodology

This chapter presents a short summary of the approaches and methodologies that have been applied in different parts of the NanoDEN project to review, describe, generate and assess information of relevance for the concluding assessment of environmental risk and possible reasons for concern, i.e. above all the two sub-projects on environmental exposure and environmental effects, respectively, as well as the risk assessment approach in the current report. These approaches and methods are applied in Chapter 3 for each of the individual ENMs included in the NanoDEN project. In Chapter 4 a number of issues beyond the more formalized risk assessment of the specific ENMs selected for this study are discussed.

2.1 Environmental exposure to ENMs

The environmental exposure model

In the NanoDEN project estimations of environmental exposure for a number of scenarios have been provided for the selected nanomaterials based on the results of a modelling study. The basis for obtaining the results was a comprehensive search on the manufacturing processes and the total content of the nanomaterials in mixtures and articles for end-applications in Denmark. The data fed into the modelling included total use and releases of nanomaterials to the environment in Denmark based on literature and interview information, as well as dispersion and environmental fate for the selected ENMs obtained through a critical review of recent scientific literature.

The model used was derived from a model of the flows and fate of nanomaterials in the EU and in Switzerland developed by the Swiss Nano Modelling Consortium (SNMC). The modelling approach was to estimate the release of nanomaterials from the technosphere to different environmental compartments by summing up such release over the complete lifecycle of the investigated ENM, including production, formulation and industrial processes, private or professional applications and waste treatment. The basic inputs were quantities of nanomaterials used for industrial processes and the total content of nanomaterials in imported mixtures and articles. The flow of the ENMs through a series of technosphere compartments/processes was determined by a series of transfer coefficients, which, for example, described the percentages of nanomaterials passing through sewage treatment plants (STPs) that are eliminated by the treatment processes or released into environmental compartments.

For four of the nanomaterials addressed by this study, a detailed analysis for the EU and Switzerland was published in 2014 (Sun *et al.*, 2014). These were nano-TiO_2, nano-ZnO, nano-Ag and CNT. In relation to these nanomaterials, the Swiss data on import in mixtures and articles were partially used as a first estimate for the use in Denmark (on a *per capita* basis), and the transfer coefficients developed to describe the Swiss situation was also partially applied to Denmark, considering that the differences between the two countries with regard to the use of the mixtures and articles and waste treatment processes are small compared to the uncertainties on the use of data and transfer coefficients. However, a critical evaluation of the Swiss data was performed, and the report updated outdated data by replacing them with new findings. For the other substances addressed by the study, data on uses and transfer coefficients were completely collected as part of this study. These substances were nano-CuCO3, nano-CuO, nZVI, nano-CeO2, quantum dots (QD) and carbon black (CB).

The probabilistic, stochastic approach[7]
The data collected were not fed into the model by means of single deterministic values, but instead by computing probability distributions based on the data. For missing knowledge, assumptive information based on expert indications had to be used. Thus, the model used was based on probabilistic nanomaterial production and use parameters, as well as release and mass transfer coefficients at all stages of the nanomaterial lifecycle. One type of parameter reflected tonnes or kilogrammes of use or release of nanomaterial each year. The second type represented transfer coefficients which indicated the percentage of the nanomaterial going into a further compartment (nanoproducts, natural or technical environments), or being dissolved/eliminated or finally deposited. The stochastic model combined a large number of scenarios (100,000) for each mass transfer and mimicked a complex system of engineered nanomaterial transport and fate. This comprehensive and explorative procedure allowed us to capture as far as possible the complete environmental exposure spectrum based on the current knowledge.

Nanomaterial sources
None of the selected nanomaterials are manufactured in Denmark. Information on the use of nanomaterials for formulation processes was obtained from companies involved in the manufacture of pigments, paints and varnishes (including antifouling paints), plastics and cosmetics. As a general trend (with a few exceptions), companies in the chemicals sector have moved production to Eastern Europe or Asia over the past few decades, and the activities in Denmark are limited mainly to research and some formulation of chemical products into ready-to-use products. Specific data from industry in Denmark turned out to be hard to retrieve and due to confidentiality reasons, details obtained on the use of nanomaterials for different formulation processes cannot be published.

Sewage treatment plants (STPs)
STPs are considered one of the main sources of release of some of the nanomaterials studied. However, no specific and empirical data on actual discharges of nanomaterials to the Danish municipal sewer system or on the releases from these STPs are available. Therefore, assumptive information and, where available, literature data for transfer coefficients were used to calculate the quantities discharged to the sewer system from manufacturing processes, from the use of the materials in mixtures and articles and from waste handling.

2.2 Environmental effects of ENMs

The approach to and methodology applied for the description and assessment of environmental effects of ENMs was based on a critical literature review with the following sub-categories of information searched for:

- Current approaches to estimation of PNECs (Predicted No Effect Concentrations) and data evaluation for traditional chemicals
- Currently existing PNEC values for nanomaterials (in scientific literature and REACH registrations)
- Biological effects of the nanomaterials selected for the NanoDEN study.

For the assessment of the literature pertaining to the latter category a concept for evaluation of ecotoxicological data for ENMs was developed specifically in this project based on the so-called Klimisch score used under REACH, i.e. an evaluation of the reliability, relevance and adequacy of each of the studies reviewed. Thus, the reliability assessment considers the test setup as such, the identification and (physic-chemical) properties of the test compound, the test organism used, the exposure conditions and the statistical design for evaluation of test results. The relevance

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[7] The term stochastic (random) process stands for the computing procedure that produces random numbers used to form the probability distributions (probability density functions) of the model input and output data.

assessment considers the biological relevance of the species, the endpoints studied and the experimental conditions, as well as the exposure relevance.

Subsequently, to find scientific articles covering effect assessment of nanomaterials in relation to risk assessment, a literature search strategy was developed. The name of each of the selected nanomaterials was combined with “tox*” and “nano*” and “alga*/crustacean*/daphni*/fish*” in Web of Science and resulted in 1,208 hits in total. A number of the articles were in fact replicates, as some articles were retrieved for the same ENM, but for different organisms, or because a single article reported data for several ENMs and therefore were retrieved for several ENMs. Across all searched nanomaterials and search strategies, there were 677 unique articles.

Using the developed concept for data quality and relevance evaluation, the identified scientific articles were reviewed and grouped into different categories of adequacy by combining their reliability and relevance scores. The adequate studies were used as basis for identifying the species and endpoints relevant for the derivation of a Predicted No Effect Concentration (PNEC) to be used later (in the current sub-project) for calculating a risk quotient (PEC/PNEC) for each ENM by combining the PNEC with relevant Predicted Exposure Concentrations (PECs) obtained by the exposure modelling. In this project, the so-called Assessment Factor (AF) approach, also used e.g. in REACH for traditional chemicals, was applied to derive the PNECS based on the ecotoxicity data taking into account the amount of effect information available for each ENM.

2.3 Environmental risk assessment of ENMs

The assessment of the environmental risks associated with the ENMs selected for the NanoDEN project was, in accordance with international standard procedures, carried out by comparing the environmental exposure results (PECs) obtained for various compartments and scenarios with the PNEC values for the aquatic environment obtained from the environmental effects sub-project. Specifically, a risk quotient (= PEC/PNEC) was calculated for the undiluted effluent from sewage treatment plants (STPs) and for surface waters after full dilution (i.e. a regional scenario). A risk quotient at or above 1 indicates a risk of effects on organisms in the (aquatic) environment while a value below indicates that this risk by convention is considered acceptably low.

The PEC values used in this study for the risk assessment were the most probable values from the exposure modelling as well as the max. value within the 95% confidence interval as described in more detail in Gottschalk *et al.* (2015a) while the PNEC values used were derived from laboratory toxicity test data using the assessment factor (AF) method (see Lützhøft *et al.* (2015) for more details).

In principle, an environmental risk assessment should include all relevant environmental compartments, i.e. the aquatic environment (fresh and marine waters), soils, sediments, and sewage treatment plants (STPs). However, while the exposure estimations carried out provide the predicted exposure concentrations needed for the risk assessment the comprehensive review of the currently available ecotoxicological studies on ENMs revealed that only for freshwater organisms there is a sufficient amount of adequate data. Further, extrapolations from effect levels in freshwater organisms to sewage treatment, marine, soil and sediment organisms are not straightforward for ENMs and it was assessed not to be scientifically correct to use the extrapolations described in the current guideline for chemical safety assessment in REACH. Therefore, quantitative risk quotients were currently considered possible to derive only for the aquatic environment.

3. Environmental risk assessment of selected ENMs

3.1 Introduction

This chapter presents for each of the nanomaterials selected for this study a brief summary of information on sources, exposure and effects relevant for environmental risk assessment and subsequently a calculation of risk quotients (PEC/PNEC values) for each material in the aquatic environment (as possible/relevant) while a broader discussion of environmental risk can be found in chapter 4.

The material-specific summaries regarding sources, exposure, environmental fate and environmental effects of the investigated ENMs are extracts of the more comprehensive texts with information and data presented in the previous thematic reports prepared for other sub-projects under NanoDEN, i.e. Hartmann *et al.* (2014) on environmental fate and behaviour, Gottschalk *et al.* (2015a) on sources and environmental exposure, and Lützhøft *et al.* (2015) on environmental effects and PNECs. For detailed descriptions of the respective themes and associated references to original literature, please consult the mentioned reports.

An environmental risk assessment should in principle include all the compartments listed in the REACH guidance for chemical safety assessment (Part E), i.e. the aquatic environment (fresh and marine waters), soils, sediments, and sewage treatment plants (STPs). The exposure estimations carried out in SP4 (Gottschalk *et al.*, 2015a) provide the predicted exposure concentrations needed for the risk assessment. However, the comprehensive review of the currently available ecotoxicity studies of ENMs has revealed that only for freshwater organisms there is a sufficient amount of data adequate for risk assessment (Lützhøft *et al.* (2015)). As will be discussed in this report, the extrapolations from effect levels in freshwater organisms to sewage treatment, marine, soil and sediment organisms are not straightforward for ENMs. It is in fact evaluated not to be scientifically correct to use extrapolations described in the current guideline for chemical safety assessment in REACH (R.10). Therefore, the risk assessments in this report will be focused on freshwater organisms.

The actual risk assessments for each of the ENMs in this study are carried out for the aquatic environment by calculating the risk quotient (= PEC/PNEC) in the effluent from sewage treatment plants (STPs) and in surface waters after full dilution (i.e. a regional scenario). A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the (aquatic) environment, while a value below indicates that this risk by convention is considered acceptably low.

The PEC values used in the risk assessment are the most probable values from the modelling as well as the max. value within the 95% confidence interval as described in more detail in Gottschalk *et al.* (2015a), while the PNEC values have been derived from laboratory toxicity test data using the assessment factor (AF) method in accordance with the general principles for ecotoxicological evaluation of chemicals as described by ECHA (see Lützhøft *et al.* (2015) for more details).

3.2 Risk assessment of photostable nano-titanium dioxide

In this study, the use of nano-TiO_2 has been divided into two groups of applications; the photostable TiO_2 and other applications group (main use UV absorbers) and the photocatalytic TiO_2 group. The anatase crystal form of TiO_2 is a more efficient photocatalyst than the rutile form of TiO_2 and, consequently, most photocatalytic consumer products contain nanosized anatase TiO_2. However, mixtures of both crystal forms are often used, and the two groups of applications do not fully reflect a division between the two crystal forms.

This section is about risk assessment of the photostable form of nano-TiO_2 while the photocatalytic form is assessed in section 3.3.

3.2.1 Sources and releases

The main applications of nano-TiO_2 listed in a Swiss modelling study by Sun *et al.* (2014) were cosmetics, cleaning agents, plastics and consumer electronics. Cosmetics accounted for more than half of the total consumption and as the major part is released directly to the environment or to STPs this application is likely the major source of nano-TiO_2 in the environment. Its use in sunscreens in Denmark may likely be lower than the EU average, as the Nordic ecolabel (the Swan) does not allow the use of TiO_2 in sunscreens.

Photostable nano-TiO_2 is used in Denmark also for production of pigments, cosmetics and possibly paints. An ongoing survey has indicated that the use of TiO_2 for the production of sunscreens in Denmark has decreased in recent years, but nano-TiO_2 may be used in other types of cosmetics.

The annual use volume in Denmark of photostable nano-TiO_2 in different products and applications has been modelled to be approx. 190 t/year (most probable value) of which a total of approx. 35-40% are released into environmental compartments. However, the modelling show a range of very likely use volume values from 100 to 300 t/year and a total spectrum of use volumes ranging from a few tonnes per year to almost 400 t/year.

Table 3-1 summarises the modelled release concentrations (PECs) for the technical compartments of relevance for nano-TiO2. The results are presented as the most probable values and the 95% probability range for the lower and upper release concentrations.

TABLE 3-1
PREDICTED CONCENTRATIONS FOR PHOTOSTABLE AND OTHER NANO-TiO_2 IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95 % PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	Concentrations				
	Unit	Most probable value	95% range		
Sewage treatment effluent	µg/L	12.6	3.4	-	91.9
Sewage treatment sludge	mg/kg	775	68.7	-	1480
Waste mass incinerated	mg/kg	15.0	1.4	-	32.1

Bottom ash of waste incineration	mg/kg	33.0	3.4	-	87.6
Fly ash of waste incineration	mg/kg	169	16.9	-	433

Figure 3-1 below shows the relative distribution of the total amount released annually from the technosphere to the environment (35-40% of 190 t/y i.e. approx. 65-70 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

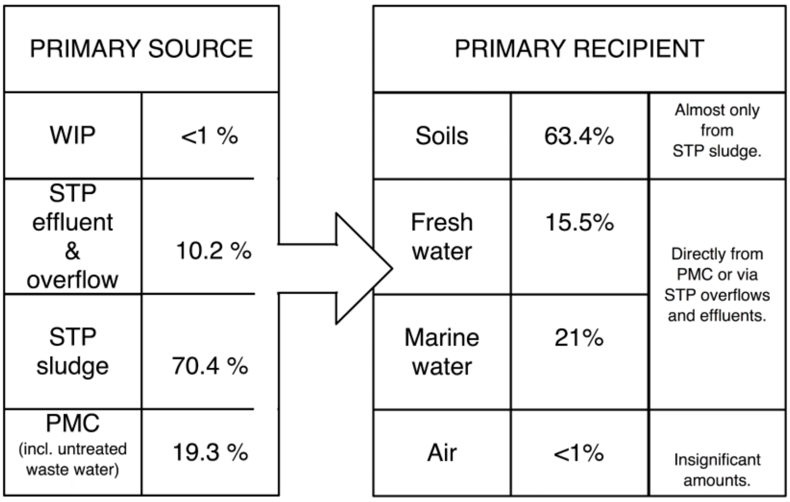

FIGURE 3-1
OVERVIEW OF THE MOST IMPORTANT SOURCES AND RECEIVERS FOR PHOTOSTABLE AND OTHER NANO-TiO_2. THE PERCENTAGES REFLECT THE MOST PROBABLE VALUES. ONLY APPROX. 35-40 % OF THE TOTAL USE VOLUME OF NANO-TiO2 IS RELEASED FROM THE TECHNICAL COMPARTMENTS TO ENVIRONMENTAL COMPARTMENTS.
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

Thus, only a limited part of the use volume (approx. 10% of the mass, at the most 20-25%) is expected to reach the aquatic environment, ending up in sediments. The amount released into the air is considered insignificant. Direct release into the aquatic environment also seems minimal (a few tons annually), and the amount released directly to soils is assumed to be almost zero while use of STP sludge on (agricultural) soils contributes considerably to the total load on the soil compartment.

3.2.2 Important environmental fate processes

TiO_2 is one of the more studied ENMs regarding environmental fate and exposure. Several processes affect the fate of TiO_2 nanomaterials, while others are less likely to occur. For example, TiO_2 ENMs do not dissolve, and the importance of dissolution for the environmental fate and behaviour or TiO_2 is hence considered to be low for all environmental compartments.

It is well-known that TiO_2 aggregates in water with a more pronounced aggregation in salt water compared to freshwater. TiO_2 aggregation behaviour in natural waters shows a formation of larger aggregates in brackish water compared to freshwater due to the higher ionic strength of the former while the difference of aggregation in freshwaters with different compositions was linked to presence of organic substances and pH. Aggregation (including hetero-aggregation, i.e. adsorption of TiO_2 to particulates present in the water) can lead to sedimentation. Therefore, aggregation and sedimentation processes are considered of high importance for TiO_2 ENMs. As regards the soil compartment, it is considered of high importance to consider adsorption/retention in soil in environmental fate modelling of nano-TiO_2.

For photostable TiO_2 photochemical transformation processes are considered to be of medium importance and as TiO_2 is considered less likely to participate in electron transfer or uptake and unlikely to dissolve to any significant extent in the environment, redox reactions and dissolution processes are considered to be of low importance. As TiO_2 is an inorganic material the process of biodegradation is considered not to be relevant, while biomodification is evaluated as having rather low importance in environmental fate modelling compared to other transformation processes.

3.2.3 Environmental exposure

Amongst all metal-containing nanosized materials studied for Denmark, the highest aquatic concentration was modelled for nano-TiO_2. This is in accordance with previous findings for other countries/regions (Gottschalk *et al.*, 2009; Sun *et al.*, 2014), although a higher nano-TiO_2 elimination in STPs was included taking recent measured data into account. .

The STP effluents showed the highest concentrations of a few to almost 100 µg/L (most probable value around 13 µg/L).For freshwater the worst case scenario concentration was 0.1 µg/L , while for marine water concentrations in the pg/L range were found. Soils and sediments were found to be the most significant nano-TiO_2 sinks, with approximate expected concentrations (most probable values) for 2020 of 3 mg/kg in sludge treated soils and a few tenths of µg/kg in non-sludge-based fertilised soils. In freshwater and marine water sediments, concentrations of 2.4 and 0.8 mg/kg were modelled.

Table 3-2 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for nano-TiO2. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations. The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-2
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR PHOTOSTABLE AND OTHER NANO-TiO_2 IN DIFFERENT ENVIRONMENTAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95 % PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATIONS.
FOR SOILS AND SEDIMENTS, THE FIGURES SHOWN ARE THE ANNUAL INCREASES IN PEC.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	µg/L	0.003	5.7E-04	-	0.1
Surface water (marine water)	µg/L	3.2E-04	4.0E-05	-	0.001
Air	µg/m3	1.1E-04	1.3E-05	-	4.9E-04
Sediments (freshwater)	µg/kg/yr	180	31.1	-	4290
Sediments (marine water)	µg/kg/yr	60.1	7.6	-	195
Soils (agricultural)	µg/kg/yr	0.013	0.002	-	0.1
Soils (natural)	µg/kg/yr	0.028	0.004	-	0.2

Soils (urban)	µg/kg/yr	0.051	0.006	-	0.2
Soils (STP sludge treated)	µg/kg/yr	205	19.9	-	473

Table 3-3 forecasts concentrations for photostable and other nano-TiO_2 after an ENM deposition period in soils beginning at zero in 2000 and ending at the beginning of the years 2014 and 2020, respectively. For non-fertilized (no sewage sludge fertilizer) soil areas, our model suggests most probable values around 80-330 ng/kg in 2014 increasing to 200-700 ng/kg in 2020 assuming an unchanged annual use volume after 2014. It should be noted that the area of sludge-treated land is only a few percent of the total cultivated area in Denmark.

TABLE 3-3
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR PHOTOSTABLE AND OTHER NANO-TiO_2 IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-TIO_2 RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	**Unit**	**Year**	
		2014	**2020**
Sediments (freshwater)	µg/kg	1170	2450
Sediments (marine water)	µg/kg	391	816
Soils (agricultural)	µg/kg	0.085	0.2
Soils (natural)	µg/kg	0.18	0.4
Soils (urban)	µg/kg	0.33	0.7
Soils (STP sludge treated)	µg/kg	1330	2780

3.2.4 Environmental effects

For nano-TiO2 more than 250 articles on environmental effects were identified but most of them did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. Around 20 articles reporting NOEC/EC_{10}-values including EC_{50} values were selected for further assessment of which six studies were found 'adequate for regulatory use' and six studies were assessed as ‘may be adequate for regulatory use'. In most cases, the reason for the lower adequacy of some studies was the poor characterisation of the inherent properties of the tested nanomaterials.

It appears that short term tests have been performed on all three trophic levels of algae, daphnia and fish. For algae, 72-h EC50 values are seen in the range of 2.53-241 mg/L while in 48-h immobilization studies of daphnia, LC50 values are found to be >10 and even >100 mg/L. Different kinds of short term studies on fish have been performed revealing 48-h LC50 values >10 mg/L, depending on the composition of the tested TiO2, and finally for embryo a 72-h LC50 value of >2,000 mg/L for a coated TiO2 NM. Among the tested organisms the algae appear to be the most sensitive on a short-term basis, with an EC50 of 2,53 mg/L. Chronic studies have been performed on both algae and daphnia revealing 72-h NOEC like data from <0.5 to 3.3 mg/L.

3.2.4.1 PNEC for nano-TiO2

As data from short-term tests are available for all three base-set test organisms as well as some long-term chronic data on the most sensitive organism group in the short term tests, an assessment factor of less than 1,000 can be applied. Chronic test revealing NOEC values have been performed on both daphnia and algae allowing an assessment factor of 50.

An EC_{10}-value of 5.02 mg/L was obtained for the chronic effects on offspring production from *D. magna*. The most sensitive test result was observed in the 72-h growth inhibition test with *P. subcapitata* with a NOEC of <0.5 mg/L. However, since this study only report that NOEC is less than 0.5 mg/L and no LOEC is given, the study is not usable for PNEC estimation. Instead the extrapolation will have to be made using the NOEC of 0.89 mg/L for C. vulgaris.

A **$PNEC_{freshwater}$ value of 18 µg/L** can therefore be derived for titanium dioxide considering the reservations mentioned in the beginning of this chapter.

Not enough or no data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.2.5 Environmental risk assessment

Table 3-4 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for photostable TiO_2 in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average regional concentrations, i.e. a regional scenario. A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-4
PEC/PNEC ESTIMATION FOR PHOTOSTABLE TIO2 IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	PEC (µg/L)		PNEC (µg/L)	PEC / PNEC	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	12.6	3.4 – 91.9	18	0.7	5.1
Surface water, fresh	0.003	5.5E-4 – 0.1	18	0.0002	0.006
Surface water, marine	0.00032	4.0E-5 – 0.001	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

As can be seen from the table, the PEC/PNEC values are far below 1 for surface water indicating a negligible risk of ecotoxicological effects of photostable TiO_2 to occur as a result of the average concentrations modelled for surface water. The concentration in the effluent itself (most probable value) is so high that the PEC/PNEC for the undiluted effluent is close to but lower than 1 (0.7) implying that the risk quotient for the most probable value is acceptably low for TiO2 in undiluted effluent from STP.

In the high end of the 95% interval a dilution factor of about 5 is required to obtain a PEC/PNEC <1, which is normally but not always possible to obtain in streams/rivers within a reasonable distance from the discharge point, in particular in small streams in Eastern Denmark during summer.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for natural, agricultural and urban soils are low and, furthermore, it is considered likely that various fate processes in the soil rather quickly will transform the photostable TiO2 into larger particles (aggregates) or sorb them to NOM or other soil particles thereby reducing the exposure to the nano-form considerably. A possible concentration in sludge-treated soils of more than 1 mg/kg (i.e. more than 1000 times higher than in the other soil categories) has been predicted but this is still considered quite low for a not very toxic material/substance like TiO_2.

3.2.6 Summary for photostable TiO2

The main findings in relation to the environmental risk assessment of photostable TiO_2 are summarised in the box below.

Summary

- In line with European studies and compared to other metallic nanomaterials, the currently highest aquatic exposure in Denmark among the ENMs in this study is found for photostable and other nano-TiO_2.
- The non-diluted effluent from sewage treatment plants shows the highest concentrations with a most probable value around 13 µg/L and a max. value of around 92 µg/L.
- The freshwater concentrations reach at most one tenth of a µg/L (worst case scenarios) while the ones in marine water are almost completely in pg/L range.
- Soils and sediments represent the most significant nanomaterial sinks with modal concentrations for 2020 of 2.4 and 0.8 mg/kg in sediments (freshwater and marine water), 3 mg in sludge treated soils and a few tenths µg/kg in untreated soils.
- The most important processes determining the environmental fate of photostable TiO_2 are aggregation/agglomeration, sedimentation and adsorption.
- Short term aquatic toxicity data on nano-TiO_2 are available for fish, daphnia and algae and chronic data exist for daphnia and algae. The effect levels are in the mg/L range with the most sensitive endpoint being a NOEC <0.89 mg/L for algae leading to a PNEC of 18 µg/L.
- Based on this PNEC the most probable risk quotient for STP effluent is 0.7 while the max. quotient is 5.1. The worst case value for freshwater is 0.006. These values suggest a very low environmental risk for freshwater in general and an acceptable local risk near STP effluent points.
- The concentrations predicted for soils not treated with STP sludge are low and it is likely that fate processes in the soil rather quickly will transform the nano-TiO_2 and thereby reduce the exposure to the nano-form considerably.

3.3 Risk assessment of photocatalytic nano-titanium dioxide

As mentioned in section 3.2 there are two main groups of nano-TiO_2 applications; the photostable TiO_2 and other applications group (by far the largest use category of the two; main uses as UV absorbers) and the photocatalytic TiO_2 group (main uses for paints and other coatings). This section is about risk assessment of the photocatalytic form of nano-TiO_2 while the photostable form was assessed in section 3.2.

UV and visible light can induce catalytic activity in photocatalytic nano-TiO_2. Via the photocatalytic effect, reactive oxygen species are formed, which can decompose organic substances and bacteria on illuminated surfaces to achieve self-cleaning/antibacterial effects.

3.3.1 Sources and releases

The main applications for photocatalytic TiO2 listed in the Swiss study by Sun et al. (2014) were paints, coatings and water and air treatment, with construction materials (self-cleaning surfaces with TiO_2) and wastewater treatment as a likely smaller application. In Denmark, photocatalytic TiO_2 is being used in the production of paints with antibacterial, self-cleaning effects.

The use volume of photocatalytic nano-TiO2 has been modelled to be approximately 45 t/year (most probable value) and it is thus more than 4 times smaller than the modelled annual use volume for photostable nano-TiO_2. About 20% is estimated to be released to the environment if the release to soil with STP sludge is included. Otherwise, around 5-6 % most probably find the way into the environment.

Table 3-5 summarises the modelled concentrations (PEC) for the technical compartments of relevance for photocatalytic nano-TiO_2. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations.

TABLE 3-5
PREDICTED CONCENTRATIONS FOR PHOTOCATALYTIC NANO-TiO_2 IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	**Concentrations**		
	Unit	Most probable value	95% range
Sewage treatment effluent	µg/L	1.6	0.4 - 14.3
Sewage treatment sludge	mg/kg	84.6	9.3 - 232
Waste mass incinerated	mg/kg	2.8	0.3 - 6.8
Bottom ash of waste incineration	mg/kg	6.0	0.7 - 18.3
Fly ash of waste incineration	mg/kg	29.7	3.3 - 90.4

Figure 3-2 below shows the relative distribution of the total amount released annually from the technosphere to the environment (20% of 45 t/y i.e. max. 10 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

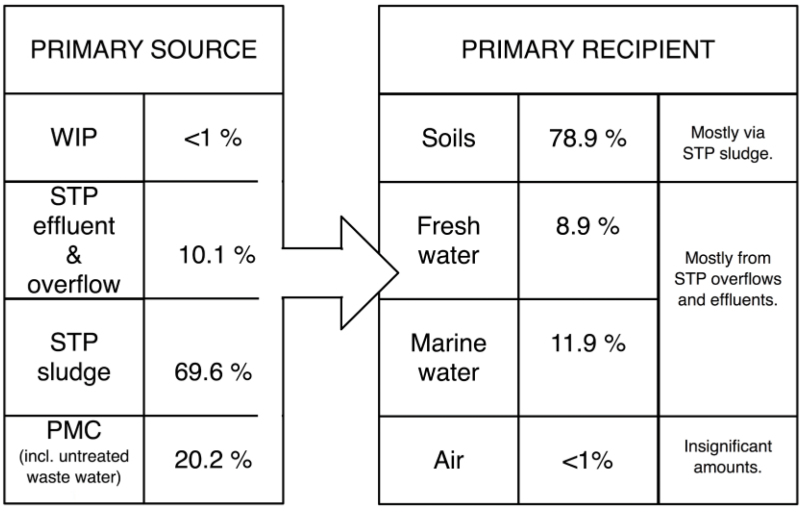

FIGURE 3-2
OVERVIEW OF THE MOST IMPORTANT PHOTOCATALYTIC NANO-TIO2 SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT THE MOST PROBABLE VALUES.
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

Thus, the figure shows that most photocatalytic nano-TiO_2 ends up in soils following application with STP sludge. A smaller portion (approx. 6 times smaller) can enter soils via air deposition of PMC-based emissions. This finding confirms that the use of photocatalytic nano-TiO_2 in various types of paints and coatings may also lead to release of nanomaterial to soils via air deposition. The results on ENM recipients here show that about 80% ends up in soils while the rest reaches the aquatic environment. The potential air contamination again seems to be insignificant for the current ENM use amounts and fields of application.

3.3.2 Important environmental fate processes

Photostable and photocatalytic nano-TiO_2 are in many respects very similar with regard to their environmental behaviour and fate and, therefore, only one common description of the relevant processes was made for this nanomaterial and is repeated here for the reader's convenience.

TiO_2 is one of the more studied ENMs regarding environmental fate. Several processes affect the fate of TiO_2 nanomaterials while others are less likely to occur. For example, TiO_2 ENMs do not dissolve and the importance of dissolution for the environmental fate and behaviour or TiO_2 is hence considered to be low for all environmental compartments.

It is well-known that TiO_2 aggregates in water with a more pronounced aggregation in salt water compared to freshwater. TiO_2 aggregation behaviour in natural waters shows a formation of larger aggregates in brackish water compared to freshwater due to the higher ionic strength of the former while the difference of aggregation in freshwaters with different compositions was linked to presence of organic substances and pH. Aggregation (including hetero-aggregation, i.e. adsorption of TiO_2 to particulates present in the water) can lead to sedimentation. Therefore, aggregation and sedimentation processes are considered of high importance for TiO_2 ENMs. As regards the soil compartment, it is considered of high importance to consider adsorption/retention in soil in environmental fate modelling of nano-TiO_2.

Overall for TiO_2, photochemical transformation processes are considered to be of medium importance and as TiO_2 is considered less likely to participate in electron transfer or uptake and unlikely to dissolve to any significant extent in the environment, redox reactions and dissolution processes are considered to be of low importance. As TiO_2 is an inorganic material the process of biodegradation is considered not to be relevant while biomodification is evaluated as having rather low importance in environmental fate modelling compared to other transformation processes.

3.3.3 Environmental exposure

The modelled load of photocatalytic nano-TiO_2 to fresh and marine water did not contribute significantly (about one-tenth) to the total aquatic load of nano-TiO_2. The concentrations in surface waters were at most in the pg/L ranges. Relatively small PECs have also been modelled for sediments and non-sludge treated soils. Most photocatalytic nano-TiO_2 ends up in recycling and landfill plants, as also seen for several other types of ENMs.

Table 3-6 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for photocatalytic nano-TiO2. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not absolute values.

TABLE 3-6

PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR PHOTOCATALYTIC NANO-TIO_2 IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95 % PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PEC.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	µg/L	2.7E-04	4.7E-05	-	0.007
Surface water (marine water)	µg/L	2.2E-05	3.6E-06	-	9.9E-05
Air	µg/m3	7.3E-04	7.7E-05	-	0.002
Sediments (freshwater)	µg/kg/yr	14.2	2.6	-	401
Sediments (marine water)	µg/kg/yr	4.1	0.7	-	18.5
Soils (agricultural)	µg/kg/yr	0.1	0.011	-	0.3
Soils (natural)	µg/kg/yr	0.2	0.027	-	0.7
Soils (urban)	µg/kg/yr	0.4	0.044	-	1.0
Soils (STP sludge treated)	µg/kg/yr	25.5	2.7	-	73.4

Table 3-7 shows the forecast concentrations for photocatalytic nano-TiO_2 after an ENM deposition period in sediments and soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively. Concentrations of approximately 200 µg/kg (freshwater sediment) and 60 µg/kg (seawater sediment) have been predicted (most probable values) for 2020. In soils fertilised with STP sludge, the model revealed about 350 µg/kg for 2020.

TABLE 3-7
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR PHOTOSCATALYTIC NANO-TIO_2 IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-TIO_2 RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	**Unit**	**Year**	
		2014	**2020**
Sediments (freshwater)	µg/kg	92.3	193
Sediments (marine water)	µg/kg	26.5	55.3
Soils (agricultural)	µg/kg	0.7	1.4
Soils (natural)	µg/kg	1.5	3.2
Soils (urban)	µg/kg	2.7	5.6
Soils (STP sludge treated)	µg/kg	166	346

3.3.4 Environmental effects

Photostable and photocatalytic nano-TiO_2 are very difficult to differentiate with regard to their environmental effects due to the lack of ecotoxicological studies distinguishing between the two different forms of TiO2. Therefore, the following effect assessment for photocatalytic nano-TiO_2 is identical with the one presented for photostable nano-TiO_2 in section 3.2.

More than 250 articles on environmental effects were identified for nano-TiO_2 but most of them did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. Around 20 articles reporting NOEC/EC_{10}-values including EC_{50} values were selected for further assessment of which six studies were found 'adequate for regulatory use' and six studies were assessed as ‘may be adequate for regulatory use'. In most cases, the reason for the lower adequacy of some studies was the poor characterisation of the inherent properties of the tested nanomaterials.

It appears that short term tests have been performed on all three trophic levels of algae, daphnia and fish. For algae, 72-h EC50 values are seen in the range of 2.53-241 mg/L while in 48-h immobilization studies of daphnia, LC50 values are found to be >10 and even >100 mg/L. Different kinds of short term studies on fish have been performed revealing 48-h LC50 values >10 mg/L, depending on the composition of the tested TiO2, and finally for embryo a 72-h LC50 value of >2,000 mg/L for a coated TiO2 NM. Among the tested organisms the algae appear to be the most sensitive on a short-term basis, with an EC50 of 2,53 mg/L. Chronic studies have been performed on both algae and daphnia revealing 72-h NOEC like data from <0.5 to 3.3 mg/L.

3.3.4.1 PNEC for nano-TiO2

As data from short-term tests are available for all three base-set test organisms as well as some long-term chronic data on the most sensitive organism group in the short term tests, an assessment factor of less than 1,000 can be applied. Chronic test revealing NOEC values have been performed on both daphnia and algae allowing an assessment factor of 50. Due to the specific catalytic action of photocatalytic TiO_2 the effect values might be expected to be lower than for photostable TiO_2 and leading to a correspondingly lower PNEC. However, the currently available data do not allow for such distinction between the two types of this nano material.

An EC_{10}-value of 5.02 mg/L was obtained for the chronic effects on offspring production from *D. magna*. The most sensitive test result was observed in the 72-h growth inhibition test with *P. subcapitata* with a NOEC of <0.5 mg/L. However, since this study only report that NOEC is less than 0.5 mg/L and no LOEC is given, the study is not usable for PNEC estimation. Instead the extrapolation will have to be made using the NOEC of 0.89 mg/L for *C. vulgaris*.

A $PNEC_{freshwater}$ value of 18 µg/L can therefore be derived for photocatalytic titanium dioxide considering the reservations mentioned in the beginning of this chapter. This value is identical to the PNEC estimated for photostable titanium dioxide.

Not enough or no data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.3.5 Environmental risk assessment

Table 3-8 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for photocatalytic TiO_2 in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations, i.e. a regional scenario. A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-8
PEC/PNEC ESTIMATION FOR PHOTOCATALYTIC TIO2 IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (µg/L)**		**PNEC (µg/L)**	**PEC / PNEC**	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	1.6	0.4-14.3	18	0.09	0.79
Surface water, fresh	2.7E-04	4.7E-05 – 0.007	18	1.5 E-5	3.9E-4
Surface water, marine	2.2E-05	3.6E-06 – 9.9E-05	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

Thus, the PEC/PNEC values are below 1 even for undiluted STP effluent and << 1 for surface waters indicating a negligible risk of ecotoxicological effects of photocatalytic TiO_2 in surface waters.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for natural, agricultural and urban soils are very low and even sludge-treated soils will contain less than 0.2 mg/kg.

3.3.6 Summary for photocatalytic TiO_2

The main findings in relation to the environmental risk assessment of photocatalytic TiO_2 are summarised in the box below.

Summary

- Contrary to photostable nano-TiO_2, and due to the different use patterns of the two nanoforms of TiO_2, the relative mass transfer of photocatalytic nano-TiO_2 into waste, recycling and landfilling processes is probably higher than that into the aquatic and terrestrial environments.

- Currently, the Danish environmental release of photocatalytic TiO_2 seems not to be too critical: the terrestrial compartments receive about 6 tons/year, while aquatic environments receive, at the most, one ton annually. I.e. the released amount is significantly lower than that of photostable TiO_2.

- Compared to photostable and other nano-TiO_2 and based on current predictions of the use amounts of photocatalytic nano-TiO_2, its aquatic exposure relevance is much lower (approx. by a factor of 10).

- The most important processes determining the environmental fate of photocatalytic TiO_2 are aggregation/agglomeration, sedimentation and adsorption.

- Short term aquatic toxicity data on nano-TiO_2 are available for fish, daphnia and algae and chronic data exist for daphnia and algae. The effect levels are in the mg/L range with the most sensitive endpoint being a NOEC = 0.89 mg/L for algae leading to a PNEC of 18 µg/L.

- Due to the specific catalytic action of photocatalytic TiO_2 the effect values could be expected to be lower than for photostable TiO_2. However, the currently available data do not allow for such distinction and the estimated PNEC values are therefore identical for the two types of nano-TiO_2.

- Based on this PNEC a most probable risk quotient for STP effluent of 0.09 and a max. value of 0.79 has been calculated and a corresponding max. value for freshwater of 0.0004. These values indicate a low environmental risk for photocatalytic TiO2 in aquatic environments.

3.4 Risk assessment of nano-zinc oxide (nano-ZnO)

3.4.1 Sources and releases

According to the study by Sun *et al.* (2014), the major application of nano-ZnO is as a UV filter in sunscreens. This application accounts for more than half of the consumption. However, nano-ZnO is not included in the list of UV filters allowed in cosmetic products in the EU and Denmark. The pigmentary grade of ZnO still allows it to be used as a white pigment, but it is not considered a nanomaterial. The only other potential major application identified was the use of nano-ZnO in paint. No actual use of nano-ZnO for production processes in Denmark was identified.

The annual use volume in Denmark of nano-ZnO in different products and applications has been modelled to be approx. 40 t/year (most probable value) of which only approx. 10% is released into environmental compartments on nano-form (mainly directly from PMC) as nano-ZnO has been found to be almost completely transformed into other forms during STP processes.

Table 3-9 summarises the modelled concentrations (PEC) for the technical compartments of relevance for nano-ZnO. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs).

TABLE 3-9
PREDICTED CONCENTRATIONS FOR NANO-ZNO IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABLITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	**Concentrations**		
	Unit	Most probable value	95% range
Sewage treatment effluent	µg/L	≈0	
Sewage treatment sludge	mg/kg	Almost no ZnO remaining in its initial nanoform.	
Waste mass incinerated	mg/kg	0.3	0.04 - 1.5
Bottom ash of waste incineration	mg/kg	0.7	0.1 - 3.9
Fly ash of waste incineration	mg/kg	3.6	0.5 - 19.2

Figure 3-3 below shows the relative distribution of the total amount released annually from the technosphere to the environment (10% of 40 t/y i.e. <5 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

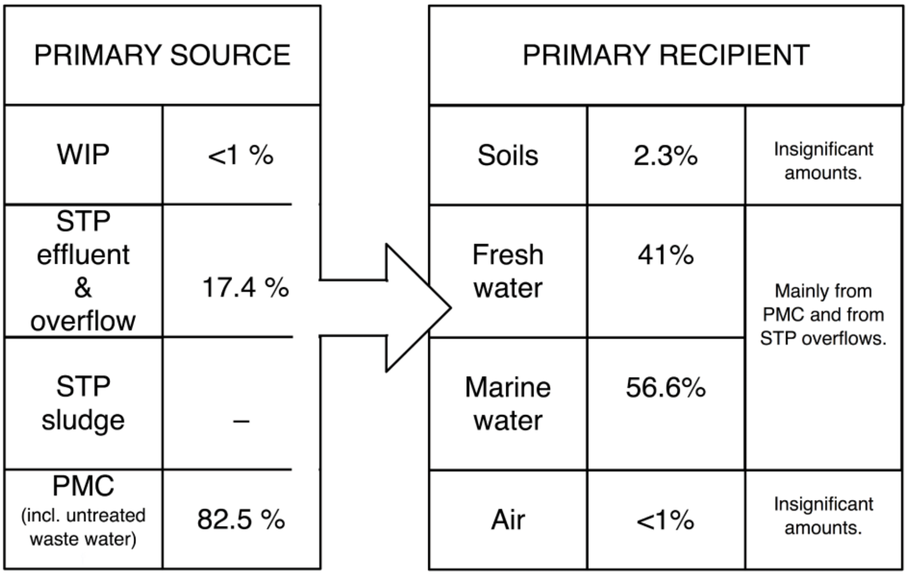

FIGURE 3-3
OVERVIEW OF THE MOST IMPORTANT NANO-ZnO SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT MODAL RESULTS (THE MOST PROBABLE VALUES).
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

The figure shows that most of the released nano-ZnO comes from PMC (production, manufacturing, consumption), which highlights the dominant use of nano-ZnO in cosmetic products. Consequently, most of the environmental flows, which are a few tonnes at the most, bring this ENM into the aquatic environment (approx. 98%). The approximately 17 % of released nanomaterial that originate from STPs have to be fully allocated to the overflow of such sewage treatment facilities. Significant air and soil contamination is not anticipated based on the current use volumes and types of nano-ZnO product applications.

3.4.2 Important environmental fate processes

Nano-ZnO has been shown to exert photocatalytic behaviour. However, compared to TiO_2 the photocatalytic activity is limited, and the importance of photochemical transformations is considered to be low.

Rapid dissolution of nano-ZnO has been demonstrated but concurrent aggregation/agglomeration will control the resulting equilibrium concentration of the suspension depending on the size of the nanoparticles. As dissolution is considered a very important process for nano-ZnO, the processes of agglomeration and aggregation will ‘compete’ with ENM dissolution. Additionally, sedimentation of larger aggregates/agglomerates will still be important. Furthermore, NOM has been shown to adsorb to nano-ZnO and is considered a relatively important fate process for this material.

Studies have shown high sorption/retention in e.g. soil columns. The sorption onto other surfaces would be evaluated medium as the sorption most likely would be either a function of retention from physical straining or through complexation of dissolved species from the ZnO NP.

As ZnO is an inorganic material the process of biodegradation is considered not to be relevant, while biomodification is evaluated as having rather low importance in environmental fate modelling compared to other transformation processes.

3.4.3 Environmental exposure

An almost complete nano-ZnO removal in the sewage treatment plants (STPs) results in low PEC values in waters and sediments for nano-ZnO (see Table 3-10). The PEC for STP sludge and soils treated with STP sludge are considered to be almost zero because nano-ZnO has been shown to be almost completely transformed and is therefore not expected to be present in its initial nanoform following sewage treatment processes.

The freshwater PECs were mainly in the pg/L to lower ng/L level; the marine water PECs were a factor 10 lower than the freshwater PECs and all in the pg/L range. Soils and sediments represented the final sinks; however, only very low quantities of nano-ZnO were modelled here.

Table 3-10 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for nano-ZnO. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-10

PREDICTED CONCENTRATIONS FOR NANO-ZNO IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABLITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS. FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PEC.

Compartment (type)	Unit	PEC Most probable value	95% PEC range
Surface water (freshwater)	µg/L	4.5E-04	8.6E-05 - 0.013
Surface water (marine water)	µg/L	4.1E-05	5.6E-06 - 1.8E-04

Air	µg/m3	3.6E-05	5.2E-06	-	2.3E-04
Sediments (freshwater)	µg/kg/yr	25.2	4.5	-	735
Sediments (marine water)	µg/kg/yr	7.6	0.9	-	33.8
Soils (agricultural)	µg/kg/yr	0.008	0.001	-	0.1
Soils (natural)	µg/kg/yr	0.017	0.003	-	0.1
Soils (urban)	µg/kg/yr	0.031	0.005	-	0.2
Soils (STP sludge treated)	µg/kg/yr	Almost no ZnO remaining in its initial nanoform			

Table 3-11 shows the forecast concentrations for nano-ZnO after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively. In 2020, a few hundred ng/kg are predicted in soils, and a few hundred µg/kg in sediments. These values are much lower than, for example, the predicted few mg/kg concentrations for photostable nano-TiO_2.

TABLE 3-11
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-ZNO IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-ZNO RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	2020
Sediments (freshwater)	µg/kg	164	342
Sediments (marine water)	µg/kg	49.4	103
Soils (agricultural)	µg/kg	0.052	0.1
Soils (natural)	µg/kg	0.12	0.2
Soils (urban)	µg/kg	0.2	0.4
Soils (STP sludge treated)	µg/kg	Almost no ZnO remaining in its initial nanoform	

3.4.4 Environmental effects

For nano-ZnO a little less than 80 scientific papers on environmental effects were identified, however, most of the identified papers did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. 18 articles reporting NOEC, EC_{10}- andEC_{50} values were selected for further assessment of which five studies were found 'adequate for regulatory use' and six studies were assessed as ‘may be adequate for regulatory use. The main reason for low reliability of the remaining studies was the lack of characterization and especially the lack of exposure quantification throughout the test duration.

For the freshwater compartment short-term acute tests for all trophic levels of the base-set have been identified, with EC_{50} for *P. subcapitata* <0.5 mg/L, LC_{50} for *D. magna* of 1-100 mg/L and LC_{50} for embryos of *D. rerio* of 1589 mg/L. Additionally chronic studies of the algae and daphnia were performed with a NOAEC for *P. subcapitata* <0.5 mg/L and a NOEC for *D. magna* of 0.125 mg/L. Cell viability studies for the fish *P. lucida* and the protozoa *T. thermophilia* were performed, revealing NOEC values of 2-25 mg/L and EC_{50} values of 4-8 mg/L, respectively.

Some articles not only reported effects for the nano-form, but also for the bulk-form and/or the ionic Zn^{2+} form. In most cases the ionic form was more toxic and often the nano-form was at least as toxic as the bulk-form.

3.4.4.1 PNEC for nano-ZnO

As data from short-term tests are available for all three base-set test organisms as well as some chronic data, an assessment factor of less than 1,000 can be applied. At the short-term acute level, algae is the most sensitive organism group. As chronic NOEC data have been derived for both algae and daphnia, an assessment factor of 50 can be applied to derive the PNEC value.

The reported growth inhibition NOEC value for *P. subcapitata* <0.5 mg/L and the reproduction NOEC for *D. magna* is 0.125 mg/L.

A **$PNEC_{freshwater}$ value of 2.5 µg/L** can therefore be derived for ZnO nanoparticles.

An assessment factor of 50 was set, based on the availability of adequate data. However, if the study used for the PNEC estimation should not be the defining study, another study with an effect data only a factor of 4 higher could be used resulting in a PNEC value of 10 µg/L.

Not enough or no data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.4.5 Environmental risk assessment

Table 3-12 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for nano-ZnO in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations, i.e. a regional scenario. A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value <1 indicates by convention that this risk is considered acceptably low.

TABLE 3-12
PEC/PNEC ESTIMATION FOR NANO-ZNO IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (µg/L)**		**PNEC (µg/L)**	**PEC / PNEC**	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	≈0	-	2.5	≈0	-
Surface water, fresh	4.5E-04	8.6E-05 - 0.013	2.5	1.8E-04	0.0052
Surface water, marine	4.1E-05	5.6E-06 - 1.8E-04	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

Thus, the PEC/PNEC values for nano-ZnO are well below 1 even for undiluted STP effluent and much below 1 for the two types of surface water indicating a negligible risk of ecotoxicological effects of nano-ZnO in surface waters.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations of nano-ZnO predicted for natural, agricultural, urban and sludge-treated soils are predicted to be 0.2 µg/kg at the most.

3.4.6 Summary for nano-ZnO

The main findings in relation to the environmental risk assessment of nano-ZnO are summarised in the box below.

Summary

- Most of the nano-ZnO is transformed into other non-nanoparticle forms during sewage treatment processes and is converted mainly to sulfides.
- The freshwater concentrations are mostly in pg/L to the lower ng/L level while the marine concentrations are a factor of 10 times lower and only reach pg/L levels.
- Soils and sediments represent the final sink for nano-ZnO, however with very low quantities of this ENM. For 2020, a few hundred ng/kg are expected in soils and the same amount in µg/kg in sediments.
- Emissions to and exposure via air are found to be insignificant.
- The most important processes determining the environmental fate of nano-ZnO are dissolution, aggregation/agglomeration, sedimentation and adsorption to NOM and other surfaces.
- Short term aquatic toxicity of nano-ZnO is documented for fish, daphnia and algae and, additionally, chronic data are available for daphnia and algae. The effect levels are in the mg/L or sub-mg/L range with the most sensitive endpoint being a NOEC of 0.125 mg/L for daphnia leading to a PNEC of 2.5 µg/L.
- The risk quotient for STP effluent has been found to be close to zero due to a very low PEC value while the max. PEC/PNEC for freshwater is 0.0052 thus indicating a very low environmental risk of this ENM.

3.5 Risk assessment of nano-silver (nano-Ag)

3.5.1 Sources and releases

Nano-Ag has a range of applications, including as antimicrobial agents in textiles, cleaning agents, paints, cosmetics and hygienic surfaces (e.g. kitchenware and med-tech). No actual use of nano-Ag in production processes in Denmark was identified; therefore, the release comes entirely from the use of products with nano-Ag as a component.

The annual use volume in Denmark of nano-Ag in different products and applications has been modelled to be approx. 0.8 t/year (most probable value). Only about 5% of the total use volume is estimated to be released to the environment on the nano-form. The rest is mostly released via PMC before being captured by recycling, waste incineration and landfilling processes or is released to sewage and subsequently being transformed into non-nano forms (silver sulphide) in sewage treatment plants.

Table 3-13 summarises the modelled concentrations (PEC) for the technical compartments of relevance for nano-Ag. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs).

TABLE 3-13
PREDICTED CONCENTRATIONS FOR NANO-AG IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE THE 95% PROBABILITY RANGE FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	Concentrations		
	Unit	Most probable value	95% range
Sewage treatment effluent	ng/L	0.5	0.012 - 58.6
Sewage treatment sludge*	µg/kg	82.1	4.2 - 254
Waste mass incinerated	µg/kg	15.3	10.1 - 23.4
Bottom ash of waste incineration	µg/kg	34.9	20.9 - 65.7
Fly ash of waste incineration	µg/kg	174	103 - 327

Figure 3-4 below shows the relative distribution of the total amount released annually (as nano) from the technosphere to the environment (5 % of <1 t/y i.e. <0.05 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

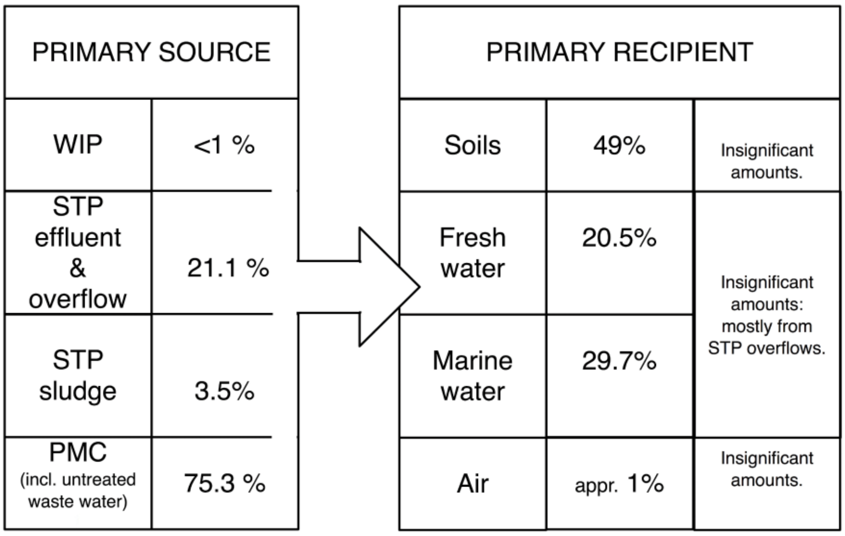

FIGURE 3-4
OVERVIEW OF THE MOST IMPORTANT NANO-AG SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT THE MOST PROBABLE VALUES.
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

Thus, three-quarters of all nano-Ag released to the environment come from the PMC compartment. The remaining quarter of the nano-Ag passes the STP treatment mostly via STP overflow. Besides untreated wastewater, such overflows are the most important sources of nanosilver in the aquatic environment. The nearly equal and complete distribution among natural nano-Ag soils and waters shows that there are also some product categories directly releasing some nano-Ag into soils (in addition to STP sludge fertilization). This phenomenon may occur via paints degradation or air transport, for example, and amount to about 3% of the total use fraction.

3.5.2 Important environmental fate processes

Nano-Ag is expected to be prone to a number of transformation processes upon release to the environment. Of these the dominant ones are reduction/oxidation, dissolution and aggregation/agglomeration. Silver salts are known to be unstable in the presence of light, but since nano-Ag most commonly occurs as metallic silver the before mentioned transformation processes are believed to be of higher importance than photochemical reactions.

Dissolution and subsequent speciation is of high importance for the fate and behaviour of nano-Ag in the environment. Upon dissolution an interaction with the anions and other constituents of the environmental media will occur. In surface waters this is dominated by silver chloride complexes and precipitates, and also silver sulphides contribute significantly to the fate and behaviour of nano-Ag. The silver sulphides are highly stable and results in significant changes in the fate and behaviour of nano-Ag. The silver sulphides are usually considered as not bioavailable.

Interaction of nano-Ag to suspended solids in surface waters is expected and this along with aggregation/agglomeration may result in a transfer to the sediments, where it may accumulated, be transformed, or depending on physical, chemical, and biological conditions. As Ag NMs is an inorganic material the process of biodegradation is considered not to be relevant, however if the particles are coated with organic coatings like PVP or PEG a degradation of these may influence the behaviour of the core nano-Ag significantly.

3.5.3 Environmental exposure

The modelling results show that generally freshwater PECs are expected to be practically zero as almost all modelled values with high probabilities lie between zero and 30 pg/L. Given the current estimated use quantities of nanosilver in Denmark, the model predicts that concentrations above 60 pg/L do not occur in freshwater. The estimated concentrations for the marine environment are all within a much more narrow range and all inpg/L and lower levels.

These results indicate that the contribution of nano-Ag to the total silver contamination in Danish surface water is marginally low at current use and release amounts. Consequently, the same low contribution to the total silver contamination is seen for sediments that will contain only a few µg/kg sediment of nano-Ag, even in 2020.

Regarding the soil concentrations, the concentrations of nano-Ag for non-fertilized soils show annual concentration increases of a few ng/kg soil. The increases in sludge-treated soils are a bit higher, with most probable values around 25 ng/kg soil and will lead to a maximum environmental concentration of about 1 µg/kg soil in sewage-sludge fertilized areas in 2020.

Table 3-14 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for nano-Ag. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-14
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-AG IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.
FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PECS.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	ng/L	0.015	≈0	-	0.044
Surface water (marine water)	ng/L	2.5E-04	≈0	-	5.8E-04
Air	ng/m3	0.8	≈0	-	2.4
Sediments (freshwater)	µg/kg/yr	0.046	≈0	-	0.1
Sediments (marine water)	µg/kg/yr	1.6	0.9	-	3.2
Soils (agricultural)	ng/kg/yr	3.7	2.0	-	9.3
Soils (natural)	ng/kg/yr	6.1	3.5	-	12.5
Soils (urban)	ng/kg/yr	25.7	3.0	-	81.6
Soils (STP sludge treated)	ng/kg/yr	0.007	0.004	-	0.011

Table 3-15 shows the forecast concentrations for nano-Ag after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-15
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-AG IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-SILVER RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	2020
Sediments (freshwater)	µg/kg	5.4	11.4
Sediments (marine water)	µg/kg	0.3	0.6
Soils (agricultural)	ng/kg	10.3	21.4
Soils (natural)	ng/kg	23.9	49.8
Soils (urban)	ng/kg	39.9	83.2
Soils (STP sludge treated)	ng/kg	167	350

3.5.4 Environmental effects

For nano-Ag more than 260 articles on environmental effects were identified, however, many of the identified articles did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. No long term studies reporting chronic NOEC values were identified through the literature search, however, a number of studies reported significant long term effects on reproduction and or growth. Only two studies were found to be 'adequate for regulatory use' and three studies were assessed as 'may be adequate for regulatory use'. The main reason for low reliability of the remaining studies was the very limited characterisation of the inherent properties of the studied nanomaterial.

In total, four studies were found adequate for risk assessment. In two of these, results from short-term tests are reported i.e. effects on algal growth rates with EC_{50}-values of 34 µg/L (72 hours) and 190 µg/L (96 hours). Along with the EC_{50} value, an EC_{10} value of 10 µg/L for the short-term chronic study on algae was also reported. The same two studies also report on 48-hour acute effects on daphnia with EC_{50} values of 1.2 and 40 µg/L for adults and 67 µg/L for neonates. In a mortality study with fish, 48-hour LC_{50}-values of 7,070 µg/L for and 7,200 µg/L were found for adults and juveniles, respectively.

3.5.4.1 PNEC for nano-Ag

The literature review and following evaluation of studies according to regulatory adequacy revealed that short-term data on each of the three base-set test organisms are available for nano-Ag, but also that no long-term NOEC values could be found. Therefore, an assessment factor of 1,000 has to be applied for PNEC estimation. The lowest effect value among the base-set test organisms was the EC_{50}-value for daphnia of 1.2 µg/L. However, also long-term data exist with $EC_{10\text{-}30}$ values for the daphnia of 1-50 µg/L. This, along with a reported EC_{10} value for algae of 10 µg/L, may be considered to fulfil the requirement for two available chronic studies. However, since the EC50-values of 1.2 µg/L still would be the lowest value in the data set, an assessment factor of 100 has to be applied according to the REACH guidance.

Using this assessment factor with the lowest reported effect value **result in a $PNEC_{freshwater}$ value of 12 ng/L**.

If the study used for the PNEC estimation should not be the defining study, an effect value one order of magnitude higher would have been the defining value (40 µg/L for daphnia), resulting in a PNEC of 40 ng/L. Further, if other chronic endpoints than the traditionally accepted ones are included in the PNEC estimaton, the assessment factor could be set at 50. The use of such studies for the risk assessment would result in a PNEC of 100 ng/L indicating that such effects are covered by the proposed PNEC of 12 ng/L.

Not enough or no data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

ENVIRONMENTAL RISK ASSESSMENT

Table 3-16 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for nano-Ag in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations, i.e. a regional scenario. A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-16
PEC/PNEC ESTIMATION FOR NANO-AG IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (ng/L)**		**PNEC (ng/L)**	**PEC / PNEC**	
	Mode	95% int.		Mode	95% int.
STP effluent	0.5	0.012 – 58.6	12	0.042	Max. 4.88
Surface water, fresh	0.015	≈0 – 0.044	12	0.001	Max. 0.004
Surface water, marine	2.5E-04	≈0 – 5.8E-04	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

The most probable PEC/PNEC value is considerably below 1 for undiluted STP effluent but close to 5 based on the max. PEC. The PEC is considerably below 1 for the two types of surface water (even in the worst case) indicating a very low risk of toxic effects of nano-Ag in surface waters. A dilution factor < 5 is required to fulfil the risk acceptance criterion (1) for the highest concentration modelled for STP effluent (95% confidence interval). A dilution of this magnitude will normally be possible to achieve within an acceptable mixing zone upon discharge to receiving fresh, surface waters in Denmark and almost certainly upon discharge to marine waters.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for natural, agricultural and urban soils are very low and even sludge-treated soils will contain less than 0.2 µg/kg.

It should, however, be kept in mind that though the release of and exposure to nano-Ag is currently small, it still contributes to the total silver release and exposure in both the water and soil environments. Knowing the high toxicity of silver in both dissolved and particulate forms, a full risk assessment should comprise the total silver concentrations in such compartments, which may be significantly higher than the ones modelled for nano-Ag.

It should also be noted that a continued discharge of nano-Ag will lead to annual increases in sediment concentrations in the µg/kg range (see Table 3-14) leaving a potential for bioaccumulation in sediment-living organisms and potentially further up in the food web. Taking the high toxicity of nano-Ag into account the use and potential bioaccumulation of nano-Ag should be evaluated continuously (see opinion by SCENIHR, 2014[8])

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[8] Extract of the SCENIHR opinion: "it is clear that Ag-NPs are taken up by organisms, translocated from the gut, in certain conditions, and also depurated, to a point. There are also indications that in certain conditions body burdens are larger following exposures to Ag-NPs, compared to the equivalent levels of dissolved silver. It must be stressed, however, that much work is still required in this area of research."

3.5.5 Summary for nano-Ag

The main findings in relation to the environmental risk assessment of nano-Ag are summarised in the box below.

Summary

- The model for nano-Ag shows a low environmental release that does not exceed the ton/year level for waters or for soils in Denmark.
- During sewage treatment, most of the nano-Ag is expected to be converted into silver sulfide (Ag_2S), which is much less toxic than silver in ionic or nanoparticulate forms.
- All modelled concentrations for nano-Ag in freshwater and marine water are in the pg/L range reaching values of almost zero. Furthermore, all estimated the soil concentrations are very low.
- Although the aquatic concentrations of nano-Ag are marginal, they do contribute to the total silver exposure concentrations in waters and soils. However, current silver concentrations are significantly higher than nano-Ag concentrations.
- As found for all other nano-metals and nano-metal oxides in this study, the modelled air concentrations of nano-Ag are insignificant.
- The most important processes determining the environmental fate of nano-Ag are redox processes and dissolution but also aggregation/agglomeration, sedimentation and adsorption to NOM are relevant.
- Valid short term aquatic toxicity data for nano-Ag exist for fish, daphnia and algae as well as chronic data for daphnia and algae (valid with reservations). The most sensitive acute endpoint is an EC50 for daphnia of 1.2 µg/L yielding a PNEC of 0.012 µg/L.
- The risk quotient (PEC/PNEC) based on most probable exposure concentrations are all considerably below the acceptability criterion (1), but the high end value (95% conf. interval) for STP effluent results in a PEC/PNEC of almost 5 and, hence, a need for a small dilution to meet the risk acceptability criterion.
- The high toxicity of nano-Ag calls for attention even though current and near-future estimates predicts no unacceptable risk for freshwater. Increases in use of nano-Ag as well as potential bioaccumulation are factors that should be monitored as precautionary actions.

3.6 Risk assessment of carbon nanotubes (CNT)

3.6.1 Sources and releases

The main applications of CNTs are as a composite and polymer additive and as a component of batteries. End applications include flat panel displays, super composite fibres, conductive plastics, field storage batteries, micro-electronics based on semiconductors and other conductive material. No information on the use of CNTs in the Danish plastics industry has been obtained, but the possibility that they are used cannot be excluded. It should be emphasized that carbon nanotubes exist in multiple configurations, forms and sizes, but that for the purpose of this risk assessment the CNTs has been considered as one group.

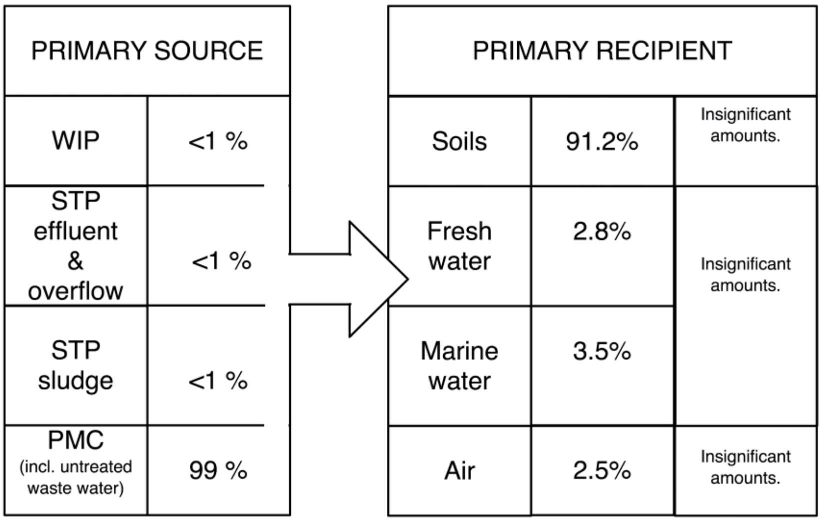
The annual use volume in Denmark of CNT in different products and applications has been modelled to be slightly less than 10 t/year (most probable value; 95% probability range 1-18 t/year) of which a few percent at the most are expected to be released to environmental compartments.

Table 3-17 summarises the modelled concentrations (PEC) for the technical compartments of relevance for CNT. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations.

TABLE 3-17
PREDICTED CONCENTRATIONS FOR CNT IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE A 95% PROBABILITY RANGE FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	**Concentrations**		
	Unit	Most probable value	95% range
Sewage treatment effluent	ng/L	0.3	0.1 - 3.5
Sewage treatment sludge	µg/kg	7.6	2.7 - 61.6
Waste mass incinerated	µg/kg	802	436 - 1320
Bottom ash of waste incineration	µg/kg	75.7	26.5 - 711
Fly ash of waste incineration	µg/kg	326	87.9 - 4770

Figure 3-5 below shows the relative distribution of the total amount released annually from the technosphere to the environment (max. 5 % of 10 t/y i.e. <0.1 t/y) and the resulting distribution of the amount in the receiving environmental compartments.



PRIMARY SOURCE	
WIP	<1 %
STP effluent & overflow	<1 %
STP sludge	<1 %
PMC (incl. untreated waste water)	99 %

PRIMARY RECIPIENT		
Soils	91.2%	Insignificant amounts.
Fresh water	2.8%	Insignificant amounts.
Marine water	3.5%	
Air	2.5%	Insignificant amounts.

FIGURE 3-5
OVERVIEW OF THE MOST IMPORTANT CNT SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT MODAL RESULTS (THE MOST PROBABLE VALUES).
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

The low CNT use volume and a high mass fraction (up to 90-95%) results in a kind of one-way transport subsystem into recycling, waste incineration and landfilling processes. This leads to marginal environmental exposure to this ENM for aquatic and terrestrial environments (5% at the most). This environmental release almost exclusively occurs from direct nanoproduct use (99%). This small material fraction reflects diffusive mass transport mostly into soils. The rest is equally distributed to fresh and marine waters and air.

3.6.2 Important environmental fate processes

CNTs are capable of absorbing sunlight and photochemical surface modifications may also occur, and photochemical transformation is considered to be of medium importance in fate modelling. While pristine CNTs in general are more reactive than functionalized CNTs, their high hydrophobicity makes them difficult to disperse in water and aggregation/agglomeration will be of major importance when assessing their environmental fate. The agglomeration/aggregation is influenced by pH and ionic strength of the surroundings and sedimentation is likely to occur.

The surface properties of CNTs can be greatly influenced by interaction with natural organic matter and the formation of “natural coatings” may prolong the residence time of CNT in the water column. CNTs are generally considered resilient to biotic degradation by microorganisms as a result of their closed-cage structure but they may be transformed by abiotic processes. Biomodification is likely to account only for a smaller part of the total transformation processes.

3.6.3 Environmental exposure

The estimated CNT concentrations in freshwater waters are all in the pg/L range freshwater; for marine water, the model also revealed concentrations in fg/L levels. The sediment (fresh and marine water) PECs are very low. Even by 2020, no more than a few µg/kg is expected in freshwater sediments and for marine sediments it is predicted that the level will remain in the ng/kg range until 2020 based on current CNT use amounts.

The forecasted concentration for urban soil for 2020 is in the range of a few hundred ng/kg soil. The results for natural and agricultural areas are a bit lower due to varying soil depths and the subsequent varying volumes for potential soil contamination. Soil fertilization with sludge has negligible importance in this case, since it is estimated that almost no CNT ends up in such sludge.

Table 3-18 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for CNTs. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-18

PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR CNT IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS. FOR SOILS AND SEDIMENTS, THE FIGURE IS THE ANNUAL INCREASE IN THE PECS.

Compartment (type)	Unit	PEC Most probable value	95% PEC range
Surface water (freshwater)	ng/L	0.001	1.8E-04 - 0.015
Surface water (marine water)	ng/L	4.9E-05	2.2E-05 - 2.0E-04
Air	ng/m3	0.042	0.022 - 0.091
Sediments (freshwater)	µg/kg/yr	0.1	0.011 - 0.9
Sediments (marine water)	µg/kg/yr	0.009	0.004 - 0.038

Compartment (type)	Unit	PEC Most probable value	95% PEC range
Soils (agricultural)	ng/kg/yr	5.3	2.8 - 11.5
Soils (natural)	ng/kg/yr	12.7	6.3 - 33.1
Soils (urban)	ng/kg/yr	20.6	11.0 - 44.8
Soils (STP sludge treated)	ng/kg/yr	9.2	4.6 - 27.4

Table 3-19 shows the forecast concentrations for CNTs after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-19
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR CNT IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF CNT RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	**2020**
Sediments (freshwater)	µg/kg	0.5	1.0
Sediments (marine water)	µg/kg	0.1	0.1
Soils (agricultural)	µg/kg	34.5	72.0
Soils (natural)	µg/kg	82.5	172
Soils (urban)	µg/kg	134	279
Soils (STP sludge treated)	µg/kg	59.8	125

3.6.4 Environmental effects

For CNT about 180 articles on environmental effects were identified but most of them did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. A total of 20 articles were found to report ecotoxicological information for CNTs and of these, 15 were selected for further assessment due to their reporting of IC_{50}, LC_{50}, EC_{50}, EC_{10} or NOEC data or other relevant toxicological information. Ten studies were assessed as 'may be adequate for regulatory use' while the remaining five studies were evaluated as 'not adequate for risk assessment'. In most cases, the assessment of low adequacy was mainly due to the general lack of material characterisation.

If one of the studies assessed as 'not adequate for risk assessment' is included, data for all three trophic levels of the base-set organisms is available. However, no traditional 96-h LC_{50} on fish or 48-h LC_{50} on daphnia were reported, but a 96-h EC50 on growth inhibition on algae. The EC_{50} values reported were in the range of 1.8-63 mg/L. Two chronic studies on algae and daphnia revealed a NOEC for the algae of 0.042 mg/L and a significantly reduced reproduction for the daphnia at a concentration of 0.125 mg/L.

3.6.4.1 PNEC for CNT

Provided that slightly non-standard exposure times are accepted in the data selections, and that the fish study scored as 'not adequate for risk assessment' is accepted as well, data from short-term tests are available for all three base-set test organisms. Furthermore, “a significant effect on daphnia reproduction” is considered a chronic effect value, and therefore it can be argued that chronic data for two trophic levels are available. This results in the use of an assessment factor of 50 for PNEC estimation. The reported growth inhibition NOEC value for *C. vulgaris* of 0.042 mg/L constitutes the lowest of all reported effect values.

A **PNEC$_{\text{freshwater}}$ value of 0.84 µg/L** can therefore be derived for carbon nanotubes.

Not enough or no data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.6.5 Environmental risk assessment

Table 3-20 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for CNT in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations (i.e. a regional scenario). A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-20
PEC/PNEC ESTIMATION FOR CNT IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (ng/L)**		**PNEC (ng/L)**	**PEC / PNEC**	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	0.3	0.1 – 3.5	840	3.57E-04	Max. 4.17E-03
Surface water, fresh	1.0E-03	1.80E-04 – 0.015	840	1.19E-06	Max. 1.79E-05
Surface water, marine	4.90E-05	2.20E-05 - 2.00E-04	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

The calculated PEC/PNEC value is more than a factor of 100 below 1 even for max. concentration in undiluted STP effluent, and a factor of >10,000 below 1 for the two types of surface water indicating a very low risk of toxic effects of CNT in surface waters. It is noted that the PNEC value was derived on a quite weak data basis.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for all categories of soils are very low and only reach 0.13 µg/kg at the most in any soil (most probable value for 2014).

3.6.6 Summary for CNT

The main findings in relation to the environmental risk assessment of CNT are summarised in the box below.

Summary

- The estimated CNT use volume in Denmark is estimated to <10 tons/year of which the most (95-99 %) ends up in technical compartments, such as waste mass and waste incineration residues, recycling plants and landfills.
- The estimated CNT concentrations in freshwater only reaches pg/L concentration levels and even lower concentrations were estimated for freshwater and marine water.
- Until 2020, no concentrations of more than a few µg/kg of CNT in freshwater sediment are forecasted; the marine concentrations are expected to be a little lower.
- STP sludge-treated soil is estimated to have negligible CNT concentrations, since almost no CNT is expected to enter STP and thus end up in STP sludge.
- Aggregation/agglomeration, sedimentation and adsorption to NOM and other surfaces are found to be the most important processes determining the environmental fate of CNT.
- A fully valid aquatic toxicity data set is not available for CNT, however acute data valid with reservations exist for fish, daphnia and algae as well as adequate chronic data for daphnia and algae. The most sensitive endpoint was a chronic NOEC of 42 µg/L for algae which was used to derive a PNEC of 0.84 µg/L.
- The aquatic risk quotients (PEC/PNEC) based on most probable exposure concentrations are all a factor of >10,000 below the acceptability criterion (= 1) and, hence, the risk of toxic effects of CNT in surface waters is considered to be negligible.

3.7 Risk assessment of nano-copper carbonate (nano-$CuCO_3$)

The following risk assessment of nano-copper carbonate (nano-$CuCO_3$) is partially made using nano-copper oxide (nano-CuO) as a surrogate, mainly due to lack of environmental fate and effects data for nano-$CuCO_3$ but also because nano-CuO is seen as a possible substitute in the future for some nano-$CuCO_3$ applications.

3.7.1 Sources and releases

Three different applications of nano-copper have been considered. Of these, the use of micronized particles of copper carbonate ($CuCO_3$) for wood preservatives was considered to potentially account for the major portion. Micronised $CuCO_3$ is not intentionally a nanomaterial, but would probably currently fall within the definition with the current particle size distribution. It is expected that in the next few years, micronised $CuCO_3$ may substitute for a significant part of the $CuCO_3$ used for wood treatment in Denmark today.

Copper carbonate is used for the production of pressure-impregnated wood in Denmark, and micronised $CuCO_3$ may already be present in imported treated wood. The current use volume is estimated to be around 190 tons/year, composed of 90 tons used for wood impregnation in Denmark and 100 tons imported with impregnated products.

The use and release of $CuCO_3$ was modelled for a future situation scenario where the micronized $CuCO_3$ has substituted the $CuCO_3$ used today. Nano-CuO may be used as an alternative to $CuCO_3$ for various applications discussed in the report, but no attempt was made to estimate the total quantities in imported mixtures and articles. Copper oxides are widely used for antifouling paints, and these paints may potentially become an application area for nano-CuO. Other known use areas include solar cell technology, photovoltaic applications, gas sensors, combustion catalysts, ceramic resistors and semiconductor applications.

Table 3-21 summarises the modelled concentrations (PEC) for the technical compartments of relevance for nano-$CuCO_3$. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations.

TABLE 3-21
PREDICTED CONCENTRATIONS FOR NANO-COPPER CARBONATE IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE A 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	Concentrations				
	Unit	Most probable value	95% range		
Sewage treatment effluent	µg/L	1.3	0.3	-	4.1
Sewage treatment sludge	mg/kg	9.1	5.2	-	17.2
Waste mass incinerated	mg/kg	2.0	1.3	-	3.0
Bottom ash of waste incineration	mg/kg	4.4	2.7	-	8.5
Fly ash of waste incineration	mg/kg	22.1	13.2	-	42.3

Figure 3-6 gives an overview of the most important sources and receiving compartments for nano-$CuCO_3$. The greatest portion of nano-copper environmental release (approx. 98% of theoretically up to 190 t/y) covers direct discharge from the impregnated wood into soils. This occurs via subsurface soil contact with such wood as well as via deposition of dust and wash-off from the impregnated wood.

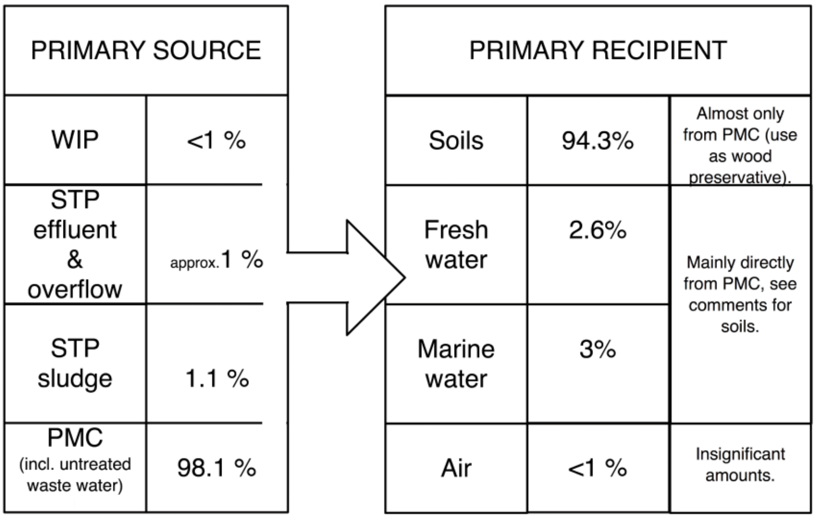


FIGURE 3-6
OVERVIEW OF THE MOST IMPORTANT NANO-CUCO3 SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT MODAL RESULTS (THE MOST PROBABLE VALUES).
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

The annual environmental release of nano-$CuCO_3$ into the aquatic environment probably does not exceed 1% of the total annual nano-$CuCO_3$ mass transfer volume. A very large portion (approx. 70%) of the annual nano-$CuCO_3$ mass ends up in technical treatment processes for nanomaterial-containing products, such as recycling, waste incineration and landfilling while about 30% end up in soils.

3.7.2 Important environmental fate processes

CuO is a metal oxide ENM with photo-catalytic properties. Binding of nano-CuO to dissolved organic matter may be altered by photo-activation, as it is the case with nano-TiO_2. However, the importance of photochemical transformations as well as of redox reactions for fate assessment is considered to be low.

Dissolution is known to be relevant for nano-CuO and therefore the processes of agglomeration and aggregation will to some extent ‘compete’ with ENM dissolution. Also sorption/retention of nano-CuO to soil is considered a relevant process. Dissolution will also influence the actual overall relative importance of this process. As CuO is an inorganic material the process of biodegradation is considered not to be relevant while biomodifications is not very well investigated but is assigned a low-medium importance.

3.7.3 Environmental exposure

The results from the modelling of environmental exposure to nano-$CuCO_3$ revealed concentrations of a few ng/L for freshwater and values in pg/L levels for marine water. The annual increase in freshwater sediment concentrations is about 140 µg/kg sediment while for marine water sediments it is about 7 µg/kg. Such annual increases would lead to PEC values of around 2 mg/kg and 0.1 mg/kg for freshwater and marine water sediments in 2020.

The high exposure scenarios for 2020 showed values up to approximately 200 µg/kg of soil. However, these high concentration values originate from the worst case exposure model, which assumed a high micronized fraction of the total nano-$CuCO_3$ used in wood impregnation.

Table 3-22 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for nano-copper carbonate. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-22
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-CUCO3 IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.
FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PECS.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	µg/L	0.002	1.2E-04	-	0.006
Surface water (marine water)	µg/L	3.5E-05	2.0E-05	-	6.8E-05
Air	µg/m3	2.1E-05	4.6E-06	-	3.9E-05
Sediments (freshwater)	µg/kg/yr	136	6.7	-	322
Sediments (marine water)	µg/kg/yr	6.5	3.8	-	12.8
Soils (agricultural)	µg/kg/yr	4.4	2.8	-	6.3
Soils (natural)	µg/kg/yr	9.3	5.9	-	19.4
Soils (urban)	µg/kg/yr	17.0	10.8	-	24.4
Soils (STP sludge treated)	µg/kg/yr	7.4	5.0	-	10.7

Table 3-23 show the forecast concentrations for nano-CuCO$_3$ after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-23
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-CUCO3 IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-CUCO3 RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	2020
Sediments (freshwater)	µg/kg	884	1850
Sediments (marine water)	µg/kg	42.2	88.0
Soils (agricultural)	µg/kg	28.4	59.3
Soils (natural)	µg/kg	60.3	126

Compartment (type)	Unit	Year	
Soils (urban)	µg/kg	110	230
Soils (STP sludge treated)	µg/kg	48.0	100

3.7.4 Environmental effects

For nano-CuO about 50 articles on environmental effects were identified but most of them did not meet basic quality or validity criteria for inclusion in the data set for derivation of a PNEC value. Of the retrieved articles for nano-CuO, 16 reported IC/LC/EC_{50} or NOEC/LOEC/EC_{10} data of mainly acute but also a few chronic effects, relevant for regulatory purposes. Only one study was found adequate for regulatory use and nine studies were assessed as 'may be adequate for regulatory use'. The main reason that several of the studies have been assigned with lower adequacy was the lack of stable and quantifiable exposure conditions.

Short-term ecotoxicity tests have been conducted for all three trophic levels of algae, daphnia and fish. For algae, 72-h EC_{50} value of 150 mg/L for *C. reinhardtii* and a 96-h EC_{50} value of 0.54 mg/L for *P. subcapitata* were found. For daphnia the 48-h LC_{50} ranged from 0.034-0.42 mg/L. 48-h and 120-h acute tests on the fish *D. rerio* (embryos, juveniles and adults) showed LC_{50} values in the range 0.71-1.56 mg/L, with one study >10 mg/L.

Chronic NOEC values have been published for both algae and daphnia. A 72-h NOEC value of ≤100 mg/L was observed for *C. reinhardtii* , but no NOEC value was obtained for *P. subcapitata*, which was found to be the more sensitive of the two, based on EC50 values. The one study found adequate for regulatory use investigated long-term chronic effects on daphnia (*D. magna*) gave a NOEC value of 0.06 mg/L (mortality and reproduction as endpoints).

In all of the reviewed studies where a metal salt ($CuCl_2$ or $CuSO_4$) has been included as a reference for free ion toxicity, the metal salt showed higher toxicity than the CuO NP. In general, the toxicity of Cu-compounds can be ranged in the following order: bulk CuO < CuO NP < Cu^{2+} based on the results from the reviewed studies.

3.7.4.1 PNEC for nano-CuO

As data from short-term tests are available for all three base-set test organisms as well as some chronic data, an assessment factor of less than 1,000 can be applied. At the short-term acute level, the daphnia is the most sensitive organism. As chronic NOEC data have been derived for both the daphnia and algae, an assessment factor of 50 can in principle be applied to derive the PNEC value. The reported growth inhibition NOEC value for *C. reinhardtii* was ≤100 mg/L and the NOEC value (mortality and reproduction) for *D. magna* was 0.06 mg/L. The lowest effect value in the data set was, however, found for daphnia with a 48-h LC_{50} of 0.034 mg/L. Since this is a result from a short-term acute test, an assessment factor of 100 has to be applied according to the REACH guidance.

A **$PNEC_{freshwater}$ value of 0.34 µg/L** can therefore be derived for nano-CuO.

Not enough or no adequate data were available to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.7.5 Environmental risk assessment

Table 3-24 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for nano-CuO in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations (i.e. a regional scenario). A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-24
PEC/PNEC ESTIMATION FOR NANO-CUO IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (µg/L)**		**PNEC (µg/L)**	**PEC / PNEC**	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	1.3	0.3 – 4.1	0.34	3.8	12.1
Surface water, fresh	0.002	1.2E-04 – 0.006	0.34	0.0059	0.018
Surface water, marine	3.5E-05	2.0E-05 – 6.8E-05	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

The PEC/PNEC value for nano-CuO is significantly higher than 1 for undiluted STP effluent (max. value approx. 12) but below 1 for surface waters (even as max. value) indicating a low risk of toxic effects of nano-CuO in surface waters outside a limited mixing zone close to the discharge point for STP effluents. A dilution of a factor of 3.8 for the most probable STP effluent concentration value, and a factor 12 for the highest concentration modelled for STP effluent (95% confidence interval), is required to fulfil the risk acceptance criterion (1). While a 3.8 times dilution is normally achievable, a dilution of a factor of 12 will under some circumstances (summer minimum flow) be difficult to meet in some streams, mainly in Eastern Denmark. Thus, a risk of toxic effects beyond a typical mixing zone cannot be completely excluded.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for all categories of soils are low and no more than 0.11 µg/kg in any soil based on most probable value.

It should, however, be kept in mind that nano-CuO contributes to the total release of and exposure to copper in the water and soil environments and the high end nano-CuO concentration in STP effluent is about at the same level as "ordinary" copper.

It should also be noted that a continued discharge of nano-CuO will lead to annual increases in sediment concentrations in the µg/kg range (see Table 3-23). As described for nano-Ag there may be a potential for bioaccumulation in sediment-living organisms and potentially further up in the food web, though this has not be studied to the same extent as for nano-Ag. Considering the high toxicity of nano-CuO the use and potential bioaccumulation of this ENM should be evaluated continuously.

3.7.6 Summary for nano-copper carbonate/oxide

The main findings in relation to the environmental risk assessment of nano-copper carbonate/oxide are summarised in the box below.

Summary

- The main environmental release (approx. 98%) of nano-copper carbonate occurs directly from impregnated wood into soils.
- However, the current use of $CuCO_3$/CuO for wood impregnation and environmental release amounts do not lead to significant exposure to nano-Cu in surface waters and sediments. The predicted concentrations show some few ng/L levels in freshwater and equivalent values for pg/L levels in marine water.
- Significant future exposure of soils to nano-copper carbonate should be viewed as possible. In 2020, the high exposure scenarios do not exclude values of up to approx. 200 µg/kg soil.
- Aggregation/agglomeration and sedimentation are found to be the most important processes determining the environmental fate of nano-$CuCO_3$/CuO.
- Valid aquatic toxicity data are available for nano-CuO covering acute data for fish, daphnia and algae as well as chronic data for daphnia and algae. The most sensitive endpoint was an acute LC50 for daphnids of 34 µg/L which was used to derive a PNEC of 0.34 µg/L.
- The risk quotients (PEC/PNEC) for nano-CuO in surface waters based on most probable exposure concentrations are a factor of 50 or more below the acceptability criterion (1) while in STP effluent the most probable PEC/PNEC is 3.8 and the high end PEC/PNEC for nano-CuO is 12. Thus, some dilution is required to fulfil the acceptability criterion near discharge points of STP effluents and this may in some cases require a somewhat extended mixing zone to be established.
- The high toxicity of nano-CuO calls for attention, and increases in use of nano-CuO as well as potential bioaccumulation are factors that should be monitored continuously to prevent effects in Danish surface water systems.

3.8 Risk assessment of nano-zero valent iron (nZVI)

3.8.1 Sources and releases

For nanosized zero-valent iron (nZVI), only a local soil remediation scenario has been considered. A few medical applications have been reported but it is the application for *in situ* soil/groundwater remediation that currently seems to be the only relevant use of nano-ZVI. Data from a well-documented, large pilot study in Denmark have been used as basis for the exposure estimation.

The nano-ZVI application for soil remediation is by nature a local event because of the high reactivity of nano-ZVI. Through oxidation, this iron reacts with its surroundings, such as air, soil components, and water, and forms iron oxides or hydroxides. In remediation, electrons released from these reactions allow the reduction of different organic and inorganic soil or groundwater contaminants.

The use volume of nZVI in Denmark is highly uncertain and probably largely theoretical at present (although some full scale tests have been made). For the exposure estimations in this study it was assumed that the current uses of other iron products for soil/groundwater remediation are replaced by nZVI and it was estimated that a volume of about 100 tons/year is required for this purpose.

3.8.2 Important environmental fate processes

The use of nZVI in soil/groundwater remediation has created a great demand for information on the environmental fate and behaviour of this ENM. Through oxidation nZVI reacts with air and water and forms iron oxides and hydroxides. The release of electrons from this reaction is utilized in remediation to reduce several types of contamination. As nZVI is engineered to undergo this oxidation, redox processes and conditions are important parameters for fate modelling, as they control the chemical composition of the particles.

Uncoated nZVI exhibits little to no mobility in groundwater or soil because of its rapid agglomeration/aggregation and subsequent straining but coating can result in greater mobility. The importance of agglomeration is considered as high whereas the importance of solubility is considered to be low. Sorption of pollutants to nZVI is part of the remediation process and thus sorption to other chemicals and surfaces is relevant for nZVI.

3.8.3 Environmental exposure

Several of the soil samples from the mentioned Danish pilot study showed total iron concentrations between 0.95% and 3.98% according to soil weight. The samples tested for background iron concentrations contained between 0.99% and 2.14% iron. A statistical evaluation did not reveal any difference between the background and the sample mean results.

The pilot study went beyond the general analysis and included a mass spectrometry (MS) reading collected for the target soil areas. In contrast to the total iron analysis, the background results of the MS reading and the one of the soil fracture sample of MS readings differed significantly. An estimation of the absolute concentration of nZVI in the injected slurry component showed a concentration of 14.2 g nZVI/L slurry. This concentration is, however, considered an exposure concentration that is unlikely to be reached ever for homogeneous distribution of nZVI in Danish soil or groundwater aquifers.

If the estimated (potential) use volume of around 100 t/y were to be distributed (and not degraded after such distribution) into the total soil volume by an average annual concentration, an increase of 20 micro/kg would be modelled. In 2020, average nano-ZVI soil concentrations of 270 µg/kg soil would be reached based on the same increase model used in the other ENM cases. If this worst-case nZVI scenario of 270 µg/kg is compared to the total (natural) iron content in soil it is evident that on a mass basis this hypothetical highest possible nZVI concentration is insignificant compared to the naturally occurring iron content in Danish soils.

In summary, even hypothetical worst-case nZVI scenarios of some hundred µg/kg soil do not represent a significant amount when compared to the natural concentrations of 20 g/kg to 40 g/kg. In order to get a significant exposure, the nZVI in slurry should be directly converted into soil concentrations, which currently seems unrealistic.

3.8.4 Environmental effects

Out of 44 hits in the literature search using "iron" and "nano" as a search terms, 7 studies were with nZVI as a test compound. Out of the seven studies with nZVI , only two studies were reporting EC50 or LOEC-values. Additionally, six studies with other iron-based nanomaterials as test compounds were reporting IC50, LC50, EC50 or NOEC/LOEC, and these are also included in the assessment in order to increase the amount of data to support the PNEC estimations. Two studies were found 'adequate for regulatory use' and three studies were assessed as ‘may be adequate for regulatory use’. The main reason for a lower reliability score is the lack of verification of exposure and exposure conditions during the tests and lack of appropriate controls.

Varying types of short-term ecotoxicity tests have been carried out for all three trophic levels of algae, daphnia and fish. Chronic LOEC values have also been obtained for algae. One study investigated the toxicity of zero-valent iron nanoparticles against several aquatic organisms (algae, daphnia, fish, bacteria and plants). nZVI was found to be harmful to cyanobacteria (EC_{50} = 50 mg/L). The toxicity of nZVI towards the tested organisms was in the following order: cyanobacteria > aquatic plant > algae > daphnia > fish > terrestrial plant. Besides the EC50 values for cyanobacteria, no EC_{50} values were provided for other organisms, but it was mentioned that EC_{50} is > 1,000 mg/L and > 2,500 mg/L for daphnia and fish, respectively.

96-h toxicity tests with different marine and freshwater microalgae and freshwater crustacean (*D. magna*) was investigated in another study and resulted in a growth LOEC for *P. subcapitata* of 8.24 mg/L. The LOEC values towards the marine organisms were much lower, ranging from 0.42-3.1 mg/L. For *D. magna*, the survival LOEC was 0.5 mg/L.

3.8.4.1 PNEC for nZVI

Before PNEC estimation is carried out, it is important to realize that nZVI will react immediately after application and will under aerobic conditions change physical and chemical form to larger than nano-sized particles consisting of iron-oxides. Therefore, a traditional PNEC for the aquatic environment (based on tests carried out under aerobic conditions) is more or less meaningless and it may be more sensible to apply the principles for deriving an intermittent PNEC, where an assessment factor of 100 is applied to the lowest acute EC_{50} value (ECHA, 2008).

Provided that the present data are treated as traditional effect data, the lowest acute effect data is a 96-h daphnia survival test resulting in a LOEC of 0.5 mg/L.

A **$PNEC_{freshwater, intermittent}$ value of 5 µg/L** can therefore be derived for nano zero valent iron on an intermittent basis.

Not enough or no adequate data were available to derive PNEC values for STP, air, soil/terrestrial, sediment or the marine environment.

3.8.5 Environmental risk assessment

nZVI differs from all the other nanomaterials assessed in this study, above all with regard to its use and type of release to the environment. The currently only significant use of nZVI is for remediation of soil and groundwater pollution by direct injection into the polluted subsurface soil/groundwater. Here the material reacts rapidly with substances and surfaces in its surroundings and hereby loses its original character by transformation into less reactive iron species and sorption to surfaces.

Hence, it is considered unlikely that nZVI should be dispersed in the environment outside a narrow zone around the application point and, consequently, the environmental risk associated with the current use of this ENM is considered to be low and very local.

Consequently, no modelling of exposure of surface waters has been attempted and no PEC/PNEC values calculated.

3.8.6 Summary for nZVI

The main findings in relation to the environmental risk assessment of nZVI are summarised in the box below.

Summary

- The use of nano zero-valent iron (nano-ZVI) in soil and groundwater remediation has led to a need for information on the environmental fate and behaviour of such iron.
- Due to the intended use of these nanoparticles, they will be discharged to the natural environment, where some reactivity is expected.
- The nano-ZVI-based remediation is a local event since it is driven by high and rapid reactivity of the nano-ZVI. For more about such reactivity and transformations, see the details in NanoDEN Report 1.
- Even hypothetical worst-case nano-ZVI scenarios of some hundred µg/kg in soil do not represent a significant amount when compared to the natural concentrations of g/kg soil concentrations.
- The most important processes determining the environmental fate of nZVI are redox processes, aggregation/agglomeration, sedimentation and sorption to surfaces.
- Acute aquatic toxicity data for nZVI include fish, daphnia and algae. As nZVI reacts very rapidly it was only considered relevant to determine a short term PNEC. The most sensitive endpoint was a 96 h LOEC = 500 µg/L for daphnia, which was used to derive a short term (intermittent) PNEC of 5 µg/L.
- The potential environmental risk related to the use of nZVI is very different from all the other ENMs studied as nZVI is injected directly into subsurface soils and groundwater. As nZVI reacts rapidly upon injection, the material is unlikely to be dispersed in the environment outside a narrow zone around the application point. Hence, the environmental risk is considered to be low and very local.

3.9 Risk assessment of nano-cerium dioxide (nano-CeO_2)

3.9.1 Sources and releases

Data on the use of nano-CeO_2 were derived from a survey of the use of nanomaterials in the EU. The main uses in the EU were estimated to be diesel fuel additives, automotive catalyst converters, glass polishing, paint and coatings. For each of these applications, transfer coefficients were estimated. The use of nano-CeO_2 as a UV filter in wood oil manufactured in Denmark was confirmed.

The modelled nano-CeO_2 Danish use volumes were relatively low with modelled most probable values of around 3-5 tons/year with a release fraction to the environment of less than 10%.

Table 3-25 summarises the modelled concentrations in the technical compartments relevant for nano-CeO_2. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations.

TABLE 3-25

PREDICTED CONCENTRATIONS FOR NANO-CEO2 IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE A 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartment (source)	Concentrations		
	Unit	Most probable value	95% range
Sewage treatment effluent	ng/L	9.3	1.1 - 59.6
Sewage treatment sludge	µg/kg	352	44.1 - 2310
Waste mass incinerated	µg/kg	179	21.0 - 931
Bottom ash of waste incineration	µg/kg	359	49.7 - 2460
Fly ash of waste incineration	µg/kg	2200	243 - 12300

Figure 3-7 below shows the relative distribution of the total amount released annually from the technosphere to the environment (max. 10 % of 3-5 t/y i.e. <0.5 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

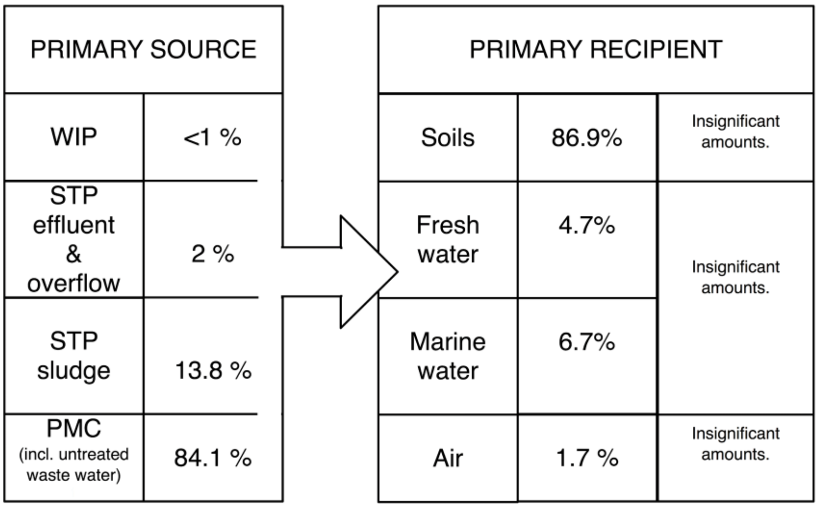


FIGURE 3-7
OVERVIEW OF THE MOST IMPORTANT NANO-CEO_2 SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT THE MOST PROBABLE VALUES.
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

As can be seen from the table, up to approx. 10% of the total mass is released to the environment, hereof most (approx. 84%) released directly from PMC. This can be explained by the character of main the nano-CeO_2 applications (use as fuel additives/fuel-borne catalysts or for glass polishing). The use in paints and coatings and probably in some cosmetics and medicinal products (though not quantified due to missing data) also leads to some fractions released to STPs.

3.9.2 Important environmental fate processes

Nano-CeO_2 is a metal oxide ENM with photo-catalytic properties. Although the activity of CeO_2 is lower than that of TiO_2, photochemical reaction may also be a relevant process for nano-CeO_2.

CeO_2 is an inert material and not likely to dissolve to any relevant extend under normal conditions. Therefore, the importance of redox reactions and of dissolution for environmental fate and behaviour or nano-CeO_2 is considered to be low.

It has been found that CeO_2 NPs aggregate in soil and are retained in the soil column and the importance of sorption and retention for CeO_2 is therefore evaluated to be relatively high.

As CeO_2 is an inorganic material the process of biodegradation is considered not to be relevant and, compared to other transformation processes, also biomodification (of the uncoated form) is likely to account only for a smaller part of the total transformations.

3.9.3 Environmental exposure

The current releases of nano-CeO_2 to the Danish environment appear to be small, not exceeding 0.5 ton per year. The environmental exposure mostly occurred via STP sludge fertilisation of soils and some air emissions.

The modelled freshwater and marine water concentrations of nano-CeO_2 were at pg/L levels (some fg/L scenarios were also modelled) while sediment concentrations only reached a few µg nano-CeO_2 per kg sediment (freshwater) until 2020. The ones for marine water sediments only reach µg/kg levels in a few scenarios.

In 2020, non-STP-sludge-fertilised soil concentrations of about a few hundred ng/kg are expected, while they will be a few µg/kg for STP-sludge-treated soils.

Table 3-26 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for nano-CeO_2. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs). The figures for soils and sediments represent the annual increase in concentrations, not the absolute values.

TABLE 3-26
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-CEO_2 IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.
FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PECS.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	ng/L	0.004	5.8E-04	-	0.1
Surface water (marine water)	ng/L	2.6E-04	3.2E-05	-	0.002
Air	ng/m3	0.1	0.010	-	0.6
Sediments (freshwater)	µg/kg/yr	0.2	0.033	-	6.9
Sediments (marine water)	µg/kg/yr	0.049	0.006	-	0.3
Soils (agricultural)	ng/kg/yr	11.7	1.5	-	81.6

Soils (natural)	ng/kg/yr	26.9	3.7 - 224
Soils (urban)	ng/kg/yr	45.6	6.0 - 316
Soils (STP sludge treated)	ng/kg/yr	232	14.5 - 781

Table 3-27 show the forecast concentrations for nano-CeO$_2$ after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-27
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR NANO-CEO$_2$ IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF NANO-CEO$_2$ RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	**Unit**	**Year**	
		2014	**2020**
Sediments (freshwater)	µg/kg	1.6	3.3
Sediments (marine water)	µg/kg	0.3	0.7
Soils (agricultural)	ng/kg	76.3	159
Soils (natural)	ng/kg	175	364
Soils (urban)	ng/kg	296	619
Soils (STP sludge treated)	ng/kg	1510	3140

3.9.4 Environmental effects

Of the 19 hits in the literature search, 16 studies on nano-CeO$_2$ have reported IC50, LC50, EC50 or NOEC/LOEC data or other toxicological information. Two studies were found 'adequate for regulatory use' while eight were assessed as ‘may be adequate for regulatory use'. In most cases, the lower adequacy of some studies was due to the lack of verification of exposure and exposure conditions during the tests.

Short-term ecotoxicity tests have been carried out for all three trophic levels of algae, daphnia and fish. The resulting toxicity values are, however, very scattered. One study investigated the toxicity of nano-CeO$_2$ to zebrafish embryos and found a LC$_{50}$-value of > 2,000 mg/L, which corresponds with the results from another study, where an EC50 value of > 200 mg/L (i.e. the highest test concentration) for the same test species .

One of the 'adequate' studies carried out acute and chronic exposures of *D. magna*, and found no acute toxicity of nano-CeO$_2$ up to the highest test concentration (10 mg/L). Up until 10 mg/L no chronic toxicity was observed either, however, 100% mortality was found in the 10 mg/L test concentration after 7 days exposure. However, the results from another study suggests high variation in intraspecies sensitivity and indicates that *D. magna* may not be the most sensitive species of crustaceans as an EC$_{50}$-value of 0.26 mg/L was found for *D. similis*.

In six studies, the toxicity of micro-sized CeO$_2$ was compared to that of nano-CeO$_2$ and in all cases nano-CeO$_2$ was found to be more toxic that the micro-sized particles. The toxicity of nano-CeO$_2$ to aquatic organisms is assumed to be due to direct physical interaction between the particles and the organism, possible causing membrane disruption.

3.9.4.1 PNEC for nano-CeO_2

As data from short-term tests are available for all three base-set test organisms as well as some chronic data, an assessment factor of less than 1,000 can be applied. At the short-term level, the daphnia is the most sensitive organism with an LC_{50} value of 0.26 mg/L. As chronic NOEC data have been derived for both algae and daphnia, an assessment factor of 50 can be applied to derive the PNEC value. The reported growth inhibition EC_{10} value for *P. subcapitata* was 0.7 mg/L and the reproduction EC_{10} for *D. magna* was 8.8 mg/L. The EC_{10}-values are used as surrogates for NOEC values.

As the acute LC_{50} value for the most sensitive organism is lower than the short-term EC_{10} value for the algae it is considered justifiable to use the acute LC50 value for daphnia of 0.26 mg/L, resulting in a **$PNEC_{freshwater}$ value of 5.2 µg/L** for nano-CeO_2.

An assessment factor of 50 was set, based on the availability of adequate data. However, if the study used for the PNEC estimation should not be the defining study, the daphnia study would have to be applied an assessment factor of 100 resulting in a PNEC value of 88 µg/L, i.e. 17 times higher.

Because of lack of relevant data it is not possible to derive PNEC values for STPs, air, soil/terrestrial, sediment or the marine environment.

3.9.5 Environmental risk assessment

Table 3-28 below shows the calculation of the aquatic risk quotient (= PEC/PNEC) for nano-CeO_2 in the effluent from sewage treatment plants (STPs) and in surface waters (freshwater and marine water) as average concentrations (i.e. a regional scenario). A risk quotient (PEC/PNEC value) at or above 1 indicates a risk of effects on organisms in the environment (here the aquatic environment) while a value below indicates that this risk by convention is considered acceptably low.

TABLE 3-28
PEC/PNEC ESTIMATION FOR NANO-CEO2 IN STP EFFLUENT AND SURFACE WATERS (FRESH AND MARINE).

Compartment	**PEC (ng/L)**		**PNEC (ng/L)**	**PEC / PNEC**	
	Mode	95% int.		Most probable value	Max. (95% int.)
STP effluent	9.3	1.1 – 59.6	5200	1.79E-03	Max. 1.15E-02
Surface water, fresh	0.004	5.80E-04 – 0.1	5200	7.69E-07	Max. 1.92E-05
Surface water, marine	2.60E-04	3.20E-05 – 0.002	*	*	*

* Marine PNECs not possible to estimate due to lack of data. Extrapolation of data from freshwater organisms not considered scientifically valid for ENMs due to significant differences in freshwater and marine bioavailability. However, due to significant additional dilution marine PEC/PNECs will typically be lower than freshwater values.

The PEC/PNEC value for nano-CeO_2 is considerably below 1 even for undiluted STP effluent (most probable value as well as max. for 95% interval) and way below 1 for the two types of surface water indicating a very low risk of toxic effects of nano-CeO_2 in surface waters.

Due to lack of ecotoxicological effect data for other environmental compartments than the aquatic, it is not possible to make a quantitative risk assessment for e.g. the soil compartment. However, the concentrations predicted for all categories of soils are low with a maximum value 1.5 µg/kg in soil amended with STP sludge (2014, based on most probable value).

3.9.6 Summary for nano-CeO_2

The main findings in relation to the environmental risk assessment of nano-CeO_2 are summarised in the box below.

Summary

- The Danish use volume of nano-CeO_2 seems relatively low with a most probable value of around 5 tons/year. The model shows a low annual load of cerium into the natural environment, not exceeding 0.5 tons/year.

- Most of the nano-CeO_2 water and marine water concentrations are in pg/L concentration levels; partially, some fg/L were also computed.

- For non-STP sludge fertilized areas, the soil concentrations in 2020 are expected to be at a few hundred ng/kg levels (most probable values). A few µg/kg are expected at this time for STP sludge-treated areas.

- Aggregation/agglomeration and sedimentation are found to be the most important processes determining the environmental fate of nano-CeO_2.

- Valid aquatic toxicity data are available for nano-CeO_2 covering acute data for fish, daphnia and algae as well as chronic data for daphnia and algae. The endpoint used for risk assessment was the acute EC_{50} for daphnia of 0.26 mg/L from which a PNEC of 5.2 µg/L was derived.

- The risk quotients (PEC/PNEC) for nano-CeO_2 based on most probable exposure concentrations are at least a factor of 500 below the critical level (PEC/PNEC = 1) even in STP effluent (a factor of about 100 for the 95% interval maximum) while in surface waters the PEC/PNEC is an additional factor of 1,000 lower indicating a very low risk for effects of nano-CeO_2 in the aquatic environment.

3.10 Risk assessment of quantum dots (QD)

3.10.1 Sources and releases

Data on quantum dots for this study were derived from a survey of the use of nanomaterials in the EU. Quantum dots are semiconductor materials of metals such as Cd and Zn ranging from 2-10 nm in diameter. Applications of quantum dots in products and commodities include semiconductors transistors, solar cells, light-emitting devices (e.g. LEDs), diode lasers and medical imaging devices. No information on the use of QDs for manufacturing in Denmark has been found, but such a use cannot be excluded. The current Danish QD use volume is most likely well below 0.5 tons per year and less than 1% of this volume is expected to be released to the environment.

Table 3-29 summarises the modelled concentrations for the technical compartments of relevance for QDs. The results are presented as the most probable values and the 95% probability range for the lower and upper concentrations.

TABLE 3-29
PREDICTED CONCENTRATIONS FOR QUANTUM DOTS (QD) IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE A 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATION LIMITS.

Compartments (sources)	**Concentrations**		
	Unit	Most probable value	95% range
Sewage treatment effluent	ng/L	3.0E-05	5.2E-06 - 0.001
Sewage treatment sludge	µg/kg	2.4E-04	3.6E-05 - 0.003
Waste mass incinerated	µg/kg	0.9	0.1 - 4.4
Bottom ash of waste incineration	µg/kg	2.2	0.2 - 11.4
Fly ash of waste incineration	µg/kg	10.3	1.0 - 56.7

The majority of the QD nanomaterial will end up in the waste management compartments or recycling at the end of its life cycle and only about 1% goes into waste incineration or is deposited in landfills. The remaining max. 10% of the negligibly small total mass (kg/year level) QD material is distributed about equally to the different natural compartments. Due to the very low quantities, we have not conducted a quantitative distinction between the aquatic, terrestrial, or air environments. The currently more or less insignificant environmental release is a reflection of two main factors: 1) the current low use volumes and 2) applications that do not lead to direct releases to the natural environment.

The modelled STP effluent concentrations were almost completely in fg/L levels. Therefore, the modelling was limited to this compartment and did not attempt to further quantify surface water concentrations in much more diluted surface waters. The STP sludge concentrations showed values ranging from 40 pg/kg to some ng/kg (95% value range).

3.10.2 Important environmental fate processes

Some QDs have a core-shell structure composed of a core and a shell of two different semiconductor materials, where the shell semiconductor has a wider band gap compared to the core material. Examples include CdS/ZnS, CdSe/ZnS and CdSe/CdS. These are high-efficient photoluminescence materials and their photoluminescence properties can be modified by adjusting the shell thickness.

Due to their varying chemical compositions and the possibility of ‘tuning’ the specific properties of QDs their ability to take part in environmental transformation processes cannot be generalised but will depend on a case-by-case evaluation.

The processes of agglomeration/aggregation, sedimentation, and NOM adsorption are however considered to be important processes for ENMs in general – and hence also for QDs. Similarly, some sorption to other surfaces is likely to be relevant.

3.10.3 Environmental exposure

As stated above, the modelled PECs for water were so low that a realistic quantification and evaluation of the probability distributions at fg/L and lower levels seemed meaningless and, hence, we restrict ourselves to stating that, based on the current QD use volumes, the aquatic PEC values are most likely negligible. In principle, similar findings apply to sediments as well; however, due to a two-decade time period of marginal QD deposition, some values could be derived. After 20 years of sedimentation, about 3 µg/kg in freshwater sediments are predicted while marine water sediments does not fully reach µg/kg concentrations in 2020.

Table 3-30 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for quantum dots.

TABLE 3-30
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR QD IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES) AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATIONS (WHERE POSSIBLE).

Compartment (type)	Unit	PEC Most probable value	95% PEC range
Surface water (freshwater)	-	Below fg/L	nq*
Surface water (marine water)	-	Below fg/L	nq
Air	-	nq	nq
Sediments (freshwater)	-	fg/kg to some pg/kg	nq
Sediments (marine water)	-	fg/kg	nq
Soils (agricultural)	-	nq	nq
Soils (natural)	-	nq	nq
Soils (urban)	-	nq	nq
Soils (STP sludge treated)	pg/kg/yr	0.1	0.010 - 1.0

* nq = not quantified

Table 3-31 show the forecast concentrations for QD after a deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-31
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR QD IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF QD RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	**2020**
Sediments (freshwater)	µg/kg	1.6	3.3
Sediments (marine water)	µg/kg	0.3	0.7
Soils (agricultural)	ng/kg	nq	nq
Soils (natural)	ng/kg	nq	nq

Compartment (type)	**Unit**	**Year**	
Soils (urban)	ng/kg	nq	nq
Soils (STP sludge treated)	ng/kg	4.5E-04	0.001

3.10.4 Environmental effects

Only 6 out of 65 articles on QDs were found to contain ecotoxicological information relevant for risk assessment, five articles reported LC$_{50}$ or EC$_{50}$ values. Four studies were assessed as 'may be adequate for regulatory use'.

For the freshwater compartment, a long-term EC$_{50}$-value on the algae *C. reinhardtii* of 5 mg/L and a significant effect from 0.1 mg/L towards stress response genes was found. Two other studies reported effects from irradiation activation of different coatings of the Cd/Se-QDs on *D. magna*. Except for the mercaptopropionic acid coating and white fluorescence irradiation giving an EC$_{50}$-value larger than 2.5 mg/L, all 48-h EC$_{50}$ mortalities ranged from 0.01-0.4 mg/L, dependent on coating and light conditions. Sub-lethal effects were reported down to 0.003 mg/L for significant effects on mRNA.

Based on the MetPLATE^{TM} bioassay, measuring the β-galactosidase inhibition of *E. coli* in the terrestrial compartment, up to 100 mg/L of the octadecylamine coated Cd/Se-QD nanomaterial tested only exerted a 34% inhibition.

3.10.4.1 PNEC for QD

Given the very limited data set, the low diversity of organisms tested (two algae species, two crustacean species, one nematode and a bacteria) and the regulatory adequacy of the studies, it is not considered justifiable to apply the assessment factor approach for PNEC derivation. Hence, no PNEC can be estimated for QD nanoparticles at the present state-of-knowledge.

3.10.5 Environmental risk assessment

Due to lack of reliable data it was not considered possible to derive a PNEC value for QD, which in any case is not one, well-defined material but a type of materials with special properties that can be based on a number of different metals/elements. However, the few existing data do not indicate high acute aquatic toxicity of QD.

The use volumes of QDs are very low (< 1 ton/year) and the use pattern not likely to result in significant environmental exposure (max. 10% is assessed to be released to the environment). Thus, modelled STP effluent and surface water concentrations are at or below fg/L levels.

Consequently, with the current uses and use volumes of QD there are no indications that the use of this ENM should be of significant environmental concern.

3.10.6 Summary for QD

The main findings in relation to the environmental risk assessment of QD are summarised in the box below.

Summary

- The use volumes of QDs are most likely well below 0.5 tons/year levels.
- About 90% of the total mass is assumed to be used for light emitting devices (e.g. LEDs) that mostly end up in recycling processes at the end of their lifecycles. Only max. 10% is assessed to be released to the environment.
- The surface water and air concentration probability distributions were not quantified and evaluated because all values were lower than fg/L (water) or fg/m^3 (air).
- The modelling results for STP sludge-fertilized soils in 2020 are even for high exposure only at some pg/kg levels.
- Aggregation/agglomeration and sedimentation are found to be the most important processes determining the environmental fate of QD but also sorption to NOM and other surfaces is relevant.
- Only few reasonably adequate ecotoxicity data are available for QD. The ecotoxicity studies do indicate some toxicity of QD but it was not considered justifiable to derive a PNEC based on the available data.
- Consequently, no risk quotients (PEC/PNEC) have been derived for QD but the presumably moderate toxicity and low environmental exposure to this ENM do not indicate that it should be of significant environmental concern.

3.11 Risk assessment of carbon black (CB)

3.11.1 Sources and releases

Carbon black (CB) is elemental carbon in the form of an extremely fine black powder, which is widely used as a black pigment. The primary particles of CB meet the definition of nanomaterials. During the production process, however, the primary particles form larger aggregates and later agglomerates of 1–100 mm in diameter may be formed. Although the aggregation, which spontaneously occurs in manufacturing processes, produces unbreakable aggregates of an average size that is generally over 100 nm, CB can include a limited fraction of nanoscale materials. On a global scale, 90% of CB is used in the rubber industry as a reinforcing filler in a variety of products, with tyres as the major application area.

In Denmark, the use of CB as a black pigment in the manufacture of inks, paints, plastics, cosmetics and textiles has been identified and the total volume in manufacturing processes in Denmark is estimated to be 100-400 tons/year. The use volume of CB in Denmark is, however, assessed to be considerably larger, approximately 36,000 tons per year (most probable value) of which around 10% is expected to be released to the environment.

Table 3-32 summarises the modelled concentrations for the technical compartments of relevance for CB. The results are presented as the most probable values and the 95% probability range for the lower and upper concentrations.

TABLE 3-32

PREDICTED CONCENTRATIONS FOR CARBON BLACK IN DIFFERENT TECHNICAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% RANGES THAT ALSO ILLUSTRATE A 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATIONS.

Compartments (sources)	Concentrations				
	Unit	**Most probable value**	**95% range**		
Sewage treatment effluent	µg/L	1241	292	-	3900
Sewage treatment sludge	mg/kg	2455	582	-	7680
Waste mass incinerated	mg/kg	1418	659	-	2520
Bottom ash of waste incineration	mg/kg	140	44	-	1310
Fly ash of waste incineration	mg/kg	539	146	-	8630

Figure 3-8 below shows the relative distribution of the total amount released annually from the technosphere to the environment (approx. 10 % of 36,000 t/y i.e. 3,600 t/y) and the resulting distribution of the amount in the receiving environmental compartments.

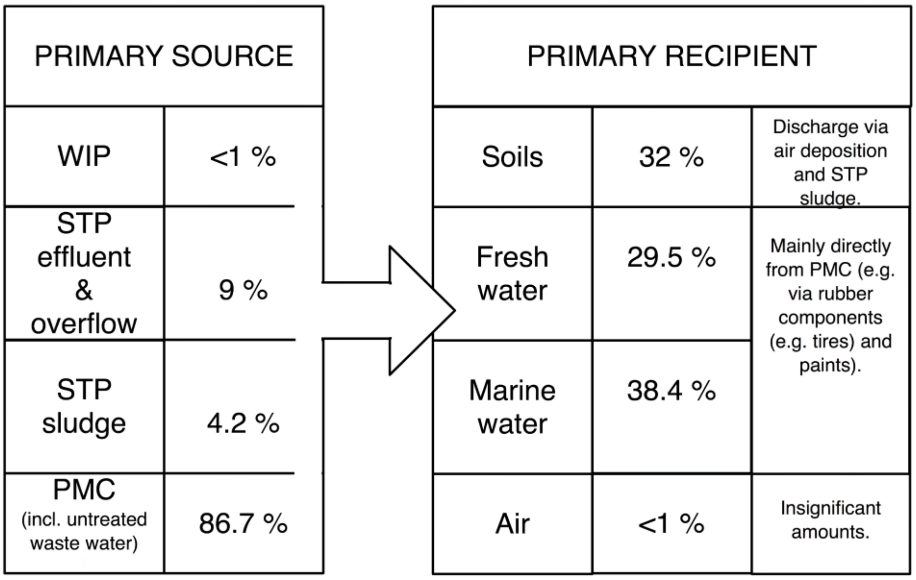


FIGURE 3-8
OVERVIEW OF THE MOST IMPORTANT CARBON BLACK (CB) SOURCES AND RECEIVERS. THE PERCENTAGES REFLECT MODAL RESULTS (THE MOST PROBABLE VALUES).
WIP = WASTE INCINERATION PLANTS;
STP = SEWAGE TREATMENT PLANTS;
PMC = PRODUCTION, MANUFACTURING AND CONCUMPTION (DIRECT RELEASE WITHOUT PRIOR TREATMENT).

Regarding the amounts of carbon black ending up in natural environments, we note that approx. 6-7% of the total annual mass reach surface waters and 3-4% find their way into soils. The aquatic pathway is dominated by direct release into surface waters e.g. originating from road runoff (from wear of car tyres) systems not connected to any water treatment. Release to soils occurs more or less directly or via previous air transport and subsequent dry or wet (rain-based) deposition.

3.11.2 Important environmental fate processes

CB has no functional groups and therefore it is insoluble in water and cannot be further degraded by light or by photo-degradation in air or in surface water. Parameters like water solubility, octanol/water partition coefficient, dissociation constant or adsorption/desorption cannot be analytically measured. However, deposition in soil or sediments is considered to be most relevant compartment for CB in the environment. This is likely to be due to aggregation/agglomeration leading to sedimentation. Due to the high carbon content it is expected that CB will interact with NOM and other organic compounds and have a high potential to adsorb to other surfaces.

As an inorganic compound with the chemical structure "C", carbon black will not be further biodegraded by microorganisms OECD (2006). Potential biomodifications may include changes in agglomeration state as a result of organism uptake but in our literature study no studies of biomodification of CB were identified.

3.11.3 Environmental exposure

For CB the model reveals by far the highest water concentrations compared to all other nanomaterials included in this study (assuming that all 36,000 tons are on nanoform, which may not be the case in reality). In freshwater, values around 0.5 µg/L are predicted (most probable value), in some high exposure scenarios up to 6 µg/L. The marine water loads show values ranging from 15 to 80 ng/L. Consequently, the sediment loads are, according to high water exposure, also higher than that of all other substances studied. In 2020, the modelled values are approx. 100-1000 mg/kg and 85 mg/kg for fresh and marine water sediments, respectively.

In STP-sludge-treated soils, the CB load has an expected value of 10 mg/kg in 2020. In non-fertilized soils (agricultural, natural and urban soils) the modelled values are 1-5 mg/kg in 2020.

In line with the results presented above, the air concentrations in the model reveal the highest values for CB among the ENMs studied in this report. Concentrations ranging from 100 to 300 ng/m^3 are predicted (95% confidence range).

Table 3-33 summarises the modelled concentrations (PEC) for the environmental compartments of relevance for CB. The results are presented as the most probable values and the 95% probability range for the lower and upper environmental concentrations (PECs).

TABLE 3-33
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR CB IN DIFFERENT NATURAL COMPARTMENTS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES AND 95% PROBABILITY RANGES FOR LOWER AND UPPER CONCENTRATIONS.
FOR SOILS AND SEDIMENTS, THE FIGURE SHOWN IS THE ANNUAL INCREASE IN THE PECS.

Compartment (type)	Unit	PEC Most probable value	95% PEC range		
Surface water (freshwater)	µg/L	0.5	0.1	-	6.0
Surface water (marine water)	µg/L	0.034	0.015	-	0.080
Air	µg/m3	0.2	0.1	-	0.3
Sediments (freshwater)	mg/kg/yr	112	5.5	-	336
Sediments (marine water)	mg/kg/yr	6.3	2.8	-	14.9

Soils (agricultural)	mg/kg/yr	0.1	0.047	-	0.2
Soils (natural)	mg/kg/yr	0.2	0.1	-	0.6
Soils (urban)	mg/kg/yr	0.4	0.2	-	0.8
Soils (STP sludge treated)	mg/kg/yr	0.8	0.2	-	2.6

Table 3-34 show the forecast concentrations for CNTs after an ENM deposition period in soils beginning in 2000 and ending at the beginning of the years 2014 and 2020, respectively.

TABLE 3-34
PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) FOR CB IN SEDIMENTS AND SOILS. THE RESULTS ARE SHOWN AS MOST PROBABLE VALUES.
THE PEC VALUES FOR SOILS AND SEDIMENTS REFLECT THE CONCENTRATIONS OF CB RESULTING FROM ACCUMULATION IN THE PERIOD 2000 UNTIL THE BEGINNING OF 2014 AND 2020, RESPECTIVELY.

Compartment (type)	Unit	Year	
		2014	2020
Sediments (freshwater)	mg/kg	730	1520
Sediments (marine water)	mg/kg	40.8	85.2
Soils (agricultural)	mg/kg	0.7	1.4
Soils (natural)	mg/kg	1.5	3.2
Soils (urban)	mg/kg	2.6	5.4
Soils (STP sludge treated)	mg/kg	5.0	10.5

3.11.4 Environmental effects

Of the identified 14 articles for CB only 6 were found to contain relevant ecotoxicological information. Only two articles have reported LC_{50} or EC_{50} values and three reported sub-lethal effects, however, all of these studies were evaluated to be 'not adequate for regulatory use'. All studies lacked nanomaterial characterization and exposure evaluation and this is the main reason for the low regulatory reliability assessment.

One study investigated effects of CB with three different surfaces on larvae of a marine crustacean (*A. franciscana*) and it was found that the commercially produced had the lowest LC50-value (370 mg/L), while the two surface modified particles were less toxic with LC50-values of 1000 and >1000 mg/L, respectively. For the sub-lethal effects, both the commercial and the more hydrophobic of the modified materials showed significant effects already at 50 mg/L, whereas the less hydrophobic did not show significant effects.

3.11.4.1 PNEC for CB

Given the very limited data set and the diversity of organisms tested (one species of crustaceans, molluscs, and insects) it is at present not possible to apply the assessment factor approach for PNEC derivation. Thus, no PNEC can be estimated for CB nanoparticles at the present state-of-knowledge.

3.11.5 Environmental risk assessment

Due to a limited data set with questionable validity it was not considered possible to derive a PNEC value for CB. However, the few existing data do not indicate a high acute aquatic toxicity of CB.

On the other hand the use volume of CB in Denmark (and worldwide) is significant, estimated to be approx. 36,000 tons/year (most probable value), which is by far the highest among the ENMs studied, and the modelled most probable concentration in STP effluent is 1.2 mg/L leading to average surface water concentrations (freshwater) of about 0.5 µg/L. The presently reported lowest sub-lethal effects were at 50 mg/L corresponding to a factor of 100 between the effect level and the PEC for STP. Thus, it can at present not be excluded that the risk quotient (PEC/PNEC) for STP effluent could exceed 1. On the other hand, a very low risk for effects in freshwater is expected given the currently available toxicity data (a factor of 100,000 is found between the effect level and PEC for freshwater).

Current soil concentrations of approx. 1 mg/kg soil are presumably not an additional carbon mass concentration that should be of concern. In 2020 up to 5 mg/kg soil can be expected and twice as much in STP sludge-treated soils.

3.11.6 Summary for CB

The main findings in relation to the environmental risk assessment of carbon black are summarised in the box below.

Summary

- The modelled annual use volume for CB display kilo-tons values with a most probable value of about 36,000 tons/year.
- The model for CB shows for waters and soils an annual load into the environment exceeding 1,000 tons/year levels. Most of the CB (approx. 90%) is, however, caught by recycling and other waste mass treatment (landfilling and waste incineration).
- The most probable freshwater concentrations are around 0.5 µg/L. The predicted concentrations in marine waters are about ten times lower.
- For terrestrial exposure significant contributions to the total carbon load are predicted. Concentrations for 2020 of about 10 mg/kg (STP sludge-treated soils) and 1-5 mg/kg (other soils) cannot be excluded.
- Among the ENMs in this study, the air concentrations for CB are the highest, ranging from 100 to 300 ng/m^3.
- The reported exposure concentration for CB are to be considered as conservative values since it has been neglected that CB may also be released partly as non-nano particles.
- For environmental fate assessment, aggregation/agglomeration, sedimentation and adsorption to NOM and other surfaces are found to be the most important processes for CB.
- Only a very limited data set on environmental toxicity of CB is available and none of the studies are considered adequate for regulatory use. The few existing studies showed sub-lethal effects at 50 mg/L.

- No risk quotients (PEC/PNEC) have been derived for CB but due to the lack of toxicity data and an estimated significant environmental release volume, it cannot be excluded that some dilution of STP effluents are required to obtain a PEC/PNEC value lower than 1.

3.12 RA summary

The risk quotients (RQ = PEC/PNEC values) calculated in this chapter for the selected nanomaterials in STP effluents and fresh surface waters are summarized in Table 3-35 on the following page.

In relation to the summary table, it should be noted that although some of the materials exist both on the nano-form and as bulk/ionic materials (e.g. silver), the risk quotients only reflect the risk associated with the release of the nano-form. Likewise, the risk quotients only reflect the risk posed by the engineered nanomaterials although for some of the materials there may also be a contribution from natural sources.

TABLE 3-35
SUMMARY OF RISK QUOTIENTS (PEC/PNEC VALUES) FOR THE SELECTED ENMS IN STP EFFLUENTS AND IN THE FRESH SURFACE WATERS (REGIONAL VALUES).

ENM	**Compartment**	**PEC / PNEC**	
		Most probable value	Max value (95% int.)
TiO2 (photostable)	STP effluent	0.7	5.1
	Surface water, fresh	0.0002	0.006
TiO2 (photocatalytic)	STP effluent	0.09	0.79
	Surface water, fresh	1.5 E-05	3.9 E-04
ZnO	STP effluent	≈0	-#
	Surface water, fresh	1.8E-04	0.0052
Ag	STP effluent	0.042	4.88
	Surface water, fresh	0.001	0.004
CNT	STP effluent	3.57E-04	4.17E-03
	Surface water, fresh	1.19E-06	1.79E-05
CuO	STP effluent	3.8	12.1
	Surface water, fresh	0.0059	0.018
nZVI	STP effluent	-##	-##
	Surface water, fresh	-##	-##
CeO2	STP effluent	1.79E-03	1.15E-02
	Surface water, fresh	7.69E-07	1.92E-05
QD	STP effluent	-##	-##
	Surface water, fresh	-##	-##

ENM	Compartment	PEC / PNEC	
		Most probable value	Max value (95% int.)
CB	STP effluent	-*	-*
	Surface water, fresh	-**	-**

-: estimation of PEC/PNEC not possible
* possibly >1; **possibly <<1
\# possibly <1; ## possibly <<1

NOTE: The PNEC estimations given throughout this report are based on the assumptions that 1) the current test methods are applicable to nanomaterials, and 2) that the current extrapolation methodologies are valid for nanomaterials. Both of these assumptions are questionable (for a further discussion of this see Lützhøft *et al.*, 2015). The PNEC values given in this report should therefore be taken as indicative for the order of magnitude for PNEC given the current regulatory recommendations for the PNEC estimation methodology.

The table shows that the risk quotient (RQ) calculated for nano-CuO exceeds 1 in STP even for the most probable value (RQ = 38) while for all the other ENMs the RQs based on the most probable value is <1. The max. concentrations in STP effluent for photostable TiO2 and for nano-Ag also leads to an RQ >1 for both (approx. 5). All other RQs are <1.

In surface waters (regional scenario) all RQ's are well below 1, most of them way below.

Based on the summary of PEC and PNEC values estimated in this chapter, the ENMs can tentatively be placed some rough "risk categories" based on the current level of use in a way similar to what could be done for chemicals:
(1) *Critical ENMs: Those that have a high PEC as well as a low PNEC*
(2) *Potentially critical ENMs (if use/PEC increases significantly)*
(3) *ENMs unlikely to be critical as an ENM.*

TABLE 3-36
ROUGH RISK CATEGORIES FOR SELECTED ENMS IN THE FREHWATER ENVIRONMENT.

		PEC	
		Low	High
PNEC	Low	*Potentially critical ENMs** Ag, CuO, CNT	*Critical ENMs* None of the ENMs included in NanoDEN
	High	*Unlikely to be critical ENMs* ZnO, nZVI**, CeO2, QD	*Potentially critical ENMs** CB, TiO_2

* If the consumption/release (PEC) increases significantly.
** Because the exposure is very local.

4. Nanomaterials in the environment – discussion of main findings

This chapter is dedicated to discussing central elements of the overarching question behind the NanoDEN project– is there a reason for concern regarding nanomaterials in the Danish environment? The chapter is divided into four main sections, all considered to be of high importance for evaluating the possible concerns relating to nanomaterials in the environment:

- Sources and releases to the environment
- Environmental exposure
- Environmental effects
- Environmental risk assessment.

This chapter scrutinizes the central findings in the previous sub-projects, the outcome of the risk assessment in the previous chapter as well as other concerns identified through the literature reviews, the modelling, and the evaluations carried out in the NanoDEN project. The chapter aims on this basis to evaluate the state of our knowledge, and the limitations herein, in relation to potential concerns and risks related to nanomaterials in the Danish environment.

4.1 Sources and releases of ENMs

Nanomaterials are invented and produced because it has been realised that not only the chemical composition of a material matters but also the size and other physical aspects play an important role in defining a range of technologically interesting properties and possibilities that are new compared to those know from traditional "macro"-materials and chemical compounds.

The functionalities characterising the materials selected for this study have a quite wide span, i.e. they differ significantly between the individual materials, but together they represent typical types of functionalities of many engineered nanomaterials. Examples of such functionalities are:

- Absorption of UV irradiation ("UV filters") (e.g. photostable TiO_2, ZnO, CEO_2)
- Antimicrobial/biocidal effect (e.g. Ag, CuO, photocatalytic TiO_2)
- Conducting/semi-conducting properties (CNT, QD, ZnO)
- Chemical reactivity (nZVI)
- Catalytic properties (CeO_2, photocatalytic TiO_2)
- Pigment (CB, TiO_2)
- Mechanical strength (CNT).

These functional properties are interesting and useful in many types of applications and, hence, ENMs (including those selected for this study) find their way into a wide range of uses and product categories of which many are consumer products. Examples of product types where nanomaterials are applied are: cosmetics (including sunscreens), paints, various other coatings including hygienic surfaces, cleaning agents, textiles, plastic and rubber (additives), batteries, electronics, solar cells, LED displays, wood impregnation, fuel additives, catalysts etc.).

The basic nanomaterials and most of the mentioned product types with nanomaterials are not manufactured in Denmark but are used in this country mainly as imported articles. With the notable exception of carbon black (CB), having an estimated annual use volume of about 36,000 tons (if assuming that it all is on nano-form), the current use volumes of nanomaterials in Denmark are presumably rather small and not exceeding 200 tons/year for any material. Photostable TiO_2 and $CuCO_3$ come close to 200 tons/year, while photocatalytic TiO_2, ZnO and (potentially) nZVI are in the range 10-100 tons/year and the annual consumption of the remaining materials is lower than that (Ag and QD even lower than 1 ton/year). Some of the non-nano parallels to these materials (e.g. TiO_2, Zno and $CuCO_3$) are used in much higher amounts in Denmark and worldwide. It should be noted here that in general it has been difficult to obtain exact information on the use and release of the studied ENMs in Denmark. This constitutes a serious limitation for the precision of the modelling of the environmental concentrations and in most cases conservative (worst-case) estimates had to be included.

Four main types of sources of ENM release to the environment are considered in this study:

- Emissions from waste incineration plants
- Effluents from sewage treatment plants and overflows of wastewater
- Sludge from sewage treatment plants, and
- Direct releases from production, manufacturing and consumption.

Waste/residuals in landfills or similar have in this study been considered to be sinks not leading to environmental exposure although in Denmark reuse of a more open character of waste incineration slags etc. take place (road fill etc.).

The extent of release to the environment differs significantly between the ENMs included in this report, from 100% for nZVI (due to its use by injection directly into soil/groundwater), over significant fractions (>25%) of environmental release for TiO_2 and copper, to release fractions at or lower than 10% for ZnO, silver, CNT, CeO_2, QD and CB. As there appears to be only very limited basic production or manufacturing of nano-containing materials in Denmark, most of the ENM volumes released to the environment in this country are assessed to originate from the direct use (consumption) of articles/materials coated with ENMs or with ENMs incorporated.

For more than half of the studied nanomaterials, a dominant part of the total environmental release has been modelled to result directly from the use/consumption of the materials, i.e. without prior elimination/retention in waste incineration plants (WIPs) or sewage treatment plants (STPs) or because they are transformed into non-nano forms before release (e.g. in STP processes). This goes for ZnO, silver, CNT, copper, nZVI, CeO_2, and CB. For the two forms of TiO_2, sludge from STPs is the dominant release source while the release from the use of QDs is so small that quantification of the distribution to source categories has not been attempted. Emissions to air are for all the studied materials a release source of minor or negligible environmental significance.

Unfortunately, no quantifications exist for the relevant ENMs that relate the anthropogenic nanomaterial (ENM) release to the amounts of the same nanomaterials already occurring in nature such as e.g. organic carbon based particles and some inorganic oxides (Nowack & Bucheli, 2007). The most prominent example of nanomaterial of natural origin presented by these authors are the carbon based ones that have some biogenic, pyrogenic and geogenic or atmospheric sources. Farre et al. 2010 state that the natural origin of such nanoparticles could be a significant one due to relevant sources such as forest fires, volcanic eruptions as well as any kind of fires that also include emissions via many industrial combustion processes.

A recent Danish review study of human exposure to nanomaterials via the environment (Larsen *et al.*, 2015) mainly focused on exposure to ultrafine particles (i.e. size <100 nm) in ambient air and did not address specific nanomaterials (due to lack of data). The authors conclude that ultrafine particles are very unstable in air (agglomerate fast) and therefore the concentrations decline rapidly with the distance from the sources (mainly ovens, traffic and other combustion sources). Carbon based nanoparticles are the most important while it is highly uncertain how much inorganic materials contribute.

4.2 Environmental exposure

With the exception of the special case of nZVI being injected directly into soil/groundwater, the two main environmental compartments being exposed to ENMs are soils (highest concentrations typically reached in those fertilized with STP sludge) and surface waters (exposed to STP effluents or direct discharges of e.g. untreated road runoff). While release to air is of significant importance for indoor environment and work-place exposure, the release of ENM to outdoor air is in all cases found to be insignificant.

The concentrations of the ENMs in soil estimated for 2014 (i.e. accumulated from 2000 to 2014) are generally at the sub-µg/kg or ng/kg level in agricultural, natural and even urban soils while for STP sludge treated soils much higher levels are estimated for a few materials, i.e. photostable and photocatalytic TiO_2 (approx. 1.3 and 0.17 mg/kg, respectively) and CeO_2 (1.5 µg/kg). Also for some other materials the concentrations in sludge treated soils are higher but not exceeding 1 µg/kg.

In effluents from STPs only one of the studied ENMs is estimated by modelling to reach a level above 1 mg/L, namely carbon black with 1.2 mg/L, while the second highest effluent concentration is found for photostable TiO_2 with 12.6 µg/L. Photo-catalytic TiO_2 and $CuCO_3$ are the only two other ENMs reaching modelled levels above 1 µg/L in STP effluents (1.6 and 1.3 µg/L, respectively). All other ENMs appear at ng/L level or even lower.

In surface waters after dilution none of the studied ENMs have been modelled to reach a concentration of even 1 µg/L (CB highest with 0.5 µg/L) and mostly the concentrations are at the low ng/L or even fg/L level.

A variety of chemical, physical and biological processes can affect the behaviour and fate of ENMs on their pathway from the point of release to the end destination in an environmental compartment. Some of the processes may transform the materials in a way that they no longer can be regarded nano-particles and thereby the basis of the exposure scenarios can be completely changed. Such, almost complete transformation has been demonstrated e.g. for nano-ZnO in STP processes.

For most of the studied ENMs (and ENMs in general) the processes of aggregation and agglomeration are considered the most important to understand and have data on when modelling environmental fate. Also other processes affecting the size of the original nanomaterial such as sedimentation and sorption to natural organic matter (NOM) or other surfaces/soil are important in the understanding the environmental fate of most ENMs. The interaction with particles naturally occurring in the environment is furthermore of great importance for the transport and fate of ENMs in the environment. This process is strongly linked to agglomeration of particles of different origin, a process referred to as hetero-agglomeration. An increase in particle size will generally result in increased agglomeration and sedimentation in water and a higher retention of ENMs in soil, independent of the chemical composition of the material. When functionalization of ENM occurs, whether it is through natural compounds or chemically intended stabilizers, the surface charge of that coating or stabilizer determines the interaction of the ENM with naturally occurring substances and particles. In this respect the core composition of the ENM is of less importance. Under environmentally more realistic conditions it is suggested that particle-to-particle agglomeration (homo-agglomeration) plays a minor role and that the interaction with soil colloids (hetero-agglomeration) will have the largest impact on the overall sorption behaviour of ENM. Thus soils with a higher fraction of natural colloids, e.g. soils with high content of clay, will generally show the highest retention of ENMs. Even given the complexity of environmentally relevant soil matrices it seems possible to describe a large part of retention and adsorption behaviour of ENM with currently available models.

Furthermore, for some ENMs (e.g., Ag, ZnO and CuO) dissolution and chemical speciation is of importance for their environmental fate. The process and kinetics of dissolution of ENMs in environmental media is not yet fully understood, but is expected to be inversely correlated to the size of the particles. In general, links between physico-chemical properties and environmental fate and behaviour of ENMs are not yet established. This hampers the development of chemical fate models like the ones that are currently in use in the safety assessment of chemicals (e.g. in the EUSES models). The models for prediction of the environmental fate and behaviour of ENMs depend on actual measured data on distribution and transformation in laboratory, pilot or field scale experiments. Since each ENM may show a unique fate and behaviour pattern, this dependency of experimental data constitutes a serious bottleneck for the development and implementation of generic models for ENM exposure assessment.

Sampling and analytical-chemical determination constitutes another approach to obtaining estimates of the environmental concentrations of ENMs. However, at present the analytical-chemical possibilities for obtaining reliable concentrations of ENMs in complex environmental samples are so limited that is not considered a feasible strategy to rely on environmental monitoring. For several of the ENMs included in this report the natural background levels or background levels originating from other uses of the materials in question (i.e., other forms than as nanomaterials) will make a quantification of the added amount of ENM impossible.

Examples of this is the natural occurrence of iron in soils versus the addition of nZVI, or the occurrence of larger TiO2 particles in wastewater due to its use as pigment in white paint versus the nano-TiO2 from sun-screens. As pointed out in this report, carbon black constitutes a potential problem due to the high loading predicted using conservative assumption in the modelling. However, it is a significant analytical-chemical challenge to monitor and identify the contribution of the nano-scale CB to the total organic carbon found in complex environmental samples.

It must be stated that the reliability of all predictive exposure and risk estimations directly depends on the data used to feed the predictive algorithms. This applies also to our comprehensive studies based on stochastic and probabilistic methods that aim at covering the whole possible spectrum of risk events. Especially for such a full spectrum approach, a more precise overview on the future use volumes and the life-cycle fate and environmental release of the ENMs when used in consumer products and industrial processes would significantly improve the exposure assessment.

The identifications of the most important processes for each ENM have been used in the modelling of environmental exposure as far as the data allowed it. To give an example, aggregation and agglomeration with subsequent sedimentation of engineered nanomaterial in surface waters was recognised in Sub-report 1 (Hartmann *et al.*, 2014) as having high importance for the environmental fate and exposure of the studied ENMs. This conclusion has fully been considered in our exposure model but due to lack of quantitative data our model had to include probability distributions for those factors that range from immediate and complete aggregation with subsequent sedimentation to theoretical scenarios of no aggregation/sedimentation. On the other hand, the full spectrum applied for aggregation etc. has allowed us to exclude that significant exposure concentration events were not captured by our model. The stochastic approach does therefore guarantee that based on the current knowledge all possible exposure/risk events have been considered, it can however not replace not available precision in the environmental fate data themselves.

4.3 Environmental effects

The result of the literature review on ecotoxicity (the studies considered adequate for regulatory use) showed effect concentrations in the range from about 1 µg/L to 900 µg/L for Ag and TiO_2, respectively. This data availability for each material was assessed and yielded PNEC values in the range from 0.012 µg/L to 18 µg/L for the ENMs used as case materials in this study. Hence, the PNEC values span more than three orders of magnitudes, depending on the ENM. This is not surprising as the materials are very different in chemical composition. It should be noted that it was not possible to identify any pattern in organism sensitivity across materials for the chronic studies included for PNEC estimations.

The metal-containing ENMs may be grouped with respect to the order of magnitude of the estimated PNEC values: For TiO_2 and CeO_2 the PNEC values were in the order of 10 µg/L, for ZnO around 2 µg/L, and for Ag and CuO at a level of a 30 ng/L. Furthermore, the PNEC estimated for CNTs was in the order of 1 µg/L. It is important to note that CNTs are truly novel materials that exist only in a nano form and for which no comparable larger (or smaller) size analogues exist. The PNEC values found in this study for nano-ZnO, nano-Ag and nano-CuO are lower than the PNEC values for the bulk forms of the materials in the REACH registrations. Given the relatively low AF of 50 used for the PNEC estimations in this report, it does not seem to be the AF that is causing these lower PNEC values for ENMs compared to other forms of the materials.

In comparison to PNEC values found in the open literature for the nano form a less clear picture emerges. For Ag and ZnO the PNEC values found in the literature span several orders of magnitude. For TiO_2, CNTs and CeO_2 the variation is much less. This reflects the availability of PNEC values found in the literature, where only few studies have published PNEC values for the last mentioned ENM but a range of studies have published PNEC values for the first mentioned ENM. Not surprisingly, for Ag and ZnO the PNEC values derived in this report fit well within the large interval published in the literature, but are in the lower end compared to literature values. For TiO_2 the PNEC values are within the same order of magnitude, but for CNTs and CeO_2 the PNEC values derived in this report are about 1-2 orders of magnitude lower than found in the literature.

While it is tempting to conclude that the lower PNEC values found for ENM in this report compared to REACH registration is due to nano-specific effects, this is not supported by the findings of the studies used to estimate the PNECs. Only in a few studies nano-specific effects were included and found to be more sensitive endpoints compared to traditional endpoints for ecotoxicity tests.

The current regulatory paradigm is that in principle the existing risk assessment methodologies are applicable to ENM. This means that, at present, no nano-specific arguments are included in the estimation methods for PNEC estimation, i.e. that it is possible to extrapolate from effect concentrations obtained in the laboratory to concentrations protective for the environment by dividing with an assessment factor. It is, however important to note that the validity of the fundamental assumption, i.e. that PNECs for ENMs can be estimated as though they were dissolved chemicals, has not been evaluated. Given the range of nano-specific concerns discussed by Lützhøft *et al.* (2015) it is at present not possible to claim that the use of the current approaches ensure that organisms will be protected at concentrations below the derived PNEC. In other words, specific circumstances related to ENMs, which differ from conventional chemicals, could likely affect the validity of the approach for deriving PNEC in an unpredictable manner (Baun *et al.*, 2009).

A number of transformation processes occurring during testing may furthermore influence the effective concentrations experienced by the test organisms These are processes such as dissolution and agglomeration that may change the properties (and concentrations) of the tested material (Hartmann *et al.* 2014). These processes depend among other things on the ionic strength of the testing medium, choice of medium constituents, time, presence of test organism (Hartmann *et al.*, 2014). While these processes are expected also to take place in the natural environment, they constitute a problem for the tests carried out as a part of a risk assessment as these tests are not designed for particles and the processes related to particles may hamper interpretation of test results and hence data reliability. It may be tempting to call for increasing the environmental realism of standard tests; however, this will most likely be at the expense of reproducibility and comparability violating the fundamental prerequisite of constant exposure during standard ecotoxicity testing.

Five of the ENMs included in this report may be grouped into two groups related to which of the transformation processes raises concerns with respect to the toxicity data used for PNEC estimation:

- For all ENMs aggregation and agglomeration is of very high importance, but especially for TiO_2 and CeO_2 it is in practice difficult to maintain these particles in stable suspension. Sedimentation of TiO_2 and CeO_2 ENMs are often reported, and there is a risk of biological endpoints being influenced by this behaviour. This may lead to physical effects on test organisms, e.g. entrapment of daphnids in larger agglomerates causing immobilization.

- For the Ag, ZnO, and CuO ENMs the possible dissolution in the test medium and release of ionic metal is often used to explain the observed toxicity. The dissolved metal ion is most often found to be more toxic than the corresponding ENM, though exceptions have been reported. It is not trivial to quantify this dissolution under actual test conditions, since the dissolution depends on ENM properties like size, surface area and coating, as well as on media composition (e.g. ionic strength and pH). Furthermore, the dissolution is time-dependent and thus ongoing from preparation of stock suspensions and throughout the test duration.

For the remaining four materials (CNT, nZVI, QD, CB) the evaluation of transformation processes must rely on case-by-case evaluation.

Furthermore, the dose metric applied represents a challenge to the current procedures for PNEC estimation. As mentioned in the Best Practice for REACH nano registrants (ECHA, 2014), a single measure of mass is not adequate for quantifying the exposure of ENMs. Particle number or specific surface area have been suggested as more appropriate dose-metrics (van Hoecke et al., 2008; Arvidsson et al., 2011)), but the number of studies that have applied these metrics is so far much too few to draw any conclusions on whether these novel metrics represent better alternatives to expressing the effective concentrations compared to the traditional mass-based concentrations.

On a final note, it must therefore be emphasized that PNEC estimations given throughout this report are based on the assumptions that 1) the current test methods are applicable to nanomaterials, and 2) that the current extrapolation methodologies are valid for nanomaterials. Both of these assumptions are questionable (for a further discussion of this see Lützhøft *et al.*, 2015). The PNEC values given in this report should therefore be taken as indicative for the order of magnitude for PNEC given the current regulatory recommendations for PNEC estimation.

4.4 Risk assessment of the ENMs

Environmental risk quotients in the form of PEC/PNEC values have been determined for the aquatic environment for seven of the studied ENMs. Due to lack of reliable ecotoxicity data it was not possible to determine a PNEC value for Quantum Dots (QD) and carbon black (CB) while for nZVI such a calculation was found to be meaningless because of the high reactivity, and thereby transformation, of this nanomaterial under the conditions of use.

The PEC/PNEC values for the average concentrations in surface waters (based on most probable PECs) are all considerably below the critical value of 1, actually they range from about 1/1000 hereof and downwards (with the exception of nano-CuO only being 20 times lower). The PEC/PNECs for STP effluents are all well below 1, with the exception of photostable nano-TiO_2 that is only slightly below 1 and nano-CuO that is somewhat higher.

Thus, for nano-CuO a 3.8 times reduction of the most probable STP effluent value must be obtained in the receiving water to give a risk quotient lower than 1. The PEC/PNECs for STP effluents calculated for the max. concentration within the 95% confidence interval show that in the worst case three of the ENMs (photostable TiO2, Ag, CuO) could require between 5 and 12 times reductions in the risk quotients to achieve a value below 1 (factor 5 required for both photostable TiO_2 and Ag, and 12 for CuO).

Possibly, also CB could require a small reduction of the PEC/PNEC for STP effluents to meet the acceptability criterion, however this is based on a very conservative assumption on the release of CB (and fraction occurring on nano form) and, further, the reduction factor cannot be quantified due to the lack of effect data for PNEC estimation. Since none of the ENMs are expected to undergo biological degradation, it is important to emphasize that dilution in receiving surface waters bringing down the risk quotient to an immediately acceptable level is not sufficient. The total load to the environment has to be taken into account and the risk of (bio)accumulation of ENMs must be evaluated further. The possible transfer of ENMs from the water phase to sediments through processes of aggregation/agglomeration and sedimentation calls for special attention.

It should, however, also be mentioned that in general the basis for calculating the exposure (the PECs) has been conservative. Due to lack of quantitative information on central fate processes such as aggregation/agglomeration etc., it has been assumed that all the nanomaterial released would be present in the environmental compartments on the nano form, i.e. no transformation into other, non-nano forms. This has presumably lead to an overestimation of the exposure, which in some cases could be significant.

For the soil environment, and for sediments, it has not been possible to derive risk quotients for any of the ENMs studied. This is partly due to lack of experimental data on effects on soil/sediment-dwelling organisms and partly because extrapolation from aquatic data is not considered possible for ENMs at the current level of knowledge about the environmental fate processes that need to be taken into account. It is, however, noted that the (conservatively) estimated concentrations of the studied ENMs in soils are generally very low, below 1 µg/kg soil. This with the exception of TiO_2 in soils treated with STP sludge where the modelled concentrations (most probable values) reach 1.3 mg/kg soil and 0.17 mg/kg soil for the photostable and photocatalytic form, respectively, and CeO2 exceeds 1 µg/kg soil. As mentioned above for ENM in sediments, the potential bioaccumulation of ENM in soil-living organisms is also an area that needs to be further investigated and for this purpose the estimated and forecasted concentrations in the report may serve as a starting point for defining environmentally realistic exposure conditions.

In summary, based on the existing information about the properties of the studied ENMs and their current use volumes and applications, only the most probable value-based risk quotient for nano-CuO in undiluted STP effluent exceeded the acceptability criterion of 1 while the risk quotient based on the max. concentration exceeded 1 also for photostable TiO2 and nano-Ag. Soils and sediments are for many of the ENMs the dominant environmental sinks but, with the exception of a few ENMs in sludge-treated soils, the calculated concentrations are very low.

4.5 Broader considerations regarding environmental risks

The preceding sections of the discussion have focused on the specific elements of the NanoDEN project that have provided the basis for the assessment of environmental risks related to the use and occurrence of the selected ENMs. These specific assessments provide an important contribution to the perspective regarding the environmental implications of ENM use as such, with emphasis on the situation in Denmark. The following discussion intends to address other relevant questions in relation to assessing the general environmental implications of the use of ENM.

Were the right ENMs selected for the study?
One of the activities in the start-up phase of the project was to identify possible candidates and among these select 10 engineered nanomaterials (ENMs) for closer examination and assessment. The selection process was risk based and considered potential toxicity as well as expected (relevant) exposure (tonnage and emission) of the environment. Furthermore, only ENMs applied in various consumer and/or industrial products and ENMs under current scientific evaluation/discussion regarding human and environmental exposure and effects were considered.

The result hereof was that the 10 selected ENMs represented partly materials currently being used in some of the highest tonnages, partly materials that together had a wide range of applications, partly materials of very different character (metals, other inorganics, carbon-based ENMs) and partly materials with a significant span regarding toxicity and behaviour in the environment.

Hence, the selection made is considered to be representative considering the purpose of the NanoDEN project and has provided a good basis for the subsequent risk assessment and considerations on possible environmental concerns. Among the high tonnage ENMs, only silica (SiO_2) was omitted while among the metal-based ENMs e.g. those based on cobalt and nickel were left out and also the carbon-based fullerenes were de-selected.

Did the study reveal any especially critical ENMs?
Based on the calculated PNEC values and risk quotients (PEC/PNEC) for the aquatic environment none of the 10 selected nanomaterials appear to be critical at the present level of use and release. A few of the materials, CuCO3/CuO and to some extent TiO2, Ag and CB, revealed risk quotients at the discharge point for STP effluents above 1 (with a max. of 12) and would, thus, in principle require some risk management to implemented. If no other management measures are implemented the discharge of these ENMs would require some initial dilution (up to 12 times) in receiving waters to achieve an acceptably low risk level. This is within the same order of magnitude as for a number of anthropogenic chemical substances. Increasing consumption, and thereby release, and/or new application areas could change the picture, however, the PEC part of the calculations were conservative in the sense that possible transformation into non-nano forms after release to the environment was not taken into account.

A few of the materials, especially nano-Ag and nano-Cu (and maybe a few other metal-based ENMs), may be relevant to consider with regard to possible bioaccumulation.

With regard to (aquatic) toxicity, comparison with the corresponding chemicals is possible is possible for a few of the ENMs (TiO2, ZnO, Ag and CuO). The general picture seems to be that the toxicity of the nanomaterial is higher than the bulk form of the corresponding chemical but lower than or equalling the toxicity of ionic form. However, it is important to note that the metals Ag and Cu are well-known environmental toxicants independent of the form in which they are used and emitted to the environment, and therefore increases or changes in the use of these metals should be followed closely.

Were particularly critical uses and sources/releases identified?
The 10 selected ENMs represent a wide range of uses and thereby potentially also many release pathways. A detailed study of these many uses has not been possible within the framework of this study but as no basic production and apparently only little manufacturing of nano-products and materials take place in Denmark, the main environmental releases are considered to be due to the consumption and use of nano-containing products and materials.

Sewage treatment plants (STPs) appear as a general rule to be capable of retaining (and possibly transforming) the major part of nanomaterials that are released into the public sewage systems (e.g. ENMs used in various cosmetics or hygienic articles), thus reducing the exposure of the aquatic environment very significantly compared to direct discharges. Likewise, waste incineration plants (WIPs) also normally retain most of the materials in the solid waste products. However, some STP sludge is applied onto cultivated land and a quite large fraction of WIP residuals is used for construction works (e.g. as road base) and therefore these retained ENM fractions could still give rise to environmental – if they are still on nano form after passing the STP or WIP processes (which is probably questionable).

The critical uses are in principle all those leading to direct release to and exposure of one or more environmental compartments, i.e. without passing through e.g. STP or WIP treatment processes, but of course the actual risk will also depend on the use amount/volume and on the toxicity of the ENM in question. Examples of such critical uses could be outdoor paints and other coatings for building facades or other constructions, impregnation of wood for outdoor constructions (on land or in water), anti-fouling paints for ships, fillers for tyres, and artificial lawns (rubber filler made from used tyres). The potential for direct release to the environment may result in locally high exposure concentrations, which for some of the most toxic ENMs (like Ag and CuO/CuCO3) may imply risk levels that are not acceptable.

Are there any special Danish conditions?
There seems to be very little production/manufacturing of ENMs in Denmark and this significantly reduces the part of the total environmental exposure originating from point sources compared to some other countries with higher degree of industrial production, while the part originating from diffuse sources (general use) is probably the same.

In Denmark the degree of re-cycling of waste materials from waste incineration plants for outdoor construction works (e.g. roads) is significant, and higher than in many other European countries. This implies potentially a higher risk of environmental exposure than in many other countries (in particular of soil and groundwater) and, hence, these material cycles could deserve a closer examination in a future study.

5. Uncertainties and data gaps

In this chapter the main uncertainties and data gaps encountered in the different NanoDEN sub-projects are summarized and discussed in thematic sections on environmental fate, environmental exposure and environmental effects, respectively. Specific data gaps/uncertainties relating to the individual ENMs are described in detail in the previously published sub-project reports (Hartmann *et al.*, 2014; Gottschalk *et al.*, 2015a; Lützhøft *et al.*, 2015).

5.1 Environmental fate and exposure modelling

A number of gaps have been identified in the current knowledge on transformation processes for ENMs in environmental matrices. The identified gaps fall into four categories: Environmental fate processes, characterisation and measuring methods, availability of data and information, and issues related to environmental exposure modelling.

Environmental fate processes. Once in the environment, the fate and behaviour of ENMs will be determined by the intrinsic properties of the ENM, the transformation processes and the specific environmental conditions. By comparing the identified knowledge gaps related to specific environmental transformation processes with the relative importance of these processes for environmental fate modelling of ENMs the following prioritisation can be made:

- Aggregation/Agglomeration: Understanding and describing the kinetic changes in aggregation/agglomeration including rate of growth. Possible adaptation of the DLVO theory

- Sedimentation : Understanding the role of chemical compositions, sizes, and shapes on ENM sedimentation in surface waters

- NOM adsorption : Investigation of parameters influencing the stabilising (or de-stabilising) effects of NOM on ENM stability

- Sorption to other surfaces: Determining the role and extent of sorption as a distribution process for ENMs in solid-water matrices

- Dissolution: Development of models to describe dissolution process. Develop appropriate tests to measure dissolution kinetics.

Characterisation and measuring methods. This covers gaps related to the distinct limitations of methods to characterise and measure concentrations of ENMs. Currently, the advancement of the understanding of ENM fate and behaviour is hampered or limited by the lack of appropriate ENM characterization, and measuring methods and methods for measuring dissolution, redox potential, sorption as well as aggregation in environmentally realistic samples are needed.

Availability of data and information. Common for all mass-based models and frameworks is that they need a basic set of experimental/empirical data and information in order to be useful for environmental exposure estimation. This includes data on quantities of ENM produced, specific uses and use concentrations as well as data quantifying release during production, transport, use and disposal. Currently the access to such information and data is very limited and little known about the routes of entry for ENMs to the environment.

Environmental exposure modelling. The reliability and relevance of all mass-based models and frameworks are dependent on the availability and quality of the input data. Common for all ENMs is the need for knowledge on real Danish ENM use quantities as well as a better understanding for the fate and behaviour of engineered nanomaterials in all environmental compartments. The input data for the models are based on the newest and most recent knowledge on use quantities and applications for Denmark, or when no data specific to Denmark were available, data and knowledge of nanomaterial use gathered in the general European (or Swiss) context, adapted to Danish conditions, have been used. However, due to the lack of empirical Danish data and gaps in the knowledge on Danish use quantities and fate and behaviour of the ENMs in the different compartments, the resulting PEC values in this study are associated with very wide 95% probability ranges. It is assessed that such empirical Danish data would be, by far, the most important factor for improving the environmental exposure model.

The exposure estimation model applied in the current project is in principle able also to estimate local exposure concentrations. However, within the framework of the NanoDEN project it was not possible to develop an adequate local Danish model e.g. for river sections and coastal waters. Such scenarios would have to include local ENM release knowledge that is almost not available. A Swiss study by Gottschalk *et al.* (2011), where local scenarios for rivers were presented, could benefit from an already available hydrological model on water level geography and sewage treatment plant performance dynamics that is not possible to link directly to Danish ENM release situations. Despite this, it is still our opinion that in the Danish model based on the same approach for regional predictions, the highest local exposure in fresh surface waters should be covered by the model through the results of non-diluted water as we find it in sewage treatment effluents.

Another factor, which affects the modelling results and add to the uncertainties regarding the predicted environmental concentrations, is the lack of information on the fate and behaviour of the ENMs in the different compartments. The results from sub-project 1 under NanoDEN (Hartmann *et al.*, 2014) revealed that the current database for incorporating, for example, photochemical degradation, oxidation, reduction, adsorption, desorption and biotransformation in detail into the generic mass transfer model is not satisfactory. Also knowledge about sedimentation is crucial for estimating the residence time of the nanomaterials in water, including sewage, as well as their subsequent discharge into sediments and sewage treatment sludge. The results from Hartmann *et al.* (2014) confirmed that currently accurate scientific information is missing, and therefore a large spectrum of materials have been modelled as having either immediate or almost no sedimentation (water-sediment transport).

It is important to mention that for all of the predicted environmental exposure concentrations it is still unknown whether the materials will be on nano form after being incorporated into soil and sediments. Common to all the case-studies, the modelling stops at the point where the materials reaches these compartments, assuming no ENM transformation, since data for ENM fate and behaviour here is lacking. The same is the case for landfills and recycling plants where the fate of the ENM in these processes were not studied, also due to lack of data.

As mentioned in chapter 4, even only more precise and robust knowledge on the nanomaterial production quantities in a particular region would significantly help improving this kind of predictive modelling. In the absence of these, we have aimed at filling most data gaps by considering very large assumptive data ranges that should enable capturing as far as possible the whole possible model data spectrum, which is essential to ensure that significant risk scenarios are not missed in the analysis.

5.2 Ecotoxicity and determination of level of protection

From the literature and the gap analysis carried out for each ENM in this study (as reflected in the discussion about environmental effects in section 4.3), a number of general gaps with respect to establishing PNEC values for nanomaterials have been identified:

- *Limited number of studies at different trophic levels*: It is generally found that the diversity of organisms tested is very low with respect to the trophic level, resulting in use of the AF approach alone for PNEC estimation.

- *Lack of studies from different environmental compartments*: For all of the ENMs studied there is a pronounced lack of studies from other environmental compartments than freshwater. This includes studies on marine organisms, as well as soil- and sediment-living organisms. For conventional chemicals there is an option of extrapolating from freshwater tests to other compartments by the use of partitioning coefficients. However, interpolation between compartments is not possible for ENMs. Thus, this data gap can only be closed by additional testing in the compartments at question.

- *Most studies focus on acute toxicity*: It was revealed from literature that the vast majority of ecotoxicity data are short-term tests. For the ENMs for which it was possible to establish a PNEC value, results from chronic tests were typically available from two studies at two different trophic levels, i.e. algae and daphnia, resulting in the use of 50 as the AF. To obtain less uncertainty in the determination of PNEC more studies focused at chronic endpoints are needed.

- *Testing of high ENM concentrations*: Often high and environmentally unrealistic concentrations are used in standardized ecotoxicity tests influencing the ENM behaviour and bioavailability. This may lead to either false-negative or false-positive results, which may influence the validity of the PNEC estimation.

Regarding the estimation of PNEC values for nanomaterials the major gap is the lack of underlying proper data suited for risk assessment. There is a general lack of reliable data, in the sense that despite a wide range of tests have been performed according to accepted international guidelines (or modification thereof), they cannot be fully trusted to yield accurate and conservative estimates of the toxicity of an ENM. This is by far due to varying exposure conditions during the ecotoxicological testing, which is a violation of the underlying assumption of constant exposure concentration, leading to constant organism concentrations and further to constant target location/organ concentrations.

Another factor, common for most of the ENMs, which influenced the adequacy of the studies already performed for regulatory risk assessment, was the general lack of material characterisation, especially measurements of the dose metric during and at the end of the test, but in fact also for basic data on inherent properties, e.g. for material identification and characterisation.

Finally, it should be emphasized that the PNEC estimations given throughout this report are based on the assumptions that 1) the current test methods are applicable to nanomaterials, and 2) that the current extrapolation methodologies are valid for nanomaterials. Both of these assumptions are questionable (for a further discussion of this see Lützhøft *et al.*, 2015). When ENMs are tested in standard ecotoxicity tests the validity criteria of monotonous concentration-response curves and stable exposure conditions are challenged due to the behaviour of ENMs in testing media and in different test concentrations. The links between transformed states of the ENMs (e.g. dissolved or agglomerate forms) and biological effects are at present unknown. However, it is known that transformation during testing influence the test results. For PNEC estimation by application of an AF this constitutes a major problem for the validity of the extrapolation from standardized tests, since environmental effects may occur at lower concentrations than those used in the standardized tests. Thus, it is questionable whether the PNEC established by application of an AF will indeed be protective for organisms in the environment.

5.3 Other environmental hazards related to ENMs

As described by Hansen et al. (2013) a range of "warning signs" for ENMs should be considered besides the possible toxicity, which is already included in the estimation of PNEC values in this report. These warning signs are not specific for ENMs and were in fact proposed by the European Environment Agency already in 2001 as an aid to identify new, potentially problematic substances (EEA, 2001). Related to environmental hazards of ENMs also the topic of novelty, bioaccumulation, and persistency may play an important role.

Novelty. The assessment carried out in this report is mainly based on traditional biological endpoints for toxicity such as mortality, growth rate inhibition and reduced reproduction. The novel properties of ENMs may result in novel effects that are not addressed when using tests developed for dissolved "ordinary" chemicals. In the few cases where nano-specific effects were reported for ENMs they were considered in the PNEC estimations, but did not result in lower PNEC values.

Bioaccumulation. Bioaccumulation studies are needed for ENMs. Though bioaccumulation is not a part of PEC/PNEC estimation, it is a crucial parameter for ERA used for classification, labelling and PBT assessment. In the current guidance documents, criteria for assessing bioaccumulation are given as well as estimation methods if data is lacking, however based on processes relevant for dissolved organic chemicals (mainly molecular diffusion as the main passive uptake route). These processes are not relevant for particles and therefore only actual measured values taking uptake and depuration into account will be valid for ENM. Such studies are presently rare (Skjolding *et al.*, 2014a and 2014b).

Persistency. Another essential part of a chemical safety assessment is the question of degradability or persistency in the environment. For this the current guidance documents provide test recommendations and cut-off values to classify the degradability. However, as discussed in detail by Hansen et al. (2013) these are not relevant for most ENMs since many are inorganic compounds. As stated by Hansen et al. (2013) this could lead to a classification of all inorganic ENMs as persistent, but the definition of persistency in the guidance documents do also include "chemical transformations". As discussed in this report, ENMs are likely to undergo a range of transformation processes depending on the redox conditions and ionic strength of the environmental media. The transformed ENMs may or may not be nanoscale. Thus, Hansen et al. (2013) proposed that only for non-reactive inorganic nanomaterials the term "persistent" should be considered. It should be noted that for trace organic contaminants the term "pseudopersistency" has been introduced to describe the occurrence of readily biodegradable compounds that, because of a continuous (low) discharge, remain in the environment (Diamond et al., 2010). This may be the situation for some of the ENMs included in the present study as reflected in the results of the exposure (PEC) modelling.

Conclusions and perspectives

The NanoDEN project, of which this report constitutes the result of the final sub-project, has partly provided state-of-the-art reviews of the current knowledge on engineered nanomaterials (ENMs) in the environment and partly generated new data relating to environmental exposure and effects as reflected in the following previously published sub-project reports:

- Environmental fate and behaviour of nanomaterials – a critical review of new knowledge on important transformation processes (Hartmann *et al.*, 2014)

- Modelling releases to and exposure of selected nanomaterials in the Danish environment (Gottschalk *et al.*, 2015a) with two comprehensive separate annex reports on sources of selected ENMs in Denmark (Lassen *et al.*, 2015) and detailed exposure modelling results (Gottschalk *et al.*, 2015b), respectively.

- Environmental effects of selected nanomaterials with emphasis on derivation of PNEC values (Lützhøft *et al.*, 2015)

The current, final sub-project under NanoDEN has aimed partly to specifically perform an environmental risk assessment for the ENMs selected for this study (based on the results of the previous sub-projects), and partly to address the overarching question behind the NanoDEN project as such: is there reason for concern with regard to nanomaterials in the Danish environment?

We have chosen to address the latter question by posing, and trying to answer, the following more concrete questions:

- *Do some individual ENMs, or groups of ENMs, constitute an environmental risk (now or in the future, how far are we from critical levels)?*

A few ENMs are already used in quite large quantities (e.g. carbon black and TiO_2) and could for that reason potentially constitute a risk, despite not being very hazardous, if/when their use volumes increase further. On the other side nano-Ag is the most toxic of all the ENMs studied but is not used or released in very big quantities. With the current use patterns and use volumes none of the selected ENMs appear to be very critical from an environmental point of view and in general the levels are quite far from concern. TiO_2 and CuO do, however, in some cases come relatively close to levels of concern, i.e. a significant increase in the use of these ENMs could increase the risk to levels where mitigating measures could be considered.

With regard to general environmental concern, the ENMs can be placed in some rough "risk categories" in a way similar to what could be done for traditional chemicals.

1. *Critical ENMs: Those with high PEC* ***as well as*** *low PNEC:*
   At present there appears to be none with that combination in Denmark (neither among the studied ENMs nor others that the project team is aware of)
2. *Potentially critical ENMs (if/when PEC increases significantly):*

2a. Highly toxic ENMs (low PNEC):
Examples: nano-Ag, nano-CuO and maybe CNT

2b. High release volume ENMS (high PEC):
Examples: nano-CB and (photostable) nano-TiO_2

3. *ENMs unlikely to be critical:*
Examples: nano-ZnO, nZVI (because the exposure is so local), nano-CeO2 and QD

It should be emphasized that the assessment above only covers the nano form, not possible bulk/ionic forms of the same material. Further, the assessment does not include possible risk of local effects due to specific industrial point sources (presently considered to be of very limited relevance in Denmark). Risk quotients (PEC/PNEC values) for the selected ENMs calculated to be higher 1 in STP effluents could indicate a potential concern in case of (significantly) increasing use and release of these nanomaterials. Nano-CuO is the case with the highest risk quotient, it should, however, be kept in mind that the case is theoretical as currently the use of CuO (or $CuCO_3$) as a biocide is not based on the nano form.

For many of the ENMs soils and sediments will be the dominant environmental sinks but it has not been possible to derive risk quotients for any of the ENMs studied in soil or in sediment. This is partly due to lack of experimental data on effects on soil/sediment-dwelling organisms and partly because extrapolation from aquatic data is not considered possible for ENMs at the current level of knowledge. However, with the exception of a few ENMs in sludge-treated soils, the (conservatively) calculated concentrations are generally very low, below 1 µg/kg soil. For soils and sediments, the potential bioaccumulation of ENM in organisms is an area that needs to be further investigated and for this purpose the estimated and forecasted concentrations in the report may serve as a starting point for defining environmentally realistic exposure conditions.

- *Are ENMs, as a group, covered sufficiently by the ENMs studied in this project?*

The ENMs selected for this project represent partly materials which are currently used in some of the highest tonnages, partly materials that together have a wide range of applications, partly materials of very different character (metals, other inorganics, carbon-based ENMs) and partly materials with a significant span regarding toxicity and behaviour in the environment.

Hence, we believe that the selection made is representative considering the purpose of the NanoDEN project. Among the high tonnage ENMs, only silica (SiO_2) was omitted while among the metal-based ENMs e.g. those based on cobalt and nickel were left out and also the carbon-based fullerenes were de-selected. These delimitations are not considered to compromise the possibility of discussing ENMs from an overall risk perspective in this report.

- *Are certain functionalities or types of uses of ENMs more critical than others from an environmental perspective?*

The critical functionalities and use types are in principle all those leading to direct release to and exposure of one or more environmental compartments, i.e. without passing through e.g. STPs or waste treatment processes, but of course the actual risk will also depend on the use amount/volume and on the toxicity of the ENM in question. Such direct releases are estimated to be dominant for more than half of the studied ENMs. Examples of such critical uses could be outdoor paints and other coatings for building facades or other constructions, impregnation of wood for outdoor constructions (on land or in water), anti-fouling paints for ships, fillers for tyres, and artificial lawns (rubber filler made from used tyres). Some of these uses lead to aquatic exposure due to discharges of untreated runoff from roofs and paved surfaces (e.g. CeO_2 and CB) while others affect directly the soil environment (such as use of CuO for wood impregnation) or even the marine environment (ENMs in antifouling paints (no current examples)).

- *Does the Danish situation with regard to ENMs deviate from the general situation in the EU?*

In Denmark there is no basic production of nanomaterials and presumably quite limited manufacturing of nano-containing materials and products. Thereby industrial point sources are probably of less importance in Denmark than in other countries in the EU with more industrial production.

The degree of re-cycling of waste materials from waste incineration plants for outdoor construction works (e.g. roads) is high in Denmark, and higher than in many other European countries. Such "open" types of re-cycling could imply a potential risk of higher total environmental exposure, in particular of (subsurface) soil and groundwater, for some ENMs.

- *Significance of natural NMs compared to the corresponding ENMs*

No quantifications appear to exist for the relevant ENMs that relate the release of the engineered nanomaterials (ENM) to the amounts of the same nanomaterials already occurring in nature such as e.g. organic carbon based nanoparticles and some inorganic oxides. It is known that the ultrafine particles produced and released as a result of (natural) fires and/or combustion of organic material and fossil fuels comprise some of the nanomaterials that also occur as "engineered", i.e. CNT, fullerenes and carbon black. Possibly, also some inorganic nanoparticles are produced from e.g. volcanic eruptions or by wind erosion of mineral surfaces (rock, soil). It is of course debatable whether anthropogenic combustion of wood, coal etc., or exhaust from vehicles, should be considered a "natural" source or be assessed along with the engineered nanoparticles.

In a discussion of natural sources in relation to possible impacts on the environment it should be borne in mind that living organisms have for millions of years been exposed to the mentioned natural nanoparticles and have therefore been able to adapt to these in the process of evolution. Therefore, natural NPs appear to be of limited relevance to discuss in the context of environmental risk of ENMs.

- *What more do we need to know (data gaps)?*

The identified main data gaps and uncertainties within a number of themes considered central for environmental assessment of ENMs are described in chapter 5. If considering this issue from a regulatory perspective the most important gaps to be addressed are those that limit the possibilities of introducing environmental regulation of ENMs, should such regulation be deemed necessary. Among the data gaps mentioned in chapter 5 we consider the following to be the most important:

- Data that can define the actual environmental exposure to ENMs more precisely than now, e.g.:
  - more specific (Danish) data on the sources and releases from industrial facilities as well as resulting from the use of nano-products by consumers;
  - data clarifying to what extent and for how long ENMs released to the environment exist as nanoparticles in the environment before they are either dissolved or no longer are on nano form due to aggregation/agglomeration;
  - Better data on retainment of ENMs in STPs and waste incineration plants.

- Data that can verify the PECs predicted by modelling i.e. analytically measured data (which will require better analytical methods for ENMs to be developed);

- Data that can lead to more precise and reliable PNECs than today, i.e. more data (especially chronic) on individual ENMs as well as testing methods that are more suitable to ENMs than the current.

- Data that can validate that the use of current methods for deriving PNEC will lead to protective concentrations with regards to environmental effects of ENMs

- Ecotoxicity data for organisms from other compartments than freshwater (e.g. soil, marine, and sediment-living organisms) since interpolation between environmental compartments is presently not feasible.

And, finally, the fundamental question behind the NanoDEN project:

- *Is there reason for concern with regard to nanomaterials in the Danish environment?*

Similar to what would be the case if the same question was asked for chemical substances, there is not one single answer that can cover the full width of nanomaterials, which are as diverse in properties and in uses as traditional chemicals.

On the other hand, as also indicated previously, the ENMs selected for assessment in the project are considered to be fairly representative of the ENMs that are commercially available in Denmark today both with regard to their properties and actual uses. Based on the review and assessments of exposure and effects of these ENMs in this project, a significant environmental concern regarding nanomaterials in the Danish environment seems hard to justify with the present level of knowledge and the current level and character of use of these materials. It should be noted that sufficient data have only been available for an assessment for the freshwater environment.

As described earlier, there are certainly many data gaps and uncertainties but, on the other hand, our exposure assessment has been rather conservative e.g. by assuming that the nanomaterials will remain on nano-form in the final environmental compartments where even the max. concentrations in the 95% confidence interval do not lead to alarming risk quotients (although exceeding 1 for a few materials).

On the effects side, generally only few adequate effect studies are available (except for 1-2 ENMs) but it is uncertain whether more data would lead to higher or lower PNEC values (more sensitive endpoints could be found but more data could also lead to lower assessment factors). If the PNECs were a factor of 10 lower, the risk quotients for a few ENMs would be so high that it would call for regulatory action. On the other hand the ENMs in question are among the most well-studied i.e. the likelihood of obtaining a 10 times lower PNEC is considered rather low. Effects of possible accumulation in sediments and soils of a few metal-based ENMs need to be considered further.

In conclusion, the current project has, based on data relevant for the freshwater environment, not identified significant concerns related to nanomaterials in the Danish environment. However, a few ENMs should probably be kept under observation. This either because the current releases have been modelled to be quite high (TiO2, CB) or because these ENMs have release patterns and hazard properties placing them in a "possible future concern" category (Ag, Cu). In Denmark, most of the other ENMs are used only for very specific low-volume purposes and/or uses with practically no environmental release and are therefore regarded to be of "low concern" with regard to the Danish environment at the current level of use.

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Appendix 1: List of Abbreviations and Acronyms

Abbreviation/Acronym	Explanation
AF	Assessment factor
Ag	Silver
Ag_2S	Silver sulphide
CB	Carbon black
Cd	Cadmium
CeO2	Cerium dioxide
CNT	Carbon nanotube
$CuCO_3$	Copper carbonate
CuO	Copper oxide
DEPA	Danish Environmental Protection Agency
DLVO	Derjaguin, Landau, Verwey and Overbeek theory (theory on colloidal stability)
EC_{10}	The concentration where an effect occurs in 10% of the organisms in a population
EC_{50}	The concentration where an effect occurs in 50% of the organisms in a population
ENM	Engineered nanomaterial
EPA	Environmental Protection Agency
ERA	Environmental risk assessment
ERC	Environmental release categories
EUSES	European Union System for the Evaluation of Substances
Fg	Femto gram (1 fg = 0,000000000001 mg)
IC_{50}	The concentration where inhibition occurs in10% of the organisms in a population (typically algae or microorganisms)
LC_{50}	The concentration where mortality occurs in 50% of the organisms in a population
LED	Light Emitting Device
LOEC	Lowest Observed Effect Concentration
MC	Monte Carlo
MFA	Material flow analysis
MS	Mass spectrometry
µg	Micro gram (1 µg = 0,001 mg)
NanoDEN	NANOmaterials – occurrence and effects in the Danish ENvironment (this project)

Abbreviation/Acronym	**Explanation**
ng	Nano gram (1ng = 0.000001 mg)
NM	Nano material
Nm	Nano meter
NOEC	No Observed Effect Concentration
NOM	Natural Organic Matter
NP	Nano particle
nZVI	Nano(sized) Zero-valent iron
PEC	Predicted Environmental Concentration
PEG	Polyethylene glycol
pg	Pico gram (1 pg = 0.000000001 mg)
PMC	Production, Manufacturing and Consumption
PNEC	Predicted No-Effect Concentration
PVP	polyvinylpyrrolidone
QD	Quantum dot
RA	Risk assessment
REACH	Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (EU's chemical legislation framework)
RQ	Risk Quotient
S	Sulphur / sulphide
Se	Selenium
STP	Sewage treatment plants
TiO_2	Titanium dioxide
UV	Ultra Violet (irradiation)
WIP	Waste incineration plant
Zn	Zinc
ZnO	Zinc oxide
ZVI	Zero-valent iron

Environmental assessment of nanomaterial use in Denmark
This is the concluding report of the project "Nanomaterials – occurrence and effects in the Danish environment" (abbreviated NanoDEN), which part the Danish Government's initiative "Better Control of Nanomaterials" ("Bedre styr på nanomaterialer") which is administered by the Danish Environmental Protection Agency. The projects in NanoDEN have aimed to investigate and generate new environmentally relevant knowledge on of nanomaterials on the Danish market and to assess the possible associated risks to the environment. The results from the sub-projects are summarized in the current report and it is assessed whether and how nanomaterials may pose a risk for the environment in Denmark.

The assessment is based on investigations of nine selected nanomaterials, which are expected to be environmentally relevant based on knowledge of consumption quantities or how they are used. These data contribute to an overall assessment of nanomaterials risk to the environment in Denmark.

The nine investigated nanomaterials are: Titanium Dioxide, Zinc Oxide, Silver, Carbon Nanotubes, Copper Oxide, Zero Valent Iron, Cerium Dioxide, Quantum Dots and Carbon Black.

Dette er den afsluttende rapport i projektet "Nanomaterialer – forekomst og effekter i det danske miljø" (forkortet NanoDEN), som udgør en del af Miljøstyrelsens udmøntning af regeringens finanslovsaftale "Bedre styr på nanomaterialer". Projekterne i NanoDEN har haft som hovedformål, at undersøge og generere miljørelevant viden om industrielt fremstillede nanomaterialer på det danske marked. Resultaterne fra NanoDENs delprojekter sammenfattes i nærværende slutrapport som vurderer om og hvordan nanomaterialer kan udgøre en risiko for miljøet i Danmark.

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