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# Environmental fate and behaviour of nanomaterials

## New knowledge on important transformation processes

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Environmental fate and behaviour of nanomaterials

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# Preface

This summary and assessment report on new knowledge concerning the processes governing environmental fate and behaviour of engineered nanomaterials (ENMs) is the first report from the project “Nanomaterials – Occurrence and effects in the Danish Environment” (“NanoDEN”). NanoDEN was commissioned by the Danish EPA in December 2012 and runs until mid-2015 and is one among a number of projects funded by the Danish EPA on nanomaterials aiming to increase the knowledge and understanding regarding occurrence of ENMs in Denmark and the 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 on consumers and the environment.

The NanoDEN project is carried out by a project team with participation of COWI A/S (lead partner) (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).

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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 (project manager)
- Anders Baun, DTU Environment
- Fadri Gottschalk/Bernd Nowack, SNMC.

# Dansk resumé

### Baggrund og formål

Under aftalen "Bedre styr på nanomaterialer og deres sikkerhed" har Miljøstyrelsen bestilt en række projekter med det formål at undersøge og generere ny viden om forekomsten af nanomaterialer i produkter på det danske marked, og vurdere potentielle risici for forbrugerne og miljøet. NanoDEN projektet, hvor denne rapport udgør den første fase, har som overordnet formål at vurdere, hvorvidt industrielt fremstillede nanomaterialer ('engineered nanomaterials') udgør en risiko for det danske miljø.

Denne rapport har til formål at give et overblik over den eksisterende viden om relevante processer for nanomaterialers skæbne og opførsel i miljøet. De væsentligste fordelings- og omdannelsesprocesser vil blive identificeret, diskuteret og prioriteret i henhold til deres relevans som input til modellering af nanomaterialers skæbne i miljøet. Desuden vil manglende viden blive identificeret og prioriteret med hensyn til relevans i forhold til at forudsige miljømæssige koncentrationer af nanomaterialer. Ud over at være et separat projekt vil resultaterne og konklusionerne i rapporten indgå i de efterfølgende NanoDEN delprojekter, som omhandler modellering af nanomaterialers skæbne i miljøet og en eksponeringsvurdering, som i sidste ende fører til en vurdering af miljørisiko for udvalgte nanomaterialer.

### Definition og udvælgelse af nanomaterialer

I forbindelse med denne rapport defineres nanomaterialer som fremstillede materialer med en eller flere eksterne dimensioner på mellem 1 og 100 nm, og som anvendes i produkter eller artikler på grund af de nye egenskaber der opnås som følge af nanomaterialernes lille størrelse og andre manipulerede egenskaber. Følgende nanomaterialer er blevet udvalgt som casestudier:

- Sølv (Ag)
- Titaniumdioxid ($TiO2$) (rutil og anatase krystalstrukturer)
- Zinkoxid (ZnO)
- Kulstof-nanorør (CNT)
- Kobberoxid (CuO)
- Nano-skala nulvalent jern (nZVI)
- Ceriumdioxid ($CeO2$)
- Carbon black (CB)
- Kvantepunkter (QDs)

Udvælgelsen er baseret på deres forventede produktions- og anvendelsesmængder i Danmark samt deres anvendelse i relevante forbrugerprodukter, industrielle processer og miljøoprensningsprocesser. Disse materialer er anvendt til at illustrere og fremhæve forskelle og ligheder i miljøprocessernes betydning for skæbne og opførsel for forskellige materialetyper.

### Identifikation af de væsentligste relevante processer

I dag er brugen af nanomaterialer stigende på tværs af en bred vifte af sektorer. Udledning af nanomaterialer til miljøet kan forekomme i alle led af deres livscyklus: under produktion, brug og bortskaffelse. Udledningerne kan stamme fra brug af forbrugerprodukter og industrielle produkter, enten som resultat af tilsigtede udledninger (f.eks. når nanomaterialer anvendes til miljøoprensning) eller utilsigtede udledninger (fx på grund af slid og ældning af materialer, der indeholder nanomaterialer).

Udledning af nanomaterialer til miljøet kan også forekomme ved utilsigtede udslip under produktion eller transport, og når produkterne bortskaffes ved afslutningen af deres brugsfase. For eksempel er det muligt, at spildevand og slam, lossepladsperkolat og rester fra affaldsforbrænding kan indeholde nanomaterialer. Ved udledning til miljøet vil nanomaterialernes adfærd og fordeling afhænge af deres iboende egenskaber samt af de specifikke miljøforhold i recipienten. Der er et presserende behov for en øget forståelse af disse samspillende processer samt for, både kvantitativt og kvalitativt, at estimere potentielle miljømæssige eksponeringer for nanomaterialer. Mere specifikt er der et behov for at estimere forventede koncentrationer af i miljøet ('Predicted Environmental Concentrations', PEC) og for at lave faktiske målinger af koncentrationer i nanomaterialer miljøet. Disse er nødvendige for at kunne lave en miljørisikovurdering. Grundet af de nuværende begrænsninger i analysemetoder til at måle, kvantificere og karakterisere af nanomaterialer i miljømatricer er modellering en værdifuld og uundværlig metode til at estimere miljøkoncentrationer, i form af PEC værdier, på kort sigt.

Både konceptuelle værktøjer og mere kvantitative modeller til at beskrive udslip, skæbne og fordeling af nanomaterialer er dukket op inden for de sidste fem år. Disse indeholder ofte aspekter af klassisk kolloid videnskab samt principper, der anvendes i modellering af skæbne og materialeflow for konventionelle kemikalier. Ved yderligere at integrere mekanistiske modeller for nanomaterialers miljømæssige skæbne og adfærd i eksponeringsvurderingen forventes en betydelig forbedring af PEC-estimeringen. Desuden er der under model-udviklingen et behov for at foretage bevidste valg på grundlag af den tilgængelige viden med det formål at forbedre pålideligheden af de estimerede koncentrationer af nanomaterialer i miljøet. I dag er denne modeludvikling hæmmet af en mangelfuld viden om, hvordan nanomaterialers nye fysisk-kemiske egenskaber påvirker deres transformationsprocesser, og dermed deres opførsel i miljøet.

Ved litteraturgennemgangen i dette projekt blev følgende vigtige omdannelsesprocesser fundet:

- Fotokemisk nedbrydning,
- Oxidation, reduktion,
- Opløselighed
- Udfældning
- Speciering / kompleksdannelse,
- Agglomerering
- Aggregering
- Sedimentation,
- Adsorption,
- Desorption
- Biotransformation.

Ved en kritisk gennemgang af den nuværende viden er betydningen af disse processer for nanomaterialers skæbne og opførsel blevet gennemgået med særlig fokus på de ovennævnte udvalgte materialer.

*Det skal understreges, at den følgende gennemgang af omdannelsesprocesser vedrører den ikke-coatede, ikke-funktionaliserede form af nanomaterialer. For overflade-coatede eller funktionaliserede nanomaterialer kan deres miljømæssige skæbne og adfærd ikke udelukkende forudsiges baseret på egenskaber af nanomaterialets kerne. I stedet er en individuel vurdering nødvendig, hvor der tages hensyn til coating-materialet, overflademodifikationer og tilstedeværelse af stabiliseringsmidler. Hvor overflade-coatings har vist sig at være af særlig betydning for omdannelsesprocesser er dette nævnt i teksten nedenfor.*

### Hovedresultater for de væsentligste miljømæssige omdannelsesprocesser

***Fotokemiske omdannelser*** er kemiske ændringer igansat af absorption af lys. For nogle kulstofbaserede nanomaterialer det blevet fundet, at bestråling kan forårsage fotoinduceret oxidation og for metaloxid nanomaterialer, at fotoaktivering kan ændre deres bindingsegenskaber til opløst organisk stof. Miljørelevante (foto-)kemiske omdannelser af nanomaterialer kan sammenfattes som: 1) fotoinducerede ændringer i nanomaterialets overfladeegenskaber, der påvirker aggregering / agglomerering og adsorption til/fra andre overflader/forurenende stoffer, 2) fotonedbrydningsprodukter af overflade-coatings, og 3) fotonedbrydningen af selve nanomaterialet. Blandt de nanomaterialer som er udvalgt som casestudier til denne projekt betragtes følgende som mere tilbøjelige til at gennemgå eller deltage i (foto-)kemiske omdannelsesprocesser: CNT og CB (fotoinduceret oxidation), TiO2 (i mindre omfang CeO2) (binding til organisk materiale ændres ved fotoaktivering; fotokatalyse). Desuden er forskellige former for sølv er kendt for at være tilbøjelige til fotokemiske omdannelser og gælder sandsynligvis også for Ag nanopartikler. Alle NanoDEN casestudie materialer kan være overflade-coatede når de anvendes i fx forbrugerprodukter og tilstedeværelsen af overfladecoating kan ændre deres potentiale for fotokemisk transformation.

***Redox-reaktioner*** involverer overførsel af elektroner mellem kemiske stoffer. Reaktionsprocesserne oxidation og reduktion indebærer henholdsvis et tab eller optag af elektroner. Redox reaktioner er grundlaget for kemiske omdannelsesprocesser for uorganiske stoffer, herunder opløsning, og er relevante for de nanomaterialer, der deltager i elektron overførsel eller optag. Der er visse tegn på størrelsesafhængige ændringer i redoxpotentiale for nanomaterialer sammenlignet med samme materiale i større partikelstørrelser, men der er et behov for yderligere undersøgelser for kunne at bekræfte en sådan størrelsesafhængighed. Anvendeligheden af de metoder der på nuværende tidspunkt anvendes til måling af redoxpotentiale for nanomaterialer er også under debat. Af de udvalgte casestudie materialer kan nZVI og Ag NPs betragtes som mere tilbøjelige til at indgå i redox reaktioner.

***Opløsningskinetik*** (opløselighedsrate) og ligevægtsopløselighed (mængden af opløst materiale) af et nanomateriale vil påvirke dets skæbne og toksicitet. Opløseligheden af et materiale ikke er en iboende egenskab som sådan, men også afhænger af mediesammensætning (f.eks ionstyrke, ligander, pH og temperatur). For nanomaterialer er der yderligere parametre som menes at spille en rolle i opløseligheden, herunder partikelstørrelse, aggregering, partikel-coating og tilstedeværelsen af naturligt organisk materiale. De fleste tilgængelige modeller til at forudsige opløselighed er enige om, at opløsningen stiger med faldende partikeldiameter. De opløste ioner eller molekyler kan efterfølgende danne opløste komplekser med fx anioner eller organisk materiale (kompleksdannelse) i mediet eller ionerne kan danne en fast fase og udfælde. På grundlag af oplysninger fra litteraturen anses opløslighed at være af høj relevans for følgende casestudie nanomaterialer: ZnO, Ag, CuO, og QDs (afhængigt af specifik kemisk sammensætning).

***Agglomerering og aggregering*** kan forekomme som et resultat af tiltrækningskræfter mellem partikler, der forårsager dannelsen af klynger af nanopartikler. Dette kan ske under produktion, opbevaring og anvendelse samt efter emissionen til miljøet - uafhængigt af om nanopartiklerne er i opløsning, på pulverform eller i gasfase. Aggregater er defineret som klynger af partikler, der holdes sammen af stærke kemiske bindinger eller elektrostatiske interaktioner. Aggregering anses derfor for at være en irreversibel proces. Agglomererede partikler holdes sammen af svage kræfter og kan være en reversibel proces. Hvor vidt der forekommer aggregering, agglomerering eller de-agglomerering vil afhænge af forholdene i det omgivende medie. Agglomerering og aggregering har en betydelig indflydelse på nanomaterialers skæbne og opførsel i miljøet og afhængighed af partikelegenskaber (f.eks størrelse, kemiske sammensætning, overfladladning) samt miljømæssige forhold (f.eks blandingsforhold, pH og naturlig organisk stof ). I nogle tilfælde er nanomaterialer coatede for at modvirke disse processer. Agglomerering og aggregering kan føre til ændringer i biotilgængeligheden af nanomaterialer og kan være første trin i en sedimentering af nanomaterialer i miljøet. Interaktioner mellem partikler der fører til agglomerering kan beskrives teoretisk ved den såkaldte DLVO teori. Anvendeligheden af denne teoretiske tilgang til nanomaterialer præsenteres og diskuteres i denne rapport, idet den kan integreres i en fremtidige udvikling af modellerer for nanomateriales skæbne i miljøet. Aggregering og agglomerering må anses som værende relevante processer for alle nanomaterialer udvalgt som casestudier i denne rapport og er en vigtig proces for nanomaterialer i alle dele af miljøet.

***Sedimentation*** er knyttet til aggregering / agglomerering idet sedimentationshastigheden af partikler i vand afhænger både af vandets viskositet og massefylde samt af partikel radius og densitet. Dette medfører at større agglomerater og aggregater vil sedimentere hurtigere sammenlignet med mindre ikke-aggregerede/agglomererede partikler. Det betyder også, at agglomerering er den hastighedsbegrænsende faktor for sedimentering af nanomaterialer i vandmiljøet. Sedimentation er relevant for alle de udvalgte casestudie nanomaterialer og en en potentiel hoved-mekanisme for fjernelse af nanomaterialer fra vandfasen.

***Interaktioner med andre stoffer*** (makromolekyler, overfladeaktive stoffer, humussyrer etc.) vil finde sted når nanomaterialer udledes til miljøet. Disse interaktioner kan beskrives som en adsorption af andre materialer til nanomaterialets overflade (dvs. ENM fungerer som en sorbent). Dette er for eksempel tilfældet når naturligt organisk materiale (NOM) såsom humussyre binder sig til nanomaterialer. Denne adsorption af NOM vil ændre nanomaterialets overfladeegenskaber og adfærd og påvirke dets interaktioner med andre partikler og overflader (f.eks agglomerering) og dets interaktioner med det omgivende medie (f.eks opløselighed). Som følge heraf vil det være afgørende for dets transport og skæbne i miljøet (f.eks. ved at påvirke sedimentering ). Binding til NOM anses for at være relevant for alle de casestudie nanomaterialer. Den nuværende viden er dog begrænset med hensyn til hvordan NOM præcis ændrer nanomaterialers overfladeegenskaber og konsekvenserne af dette for andre omdannelsesprocesser.

***Interaktion* med faste overflader** er af stor betydning for nanomaterialers transport og skæbne i miljøet. I denne rapport er den mekanisme, hvormed nanomaterialer binder sig til andre materialer (undertiden benævnt hetero-agglomerering), beskrevet med fokus på adsorption af nanomaterialer til jordpartikler. Ii praksis kan det være vanskeligt at skelne mellem sorption og andre mekanismer der tilbageholder nanomaterialer i kolonne-eksperimenter. Tilbageholdelsen er i høj grad styret af aggregering og agglomerering idet større partikelstørrelser er mere tilbøjelige til at sidde fast i mikro-porer i jorden. Når der er tale om en egentlig adsorption af nanopartikler til jordpartikler (eller hetero-agglomerering) er DLVO teorien relevant, da den beskriver tiltrækningsenergien mellem en nanomaterialet og den overflade til hvilken de adsorberer. Anvendeligheden af DVLO teorien er blevet påvist i en række studier for større nanomaterialer (> 30 nm). For mindre partikler (<30 nm) har DVLO teorien imidlertid ofte vist sig ikke at være tilstrækkelig da også andre processer styrer sorptionen. Under miljørealistiske forhold menes det, at partikel-til-partikel agglomerering (homo-agglomerering) spiller en mindre rolle, og adsorption til jordkolloider (hetero-agglomereringsprocesser) vil spille en dominerende rollefor nanomaterialer. Således vil jord med en stor andel af naturlige kolloider, fx jord med et højt indhold af ler, generelt vil vise den højeste tilbageholdelse af ENMS. Naturlige og /eller tilsigtede overfladecoatings vil kraftigt påvirke nanomaterialernes sorptionsegenskaberne uafhængigt af selve nanomaterialets kemiske sammensætning. Også ændringer i pH påvirker nanomaterialets sorption til faste overflader, og processen anses for at være relevant for alle de udvalgte casestudie nanomaterialer i denne rapport. Imidlertid kan relevansen være noget lavere for nanomaterialer der let opløses i miljøet.

***Bionedbrydning*** er en biologisk proces, der involverer mikroorganismers nedbrydning af et organisk stof. Det betyder, at denne proces ikke er relevant for uorganiske nanomaterialer. Selv kulstof-baserede nanomaterialer har en tendens til at være af uorganisk natur og betragtes generelt som resistente over for biotisk nedbrydning. Bionedbrydning anses derfor som værende irrelevante for de fleste af casestudie nanomaterialer (Ag, TiO2, CeO2, nZVI, ZnO, CuO og QDs). Et begrænset antal studier har undersøgt den biologiske nedbrydning af kulstof-baserede ENMS. Under visse betingelser er en delvis mineralisering af CNT observeret. Mineralisering synes imidlertid at være begrænset og processen er meget afhængig af de specifikke forsøgsbetingelser. Relevansen af bionedbrydning som omdannelsesproces vurderes derfor som lav for CB og lav/medium for CNT. Der skal tages højde for biologisk nedbrydning af overfladecoatings når dette er relevant.

***Bio-modifikation*** defineres som biologisk medierede omdannelsesprocesser. Dette omfatter processer som finder sted efter optagelse af nanomaterialer i organismer eller processer der ’indirekte’ er medieret af en organisme, som  for eksempel ved frigivelse ekssudater, der bindes til nanomaterialer og ændrer deres egenskaber. Undersøgelser af hvordan planter kan modificere metaloxid og metalhydroxid nanomaterialer indikere en generel proces hvorved nanomaterialer adsorberer til rødderne hvorefter de går i opløsning og der sker en kompleksdannelse. I vandmiljøet er to forskellige former for biologisk omdannelse beskrevet: krebsdyr Daphnia magna der fjerner CNT overfladecoating, og ændringer i størrelse og form af serumalbumin stabiliserede guld nanopartikler når de filtreres af muslinger. Det fremhæves i flere studier at biotransformationer og dets rolle i nanomaterialers skæbne og transport stadig ikke forstås til bunds. Potentielle biomodifications kan også omfatte ændringer i opløslighed (f.eks Ag, ZnO, CeO2 og CuO) og agglomerering (f.eks CNTs) som følge af optagelse i organismer. Sammenlignet med andre omdannelsessprocesser udgør biomodification (af ikke-coatede nanomaterialer) sandsynligvis kun en lille  del af de samlede omdannelser der sker i miljøet og vurderes derfor at have relativt lille betydning i modellering af nanomaterialers miljømæssige skæbne. Der skal tages højde for bio-modifikationer af coatings når dette er relevant.

### Generelle mønstre for nanomaterialers skæbne og opførsel i forskellige miljøer

På baggrund af gennemgangen af de vigtigste omdannelsesprocesser for nanomaterialer i miljøet er disse blevet rangordnet baseret på deres generelle relevans med hensyn til modellering af nanomaterialers skæbne og opførsel i forskellige delmiljøer. Denne rangordning er ikke begrænset til de nanomaterialer, som er inkluderet som casestudier i NanoDEN projektet, men beskriver relevansen af disse processer for nanomaterialer i almindelighed.

I ***vandmiljøet*** kan fotokemiske reaktioner og redox-reaktioner kan være væsentlige processer i omdannelsen af nanomaterialer. Potentialet og omfanget heraf er dog meget afhængig af den kemiske sammensætning af det pågældende nanomateriale. Opløselighed skal overvejes for nogle nanomaterialer (f.eks Ag og ZnO) mens dette ikke er relevant under realistiske miljøforhold for andre (f.eks CNT og TiO2). Aggregering/agglomerering og sedimentation er af meget stor relevans for de fleste nanomaterialer i vand. Sorption til andre overflader er relevant at overveje for de fleste nanomaterialer og ligeledes er interaktioner med NOM. Bionedbrydning skal overvejes til modellering af nan materialers skæbne i miljøet, men er irrelevant for uorganisk nanomaterialer (metal og metaloxider) samt for kulstof-basered nanomaterialer, som anses for at være uorganiske af natur.

Hvad angår mulige omdannelserprocesser i ***jord og sedimenter*** er der ikke samme grad af videnskabelig konsensus som det var tilfældet for vandmiljøet.  Det er klart at aggregering/agglomerering af nanomaterialer i vandfasen kan føre til sedimentation og hermed en ophobning af ENMS i sedimenter, hvilket gør dette til et meget relevant delmiljø for nanomaterialer.  I jord og sedimenter vil sedimentering per definition ikke forekomme. Nanomaterialer forventes at adsorbere til partikulært materiale og faste overflader i sedimenter og jord, hvilket gør sorption til en meget væsentlig process. Redoxforhold er anderledes i jord sammenlignet med sedimenter, hvor der kun er ilt tilstede i de øverste millimeter fra overfladen. Også NOM indholdet er forskelligt: sedimenter indeholder ofte meget mere NOM end jord. Disse forskelle i NOM indhold og redoxforhold kan resultere i meget forskellige omdannelsesmønstre af nanomaterialer i disse delmiljøer. Derfor NOM interaktion og redoxforhold mere relevant i modellering af nanomaterialers skæbne i sedimenter i forhold til i jorden.

I ***luften*** er processer for ultrafine partikler velbeskrevet i litteraturen. For luftbårne nanomaterialer er der stadig nogle vigtige spørgsmål, der skal besvares i forhold til at klarlægge de processer, der styrer deres adfærd, transport og skæbne. Nanopartikler vil have høj mobilitet og vil blande sig hurtigt i aerosol-systemer. ENMS suspenderet i luften vil sandsynligvis blive udsat for sollys, inklusiv lys UV bølgelængder, til en langt større grad end i andre delmiljøer (vand, sediment, jord). Dette øger mulighederne for fotokemiske omdannelser. Deponering af nanopartikler fra luften afhænger af sedimentationshastighed, som er proportional med nanopartiklens diameteren. Som følge heraf vil mindre nanopartikeler i luft deponeres i et meget langsommere tempo end større partikler. Agglomerering vil derfor øgedeponeringen af naomaterialer betydeligt. Fotokemisk reaktioner, agglomerering og deponering ('sedimentering') vurderes at være de vigtigste i dette delmiljø mens andre processer vurderes at være af langt mindre betydning, eller endog at være irrelevant, for nanomaterialer i luften.

### Materiale-specifikke vurderinger af de vigtigste omdannelsesprocesser for en forbedret forståelse og modellering af nanomaterialers skæbne i miljøet

På baggrund af gennemgangen af de vigtigste omdannelsesprocesser, blev den relative betydning af disse processer for miljømæssig skæbne modellering af casestudie nanomaterialer evalueret. Det blev konstateret, at agglomerering /aggregering, sedimentering, NOM adsorption og adsorption til andre overflader/tilbageholdelse i jord var de vigtigste processer at medtage for alle casestudie materialer. I en overordnet sammenligning af de miljømæssige omdannelsesprocesser betragtes bio-modifikationer at have relativt lav relevans for de casestudie nanomaterialer som indgår i denne rapport.

Tabel 0.1 giver et overblik over den relative betydning af miljømæssige omdannelsesprocesser i forhold til modellering for de udvalgte casestudie nanomaterialer. Begreberne 'lav', 'middel' og 'høj' beskriver hvor afgørende den specifikke proces er for at opnå en pålidelig model for det pågældende nanomateriales skæbne. En proces med "lav" betydning anses for at have en lav indvirkning på modellering af skæbnen for dette specifikke nanomateriale og at udelade processen vil derfor kun medføre en lille fejl. På samme måde vil en proces af 'stor' betydning være afgørende for en præcis og pålidelig modellering af skæbnen for det pågældende nanomateriale.

Omdannelsen af Ag, ZnO og CuO vurderes at være stærkt påvirket af opløsning. For Ag og nZVI vil også redox processer være af stor betydning. TiO2 og CNTs (og mindre grad Ag og CeO2) kan undergå nogle fotokemis- induceret omdannelser, som anses for at have middelstor betydning i modelleringen af deres skæbne i miljøet. På grund af de forskellige kemiske sammensætninger af QDs, samt det faktum at deres egenskaber kan 'tunes' alt efter deres anvendelse, kan man ikke generalisere deres omdannelsesprocesser i miljøet. Dette vil kræve individuelle vurderinger for den specifikke QD.

**TABLE 0.1.**

RELATIVE BETYDNING AF OMDANNELSESPROCESSER FOR MODELLERING AF CASESTUDIE NANOMATERIALERS SKÆBNE. DET SKAL UNDERSTREGES AT DENNE EVALUERING KUN VEDRØRER DEN IKKE-COATEDE OG IKKE-FUNKTIONALISEREDE FORM AF NANOMATERIALERNE. OVERFLADE-COATINGS VIL POTENTIELT SPILLE EN STOR ROLLE I NANOMATERIALERS SKÆBNE OG ADFÆRD. FOR OVERFLADE-COATEDE MATERIALER KAN DERES MILJØMÆSSIGE SKÆBNE OG OPFØRSEL IKKE FORUDSIGES BASERET PÅ KERNEMATERIALETS EGENSKABER OG EN INDIVIDUEL VURDERING ER NØDVENDIG HVOR DER TAGES HENSYN TIL COATING-MATERIALETS EGENSKABER. (AG: SØLV, TIO2: TITANDIOXID, ZNO: ZINKOXID, CNT: KULSTOF-NANORØR, CUO: KOBBEROXID, NZVI: NANO NULVALENT IRON, CEO2: CERIUMDIOXID, CB: CARBON BLACK, QD: KVANTEPUNKT)

| | **Proces** | **Vigtighed af transformationsprocesser i modellering af skæbne i miljøet** | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Lav* | | *Medium* | | | *Høj* |
| **(Foto) kemisk** | *Fotokemisk nedbrydning* | nZVI, CB | ZnO, CuO | Ag, CeO2 | TiO2, CNT | | |
| | *Redox* | TiO2, CNT, CeO2, CB | ZnO, CuO | | | | Ag, nZVI |
| | *Opløsning* | TiO2, CNT, nZVI, CB | CeO2 | | | CuO | Ag, ZnO |
| **Fysisk** | *Aggregering/ Agglomerering* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB, |
| | *Sedimentering* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB |
| **Interaktion med overflader og andre stoffer** | *NOM adsorption* | | | | | Ag, TiO2, ZnO, CuO, nZVI, CeO2 | CNT, CB |
| | *Sorption til andre overflader/tilbageholdelse i jord* | | | | Ag, ZnO, CuO | TiO2, CeO2 | CNT, nZVI, CB |
| **Biologisk medieret** | *Bionedbrydning* | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | | |
| | *Bio-modifikation* | | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | |

## Videnshuller og manglende data

Der er identificeret en række mangler i den nuværende viden om omdannelsprocesser for nanomaterialer som forhindrer en valid kvalitativ og kvantitativ vurdering af deres skæbne og adfærd i miljømatricer. De identificerede videnshuller kan inddeles i tre forskellige kategorier:

1) Processer der styrer nanomaterialers skæbne og opførsel. Dette dækker manglende viden om de specifikke processer, der bestemmer skæbnen for nanomaterialer i miljøet.

2) Karakterisering og målemetoder. Dette dækker videnshuller i tilknytning til de forskellige metode- begrænsninger til at karakterisere og måle koncentrationer af nanomaterialer

3) Adgang til data og informationer. Dette vedrører videnshuller om f.eks. produktion, brug og anvendelse af nanomaterialer

Med hensyn til kategori 1) bør der gives prioritet til: kinetiske ændringer i aggregering/agglomerering, herunder bestemmelser af rater, en eventuel tilpasning af DLVO teorien, betydning af kemiske sammensætning, størrelser og form for sedimentering af nanomaterialer i overfladevand, parametre der påvirker de stabiliserende (eller de- stabilisering) effekter af NOM i nanomateriale-suspensioner, betydning og omfanget af sorption som fjernelses-proces til for nanomaterialer i jord-vand matricer, og endelig udvikling af passende testmetoder til at måle opløsningskinetikken for nanomaterialer og modeller til at beskrive deres opløsningskinitik.

Vedrørende kategori 2), skal det prioriteres at opbygge viden om karakterisering og målemetoder for nanomaterialer. Disse analysemetoder er nøglen til at etablere af model inputparametre samt til at validere disse modeller ved ar foretage in-situ målinger i miljøet.

Endelig med hensyn til kategori 3), med henblik på at udvikle masse-flow baserede modeller, er datatilgængelighed af stor betydning. Nuværende huller i vores viden omfatter oplysninger om produktionsmængder, specifikke anvendelser og anvendte koncentrationer af nanomaterialer i produkter samt kvantitative data om frigivelse af disse i løbet af produktion, transport, anvendelse og bortskaffelse.

# Executive summary

**Background and Objective**

Under the Agreement "Better Control of Nanomaterials" (“Bedre styr på nanomaterialer”), the Danish EPA has commissioned a number of projects aiming to investigate and generate new knowledge on the presence of nanomaterials in products on the Danish market and assess the possible associated risks to consumers and the environment. The NanoDEN project, of which this report constitutes the first stage, has the overall objective of assessing whether engineered nanomaterials (ENMs) pose a risk to the Danish environment.

This report has the aim of providing an overview of the present knowledge regarding processes relevant for environmental fate and behaviour of ENMs. Key environmental processes will be identified, discussed and prioritized according to their relevance as inputs for fate modelling for ENMs. Furthermore, knowledge gaps will be identified and prioritised with regard to their relevance in relation to predicting environmental concentrations of ENMs. In addition to being a project output in its own right, the results and conclusions of the report will feed into subsequent subprojects on environmental fate modelling and exposure assessment, eventually leading to an environmental risk assessment of the selected ENMs.

**Definition and selection of nanomaterials**

In the context of this report ENMs are defined as manufactured materials with one or more external dimensions between 1 and 100 nm, used in applications due to their novel characteristics resulting from their small size and other engineered properties. In that respect ENMs (stemming from engineered/intentional processes) can be seen as part of a broader group of nanomaterials, which in addition can also result from natural and anthropogenic (incidental) processes. The following ENMs have been selected as case-studies:

- Silver (Ag)
- Titanium dioxide (TiO2) (rutile and anatase crystal structures)
- Zinc oxide (ZnO)
- Carbon nanotubes (CNTs)
- Copper oxide (CuO)
- Nano-scale zero valent iron (nZVI)
- Cerium oxide (CeO2)
- Carbon black (CB)
- Quantum dots (QDs)

The selection was based on expected production and use volumes in Denmark and their applications in relevant consumer products, industrial processes, environmental remediation processes etc. These materials were used to illustrate and highlight differences and similarities in environmental fate and behaviour processes for different material types.

**Identification of key relevant processes**

Today the use of nanomaterials increases across a wide range of sectors and environmental release is possible throughout the life-cycle of ENMs during production, use and disposal. Releases may originate from the use of nano-enabled consumer and industrial products either by intentional (e.g., when ENMs are used for environmental remediation) or non-intentional releases (e.g. due to wear and tear of materials containing ENMs). Emissions of ENMs to the environment may also occur by accidental spills during production or transportation, and when products are disposed of at the end of their use phase For example, wastewater treatment effluents and sludge, landfill leachates and waste incineration residuals are all likely to contain ENMs. Upon emission into the environment the behaviour and distribution of ENMs will be determined by the intrinsic properties of the ENMs as well as the specific environmental conditions. There is an urgent need to improve the understanding of these interplaying processes as well as to estimate the potential environmental exposures to ENMs in both quantitative and qualitative terms. More specifically predicted environmental concentrations (PECs) or measured environmental concentrations are needed for the purpose of environmental risk assessment. Due to current severe limitations in analytical detection, quantification and characterisation of ENMs in environmental matrices modelling of ENM fate and exposure represents a valuable and indispensable approach to estimate possible exposure concentrations, in the form of PEC values, for ENMs in the short future.

Models and frameworks to describe release, fate and distribution of ENMs have emerged within the last five years incorporating aspects of classical colloid science as well as principles used for chemical fate and material flow modelling. By further integrating mechanistic models for ENM environmental fate and behaviour in the exposure assessment a significant improvement of PEC estimations is expected. Furthermore, informed choices on the basis of available knowledge are needed during model formulation and development to improve the reliability of the predicted environmental concentrations of ENMs.

A major knowledge gap, hampering this further development, is the current lack of understanding of how the novel physico-chemical properties of ENMs influence the transformation processes and hence ENM behaviour in the environment.

In this project we have identified the following key transformation processes influencing the environmental fate and behaviour of ENMs:

- Photochemical degradation,
- Oxidation, Reduction,
- Dissolution,
- Precipitation,
- Speciation / complexation,
- Agglomeration,
- Aggregation,
- Sedimentation,
- Adsorption,
- Desorption,
- Biotransformation.

Through a critical review of current state-of-knowledge the importance of these processes for ENM environmental fate and behaviour was reviewed with a specific focus on the selected ENMs chosen for this project.

*It must be emphasized that the following review of transformation processes relate to the un-coated, non-functionalized form of the ENMs only. For surface coated or functionalised nanomaterials the environmental fate and behaviour cannot be predicted based on core nanomaterial properties. Instead a case-by-case evaluation is required, taking into account the coating properties, surface modifications and presence of stabilizing agents. Where surface coatings have been found to be of particular importance to transformation processes this is mentioned in the text below.*

**Key findings for the most important environmental transformation processes**

***Photochemical transformations*** are chemical changes induced by absorption of light. For some carbon-based ENMs it has been found that irradiation can cause photoinduced oxidation and for metal oxide ENMs photoactivation may alter their binding properties to dissolved organic matter. Environmentally relevant (photo)chemical transformations of ENMs can be summarized as: 1) photoinduced changes of ENM surface properties, influencing aggregation/agglomeration and adsorption to/of other surfaces/contaminants, 2) photodegradation of surface coatings, and 3) photodegradation of the ENM itself. Among the ENMs selected for this study the following are considered more likely to undergo, or participate in, (photo)chemical transformation processes: CNT and CB (photoinduced oxidation), TiO2 (to a lesser extend CeO2) (binding to organic matter changed by photoactivation; photocatalysis). Furthermore, different forms of silver are known to be prone to photochemical transformations and this is also likely to be true for Ag NPs. All NanoDEN materials may be coated in the form they are used in e.g. consumer products and the presence of surface coating may alter the photochemical transformation potential of the ENM.

***Redox reactions*** involve transfer of electrons between chemical species. The reaction processes of oxidation and reduction involve a loss or gain of electrons, respectively. Redox reactions are the basis for chemical transformation processes for inorganic substances, including dissolution, and are relevant for ENMs that participate in electron transfer or uptake. There is some evidence of size dependent changes in redox potential for ENMs compared to bulk materials, but further investigations are needed for confirming such dependency. The appropriateness of currently applied methods to measure redox potential of ENMs is also under debate. Of the selected materials nZVI and Ag NPs are considered more likely to participate in redox reactions.

***Dissolution*** kinetics (rate of solubility) and equilibrium solubility (amount of dissolved material) of an ENM will influence its environmental fate and toxicity. Although solubility is material-dependent, the solubility of a solid material is not an inherent property as such but also depends on the media composition (e.g. ionic strength, ligands, pH, and temperature). For ENMs additional parameters are believed to play a role in the dissolution process, including particle size, state of aggregation, particle coating, water chemistry and presence of natural organic matter. Most of the currently available models to predict dissolution agree that the dissolution rate increases with decreasing particle diameter. Upon dissolution the dissolved ions or molecules may form dissolved complexes with e.g. anions or organic matter (complexation) in the media or the ions may form a solid phase and sediment out (precipitation). On the basis of available information from the literature the following materials are considered of high relevance with regard to dissolution within the scope of the NanoDEN project: ZnO, Ag, CuO, and QD (depending on specific chemical composition).

***Agglomeration and aggregation*** may occur as a result of attractive forces between particles, causing them to cluster together. This can happen during production, storage, and use and after emission to the environment - independent of whether the nanoparticles are in solution, powder form or in the gas phase. Aggregates are defined as clusters of particles held together by strong chemical bonds or electrostatic interactions. It is thus considered an irreversible process. Agglomerated particles are held together by weaker forces that do not exclude reversible processes. The direction of such processes will depend on the conditions of the surrounding media. The processes of aggregation and agglomeration significantly influence the fate and behaviour of ENMs in the environment, with a dependency of particle properties (e.g. size, chemical composition, surface charge) as well as environmental conditions (e.g. mixing rates, pH, and natural organic matter). In some cases ENMs are coated to counteract these processes. Aggregation and agglomeration may lead to changes in bioavailability of ENMs and serve as a starting point for sedimentation of ENMs in the environment. The interactions between particles leading to agglomeration can be defined theoretically by the so-called DLVO theory. The applicability of this theoretical approach to ENMs is presented and discussed in this report, as it might be integrated in future developments of chemical fate modelling. Aggregation and agglomeration is considered relevant for all ENMs selected as case-study materials for this report– and is a key process for ENMs in all environmental compartments.

***Sedimentation*** is linked to aggregation/agglomeration as the velocity of the settling of particles in water depends on both the viscosity and density of water as well as particle radius and density. This implies that larger agglomerates and aggregates will settle more quickly compared to more disperse particles. This also means that agglomeration is the rate limiting factor for sedimentation of ENMs in the aquatic environment. As for agglomeration and aggregation, sedimentation is considered relevant for all the selected ENMs and as a potential key removal mechanism for ENMs from the water phase in the aquatic environment.

***Interactions with other substances*** (macromolecules, surfactants, humic acids etc.) will take place after the ENMs are released to the environment. These interactions can be described as an adsorption of other materials onto ENMs (i.e. the ENM acts as a sorbent). This is for example the case for the binding of natural organic matter (NOM) such as humic acid to ENMs.  This association with NOM will alter the surface properties and behaviour of the ENM and influence its interactions with other particles and surfaces (e.g. agglomeration) and the surrounding media (e.g. dissolution), and in turn be determining for its environmental transport and fate (e.g. sedimentation). Binding to NOM is considered relevant for all the selected ENMs. However, the current knowledge is limited with respect to alterations of surface properties caused by NOM, and consequences for other transformation processes.

***Interaction with solid surfaces*** is of great importance for the transport and fate of ENMs in the environment. In this report the deposition mechanism by which the ENM attach to other materials (sometimes referred to as hetero-aggregation) is described with a focus on ENMs adsorption to soil particles. It was found that that in practice it can be difficult to distinguish between sorption and other retention mechanisms for ENMs in solid column experiments. Retention can be strongly linked to aggregation and agglomeration as larger particle sizes are more likely to get stuck in the micro-pores of the soil – an effect referred to as straining. For actual ENM adsorption to soil particle surfaces (or hetero-aggregation) the DLVO theory is also applicable, as it describes the interaction energy between an ENM and a collector surface. The applicability of the DVLO theory was demonstrated in a number of studies for larger ENM (>30 nm). However, for smaller particles (<30 nm) the DVLO theory was in most cases found to be invalid due to other processes controlling the sorption process. At environmentally 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. Natural and/or intentionally engineered surface coating will strongly influence sorption properties independent of the ENM core composition properties. Also changes in pH affect the ENM sorption process that are considered to be relevant for all the selected case-study ENMs in this review. However, the relevance may be somewhat lower for ENMs that dissolve more readily in the environment.

***Biodegradation*** is a biological process that involves the decomposition of an organic substance by microorganisms, implying that this process is not relevant for inorganic ENMs. Even carbon-based ENMs tend to be of an inorganic nature and are generally considered resistant to biotic degradation. Biodegradation is thus considered not to be relevant for most of the selected ENMs (Ag, TiO2, CeO2, nZVI, ZnO, CuO, and QDs). A limited number of studies have investigated the relevance of biodegradation for carbon-based ENMs. Under certain conditions some mineralization of CNTs has been observed. However, based on available information very limited mineralisation seems to occur and the process is very dependent on the specific test conditions. The relevance of biodegradation is therefore evaluated as low for CB and as low/medium for CNTs. Biological degradation of surface coatings has to be considered whenever relevant.

***Bio-modification*** is defined as biologically mediated transformation processes. Such processes include intra-organism processes that occurs after uptake or processes that are' indirectly' mediated by an organism for example by release exudates that bind to the ENMs and change their properties. Studies of metal oxide and metal hydroxide ENM modifications by plants indicate a general process of ENM adsorption to the roots followed by dissolution and complexation. In the aquatic environment two different biological transformation processes are described: removal of CNT surface coating, as a result of ingestion by crustacean *Daphnia magna*, and changes in ENM size and shape of bovine serum albumin stabilized gold nanoparticles by filter-feeding bivalves. It is highlighted in several papers that ENM biotransformations and their role in ENM fate and transport are still poorly understood. Potential biomodifications may also include changes in dissolution (e.g. for Ag, ZnO, CeO2 and CuO) and agglomeration state (e.g. CNTs) as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. Bio-modifications of surface coatings have to be considered whenever relevant.

### General fate and behaviour patterns for ENMs in environmental compartments

Based on the review of the key transformation processes for ENMs in the environment a ranking was made of the general relevance of each process for modelling of the fate and behaviour of ENMs in different environmental compartments. This ranking is not limited to the ENMs included in the NanoDEN project but describes the relevance of these processes to ENMs in general.

In the ***water compartment*** photochemical reactions and redox reactions may be significant processes for transforming many ENMs. The potential and magnitude hereof is however very dependent on the chemical composition of the ENM in question. Dissolution must be considered for some ENMs (e.g. Ag and ZnO) whereas this is not relevant under realistic environmental conditions for others (e.g., CNT and TiO2). The processes of aggregation/agglomeration and sedimentation are of very high relevance for most ENMs in water. Sorption to other surfaces is relevant to consider for most ENMs as well as interaction with NOM. In general biodegradation must be considered for fate modelling, but is considered irrelevant for inorganic metal and metal oxide ENMs as well as for carbon ENMs, which are considered to have an inorganic nature.

Regarding possible transformations in ***soils and sediments*** there is less agreement in the scientific community compared to the consensus evolving regarding transformation processes in water. It is clear that the aggregation/agglomeration behaviour of most ENMs in the water phase may lead to sedimentation and hereby a build-up of ENMs in sediments, making this a relevant compartment for ENMs in the environment. Within the soil and sediment matrices the process of sedimentation will by definition not occur. ENMs are expected to adhere to solids and the available solid surfaces in sediments and soils makes sorption even more important than for the water compartment. Redox conditions are different in soils compared to sediments, where no oxygen is present only a few millimetres from the surface. Also NOM contents differ, with sediments often containing much more NOM than soils. These differences in NOM content and redox conditions may result in very different transformation patterns of ENMs in these two compartments. Therefore, the relevance of including NOM interaction and redox conditions in environmental fate modelling is considered to be higher for the sediment compartment compared to the soil compartment.

In ***air*** the fate processes for ultrafine particles are well described. However, for air-borne ENMs there are still some major issues to be addressed with regards to disclosing the processes governing their behaviour, transport and fate. Nanoparticles will have high mobility and will mix rapidly in aerosol systems. ENMs suspended in air will most likely be exposed to sunlight, and especially to UV wavelengths of light, to a much larger degree than for the other environmental compartments. This increases the possibilities for photochemical transformations. The deposition of nanoparticles in air depends on the gravitational settling velocity, which is proportional to the diameter of the particle. As a consequence hereof, smaller nanoparticle in air will deposit at a much slower rate than larger particles. Agglomeration will therefore significantly increase the deposition of ENMs. In comparison to photochemical reactions, agglomeration and deposition, other processes are evaluated to be of much less relevance or even to be irrelevant for ENMs in the air.

### Material specific assessments of most important transformations processes for understanding and modelling ENM environmental fate

Based on the review of the key transformation processes, the relative importance of these processes for environmental fate modelling of the selected case-study ENMs was evaluated. It was found that the processes of agglomeration / aggregation, sedimentation, NOM adsorption and sorption to other surfaces / retention in soil were important to include for all the selected case-study ENMs in this review. In an overall comparison of environmental transformations, bio-modifications are considered of relatively low relevance for the ENMs included in the current report.

Table 0.1 provides an overview of the relative importance of environmental transformation processes for modelling purposes of the ENMs selected as cases in this report. .  The terms ‘low’, ‘medium’ and ‘high’ are used to describe how crucial the specific process is in a  model prediction of the fate of the ENM in question.A process of ‘low’ significance is considered to have a low impact on the fate modelling of that specific ENM and omitting the process will therefore not result in a large error. In the same way a process of ‘high’ significance is vital to accurately model the fate and behaviour of the ENM in question.

The transformation of Ag, ZnO and CuO is evaluated to be highly influenced by dissolution. For Ag and nZVI also redox processes are of high importance. TiO2 and CNTs (and to less extend Ag and CeO2) may undergo some photochemically induced transformations, which are considered of medium importance in the modelling of their environmental fate. Due to the varying chemical compositions of QDs 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.

**TABLE 0.1.**

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF ENMS SELECTED AS CASES FOR THE CURRENT PROJECT. IT MUST BE EMPHASIZED THAT THIS EVALUATION RELATES TO THE UN-COATED, NON-FUNCTIONALIZED FORM OF THE ENMS ONLY. IT IS RECOGNISED THAT SURFACE COATINGS WILL POTENTIALLY PLAY A MAJOR ROLE IN THE ENM FATE AND BEHAVIOUR. FOR SURFACE COATED MATERIALS THEIR ENVIRONMENTAL FATE AND BEHAVIOUR CANNOT BE PREDICTED BASED ON CORE MATERIAL PROPERTIES AND A CASE-BY-CASE EVALUATION IS REQUIRED, TAKING INTO ACCOUNT THE CORRESPONDING COATING PROPERTIES. (AG: SILVER, TIO2: TITANIUM DIOXIDE, ZNO: ZINC OXIDE, CNT: CARBON NANOTUBES, CUO: COPPER OXIDE, NZVI: NANO ZERO VALENT IRON, CEO2: CERIUM OXIDE, CB: CARBON BLACK, QD: QUANTUM DOT)

| | **Process** | **Importance of the environmental process in fate modelling** | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Low* | | *Medium* | | | *High* |
| **(Photo) chemical** | *Photochemical* | nZVI, CB | ZnO, CuO | Ag, CeO2 | TiO2, CNT | | |
| | *Redox* | TiO2, CNT, CeO2, CB | ZnO, CuO | | | | Ag, nZVI |
| | *Dissolution* | TiO2, CNT, nZVI, CB | CeO2 | | | CuO | Ag, ZnO |
| **Physical** | *Aggregation / Agglomeration* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB, |
| | *Sedimentation* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB |
| **Interactions with surfaces/ substances** | *NOM adsorption* | | | | | Ag, TiO2, ZnO, CuO, nZVI, CeO2 | CNT, CB |
| | *Sorption onto other surfaces/ retention in soil* | | | | Ag, ZnO, CuO | TiO2, CeO2 | CNT, nZVI, CB |
| **Biologically mediated** | *Biodegradation* | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | | |
| | *Bio-modification* | | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | |

## Knowledge and data gaps

A number of gaps have been identified in the current knowledge on important transformation processes, hampering the understanding, and valid qualitative and quantitative estimation, of the fate and behaviour of ENMs in environmental matrices. The identified gaps were divided into three different categories:

1) Environmental fate processes. This covers knowledge gaps on the specific processes determining the fate of ENMs

2) Characterisation and measuring methods. This covers gaps related to the distinct limitations of methods to characterise and measure concentrations of ENMs

3) Access to data and information. This relates to the gaps in knowledge on e.g. production, use and applications of ENMs.

Regarding category 1) priority should be given to reduce the knowledge gaps in the following areas: kinetic changes in aggregation/agglomeration including rate of growth, possible adaptation of the DLVO theory, the role of chemical compositions, sizes, and shapes on ENM sedimentation in surface waters, parameters influencing the stabilising (or de- stabilising) effects of NOM on ENM suspensions, the role and extent of sorption as a distribution process for ENMs in solid-water matrices, development of appropriate tests to measure dissolution kinetics, and models to describe dissolution processes.

Furthermore, regarding category 2), studies to fill the knowledge gaps regarding ENM characterization and measuring methods must be prioritized. These analytical methods are key to the establishment of model input parameters as well as model validation by *in-situ* measurements in the environment.

Finally, regarding category 3), for the purpose of mass-flow based models, data availability and accessibility are of high importance and current knowledge gaps include data on ENM production quantities, specific uses and use concentrations, as well as quantitative data on release during production, transport, use and disposal.

Environmental fate and behaviour of nanomaterials

# 1. Introduction

## 1.1 Background

The current report reviews recent literature on the environmental processes governing the fate and behaviour of engineered nanomaterials (ENMs). These processes strongly influence the subsequent environmental exposure to ENMs and hereby also the environmental risk assessment of ENMs.

In the context of this project we define an ENM as a manufactured material with one or more external dimensions between 1 and 100 nm, used in applications due to their novel characteristics resulting from their small size and other engineered properties. In that respect ENMs can be seen as part of a broader group of nanomaterials, which cover materials resulting from natural, anthropogenic (incidental) and engineered (intentional) processes. While natural, incidental and manufactured nanomaterials are covered by the definition of a “nanomaterial” published by the European Commission in its “Recommendation on the definition of a nanomaterial” (EC, 2011), the current report will only focus only on nanomaterials that result from engineered processes i.e. ENMs.

In understanding their environmental fate and behaviour the aquatic phase is seen as the starting point and as a main point of potential entry and dispersion in the environment. The aquatic phase creates a link to and between the other environmental compartments such as soil, sediment and air. Furthermore, the behaviour of ENMs in more complex matrices (including soil and sediments) is not well-understood at present. For these reasons the present report will have a strong emphasis on the aquatic compartment, although other environmental compartments will be discussed where relevant.

Today the use of nanomaterials increases across a wide range of sectors and environmental release is possible throughout the life-cycle of ENMs during production, use and disposal. Figure 1.1 gives an overview of possible routes for ENMs into the environment. Releases may originate from the use of nano-enabled consumer and industrial products either by intentional (e.g., when ENMs are used for environmental remediation) or non-intentional releases (e.g. due to wear and tear of materials containing ENMs). Emissions of ENMs to the environment may also occur by accidental spills during production or transportation, and when products are disposed of at the end of their use phase For example, wastewater treatment effluents and sludge, landfill leachates and waste incineration residuals are all likely to contain ENMs. Upon emission into the environment the behaviour and distribution of ENMs will be determined by the intrinsic properties of the ENM as well as the specific environmental conditions.

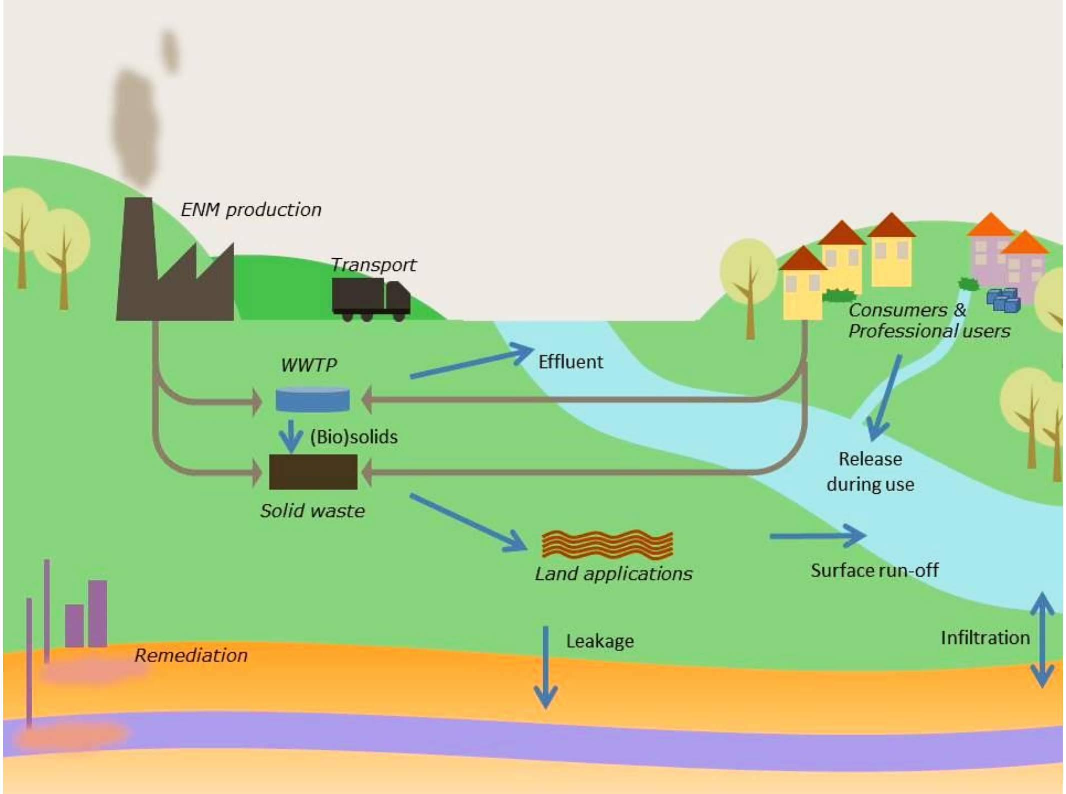


**FIGURE 1.1.**

A SIMPLIFIED OVERVIEW OF SOURCES AND EMISSIONS OF NANOMATERIALS INTO THE ENVIRONMENT. ENMS ARE POTENTIALLY RELEASED INTO THE ENVIRONMENT OVER THEIR WHOLE LIFE-CYCLE VIA A DIFFUSE AND POINT SOURCES. THIS INCLUDES RELEASE DURING MATERIAL PRODUCTION, WHEN APPLIED IN PRODUCTS OR APPLICATIONS, RELEASE DURING/RESULTING FROM WASTE TREATMENT AND FROM PRODUCT WEATHERING.

The ENMs may be present in different matrices including air (aerosol), liquid (suspension) or solid (embedded). Their route into the environment and likelihood of environmental exposure will depend on the specific matrix in which they were originally used (Hansen et al., 2008), and this matrix is likely to change during the different steps of the life span of the ENM. For example an ENM may be produced as a powder, mixed into a liquid (such as paint) applied on a surface, weathered and released into urban runoff water. Once in the environment the behaviour of ENMs is dependent both on the physicochemical characteristics of the ENM in question, as well as the characteristics of the receiving environment. Presently it is known that ENMs in the environment tend to be transformed from their pristine/as produced/as released form due to aggregation, agglomeration, or association with dissolved/colloidal/particulate matter present in the environment (Nowack et al., 2012). While some analogies can be made to the behaviour of colloids in the environment, the novel physico-chemical characteristics, which make ENMs desirable in applications and products, also present a challenge in determining how they interact with the environment. An understanding of underlying processes is crucial to gain an overview of the potential ENM transformation and distributed in the environment. This knowledge is also fundamental for developing nano-tailored models, or model approaches, and hence for improving the estimation of predicted environmental concentrations (PECs).

If ENMs are released to the environment there is a need of understanding and estimating potential human and environmental exposure to ENMs in both quantitative and qualitative terms. More specifically predicted environmental concentrations or measured environmental concentrations are needed for the purpose of environmental risk assessment. Current limitations in analytical detection, quantification and characterisation of ENMs in environmental matrices, make fate and exposure models the only feasible approach to obtain realistic estimates of current ENM exposure concentrations. As reviewed in Gottschalk et al. (2013) a handful of model-based studies and some analytical quantifications provide initial quantitative results on environmental exposure concentrations for some ENM. On this basis, rough estimates of PECs are available for nanosized TiO2, ZnO, Ag, fullerenes, CNT and CeO2 in different environmental media such as surface waters and sediments, wastewater treatment effluents and biosolids, soils and air.

Models and frameworks to describe the fate and distribution of ENMs have been emerging within the last 5 years, some of which incorporate classical knowledge of colloid science (Arvidsson et al., 2011) and others combine principles used for chemical fate modelling and material flow modelling (Mueller and Nowack, 2008; Blaser et al. 2008; Gottschalk et al., 2009: 2010a; 2010b; Praetorius et al. 2012). Attempts have been made to quantitatively determine PEC values e.g. by using a mass flow analysis approach and summing up releases over the complete life cycle of the investigated ENM (Mueller and Nowack 2008). Gottschalk et al. (2009) extended the scenario approach by running computer simulations based on a stochastic model that allowed calculating probabilities of PECs by considering, as far as possible, the uncertainty and variability of all the factors (throughout the model) leading to environmental exposure. Recently, this exposure and risk analysis has been updated based on newer model input data (Sun, 2014).

The prediction of PECs can be refined by including mechanistic description of ENM behaviour (e.g. agglomeration or sedimentation). For example, transport between different compartments (including sedimentation and re-suspension processes) was included in the model by Blaser et al. (2008). Praetorius et al. (2012) introduced nano-specific fate process descriptors in for the water column and sediment in a model for ENMs in surface waters.

A major knowledge gap hampering the further development of mechanistically-based model approaches is the current lack of understanding of the novel physico-chemical properties exhibited by many ENMs and the influence these have on particle behaviour. This applies equally to novel carbon-based nanoparticles (e.g. CNT and $C_{60}$) as well as to metal and metal oxide nanoparticles. In addition, ENMs released into the environment are likely to exist as modified forms of the material initially released (SCENIHR, 2009; Nowack et al., 2012). It has been highlighted that reliable exposure models for ENMs needs a solid data basis with respect to model input parameters (e.g. transfer and partitioning coefficients, and emission factors). Unfortunately, and important to clearly emphasize, present model input parameters are often based on crude assumptions (ITS-NANO, 2013) and not specifically designed based on novel ENM physico-chemical properties (Gottchalk, 2013).

For the purpose of rapid initial risk assessment the software program EUSES (The European Union System for the Evaluation of Substances) is applied for industrial chemicals. This tool contains models for both exposure assessment and effects assessment and can be used to derive an initial risk characterisation. The use of already established chemical fate models to predict ENM fate was discussed in the final report from the NanoImpactNet FP7 project (Hansen et al., 2009). Here it is stated that "EUSES was...()... deemed insufficient for nanoparticles for a number of reasons: a) the extent/rate of dissolution is unknown or not included in current models and the same goes for b) the extent/rate of aggregation/settling and c) the extent of association with sediment." In more general terms, environmental fate models for ENMs need to be based on descriptions of all environmental processes that take into account the specific properties of the ENMs. The physicochemical properties of ENMs are completely different from those of organic chemicals, and this requires extensive adjustments of environmental fate models before they can be applied to ENMs, as has been demonstrated by Praetorius et al. (2012). These adjustments have not yet been made for EUSES, which implies that EUSES currently cannot be applied to ENMs.

To develop a more complete understanding of the potential risks posed by manufactured nanomaterials, it is necessary to further examine their transport and fate in all environmental compartments. Although the current lack of quantitative data on ENM release hampers subsequent prediction of the expected environmental fate and concentrations, the knowledge-base in this area continues to grow and is developing fast. Also, much consideration has been given to extrapolating evidence from laboratory situations and from knowledge obtained with industrial chemicals.

For mass-flow based approaches there are substantial knowledge gaps causing a considerable reduction in the reliability of the modelled results – many of which could be addressed through an increased collaboration with industry. Such gaps include data on real quantities of ENM production, use, and release. However, as underlined by Gottschalk et al (2013), a validation of the modelled values is currently impossible as trace analytical methods for the specific detection and quantification of ENM in environmental matrices are lacking. A few combinations of filtration, microscopic, spectroscopic, chromatographic, and other approaches have been used mostly under laboratory conditions. Unfortunately, these combined techniques are not yet developed to a sufficient level for real time (and long term) sampling under natural conditions that would significantly increase the reliability of such exposure concentration results (von der Kammer, 2012).

This situation leaves a potential gap between the present state-of-science and the increased demand for reliable estimation of environmental concentration and risks of ENM. Hence, an improvement of ENM specific fate and exposure models is the only feasible way to approach to realistic PEC values for risk assessment of ENMs in the short future. However, chemical fate modelling for nanomaterials is still in its infancy. Informed choices on the basis of empirical knowledge are urgently needed for model formulation and development to improve reliability of the predictions on environmental concentrations and risks of nanomaterials.

## 1.2 Objective and scope

The main objective of this report is to provide **an overview of the present knowledge regarding processes relevant for environmental fate and behaviour of ENMs**. The focus is on the processes that transform manufactured nanomaterials from their pristine form (as produced or in a product). Hence the report will focus on degradation, aggregation, agglomeration of ENMs and investigate ENM interactions with dissolved, colloidal or particulate matter present in the environment. These **key environmental processes are identified and discussed** both for ENMs in general as well as for specific selected ENMs that are used as ‘case studies’ to highlight differences in ENM transformations.

An additional objective of this report is to **prioritize the identified processes according to their relevance as inputs for fate modelling** for ENMs. This serves as a basis to identify significant parameters relevant to exposure and risk assessment of ENMs in the Danish environment.

Furthermore, **knowledge gaps** regarding environmental fate and behaviour of ENMs are identified and prioritised with regard to their relevance in relation to predicting environmental concentrations of ENMs.

The following ENMs have been selected as case-studies for this project:

- Silver (Ag)
- Titanium Dioxide (TiO2) (rutile and anatase)
- Zinc Oxide (ZnO)
- Carbon Nanotubes (CNTs)
- Copper Oxide (CuO)
- Nano Zero Valent Iron (nZVI)

- Cerium Oxide (CeO2)
- Carbon Black (CB)
- Quantum Dots (QDs)

The selection was based on expected high production, import and/or use volumes in Denmark and their applications in relevant consumer products, industrial processes, environmental remediation procedures etc. These materials were used to illustrate and highlight differences and similarities in environmental fate and behaviour for different materials.

# 2. Environmental processes of importance for fate and behaviour of nanomaterials

## 2.1 Initial identification of processes relevant for environmental fate and behaviour of ENMs

The intended and unintended release of ENMs into the environment is likely to result from either direct emission of the ENM itself or as part of a product matrix. Upon release into the environment the behaviour and distribution of ENMs will be determined by the intrinsic properties of the ENM as well as the specific environmental conditions. It is obvious that ENMs in an environmental matrix do not form a static system, a number of transformation processes will occur. Based on existing literature (Lowry et al., 2012b; Nowack et al., 2012; Stone et al. 2010) we have identified the following key transformation processes for ENMs, determining their fate and behaviour in the natural environment:

### Key transformation processes influencing the environmental fate and behaviour of ENMs

- Photochemical degradation
- Oxidation
- Reduction
- Dissolution
- Precipitation
- Speciation – complexation
- Adsorption
- Desorption
- Biotransformation
- Agglomeration
- Aggregation
- Sedimentation

These processes have been in the focus of the present literature review and subsequent identification of data gaps. Some of these transformations may occur to the 'as produced'/'as engineered', 'product modified', or 'product-weathered' ENMs (Nowack, Ranville et al. 2012). The 'as released' ENMs will have certain physico-chemical characteristics i.e. specific sizes, shapes, surface charge, chemical composition and coating as a result of their 'as produced' characteristics and subsequent transformations along the ENM life-cycle, prior to release into the environment.

As illustrated in Figure 2.1 this report focuses on the transformation processes that occur after the ENM is released into the environment, i.e. transformations of the 'as released' ENM. In the subsequent chapters we describe how these transformations can influence the environmental fate and behaviour of the ENM in the environmental compartments. The transformation processes, as identified above and as illustrated in Figure 2.1., include chemical, physical and biological transformations.

Briefly, the chemical processes are those that change the chemical structure, composition and/or speciation of the ENM. Physical transformation processes are those that influence the physical appearance of the ENM. Finally, biological transformations are defined as transformations processes that are biologically mediated. Ultimately, the transformation processes determine the environmental fate and behaviour of the ENM as a result of both environmental conditions (ionic strength, NOM etc.) and ENM intrinsic properties (illustrated in Figure 2.1.).

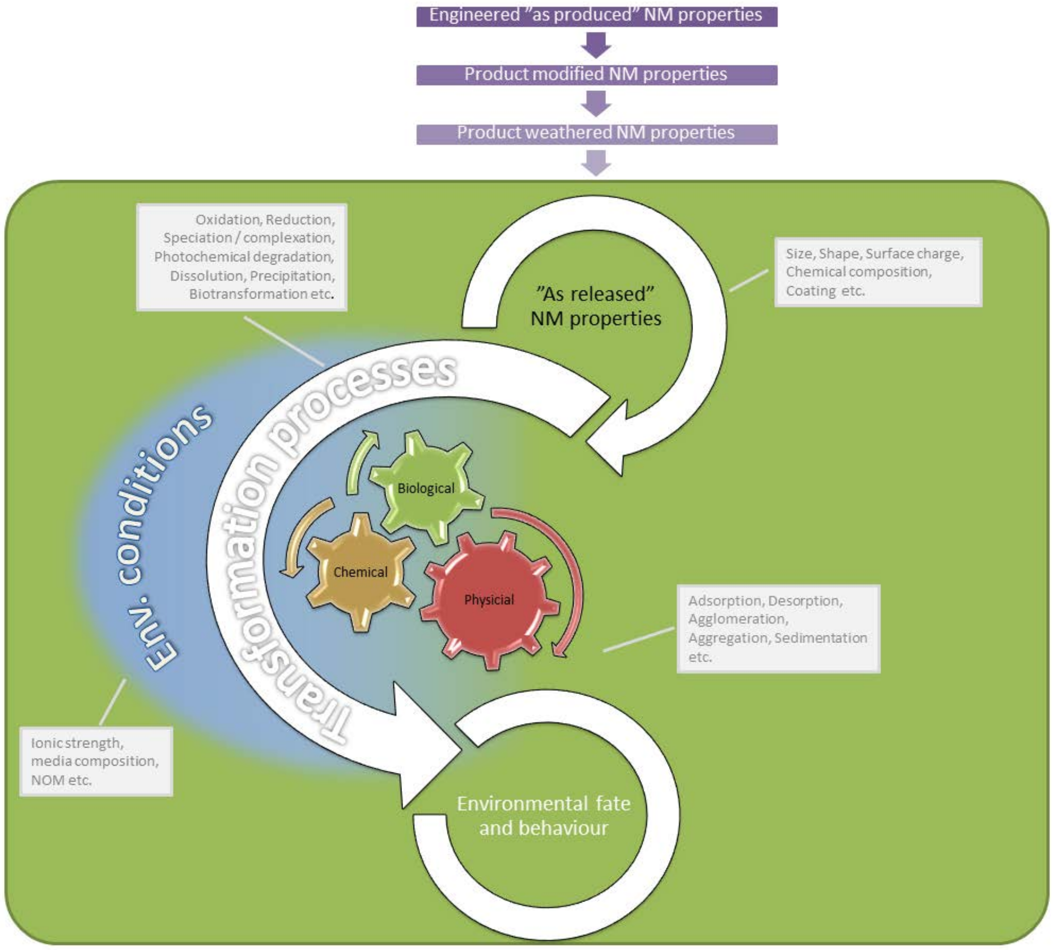


FIGURE 2.1.
A SCHEMATIC OVERVIEW OF THE POSSIBLE TRANSFORMATIONS OF ENMS IN THE ENVIRONMENT. IT IS SHOWN HOW 'AS RELEASED' ENMS ARE TRANSFORMED BY A COMBINATION OF INTERLINKED CHEMICAL, PHYSICAL AND BIOLOGICALLY MEDIATED PROCESSES. THE ENVIRONMENTAL FATE AND BEHAVIOUR OF ENMS FURTHER DEPEND ON THE PROPERTIES OF THE TRANSFORMED ENM IN COMBINATION WITH AND/OR RESULTING FROM THE ENVIRONMENTAL CONDITIONS.

## 2.2 Definitions of the key transformation processes

The definitions used in this report are listed in Table 2.1 and are mainly based on definitions in Stone et al. (2010) and Nowack et al. (2012). In our literature review the processes were grouped, in accordance with Figure 2.1, into 'chemical / photochemical transformations', 'physical transformations', and 'biological transformations'. An additional process category, 'interactions with other surfaces', was included to cover the situation when other substances adsorb onto the ENM surface as well as when ENMs adsorb onto a surface of another material.

In some cases the four categories are overlapping as the processes in reality are highly interlinked, influencing each other in an interdependent way. Hence, the above categories provide the main structure for the literature review but some processes will be described in more than one of the following sub-chapters.

**TABLE. 2.1.**
DEFINITIONS OF ENVIRONMENTAL BEHAVIOUR/DISTRIBUTION PROCESS FOR ENMS IN THE CONTEXT OF THIS REPORT BASED ON DEFINITIONS IN (STONE ET AL. 2010, NOWACK ET AL. 2012).

| Environmental process | Definition |
|---|---|
| **1. Photo)chemical transformation** | |
| 1.1.a Photocatalytic degradation | Chemical change induced by light, which includes excitation of photocatalytic ENMs (absorption of a photon causing generation of free radical species) and photolysis of the ENM or components of the ENM (e.g. decomposition of coating material). |
| 1.1.b Oxidation (chemical) | The nanoparticle surface atoms or molecules are oxidized by loss of electrons. |
| 1.1.c Reduction (chemical) | The nanoparticle surface atoms or molecules are reduced by uptake of electrons. |
| 1.2.a Speciation/ complexation | ENMs (or released ions / molecules) associating with other molecular or ionic dissolved chemical substances in the environmental matrix. This includes interactions with macromolecules (e.g. chemisorption to the ENM surface, forming a surface coating). |
| 1.2.b Dissolution | Process whereby a solid ENM dissolves (release of individual ions or molecules) in water. |
| 1.2.c Precipitation | The process of dissolved species forming a solid phase (such as metal ions released from an ENM precipitating into a solid material). |
| **2. Physical transformations and processes** | |
| 2.1.a Agglomeration | Reversible coagulation of primary particles to form clusters. |
| 2.1.b Aggregation (homo-aggregation) | Irreversible fusing of primary particles to form larger particles of the same material. |
| 2.2.a Sedimentation | Process whereby ENMs in suspension settle out of the water phase. |
| **3. Interactions with other surfaces and substances** | |
| 3.1.a Adsorption / 'hetero-aggregation' | The association of the ENM with other solid surfaces in water. This can be divided into: ENM as sorbent: When other substances adsorb onto the ENM surface. ENMs as sorbate: When ENMs adsorb onto other surfaces. |
| 3.1.b Desorption | Detachment of the ENM from other surfaces into water. |
| **4. Biological transformation** | |
| 4.1.a Biologically mediated processes | Processes whereby an ENM undergoes a transformation due to the presence of living organisms. This may include processes such as biological oxidation and degradation, interactions with bio-macromolecules excreted by organisms (e.g. leading to surface coating of the ENM). |

To further clarify these definitions, the different processes are illustrated graphically in Figure 2.2. From this figure it can also be observed that the processes occur as:

- Processes on the level of the individual particles (e.g. photochemical transformation)
- Interactions between particles (e.g. aggregation/agglomeration),

- Interactions with solid surfaces (e.g. ENM adsorption to a surface)
- Interactions with other substances (e.g. adsorption of NOM to the ENM surface).

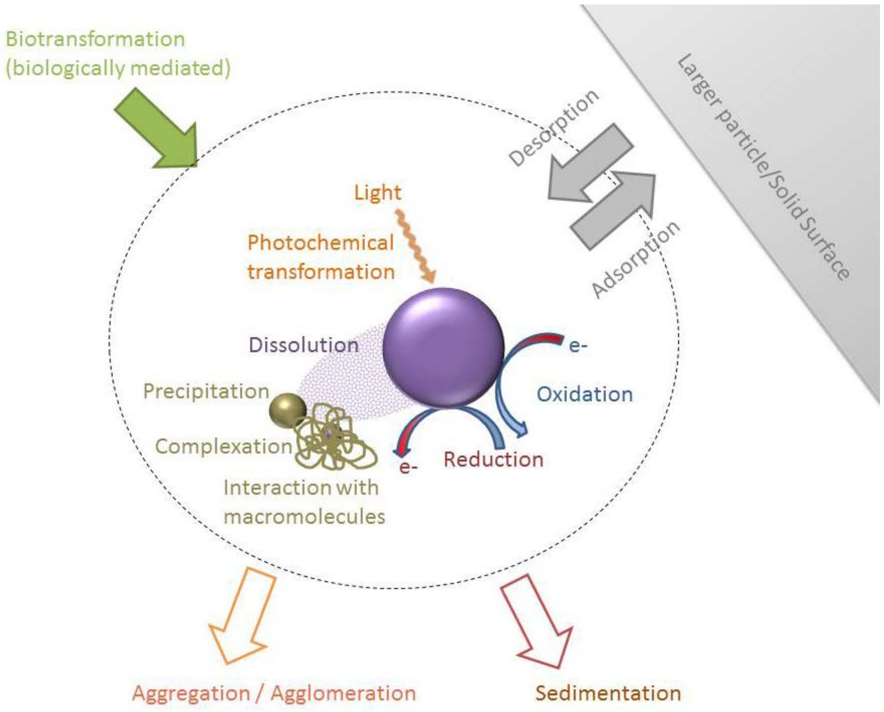


**FIGURE 2.2.**

ILLUSTRATION OF ENVIRONMENTAL TRANSFORMATION PROCESSES FOR ENMS ON THE LEVEL OF THE INDIVIDUAL PARTICLES (E.G. PHOTOCHEMICAL TRANSFORMATION), AS INTERACTIONS BETWEEN PARTICLES (E.G. AGGREGATION/AGGLOMERATION), AS INTERACTIONS WITH OTHER SURFACES (E.G. ADSORPTION) AND AS INTERACTIONS WITH OTHER SUBSTANCES (E.G. COMPLEXATION).

## 2.3 Literature search strategy

The scientific literature was searched and reviewed with focus on the transformation processes identified above. The starting point for this literature review was the work carried out in the ENHRES, covering state-of-knowledge up until December 2008 and described in the ENHRES final report project (Stone et al. 2010). As stated in the report, the ENHRES review revealed a “general paucity in data in the area of environmental fate and behaviour”, which was identified as an obstacle to understand and describe the environmental exposure potential of ENMs. It was found that most nanomaterials are expected to interact with natural organic matter in the environment and that this will influence the fate and transport of NM in water. Furthermore, sorption of other materials present within the aquatic environment was found to potentially influence the nanomaterial transport. In the ENHRES review it is stated that that nanomaterials dispersed in water are expected to behave as colloidal suspensions. These are generally expected to be unstable suspensions as particles may adhere to each other and then settle due to gravity (Baalousha et al. 2008, Ju-Nam and Lead 2008, Saleh et al. 2008). However, in the literature reviewed in the ENRHES report the documentation of these statements suffered from an extensive lack of experimental and environmental data in relation to the fate and behaviour of nanomaterials in water and soil.

The scientific literature on all aspects of nanomaterial risks has experienced a boom in recent years and a number of new studies on the environmental fate and behaviour of ENMs has been published since December 2008. In this report the ENHRES review has been updated through a literature review limited to scientific papers published in the period 2009-2013. Based on initial searches in different databases, ISI Web of Science was found to give the most comprehensive records and a final refined search strategy was therefore implemented using this database. The literature searches were carried out by combining the following search terms:

1. The transformation processes in an truncated form (e.g. photochem*, aggre*, desorp*)
2. Combinations of more general search terms, limiting the searches to records relevant for fate and behaviour of ENMs in the environment: e.g. nano*; environ*; fate; behav*

For each process an initial ‘broad’ search was carried out as a first step (“*process_truncation**” AND “nano*” AND “environ*”). Depending on the number of records from this initial step, the search was subsequently adjusted to be more specific (by addition of more search criteria) or broader (by removing some of the general search terms). The resulting list of scientific papers was subsequently screened manually for relevance and a database was created for each process. For a detailed description of the literature search strategy please see Appendix 2.

## 2.4 Chemical and photo-chemical transformations in the environment

When entering into an environmental matrix, and/or exposed to light, ENMs may undergo (photo)chemically-induced “weathering”, which in turn may alter the surface properties and/or ENM composition. This may include for example dissolution, degradation or replacement of surface coatings, changes to surface functional groups and changes in redox state. Chemical transformations can be described as the conversions of the chemical structure of an ENM through different mechanisms, and may occur as a result of chemical reactions between the ENM and components of the surrounding media. These chemical transformations are also important in relation to changes in toxic mechanisms of the ENM, which may be a result of (1) metal ions released from the ENM, (2) (photo)catalytic effects of metal and metal oxide EMNs or (3) redox changes on the ENM surface (Auffan et al 2009). At the same time these processes may also influence the fate and behaviour of the ENM.

### 2.4.1 Photochemical reactions

## Summary

Photochemical transformations are chemical changes induced by light. For some carbon-based ENMs it has been found that irradiation can cause photoinduced oxidation and changes to the π-bond electron structure. For metal oxide ENMs photoactivation may alter their binding to dissolved organic matter. (Photo)chemical transformations relevant for ENM fate and behaviour can be summarized as: 1) photoinduced changes of ENM surface properties influencing aggregation/agglomeration and adsorption to/of other surfaces/contaminants, 2) photo-degradation of surface coatings and 3) photodegradation of the ENM itself.

Some nanomaterials are considered more likely to undergo, or participate in, (photo)chemical transformation processes. For the case-study materials in this report this applies to: CNT and CB (photoinduced oxidation), TiO2 (to a lesser extend CeO2) (binding to organic matter changed by photoactivation; photocatalysis). Furthermore, different forms of silver are known to be prone to photochemical transformations and this is also likely to be true for Ag NPs. For all materials coating may alter the photochemical transformation potential of the ENM.

A photochemical transformation is defined as a chemical change induced by light. This includes photolysis (the process by which a molecule is exited to a higher energy state by absorption of photons and the excess energy results in a break-down of the molecule) and photocatalysis (accelerated reaction in the presence of a catalyst, involving absorption of a photon and generation of free radical species).

Photo-reactivity is one of the parameters that traditionally are applied in models for prediction of environmental fate and behaviour (Stone et al. 2010). For ENMs the main focus in relation to photochemical reactions has been on their potential phototoxic activity. For example some metal oxide ENMs can act as photo catalysts and, upon excitation (absorption of photons), generate reactive oxygen species (ROS) by causing degradation of organic compounds and (eco)toxic effects. For example TiO2 ENMs have been found to be more toxic (two to four orders of magnitude higher) to the crustacean *D. magna* and the fish Japanese Medaka under simulated solar radiation (Ma, Brennan et al. 2012) compared to exposure under ambient laboratory light. The absorption of photons depends on the properties of the ENM (bandgap energy) and on the wavelength of the incoming light (photon energy). In brief, the ENM can be photoactivated if the photon has a higher energy than the ENM bandgap energy. If this is the case, unpaired electrons can be formed and ROS can be generated (Clemente, Castro et al. 2012). Both TiO2 and ZnO display high photocatalytic activity under UV illumination. Traditionally the anatase crystalline form of TiO2 has been considered as being a better photocatalyst compared to the rutile crystalline form. However, recent studies point to the fact that, when synthesized using low-temperature methods, the rutile form can also exhibit photocatalytic activity for degradation of organic pollutants - even superior to that of the anatase $TiO_2$ (Luan et al. 2012). Other metal oxides, such as $CeO_2$ and CuO, may also be photocatalytically active, but to a much lower extent than TiO2 (Miyauchi et al., 2002; Yabe & Sato, 2003). The ability of the light to penetrate the outer layers of the ENM, or the matrix in which it is embedded, influences its photocatalytic activity. This means that a surface coated ENM may be less photocatalytically active than the corresponding un-coated ENM (Nowack et al. 2012).

The environmental fate and behaviour of ENMs may change due to a photochemical transformation of the properties of the ENM itself. Such a transformation may occur if the generated ROS changes and/or degrades the ENM or components of the ENM. It has been found that for $TiO_2$ ENMs photoactivation may alter their binding to dissolved organic matter (Carp et al. (2004) as cited by Nowack et al. (2012)), which in turn may influence the ENM behaviour in the environment. At the same time, a photocatalytic reaction could also cause the degradation of (organic) coating material and hereby affect e.g. the stability, agglomeration and dissolution behaviour of the ENM.

Photochemical surface modifications may also occur as a direct result of photons interacting with the ENM. For carbon-based ENMs it has been found that light can induce such photochemical transformations. For example, it was found that $C_{60}$ was oxidized when exposed to UV-light. This increased the particles' negative surface charge and hydrophilicity (Qu et al. 2012). The conjugated π-bond electron structure of $C_{60}$ and CNTs enables them to adsorb sunlight (Chen & Jafvert 2010) and undergo chemical reactions. This is supported by a study in which CNTs exposed to UV light and oxygen were found to exhibit photoinduced oxidation (Savage et al. 2003), making photochemical transformation a relevant process influencing their fate in the environment. Evidence can also be found that oxidation of CNTs (i.e. increase in the amount of oxygen and oxidized carbon) is correlated to structural changes that increases their degradation in inter-atomic conjugation (Lachman et al. 2012).

Correspondingly, in a study of modifications of $C_{60}$ in aqueous suspensions caused by simulated aging conditions, it was observed that irradiation resulted in smaller aggregates sizes. In combination with oxygen the aggregate sizes were reduced further and sorption of PAHs was reduced due to modifications to the $C_{60}$ surface properties (Hüffer et al. 2013). The changes of the $C_{60}$ structure has been found to be induced by long-wavelength light ($\lambda \geq 400$ nm). The resulting photoproducts were found to mostly retain the $C_{60}$ structure but with a variety of oxygen-containing functional groups such as vinyl ether and carbonyl or carboxyl groups. As a result the original π-bond electron structure of $C_{60}$ is changed. Thus, the photoreactivity of aqueous dispersions of $C_{60}$ in sunlight causes the formation of water-soluble $C_{60}$ derivatives (Hou & Jafvert 2010) with reduced sorption affinity and capacity for nonpolar contaminants (Hüffer et al. 2013).

Direct photolysis or photochemical degradation is also relevant for some ENMs. For organically coated ENMs the chemical structure of the coating will generally determine the ability for a photochemical degradation and information on photo-degradability of the coating material can be used as an indication for modifications of the coated ENM. In these cases the ENM core material may remain untransformed but the photodegradation of the coating may change the fate and behaviour of the ENM.

With regard to modelling of environmental fate and behaviour of ENMs, photochemical transformations are relevant when 1) photochemical transformations change the surface properties of the ENM, consecutively influencing its degree of aggregation/agglomeration and adsorption to/of other surfaces/contaminants in the environment 2) when the surface coating of the ENM is composed of a substance that is known/likely to be photodegraded resulting in changes to its surface properties and 3) when the ENM is in itself composed of a material that is known/likely to undergo photodegradation (or other transformations) upon illumination.

Photochemical transformation could be considered relevant for some ENMs such as CNT (photoinduced oxidation), TiO2 and ZnO (to less extent CeO2 and CuO) (photo-activation changing binding to organic matter). Furthermore, different forms of silver are known to be prone to photochemical transformations and hence this is also likely to be true for silver nanoparticles, though the literature is sparse on this issue. The presence of surface coatings may alter the potential for photochemical transformation of the ENM.

### 2.4.2 Oxidation & reduction (redox reactions)

#### Summary

Redox reactions involve transfer of electrons between chemical species. The reaction processes of oxidation and reduction involve a loss or gain of electrons, respectively. Redox reactions are the basis for chemical transformation processes for inorganic substances, including dissolution, and are relevant for ENMs that participate in electron transfer. The redox potential expresses the tendency of a chemical species to undergo oxidation or reduction and is influenced by several environmental conditions (pH, presence of reducing or oxidizing agents etc.). There is some evidence of size dependent changes in redox potential for ENMs compared to bulk materials, but further investigations are needed to clarify this relationship. The appropriateness of currently applied methods to measure redox potential of ENMs is also under debate.

Of the case-study materials reviewed in this report, nZVI, Ag, CuO, and ZnO are considered the most likely ones to participate in redox reactions.

Redox reactions (or oxidation-reduction reactions) play an important role in many natural phenomena ranging from cellular respiration to geological processes (Tantra et al. 2012) through simple or complex reactions involving transfer of electrons between chemical species. The half-reaction processes of oxidation and reduction involve a loss or gain of electrons, respectively. For example the oxidation of silver nanoparticles can be described by the following half-reaction: $Ag^0 = Ag^+ + e^-$. Silver here acts as an electron donor, which increases its oxidation number through the loss of one electron. This electron can then reduce another chemical species (electron acceptor) in the system. Together the electron donor and the electron acceptor are called a redox couple. The redox potential expresses the tendency of a chemical species to undergo oxidation or reduction and is measured in millivolts (mV).

Possible links between redox potential and toxicity of ENMs has been discussed in the scientific literature. For example a theoretical framework is proposed by Auffan et al (2009) in which the cytotoxicity of metallic (metal or metal oxide) nanoparticles is correlated with their potential to be oxidised, reduced or dissolved in biological *in vitro* conditions. Transfer of electrons and/or ions during oxidation-reduction reactions is considered a main source of toxicity for metallic ENMs. Chemical stability under physiological redox conditions is therefore suggested as a theoretical framework to predict their toxic effects. Also Burello & Worth (2011) have coupled redox potential of ENMs with their potential of inducing oxidative stress.

With respect to chemical transformation of ENMs it is stated in the RIP-oN2 report (3.5.264) (Hankin et al., 2011) that the "OECD WPMN have highlighted that redox reactions are the basis of chemical transformations of inorganic and organic species and the precipitation and dissolution of inorganic substances that influences their sequestration and mobility (ENV/JM/MONO(2010)46). Hence, OECD suggests that measurement of the redox potential would be potentially meaningful for ENMs which can participate in electron transfer or uptake". Thus, redox potential and reactions are important, not only for ENM transformations in the environment, but also for the interaction of ENMs with natural redox phenomena (Tantra et al. 2012). At the same time the environmental conditions (e.g. pH and the presence of electron donors and acceptors) will influence whether redox reactions will occur (Nowack et al 2012).

The influence of particle size on redox potential of ENMs is not a well-investigated area. Work by (Ivanova & Zamborini, 2010) suggests a size dependent change in the standard redox potential for the Ag/Ag+ redox couple. This is described as being in correspondence with theoretical studies predicting a large negative shift in the redox potential for nanosized Ag ENMs. This negative shift for (small) Ag ENMs has been predicted to be inversely proportional to the nanoparticle radius relative to that of the bulk metal. The size-dependent change to redox potential for ENMs is a topic that requires further investigations.

### *Measuring redox potential*

The current OECD guidelines for the testing of ENM recommends that the redox potential is measured using a potentiometer (high impedance voltmeter) with an oxidation-reduction potential (ORP) electrode. This approach was for example used by Rogers et al. (2010) to measure the redox potential of cerium oxide nanoparticles dispersed in freshwater algal medium. It should however be noted that (Tantra et al. 2012) point towards several limitations of the applicability of this technique for measuring redox potential of ENMs and they conclude that the redox potentials measured are dominated by the media constituents rather than the particles studied. This is believed to be a result of little interaction between the ENMs and the ORP probe. Hence evaluation and development of appropriate methods to measure redox potential of ENMs is another area for additional investigations.

### 2.4.3 Dissolution and Speciation of ENM

## Summary

Particle dissolution kinetics (rate of solubility) and equilibrium solubility (amount of dissolved material) of an ENM will influence its environmental fate and toxicity. Although solubility is material-dependent, the solubility of a solid material is not only an inherent property as such but depends also on the media composition (e.g. ionic strength, ligands, pH, and temperature). For ENMs additional parameters are believed to play a role in the dissolution process, including particle size, state of aggregation, particle coating, water chemistry and presence of natural organic matter. Although no single model exists that takes into account all parameters expected to play a role in the dissolution of nanosized particles, most models agree that the dissolution rate increases with decreasing particle diameter. Upon dissolution the dissolved ions or molecules may form dissolved complexes with e.g. anions or organic matter (complexation) in the media or the ions may form a solid phase and sediment out (precipitation).

On the basis of available information from the literature the following case-study materials are considered of high relevance with regards to dissolution: ZnO, Ag, Cu/CuO, and QD (depending on specific chemical composition). Conversely, for the following materials solubility is considered to be of less concern: TiO2, CeO2, CB, nZVI and CNT.

Water solubility is a key parameter for the environmental fate and behaviour of certain ENMs (Klaine et al., 2008; Stone et al., 2010). In this section we describe the process of ENM dissolution in aqueous media and touch upon the related processes of precipitation and speciation/complexation, which involve interactions of the dissolved ions with other media components including biomolecules (e.g. natural organic matter, NOM).

Dissolution can generally be defined as when “individual molecules of the solute are separated by the molecules of the solvent” (Connell, 1997). Nanoparticles are not individual molecules but instead multi-molecular objects consisting e.g. of crystalline solids or metals in their elemental state ($Me^0$). This implies that ENMs do not dissolve in aqueous media but rather disperse in a particulate form. Nonetheless, ions or molecules may be released from the ENM surface, followed by diffusion, and become truly dissolved in the surrounding media. ENMs may hence be soluble to varying extends ranging from insoluble to poorly soluble, partly soluble or completely soluble, as illustrated by Figure 2.3. The extent to which they dissolve will influence their environmental behaviour, fate and effects (Misra et al, 2012), which may be dominated by their particulate form and/or the presence of dissolved species.

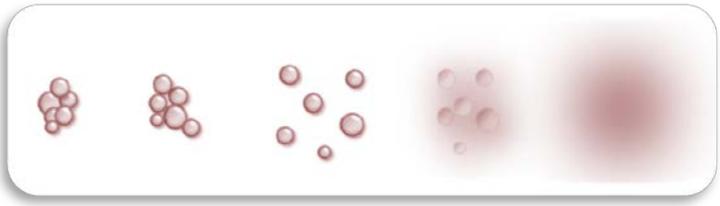


**FIGURE 2.3.**

DIFFERENT BEHAVIOURS OF NANOPARTICLES IN AQUATIC SYSTEMS. FROM LEFT TO RIGHT THESE ARE NANOPARTICLES AS SUSPENSIONS OF AGGREGATES OR AGGLOMERATES, COLLOIDAL SUSPENSIONS (SOLS), AS PARTLY DISSOLVED PARTICLES OR AS COMPLETELY DISSOLVED MATERIAL. (MODIFIED FROM HARTMANN, 2011)

The rate by which this process takes place governs which material form different environmental compartments will be exposed to. For example, it can be hypothesised that for metal-containing ENMs with a slow dissolution rate, benthic (sediment) organisms will have a higher exposure to metal ions than pelagic (water column) organisms, as the ENMs will distribute mainly into the sediment in the longer-term (Baun et al, 2008. Navarro et al. 2008). Also in a toxicity test system it is important to determine the dissolution kinetics and degree of solubility in order to correctly interpret the test results.

Particle dissolution kinetics (rate of solubility) and equilibrium solubility (amount of dissolved material) of an ENM is both influenced by media constituents and ENM properties, as outlined below. Upon dissolution into the aqueous media dissolved ions or molecules will interact with other media components, for example anions such as hydrogen carbonate, sulphate, or chloride and organic matter, to form e.g. dissolved metal complexes or precipitates. Precipitation is defined as the process by which dissolved species form a solid phase. This is the case when metal ions, released from an ENM, reassemble into a solid material. The processes of dissolution and precipitation are governed by the solubility product (Ksp), which expresses the equilibrium between a solid material and the ion concentration in solution. This is not an inherent property of the material but depends also on media composition (ionic strength, ligands, pH, and temperature) (Carp, 2004 as cited in Nowack et al. (2012). Different ENMs will behave differently in terms of these processes and the solubility product for ENM may be different from that of the bulk materials.

Details on the process of aqueous solubility of metals and metal oxides are included in this section whereas non-functionalized carbon-based ENMs will not be included due to their extremely low solubility in water. However, CNTs are known to contain metal impurities for which dissolution is important to consider – especially from an (eco)toxicity point of view. While functionalization of CNTs (e.g. hydroxylation) has been shown to increase the dispersability of CNTs in water, the CNT structure will still be maintained and they are not in true solution. This means that they keep their fibre structure and behaviour and should not be considered as dissolved species in fate modelling.

In the following we provide a general description of the dissolution of metal-containing ENMs. Nano-sized silver particles (Ag NPs) will be used as an example to illustrate the dissolution processes of metal-based ENMs and the different factors influencing these processes. The use of metallic silver particles have been registered in approximately 200 different consumer products (Nanodatabasen, 2013) and at the same time Ag NP have been shown to be toxic to living organisms (Hansen & Baun, 2012). Therefore Ag NPs are under intensive environmental and toxicological research (Nowack, 2010), including efforts to link observed adverse effects to dissolved Ag ions. It has, however, been pointed out by several studies that, upon release to the wastewater system, Ag NP will be transformed to silver sulphide (Ag2S) nanoparticles (Nowack, 2010; Kim et al., 2010). Estimates made by Blaser et al., 2008 show that biocidal use of silver (including silver nanoparticles, but also ionic and other forms) amounts to up to 15% of the total silver emissions into wastewater (Blaser et al., 2008).

In the ENRHES project, Stone et al. (2010) concluded that the environmental fate and behaviour of Ag NP was relatively unknown. This was also highlighted in a review by RIVM (Pronk et al., 2009), that described a lack of information on Ag NP dissolution kinetics and its correlation with particle and media properties. Since then some studies have focused on the environmental transformation of Ag NPs as reviewed by Levard et al. (2012). Engineered Ag NPs typically have an inner core of metallic silver ($Ag^0$) with an, usually, organic coating. These coatings will stabilize Ag NPs against aggregation and keep the particles suspended (Levard et al., 2012). Uncoated Ag NPs can be electrostatically stable because of the presence of negative charges on their surface due to hydroxide, oxide or sulphide groups (Levard et al., 2012). However, Gao et al. (2009) demonstrated that the dispersion of Ag NP is greatly influenced by the aqueous chemistry: in natural waters, Ag

NP aggregation depends on pH, Dissolved Organic Carbon (DOC) and presence of electrolytes (such as NaCl, NaNO3 and CaCl2 (Li et al., 2010).

The influence of the chemical composition of the water on particles sizes, dissolution and stability of Ag NP dispersions is however difficult to predict. A number of models have been developed to describe this dissolution process, but currently no model takes into account all parameters believed to play a role in the dissolution process, e.g. particle size, state of aggregation, particle coating, water chemistry and influence of natural organic matter. Especially the latter may play a major role in reducing the dissolution of Ag NPs in water due to a rapid coating (e.g. Gao et al., 2009).

*ENM intrinsic properties of relevance for dissolution*

The intrinsic properties of relevance for ENM dissolution include chemical composition, size, surface area, shape/curvature/morphology, crystal phase, and surface coating/chemistry (Misra et al., 2012). An overview of intrinsic and external physicochemical factors affecting dissolution of nanoparticles is seen from Figure 2.4. ENM properties can affect dissolution either directly by determining the ability of surface molecules to undergo reactions and of surface ions to be released from the particle – or indirectly by e.g. affecting aggregation which in turn influences the surface available for dissolution.

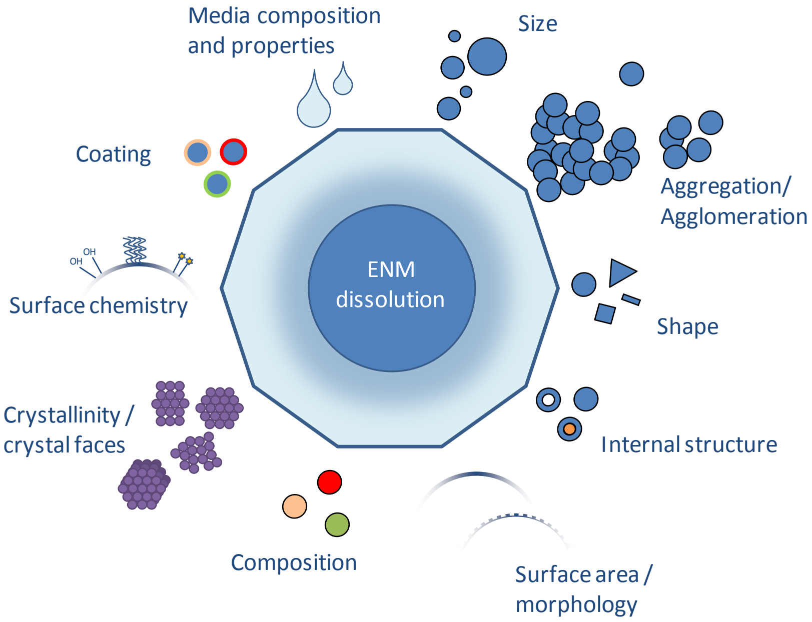


FIGURE 2.4.

VARIOUS INTRINSIC AND EXTERNAL PHYSICOCHEMICAL FACTORS AFFECTING DISSOLUTION OF NANOPARTICLES (PARTLY MODIFIED FROM MISRA ET AL 2012)

The solubility of metal oxides in water can be described by the following equation (Dyer et al., 1998):

$$MO_{\frac{Z}{2}}(s) + \frac{Z}{2}H_2O \leftrightarrow M^{Z+} + zOH^-$$

, where Z is the oxidation state of the metal

The solubility product, $K_{sp}$, can be expressed by the equilibrium equation:

$$K_{sp} = \{M^{Z+}\}\{OH^-\}^Z$$

Values of $K_{sp}$ can be found in the literature. However, it should be noted that $K_{sp}$ is temperature dependent and the appropriate case-specific value should be chosen (Dyer et al., 1998). The higher the $K_{sp}$ (or the less negative the $\log K_{sp}$), the higher is the solubility of the compound. Most metal oxides are considered to be insoluble or sparingly soluble in water and the degree of solubility depends partly on the inherent material properties including e.g. lattice energy (ionic bond strength). Specific values for $K_{sp}$ are mostly unknown for ENMs, but based on values from bulk materials it is found that metal elements are less soluble than metal oxides and within these CuO and ZnO are more soluble than $TiO_2$ and $CeO_2$.

Size and surface area have been found to substantially affect dissolution of metals and metal oxides. It is mentioned in recent ECHA guidance that "In the case of nanomaterials it is necessary to take into account that water solubility has the potential to increase for materials in the nanosize range" (EHCA, 2012b). For example, whereas bulk silver is considered insoluble in aqueous media, Ag NP releases free silver ions (Stone et al., 2010). In general, the equilibrium solubility of particles increases with smaller particle size as expressed by the Ostwald–Freundlich equation (Bian et al., 2011. See also e.g. Borm et al., 2006; Letellier et al., 2007; Mudunkotuwa and Grassian, 2011; Misra 2012):

$$\frac{S}{S_0} = exp\left[\frac{2\gamma\bar{V}}{RTr}\right]$$

, where S is the solubility of fine particles with radius r (in meters), $S_0$ is the solubility of the bulk material. $\bar{V}$ is the molar volume, $\gamma$ is the surface free energy, R is the gas constant, and T is the temperature

Although most of the presently available models agree that the dissolution rate increases with decreasing particle diameter, the relationship is not straight-forward and difficult to demonstrate. For example, the size of Ag NPs is often controlled by a coating agent, which in turn can influence solubility (Misra et al., 2012). The size dependence of ZnO dissolution was studied by Bian et al. (2011), comparing three different particle sizes: 4, 15 and 241 nm ZnO particles. It was found that the smallest particles dissolved more readily the dissolution of ZnO nanoparticles was generally enhanced for smaller particles (4 nm and 15 nm compared to 240 nm). However the 15nm ZnO particles dissolved to a larger extend compared to the 4 nm particles, and hence demonstrates a deviation from the generally assumed size-dissolution relationship expressed by the Ostwald–Freundlich equation. Possible explanations were proposed including the influence of aggregation and differences in surface tension due to different surface places and surface coatings. It was concluded that although there is a qualitative trend, surface dependent dissolution could not be quantitatively predicted by classical thermodynamics (Bian et al., 2011).

The influence of aggregation on ENM dissolution still requires further investigations. A decreased tendency of aggregated ENMs to dissolve would be explained by a decreased surface area of aggregates compared to mono-dispersed, or less aggregated, ENMs. Contradicting this hypothesis it has been found that Ag NP dissolution is controlled rather by the initial Ag NP size than by the aggregation (Kent & Vikesland, 2012). Conversely, dissolution of Ag NPs was slower in solutions with higher ionic strength, in which higher aggregation was 0bserved (Gondikas et al, 2012). Also, a seemingly shape-dependent difference in dissolution of spherical CuO and rod shaped CuO is suggested to be an indirect result of shape-dependent differences in aggregation (Misra et al 2012b). Concentration has also been found to affect equilibrium concentration of dissolved species and dissolution rate. For example Baek and An (2011) found that the dissolution rate of copper nanoparticles decreased with increasing particle concentration. Again, concentration dependent increase in aggregation could be a plausible indirect explanation for the decreased dissolution.

In spite of the complex relationship between ENM properties and dissolution some general trends can be drawn from the current state of knowledge. In qualitative terms dissolution kinetics and equilibrium solubility is likely to increase with smaller particle sizes, which should also be taken into account in modelling of ENM behaviour in the environment. In line with this, dynamic testing and modelling of metal uptake and toxicity has been proposed as the way forward, taking into account the non-equilibrium behaviour of Ag NP dissolution during toxicity testing (Veltman et al. 2010). However, until now only very few studies have focussed at this dynamic testing. At the same time it is clear that the influence of aggregation behaviour and kinetic as well as the presence of surface coatings on dissolution needs to be examined further.

### *Media properties influencing dissolution*

In addition to particle intrinsic properties also the properties of the surrounding media (pH, ionic strength, hardness, redox environment, inorganic ligands, and organic matter) can affect the dissolution of nanoparticles. As it is the case for particle intrinsic properties, the influence of media properties can be either direct (as for example the reaction between AgNPs and sulphide (Levard et al., 2012) or indirect (by e.g. affecting aggregation which in turn influences dissolution).

Several studies have identified pH as a key factor, generally resulting in an increased solubility of ENMs at lower pH. For example it was found by Levard et al. (2012) that at lower (environmentally relevant) pH there was a higher solubility of Ag NPs. The same was observed by Bian et al. (2011), where a complete dissolution of ZnO was observed at pH 1 during a 48h period compared to ~40% release of zinc ions of at pH 3 and ~15% at pH 6. At higher pH the dissolution was even lower (~2% at pH 11 and ~7 % at pH 9).

The presence of organic and/or inorganic components in the surrounding media has in some cases been found to catalyse the dissolution of nanoparticles, as in the case of cystine increasing the dissolution of AgNPs (Gondikas et al., 2012). The effect of humic acid on the dissolution of ZnO nanoparticles was investigated by Bian et al. (2011). The addition of 100 mg/L humic acid was found not to have any significant effect on dissolution at low pH (pH 1, 3 and 6). However, at high pH (pH 9 and 11) a significant increase in ZnO dissolution has been observed when humic acid was added.

At the same time, however, it is hypothesised by Misra et al. (2012) and Bian et al. (2011) that adsorption of organic matter can also cause steric protection which may reduce dissolution, analogous to a study finding inhibition of boehmite dissolution by the adsorption of Suwannee River fulvic acid (Yoon et al., 2005).

Redox conditions also play an important role in dissolution as a consequence of e.g. oxidation of metal particle surfaces. For example Ag NPs react with oxygen to form $Ag_2O$ on the particle surface followed by subsequent Ag+ dissolution into the surrounding aqueous solution (Levard et al 2012). The reaction can be described through the following equation:

$$\mathrm{Ag}_{(s)} + \frac{1}{2}\mathrm{O}_{2(aq)} + 2\mathrm{H}^{+}_{(aq)} \leftrightarrow 2\mathrm{Ag}^{+}_{(aq)} + \mathrm{H}_2\mathrm{O}_{(l)}$$

Conversely, the dissolution of Ag NPs is inhibited when dissolved oxygen is removed from the water (Liu et al 2010).

Under anoxic environmental conditions metallic silver is expected to react with sulphur. This process will both alter the surface charge and dissolution rate of Ag NP (Levard et al., 2012), and also affect their reactivity, mobility and toxicity.

### *Methods for measuring solubility*

With reference to OECD TG 105 on Water Solubility (OECD, 1995) and the 2009 preliminary review of OECD guidelines (ENV/JM/MONO(2009)21 ) (OECD, 2009) it was highlighted in the RIP-oN 2 report that “currently available standard methods for measuring dissolution may not be applicable” (Hankin et al., 2011). This is due to the fact that the OECD TG105, which includes both a micro-column method and a flask method, is developed for soluble substances. Its applicability to colloidal suspensions is not well-studied and some modifications may be required. Dissolution of metals and metal compounds in aqueous media can instead be measured by adding the metal compound to synthetic freshwater followed by agitation, solid-liquid phase separation (by filtration or ultracentrifugation) and analysis for total dissolved metal concentration in the water phase. This is also described in the OECD Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (ENV/JM/MONO(2001)9) (OECD, 2001). This guidance is recommended for the determination of water solubility of metals and sparingly soluble metal compounds in aqueous media under REACH (ECHA, 2012a). However, specific recommendations for nanomaterials are described in an appendix to the ECHA Guidance (ECHA, 2012b; ECHA, 2012c; ECHA 2012d). The potentially size-dependent solubility of nano-sized particles is highlighted and problems of distinguishing between truly dissolved and dispersed ENMs are emphasised as an analytical concern. The methods for solid-liquid phase separation may require specific considerations for nanomaterials. Historically the dissolved fraction has been operationally defined by its ability to pass through a filter of a specific pore size, e.g. 0.4-0.45µm (Nowack & Bucheli, 2007; USEPA, 2003; Mackay & Fraser, 2000). However this would allow for ENMs to pass through the filter. Other filtration methods have also been applied such as centrifugal filtration through 1-2 nm membranes (e.g. Poynton et al., 2011; Miao et al., 2010) or dialysis membranes. As described by Misra et al. (2012) sorption of heavy metals (incl. silver) may pose an obstacle for the use of filtration as a separation method for metal based ENMs. For CNTs with lengths of 0.5-2 um filtration through a microporous membrane showed a retention of >99% CNTs and could therefore theoretically be used to measure dissolution of trace metals from the CNTs. However, this method requires external pressure and the pores of the membrane tend to clog, limiting the feasibility of this method.

Methods to determine dissolution of nanoparticles in aqueous media was reviewed by Misra et al (2012). In addition to filtration and ultracentrifugation this review also highlighted the use of recombinant metal sensor bacteria and dialysis membranes. Also, monitoring the changes in particle characteristic and (number) concentrations will give some qualitative information on transformations and possible dissolution.

*Precipitation, speciation and formation of complexes*

Upon release of the metal ions from ENM it is likely that the ion will interact with the components of the surrounding media. This may lead to changes in the speciation of the metals by the formation of water soluble complexes as well as precipitates which in term may settle out of the aqueous media. The formation of specific metal complexes will depend on media constituents (including identity and amount of anions, cations, and organic matter, e.g. humic and fulvic acids), temperature and pH. Geochemical speciation models can be used to model the complexation processes when metal ion concentrations, media chemistry and relevant complexation and solubility constants are known. This is a common approach used for evaluation of metals in water and hence not specific to modelling of ENM behaviour. However, it must be emphasised that the values for e.g. solubility constants for bulk materials may be significantly different from those of the materials in its nano-forms. Further information on geochemical modelling can be found e.g. in (Louma and Rainbow, 2008).

An example of change in speciation upon release to the environment is the reaction of silver ions and sulphur under anoxic environmental conditions. A recent study by Lowry et al. (2012a) demonstrated that 18 months after addition on AgNPs to freshwater mesocosms, the speciation of Ag in sediments was indeed dominated by $Ag_2S$ and Ag complexed with reduced S in organic matter (Ag-sulhydryl). However, Lowry et al. (2012a) found that even though AgNPs were transformed to S-containing species, some of the added Ag was taken up by plants, fish and insects in the mesocosms showing that Ag originating from the NPs was bioavailable even after transformation in the aquatic environment.

## 2.5 Physical transformations of nanomaterials in the environment

### 2.5.1 Aggregation and Agglomeration

### Summary

Attractive forces between particles may cause them to cluster together, forming aggregates or agglomerates. This can occur during production, storage, use, and after emission to the environment - independent of whether the nanoparticles are in solution, powder form or in the gas phase. Aggregates are defined as clusters of particles held together by strong chemical bonds or electrostatic interactions. Aggregation is thus considered an irreversible process. Agglomerated particles are held together by weaker forces and can be a reversible process. The direction of this process will depend on conditions of the surrounding media.

The processes of aggregation and agglomeration significantly influence the fate and behaviour of ENMs in the environment, with a dependency of particle properties (e.g. size, chemical composition, surface charge) as well as environmental conditions (e.g. mixing rates, pH, and natural organic matter). In some cases ENMs are deliberately coated to counteract these processes. Aggregation and agglomeration may lead to changes in bioavailability of ENMs and serve as a starting point for sedimentation of ENMs in the environment. The interactions between particles leading to agglomeration can be described theoretically by the DLVO (Deryaguin, Landau, Verwey and Overbeek) theory, which describes the aggregation of particles in a liquid as a result of the interaction energy (sum of attractive and repulsive forces) between particle interfaces. The applicability of this theoretical approach to ENMs will be presented and discussed here, as this might be integrated in chemical fate modelling.

Aggregation and agglomeration is considered relevant for all ENMs that are part of the NanoDEN project – and is a key process for ENMs in all environmental compartments. The processes aggregation and agglomeration result from different combinations of particle properties and environmental conditions, and are highly probable to occur at some point during release from products, as well as during emission and/or residence in the environment.

The role of aggregation and agglomeration for the fate and behaviour of ENM in the environment has been highlighted in numerous studies (e.g. Navarro et al., 2008; Baalousha et al., 2009; Quik et al., 2010). Nanoparticle aggregation and agglomeration can occur at all stages of the life-cycle of the nanoparticles e.g. in production, storage and during handling. If the ENMs are not coated, or otherwise stabilized, these processes will inevitably occur, independent of whether the nanoparticles are in solution, in powder form or suspended in air (Stone et al., 2010, Lowry et al., 2012). Aggregation and agglomeration also occurs in test media of eco-/toxicological tests and in the procedures to prepare test suspensions. Aggregation and agglomeration in test media seems to depend on the nanoparticle size, the chemical composition of the nanoparticles, the surface charge of the nanoparticles as well as media composition, mixing rates, and the presence of natural organic matter, e.g. humic acid (Lowry et al., 2012b).

The interactions between particle interfaces in dispersion can be described by the DLVO theory (Deryaguin, Landau, Verwey and Overbeek). Briefly this theory states that the overall interaction energy between such particle interfaces is the sum of repulsive electrostatic Coulomb (double layer interaction) forces and attractive van der Waals forces. This will be described further in the following, which is largely focused on the applicability of the DLVO theory to ENMs, as this might be integrated in chemical fate modelling.

*Definitions*

Agglomerates are defined as clusters of primary particles held together by van der Waals forces (Figure 2.5). Agglomeration is a reversible equilibrium reaction or process and the direction of this process will depend on environmental conditions. As the primary particles still exist as individual entities, the specific surface area of the individual particle is constant. Aggregates are defined as clusters of particles held together by strong chemical bonds or electrostatic interactions, i.e. covalent or ionic bonds. Aggregation is an irreversible process in which a material in the nano-scale may be converted into a bulk material. Contrary to agglomeration, aggregation causes a decrease in specific surface area compared to the individual particle (Nichols et al. 2002; Oberdörster et al. 2007; Aitken et al. 2010).

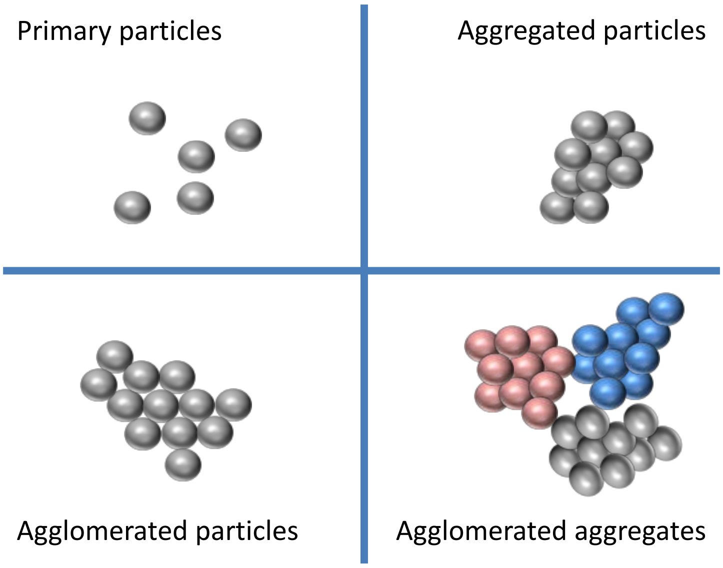


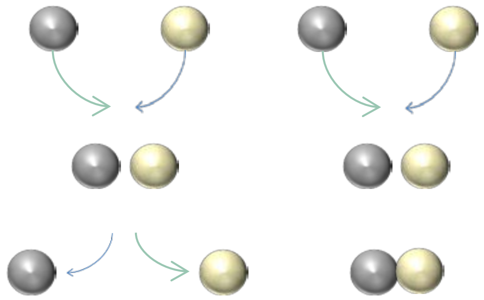
**FIGURE 2.5.**

ILLUSTRATION OF THE DIFFERENCE BETWEEN PRIMARY PARTICLES, AGGREGATED PARTICLES, AGGLOMERATED PARTICLES AND AGGLOMERATED AGGREGATES OF PARTICLES (REDRAWN FROM OBERDÖRSTER ET AL. 2007)

ENMs in the environment, in test media or elsewhere do not exist as *either* primary particles *or* agglomerates *or* aggregates, but occur simultaneously in a mix of different states. Therefore, it is difficult to distinguish between agglomerates and aggregates in practice, meaning that these two terms are often used interchangeably. In the following we will use the term agglomeration to cover both processes unless the description specifically relates to aggregation. It is hard to theoretically distinguish the two interacting processes and cumbersome to quantify them analytically. However, there are well-established theories that describe colloidal stability and the processes and forces leading to agglomeration.

*Collision rate and collision efficiency*

In the environment, ENMs will move as a result of Brownian motion, gravity and fluid motion (Allen et al., 2001). The term "Brownian motion" is used to refer to the random movement of particles suspended in a fluid. In the course of their motion in a fluid the ENMs may collide with one another, which can be described as a collision rate (frequency of collisions). Upon collision, agglomeration is determined by the sum of attractive and repulsive forces between the particles. The ratio (%) of collisions that leads to agglomeration can be described as the collision efficiency. This is illustrated by Figure 2.6. For ENMs their movement by Brownian diffusion is considered to be the predominant factor in ENM agglomeration compared to sedimentation by gravity and shear-driven fluid motion (Petosa et al., 2010).



Collision rate

**Result of:** Brownian motion, gravity, fluid motion

**Influenced by:** Concentration, Temperature etc.

Collision efficiency

**Result of:** attractive and repulsive forces

**Influenced by:** Surface potential, media composition incl. ions, NOM etc.

**FIGURE 2.6.**

IN THE COURSE OF THEIR MOTION IN A FLUID THE ENMS MAY COLLIDE WITH/COME VERY CLOSE TO EACH OTHER. THE FREQUENCY OF COLLISIONS CAN BE DESCRIBED BY A COLLISION RATE OR COLLISION FREQUENCY, EXPRESSED AS NUMBER OF COLLISIONS PER UNIT TIME. UPON COLLISION, AGGLOMERATION IS DETERMINED BY THE SUM OF ATTRACTIVE AND REPULSIVE FORCES BETWEEN THE PARTICLES. THE RATIO (%) OF COLLISIONS THAT LEADS TO AGGLOMERATION CAN BE DESCRIBED AS THE COLLISION EFFICIENCY.

Classic collision theory predicts that collision rate, and therefore potentially agglomeration and aggregation, will be more pronounced at higher particle concentrations due to a higher collision probability. This tendency has been demonstrated for ENMs by Piccapietra et al. (2011), who found that, under unstable conditions at low pH and high electrolyte concentrations, AgNP agglomeration was proportional to the particle concentration in suspension (Piccapietra et al., 2011). These findings are similar to Phenrat et al. (2007) who found that higher concentrations of iron nanoparticles (60 mg/L) resulted in higher aggregation rates and stability of aggregate size in comparison with lower concentrations (2 mg/L) and that the aggregation rate has a second-order dependence on particle concentration (Tourinho et al., 2012). Other studies have also found a non-linear concentration-aggregation relationship (Baalousha, 2009; Arvidsson et al., 2011).

Particle collision efficiency is one of the important parameters for ENM fate modelling purposes. Based on theoretical calculations of attractive and repulsive forces, the DLVO theory is often used to describe agglomeration and colloidal stability (Tourinho et al., 2012). However, it is not a trivial exercise to assign a value to the collision efficiency under environmentally relevant conditions. For example current approximation equations only account for electrostatic forces between pure particles in water not taking natural organic matter (NOM) in to account. Here we describe the current state of knowledge on the applicability of DLVO theory to ENMs including suggested modifications to the classic DLVO theory.

### *DLVO theory – principles and applicability in the real world*

Particles will only remain dispersed as individual (primary) particles if there is some mechanism to prevent them from attaching to each other upon collision. For instance, if all the particles have the same electrical charge (either positive or negative), they will repel one another as they approach one another. The system is then said to be colloidally stable (Allen et al. 2001). Colloid stability in a fluid has been described by the DLVO theory, based on the assumption that in any stabilized fluid there are two opposite directed forces: an electrostatic double layer repulsion that prevents agglomeration/aggregation and van der Waals force that binds particle together.

Whereas van der Waals attraction is negligible for particles that are far apart, particles will stick together if they come within a few nanometers from each other and van der Waals attractions can be strong at short distances (< 10 nm). Thus, the sum of these two opposite forces is important in determining the nature and kinetics of agglomerates (Allen et al., 2001, Elzey and Grassian, 2010, Loux et al., 2011).

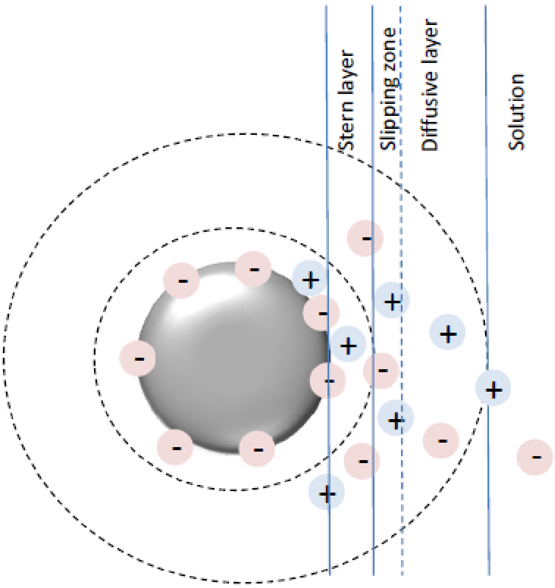


FIGURE 2.7.
ILLUSTRATION OF A PARTICLE WITH NEGATIVE SURFACE POTENTIAL SUSPENDED IN AN AQUEOUS MEDIA. ADSORBED IONS ARE PLACED IN THE SO-CALLED STERN LAYER, ADJACENT TO THE SURFACE, FOLLOWED BY A DIFFUSIVE LAYER CONSISTING OF MORE MOBILE IONS. TOGETHER THE STERN LAYER AND THE DIFFUSIVE LAYER FORM THE ELECTRIC DOUBLE LAYER (EDL).

The repulsive double layer forces are electrostatic forces that form on surfaces interfaces, for example on the interface between a particle and water in an aqueous system. This interphase is called the electric double layer (EDL) and refers to the Stern layer and the diffusive layer surrounding the particle (See Figure 2.7). The Stern layer consists of immobile ions on the surface of the particle, bound by electrostatic forces, whereas the diffuse layer consists of mobile ions. The EDLs are formed in the presence of ions: when for example ENMs are introduced into a media they will equilibrate with media constituents by adsorption or desorption of charged species (anions, cations; Lyklema, 2005), where the specific adsorption or desorption will depend on the particle surface potential. A double layer can also be formed by adsorption of anionic surfactants to a hydrophobic surface, which corresponds to the surfactant adsorption that form a coating of the ENMs. The double layer hence creates a ‘counter charge’ making the overall ENM-EDL structure electrically neutral (Lyklema, 2005). The above mechanisms are summarized in Figure 2.7. From this it can be deducted that EDL forces will be a result of ENM properties and those of the surrounding media (presence of charged species). According to the DLVO theory it follows that, if repulsive double layer forces become smaller than the attractive van der Waal forces, particles will agglomerate in the media.

Several studies have compared the DLVO theory with experimental observations of ENM agglomeration behaviour. For example for cerium oxide nanoparticles Buettner et al. (2010) found that DLVO theory was able to provide an adequate prediction for the interactions between cerium oxide nanoparticles. Liu et al. (2010, 2011b) noted that aggregation of silicon and boron ENMs satisfied classical DLVO-theory. Dissolution was observed for Ag NPs with various coatings by Li et al. (2011). However, for all coatings, the behaviour of Ag NPs was still consistent with classical DLVO- theory. This was also found by Liu et al. (2012) and Liu et al. (2010) for ZnO and Ag under various electrolyte concentrations. For CNTs, Petersen et al. (2011) have also noted that the aggregation behaviour of functionalized CNTs was in qualitative agreement with the principles of classic DLVO theory.

The composition of the environmental media highly influences agglomeration behaviour. Piccapietra et al. (2011) found that agglomeration of carbonate-coated AgNP commence at a concentration of 2 mM $Ca^{2+}$ and 100 mM $Na^+$ both in natural water and in synthetic media. This is in line with the DLVO theory since the electrical double-layer on the particle surface is strongly compressed under these conditions and the average zeta potential approaches zero. Also, transport and retention of $C_{60}$ in saturated quartz has been found to be strongly dependent on electrolyte conditions (Wang et al. 2012b). Suppression of the electrical double layer was found to explain that the increase in electrolyte concentration caused minimal changes in the diameter of $C_{60}$ aggregates in the presence of NaCl and a sevenfold increase in the presence of $CaCl_2$ (Wang et al., 2008).

In studies of behaviour and transport of $Fe_3O_4$, $TiO_2$, CuO, and ZnO ENMs in porous media, Ben-Moshe et al. (2011) found that increasing ionic strength and lower flow rates enhanced the deposition of the nanoparticles whereas changes in pH had little effect. In contrast, the addition of humic acid increased the nanoparticle mobility significantly. The findings by Ben-Moshe et al. (2011) were found to be in good agreement with DLVO-theory, when assuming that the particles were spherical. $TiO_2$ was found to exhibit the highest dispersion stability and mobility in the porous media column. This is in agreement with DLVO calculations predicting that there will be an electrostatic barrier for $TiO_2$ indicating the suspension is stable. According to DLVO, there is a small electrostatic barrier for CuO and no barrier for $Fe_3O_4$ and ZnO, suggesting rapid aggregation due to attractive van der Waals forces. In the experiments, however, $Fe_3O_4$ demonstrated higher stability than expected from the simple DLVO theory (Ben-Moshe et al. 2011).

The aquatic stability of 4 ± 1 nm ZnO ENMs as a function of pH, ionic strength and adsorption of humic acid was studied by Bian et al. (2011). DLVO interaction energy curves were calculated for ZnO ENMs as a function of the different parameters and on this basis the experimental findings were found to be consistent with the trends expected from DLVO theory.

When it comes to exposure assessment, it is important to know the rate of growth of particle aggregates as a function of time. Models have been developed that can be used to predict the equilibrium aggregate size distribution under different thermal and shear conditions. Though some studies exist (e.g. Liu et al., 2012), the literature review revealed a lack of studies focused at these kinetic changes for ENM in environmentally relevant media.

*Methods for quantifying agglomeration*

A number of methods exist to determine agglomeration of ENMs and these are often used in combination. Examples of applied methods include electron microscopy (EM) techniques (e.g. scanning EM (SEM), transmission EM (TEM) or scanning transmission EM (STEM)), scanning probe microscopy techniques (e.g. atomic force microscopy (AFM) or scanning tunnelling (STM)), centrifugation techniques (e.g. analytical ultracentrifugation, ANUC), spectroscopy techniques (e.g. dynamic light scattering (DLS), X-ray diffraction (XRD) or small angle neutron scattering (SANS)) or measurements of the zeta potential. All methods have their specific benefits and are able to provide different information on agglomeration and agglomerate properties. At the same time they also have different disadvantages, which include for example artefacts induced by sample preparation, problems in measuring polydisperse samples etc. This has been described well in the literature and for an overview of uses, applicability, pros and cons of various techniques for ENM agglomeration quantification and characterisation we refer the reader to Tiede et al. (2008).

Measurement of zeta potential is an expression of electro-kinetic potential and can be considered an ‘indirect’ way of measuring ENM agglomeration. Zeta potential corresponds to the difference in electric potential between the surrounding media and EDL at the location of the slipping plane (see Figure 2.7) (Heimann, 2010). At a low zeta potential the difference in electric potential is low and the electrostatic repulsive forces are overcome by attractive van der Waals forces meaning that the ENMs agglomerate. A higher zeta potential (in absolute terms) corresponds to a larger difference in electric potential whereby de-agglomeration will occur and the colloidal systems will remain more stable and dispersed. In suspensions zeta potential measurements can be used as an indication of agglomeration state and dispersion stability of nanoparticles (see Table 2.2) (Hankin et al., 2011). The stability behaviour of the colloid suspension is often divided in ranges from “Very unstable” to “Highly stable” based on measurements of Zeta potential.

Measurements of the surface charge or the zeta potential as a function of pH and salt concentration can and has been used to predict the extent of agglomeration (Allen et al. 2001). As the pH increases, agglomeration should occur regardless of the salt concentration.  As the salt concentration increases, the instability regime widens.

**TABLE 2.2**

ZETA POTENTIAL (MV) VERSUS STABILITY BEHAVIOUR OF COLLOID SUSPENSIONS

| Zeta potential (mV) | Stability description of the colloid suspension |
|---|---|
| 0 to ±5 | Coagulation/flocculation |
| ±10 to ±30 | Unstable |
| ±30 to ±40 | Moderate stability |
| ±40 to ±60 | Good stability |
| >±60 | Excellent stability |

Table 2.2 lists the general “rule-of-thumb” for interpretation of zeta potentials in terms of colloid suspension stabilities. However, it should be noted that zeta potential measurements alone may not be sufficient to determine suspension stability and should be accompanied by measurements of the particle size distribution and/or visual observations using other techniques such as e.g. DLS and microscopy. A study of TiO2 ENMs by Almusallam et al. (2012) is an example of combining zeta potential, DLS and AFM to measure growth kinetic of hydrodynamic diameters of aggregate/agglomerates. Also, while zeta potential is an expression of the extent of agglomeration, another key aspect of agglomeration and aggregation is the question of how strongly the ENMs are held together. For this purpose a method to measure the strength of the agglomerates is needed (NANOTRANSPORT, 2008; Hankin et al., 2011).

In addition to the above mentioned analytical techniques a number of simulations models for ENM agglomeration have been developed. For instance, Liu et al. (2011a) have developed a constant-number Direct Simulation Monte Carlo (DSMC) model for the analysis of nanoparticle agglomeration in aqueous suspensions. The model is based on the “particles in a box” simulation method which considers particle agglomeration and gravitational settling as well as particle-particle agglomeration probability. This, in turn, is based on the classical DLVO-theory and on considerations of the collision frequency as impacted by Brownian motion. A reasonable agreement was seen between model predictions and measured particle size distributions and agglomerate sizes for TiO2, CeO2, and C60 in aqueous media with a pH of 3-10 and an ionic strength of 0.01-156 mM). Based on this work it was suggested that a DSMC modelling approach, in combination with an extended DLVO theory, could potentially become a prediction-tool to calculate the agglomeration behaviour ENMs in aqueous suspensions. In this context, Handy et al. (2012) provides a review of the recent developments of user-friendly software that can be used to predict particle behaviour in test media based on DLVO theory.

*Effects of Natural Organic Matter and Humic Acid on agglomeration and aggregation of ENM*
The effect of natural organic matter (NOM) on the agglomeration of ENMS is complex, since it can both enhance and reduce agglomeration (Arvidsson et al., 2011). A large number of studies have observed that the presence of NOM and humic acid (HA) affect aggregation of nanoparticles such as iron oxide, TiO2, silicon, SWCNT, Ag  (Hu et al., 2010, Baalousha et al., 2009, Domingos et al. 2009, Liu et al., 2010, Liu et al., 2011b, Akaighe et al., 2012).

Using Field Flow Fractionation (FFF) and DLS, Baalousha et al. (2008) investigated the interactions between unpurified iron oxide 7 nm nanoparticles and standard Suwannee River humic acid (SRHA) under a range of environmentally relevant conditions. Baalousha et al. (2008) found that larger aggregates were formed with increases in both pH (from 2 to 6) and SRHA concentration (from 0 to 25 mg/L) noting that a 1nm thick SRHA surface coating was formed on the iron oxide nanoparticles and that the thickness of the coating was concentration-dependent. The presence of SRHA also affect the structure of the iron nanoparticle aggregates as they were observed to be open and porous in the absence of SRHA and compact in the presence of SRHA.

Similar to Baalousha et al. (2009), Domingos et al. (2009) studied aggregation of 5 nm bare TiO2 particles and the effects of various concentrations of the Suwannee River Fulvic Acid (SRFA) as well as pH and ionic strength. For pH values near the zero point of charge, aggregation was observed to increase and an increase in ionic strength generally resulted in increased aggregation independent of pH. Adsorption of the SRFA was furthermore observed to result in less aggregation of $TiO_2$ nanoparticles, presumably due to increased steric repulsion according to Domigos et al. (2009). Domigos et al. (2009) found that under environmentally relevant conditions of SRFA, pH, and ionic strength, ENM dispersions were often stable. This suggests that in the natural environment $TiO_2$ ENM dispersion might be more stable than what should be expected from experiments performed in synthetic media.

When studying the aggregation behaviour of nano boron and CeO2, Liu et al. (2010) and Quik et al. (2010) also found evidence that the addition of SRHA caused the boron nanoparticles to stabilize and the increase in the electrostatic repulsion is also here suggested to be the main cause of the induced stabilization.

Bian et al. (2011) studied the aquatic stability of 4 ± 1 nm ZnO ENMs as a function of pH, ionic strength and adsorption of HA. They found experimentally that addition of HA at low concentrations increases ZnO sedimentation whereas more stable dispersions may be obtained at higher HA concentrations. DLVO interaction energy curves were calculated for ZnO ENMs as a function of HA concentration. On this basis the experimental findings were found to be consistent with the trends expected from DLVO theory. In the absence of HA, ZnO nanoparticles have a net repulsive energy barrier at low ionic strength. With the addition of HA the energy barrier decreases at first and approaches zero, causing the observed increased aggregation at a dissolved HA concentration of 1.7 mg/L (10 mg/L initial mass concentration). This is followed by an increase in net repulsive energy barrier and more stable suspensions with further addition of HA (Bian et al. 2011).

In a study of the effect of HA on surface charge status and aggregation potential of magnetite ($Fe_3O_4$) NPs, Hu et al. (2010) observed rapid aggregation, independent of solution chemistry, when the pH is close to the point zero charge and the ionic strength is above the critical coagulation concentration. These authors also saw that a small dose of 2 mg $L^{-1}$ HA stabilized the suspension significantly. A subsequent DLVO analysis revealed the possible presence of secondary energy minima and the possibility of de-agglomeration of magnetite agglomerates.

Li and Huang (2010) observed that SWCNT were relatively stable in water and that their aggregation was not sensitive to pH over the range of 3–8. The effect of HA on the aggregation of SWCNT was negligible in the presence of $CaCl_2$ and $AlCl_3$.

Whereas most studies indicate that HA stabilises various types of ENM, there are exceptions to this rule. For instance, Akaighe et al. (2012) studied the formation of Ag nanoparticles formed from the reduction of Ag+ by SRHA/NOM and observed that the nanoparticles were very unstable at high ionic strength solutions and that the presence of SRNOM and SRHA contributed to this instability.

According to Akaighe et al. (2012), this is most likely due to intermolecular bridging with the organic matter which suggests that changes in solution chemistry can greatly affect nanoparticle long term stability and transport in natural aqueous environments. Similarly, Gao et al. (2012) found that a larger fraction of AgNPs remained dispersed and stabilized after 2 days in water with SRHA concentrations <10 mg TOC $L^{-1}$. Increased aggregation was induced at higher SRHA concentrations (above 10 mg TOC $L^{-1}$). This corresponds well with measurements of zeta potential that increases (in absolute values) up to 10 mg TOC $L^{-1}$ and then decreases at higher TOC concentrations (Gao et al. 2012). These findings are somewhat in contrast with the above mentioned findings of e.g. Baalousha et al. (2009) and Bian et al. (2011).

### *Shape, size and coatings and their influence on aggregation and agglomeration*

Only a limited number of studies have investigated whether other factors than HA have an influence on ENM agglomeration/aggregation behaviour. Liu et al. (2011a) investigated the sedimentation and aggregation kinetics of $TiO_2$ NPs with varying material properties (i.e., crystallinity, morphology, and chemical composition). It was found that aggregation kinetics fitted reasonably well with the DLVO equations for all the $TiO_2$ nanoparticles tested and that crystallinity and morphology are not influential factors in determining the stability of $TiO_2$ nanoparticle suspensions. Differences in concentrations of impurities, such as silicon and phosphorus, may however have influenced the sedimentation and aggregation of $TiO_2$ nanoparticles in the aqueous phase. Li et al. (2011) studied aggregation behaviour of Ag NPs coated with three different kinds of coatings e.g. trisodium citrate, sodium dodecyl sulphate (SDS), and Tween 80 (Tween). No difference in aggregation was observed when it came to citrate-coated particles and SDS-coated particles and the uncoated particles, but the Tween stabilized particles were significantly more stable which could be explained by the Tween coating – and to some extent SDS - ability to impart steric interactions of the particles. Similarly, Prathna et al. (2011) found that biological vs. chemical coating of Ag NPs affected their aggregation behaviour in in aqueous environmental matrix (lake water, sea water) and biological matrix (Potato Dextrose broth, Luria Bertani broth). Hence the type of ENM coating is very important in understanding its aggregation behaviour in aqueous matrices.

### *Deviations from DLVO theory*

Although, quite a few studies have found that the aggregation behaviour of various ENMs are in accordance with DLVO-theory, there is a growing body of literature that suggests that DLVO-theory may have to be adapted. The reason why the DLVO theory has limitations with regards to ENMs is described as a result of the novel surface chemistries and novel shapes (Lowry et al., 2010; Hotze et al., 2010). It is further emphasized that the DLVO theory has never been successfully applied to complex matrices, such as the environmental ones, but is rather a method to explain behavioural trends of particles in the environment (Lowry et al., 2010). Another questionable assumption is highlighted by (Zhou et al., 2012), i.e. the assumption that the energy-separation distance between primary particles is representative for interaction between aggregates when calculating the behaviour of ENMs in suspension, which might not be the case.

These limitations have also been demonstrated experimentally. For example Chowdhury et al. (2012) studied the transport of TiO2 ENMs in macroscopic porous media and found that, in addition to DLVO forces, the transport was controlled also by non-DLVO-type forces, aggregation, shear forces and gravitational forces. El Badawy et al. (2012) investigated the aggregation kinetics of sterically and electrosterically stabilized AgNPs, which were found not to be in agreement with the DLVO theory. It was observed that the AgNPs were resistant to aggregation “even at high ionic strength and electrolyte valence” El Badawy et al. (2012). Mudunkotuwa & Grassian (2011) found that an extended DLVO theory can be useful due to the polymeric nature of the dissolved organic matter adsorbed on ENM surfaces. Loux et al. (2011) argue that “...traditional DLVO theory can be extended through either the adoption of additional non-DLVO energies and/or describing the interaction between particles and dissimilar (environmental) surfaces".

The most significant non-DLVO forces that affect ENMs in aquatic media are considered to include steric interactions, magnetic forces (relevant for e.g. nZVI and other iron ENMs) and hydration forces (Petosa et al., 2010). Hence, there seems to be some evidence that a modified DLVO theory is needed to describe the aggregation behaviour of ENMs. However, this requires incorporating one or more additional parameters with their associated uncertainties.

### 2.5.2 Sedimentation

#### Summary

Sedimentation is linked to aggregation as larger particles tend to settle more rapidly, and the gravitational settling can be expressed by Stoke's law where settling velocity as a function of fluid viscosity and density as well as particle radius and density. Stoke's law implies that larger agglomerates and aggregates will settle more quickly compared to more disperse particles. This also means that agglomeration is the rate limiting factor for sedimentation of ENMs in the aquatic environment.

As for agglomeration and aggregation, sedimentation is considered relevant for all ENMs that are part of the NanoDEN project and as a potential key removal mechanism for ENMs in the aquatic environmental compartments.

Deposition mechanisms, such as gravitational settling, are of high importance to ENM environmental fate modelling (Quik et al., 2010).  Sedimentation is a potential major removal process whereby ENMs are removed from the aqueous phase and transported to the sediment phase. Understanding and describing these processes are therefore of key importance to reliable ENM exposure estimations.

There is a clear and logic link between agglomeration and sedimentation as a result of the shift of the particle size distribution towards larger sizes during the agglomeration process of dispersed particles. The larger particles and agglomerates will diffuse more slowly but tend to settle more rapidly under gravity.  Sedimentation may also be affected by so-called gravitational agglomeration, where the slower settling (smaller) particles/agglomerates/aggregates are captured by the more rapidly settling (larger) particles. Brownian motion and Brownian agglomeration are processes of high importance for primary nanoparticles, but as agglomerates/aggregates grow bigger the process of gravitational agglomeration becomes more important.

The gravitational settling of particles can generally be described by Stoke's law that expresses gravitational settling velocity as a function of fluid viscosity and density, particle radius and density (Lamb, 1993). The particle radius used in Stoke's law, the so-called Stoke's radius, is the radius of a hard sphere that diffuses with the same velocity as the investigated particle. Hence, it is an artificial radius size that also takes into account the deviation of the particle in shape and structure from a hard sphere, for which Stoke's law applies. Stoke's law implies that larger agglomerates and aggregates will settle more quickly compared to smaller single particles. This also means that agglomeration is the rate limiting factor for sedimentation of ENMs in the aquatic environment.

The sedimentation behaviour of NMs in aqueous systems has been described in several studies, where the complexity of the environmental matrices range from artificial media with added NOM to natural freshwater and to sea water (e.g., Keller et al.,2010; Quik et al., 2010; Von der Kammer et al., 2010). For example it was found by Quik et al. (2010) that the sedimentation of CeO2 ENMs was greatest in synthetic algae media (average particle diameter: 417 nm) compared to deionized water (average particle diameter: 301 nm). The addition of NOM was found to reduce agglomeration in algal media and sedimentation was reduced with increasing NOM concentration.

Quik et al. (2014) recently presented an overview of sedimentation/ aggregation behaviour for four ENMs ($C_{60}$, CeO2, SiO2 –Ag and PVP–Ag) in a series of different waters, ranging from a small stream to seawater. Medium to long timescale experiments provided information on sedimentation and hetero-aggregation. The results are considered to have a high reliability as they were based on different (possibly realistic) doses of ENM, varying water types and ENM dispersions and on a longer investigation period (15 days). When exposed to natural colloids, the observed ENM sedimentation rates were in the range of 0.0001 m $d^{-1}$ for $SiO_2$–Ag and 0.14 m $d^{-1}$ for $C_{60}$. The observed rates of hetero-aggregation (highest rates were found for seawater) between nanomaterials and natural colloids ranged from 0.007 to 0.6 L $mg^{-1}$ $d^{-1}$. These sedimentation results were derived from quiescent settling experiments by comparing total initial and non-settling concentrations considering sedimentation length and dissolution dynamics. Hetero-aggregation between ENM and natural colloids was quantified using a method that distinguishes hetero-aggregation and homo-aggregation by following aggregation-settling equations that were applied to data from filtered or unfiltered liquids.

## 2.6 Interactions with other surfaces and substances

Adsorption and desorption of ENM onto different solid surfaces is of great importance for understanding their transport and fate in the environment. For example ENMs may adsorb to particles in sediments, soil, or to suspended solids in water affecting their mobility and transport. In this case the ENM act as sorbate (see Figure 2.8). Also the adsorption of substances from the surrounding media onto the ENM will determine the ENM characteristics and behaviour. This is for example the case when organic matter in the environment adsorbs onto the ENM surface. In this case the ENM hence acts as sorbent (see Figure 2.8). The adsorption of organic matter (such as HA) has been touched upon in most of the previous sections. Here we will limit the description to a short summary with main focus on the adsorption of ENMs to other surfaces.

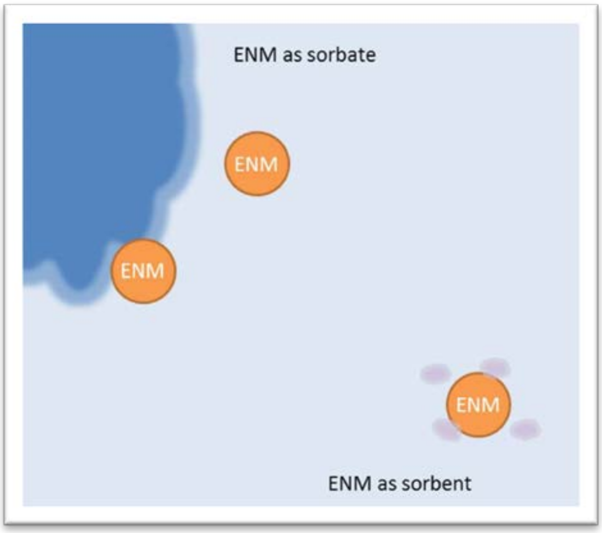


FIGURE 2.8

ILLUSTRATION OF THE ROLE OF ENM AS SORBENT (SORPTION OF OTHER SUBSTANCES TO ENM SURFACES) AND SORBATE (SORPTION OF ENM ONTO SOLID SURFACES)

### 2.6.1 Adsorption of natural organic matter onto ENMs – ENMs as sorbent

## Summary

As mentioned throughout this report ENMs may interact with other substances (macromolecules, surfactants, humic acids etc.) in the environment. Such interaction can represent an adsorption of other material onto ENM (i.e. the ENM acts as a sorbent). This is for example the case for the binding of natural organic matter (NOM) such as humic acid (HA) to ENMs. As also described elsewhere in this report, this association with e.g. HA will alter the surface properties and behaviour of the ENM and influence its interactions with other particles (agglomeration) and the surrounding media (e.g. dissolution), and in turn determine its environmental transport and fate (e.g. sedimentation) characteristics.

Binding to NOM is considered relevant for all ENMs that are part of the NanoDEN project. Knowledge is lacking on alterations to surface properties caused by NOM, and consequences for other transformation processes.

Natural organic matter (NOM) is abundant in the natural aquatic environment. NOM covers over a group of different substances including humic substances, which are complex mixtures of macromolecular substances (humic acids (HA), fulvic acids and humin) and constitute a major component of NOM. Humic substances stem from the decay of biomass from plants and animals. However, it has also been reported that 3–28% of the dissolved organic matter in wastewater treatment plants effluents are humic substances (Imai et al., 2002). Humic substances participate in processes such as acid dissociation, metal complexation, ion exchange, sorption onto mineral surfaces and redox reactions (MacCarthy, 2001). . They exhibit hydrophilic and hydrophobic properties and are known to bind to mineral surfaces (MacCarthy, 2001). The binding of HA to metal oxides has been found to influence the sorption behaviour of the metal oxide. This is explained by altered surface properties through the formation of a polyanionic organic coating on metal oxides particles (Liu et al, 2008 and references within).

It is not only the sorption properties but also the ENM transformation processes in general, that will be influenced by adsorption of humic substances (or other media/matrix constituents). For example the changed surface properties will influence interactions with other particles (agglomeration) and the surrounding media (e.g. dissolution), and in turn be determining for its environmental transport and fate (e.g. sedimentation). For more information on the influence of HA on environmental processes please refer to the corresponding sections of this report.

### 2.6.2 Adsorption and desorption of ENMs on solid surfaces – ENMs as sorbates

#### Summary

The interaction of ENM with solid environmental surfaces is of great importance for the ENM transport and fate in the environment. Here we look at the deposition mechanism by which the ENM attach to other material with focus on ENMs adsorption to soil particles. This process is strongly linked to aggregation and agglomeration. Agglomeration and aggregation between similar particles is sometimes described as homo-aggregation as opposed to adsorption of ENMs to other (non-similar) particles, which is sometimes referred to as hetero-aggregation. The DLVO theory (which describes the aggregation of particles in a liquid as a result of the sum of attractive and repulsive forces between particle interfaces) may be applied to describe the process of hetero-aggregation as it describes the interaction energy between an ENM and a collector surface. This section addresses the complexity of processes involved in disclosing the role of sorption of ENM to other surfaces in environmentally relevant matrices.

The literature points towards a number of parameters relevant for most, if not all, ENMs chosen as case study materials in this report. Not surprisingly, it is found that an increase in size generally will result in increased retention of ENM in soil independent of its chemical composition. The applicability of the DVLO theory was demonstrated in a number of cases for larger ENM (>30 nm). However, for smaller particles (<30 nm) the DVLO theory was in most cases invalid due to other processes controlling the sorption activity. When functionalization of ENM occurs, whether it is through interaction with natural compounds or via chemically intended stabilizers, it is the surface charge of the coating or stabilizer that determines the sorption properties and not the chemical composition of the ENM. At more environmentally 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 standard DVLO theory models.

The deposition of an ENM onto a surface has been found to depend on factors such as particle size, surface potential of both ENM and surface, solution chemistry and the Hamaker constant of the particle-fluid-collector. The interactions between an ENM and a surface may generally be described by the DLVO theory (see section 2.5.1). Hence, the processes of ENM sorption (deposition on a surface) is affected by the stability of the colloidal suspension, which in turn is dependent on the surface charge of the colloids. At neutral pH the net charge of natural soils is generally negative (Sparks, 1999). As described in the section on aggregation and agglomeration the zeta-potential plays a major role on in these processes and this is also true for sorption. In this case the significance of the zeta-potential is more indirect as the agglomeration of ENP makes them more likely to get stuck in the micro-pores of the soil. This effect is referred to as straining. In many cases straining can be mistaken for adsorption in experiments where examination on the micro level is not assessed e.g. in soil column experiments. Another aspect of the agglomeration in the context of sorption is that the apparent larger size of the particles may decrease the number of available sites for adsorption thus decreasing the adsorption efficiency. Consequently, the pattern of sorption will be highly dependent on both state of agglomeration and which type of ENM used in the experiments.

For regulatory purposes an OECD test guideline for sorption of chemicals (OECD 106) has been developed to assess the behaviour of compounds in association with a soil-water interface. However, this guideline lacks a direct link to environmental conditions and the applicability to ENMs remains to be tested. This is a general pattern regarding the role of ENMs as sorbates since the parameters affecting the sorption behaviour is not well described in literature so far. Consequently, it is not fully known which parameters control the sorption of ENM to soil and neither is the influence of e.g. coating, size and functionalization. Many of the parameters controlling agglomeration could also be governing the adsorption in the sense of retention as mentioned earlier. However, the parameters affecting agglomeration could also alter the sorption in other ways than the increase in size leading to straining.

The few available studies have, however, showed that changes in pH as well as the presence of natural organic matter (NOM) influence the adsorption of ENM (Baalousha (2009); Cornelis et al. (2011); Fang et al. (2013); Chen et al. (2012) ). A change in pH is known to affect dissolution as well as the colloidal stability of ENMs, the latter due to the changes in charge on the ENM surface. Since most soils are negatively charged a positively charged ENM would more easily bind to these soils due to electrostatic forces. As described for agglomeration and aggregation the presence of NOM may highly influence the adsorption of ENMs to soil. The stabilizing effect of NOM on ENM by adhering to the surface, thus creating a steric coating around the particles, could lead to an expected higher mobility of ENMs in soil with high NOM content. However, high concentrations of NOM have in some cases also been shown to decrease the stability thus leading to higher sorption. Which concentrations and types of NOM that causes stabilization and which ones cause destabilization has not yet been fully understood.

From the above it is clear that the sorption of ENM in solid-water matrices involves a range of interacting processes and a full description hereof is highly complex. However, the studies that have used soil column experiments to unveil some of the processes may be used to shed some light on the current state-of-knowledge and to identify some of the general parameters influencing the sorption of ENM.

*The role of ionic strength and pH in sorption studies of ENM*

In a study from 2011 the retention and solubility of $CeO_2$ NP in 16 different soil samples with varying physical and chemical characteristics was studied (Cornelis et al. (2011)). Ionic Ce(III), Ce(IV) and bulk $CeO_2$ was used as control. The experiment was carried out with two different spiking concentrations a low (9.29 µmol/kg) and a high (12600 µmol/kg). The initial size of the $CeO_2$ NP was 20 nm in powder form. After suspension in media the particles agglomerated rapidly increasing in size from the 20 nm initial size to 130 nm at a pH around 5. Particle sizes bigger than 1 µm were observed at pH <4.5 with the addition of citrate and phosphate. It was concluded that the addition of phosphate and citrate and the changes in pH affected the agglomeration pattern thus also the potential for sorption to the tested soil matrices. A multiple linear regression was used to estimate the parameters explaining the retention properties using parameters phosphate, clay content and pH. It was concluded that the addition of phosphate decreased the retention of $CeO_2$ NP in the soil and an increase of pH would lead to higher retention. It was emphasized that the decrease in retention due to phosphate addition was mostly due to limiting of electrostatic forces thus decreasing the sorption of $CeO_2$ NP onto natural colloids in the soil matrix and not due to the increase in surface potential of the $CeO_2$ NP (as has been proposed for other ENM e.g. Al NP and $TiO_2$ NP).

Li et al. (2011) used mathematical modelling to link the transport and deposition of $CeO_2$ NP in water-saturated porous media. The ionic strength of the media was altered by addition of NaCl at concentrations and differences in pH were also tested by using three different levels 3, 6, 9. Li et al. (2011) also observed increasing retention with increasing pH as it was also observed by Cornelis et al. (2011). An increase in attachment efficiency was also seen with increasing ionic strength. However, no information on the processes of retained $CeO_2$ NP in the sand column was given and it was not possible to deduce whether straining rather than sorption caused the retention of $CeO_2$ NP in the column. The issue of stability as function of ionic strength was also addressed by Jiang et al. (2013) who studied the initial transport and retention behaviours of ZnO nanoparticles in quartz sand porous media coated with *Escherichia coli* biofilm. Particle sizes increased with increasing ionic strength and the results suggest that the straining of particles would be more pronounced by divalent cations due to larger agglomerated particle size. With the introduction of biofilm to the sand column the straining was more pronounced due to squeezing of pore spaces thus resulting in higher retaining of NP in columns with biofilm. However, the retention was not only increased through smaller pore space: a decrease in the electrostatic repulsion of the particles was also observed. This suppression was adequately described by DLVO and non-DLVO interactions. Consequently, it was concluded that in an environmentally relevant soil matrix where biofilms would be present an increase in the retention of ZnO NP would be observed.

In the study by Fang et al. (2013) the importance of ionic strength and pH was investigated for $TiO_2$ NP aggregates in saturated and unsaturated granular media. Initial size of the tested $TiO_2$ NP were 15 nm, however as soon as dispersed in solution rapid agglomeration occurred. The range of agglomerates depended on the suspensions ionic strength and pH with larger agglomerates forming at higher ionic strength. It was found that $TiO_2$ agglomerates were highly mobile in both saturated and unsaturated conditions as long as the ionic strength was < 50 mM NaCl and > pH 7. It was suggested that the agglomeration at high ionic strength and low pH resulted in increased straining. The presence of air in the unsaturated test setup had little effect on the retention. Both findings were to be expected by the DVLO predictions made.

*The role of concentration and particle size in sorption studies of ENM*

In a paper by Wang et al. (2012a) the effect of concentration and particle size on retention and transport of silica nanoparticles was studied. In the study two sizes of silica nanoparticles (8±2 nm and 52±1 nm) were used and for the smallest nanoparticle it was found that higher concentrations gave rise to lower relative retention. The larger nanoparticle showed a lower retention capacity in the column. For the two sizes a difference in retention was observed which could be attributed to general DLVO theory describing the interaction energy between the ENM and the sand matrix. Also, the zeta-potential is affected by size thus indirectly affecting the interaction energies as described in previous section. In summary it was found that smaller NP showed higher relative retention, faster deposition and lower surface coverage. This is in contradiction with the previous studies mentioned here on e.g. metal oxides showing less relative retention for smaller particles. However, this effect could be explained by using mass concentrations instead of number concentrations. Additionally, metal oxide NP has shown a general tendency to agglomerate unless coated thus making comparison of sizes difficult. Furthermore, it was concluded that DVLO theory could not account for behaviour of the smaller NP (8 nm) possibly due to short-range interactions e.g. hydration and solvation. The study also emphasized that release of deposited NP could be caused by a decrease in ionic strength which in the context of the environment could be caused by rainfall events. Similar conclusions were given in previous reviewed papers describing the effect of ionic strength on the retention and transport of ENM (Cornelis et al. (2011); Fang et al. (2013); Chen et al. (2012)).

*The role of engineered and natural particle coating in sorption studies of ENM*

The study by Cornelis et al. (2011) clearly illustrates that particle coatings are crucial for the mobility and adsorption properties of ZnO ENMs. This is also illustrated by Kim et al. (2009) who studied the mobility of nZVI in porous media after aging. Bare nZVI showed no mobility in sand columns used at concentration of 1g/L whereas the adsorption of polyelectrolytes forming a coating increased the mobility of nZVI. It was concluded that even though the coating of the nZVI was poor in stabilizing the particles the repulsion to sand grains was still larger than that of bare nZVI.

Chen et al. (2012) investigated the effect of HA on transport and retention of $TiO_2$ rutile nanoparticles in saturated sand columns. When considering the retention of the $TiO_2$ NP it was clear that humic acid markedly increased the mobility and stability. It was found that a modified DLVO theory was in qualitatively agreement with the retention and transport behaviour. Furthermore, it was emphasized that in acidic surface waters with abundance of NOM, $TiO_2$ NP could be expected to be stable thus having a high mobility while in alkaline solution adsorption of NOM would not be expected to cause a change in the stability and retention due to limited or no adsorption under these conditions. However, electrostatic repulsion of $TiO_2$ NP to sand is increased at alkaline conditions thus naturally enhancing their mobility.

## 2.7 Biological transformation

Biological transformation processes (biodegradation and biomodification) are in principle similar to the transformation processes described in previous sections (interactions with macromolecules (formation of surface coatings), reduction and oxidation etc.). However, in contrast to these, biological transformations require the presence of and interaction with living organisms.

### 2.7.1 Biodegradation

#### Summary

Biodegradation is a biological process that involves the decomposition of an organic substance by microorganisms. This implies that this process is not relevant for inorganic ENMs. Even carbon-based ENM, like CNTs and CB, tend to be of an inorganic nature and generally considered resilient to biotic degradation.

Biodegradation is therefore considered not to be relevant for Ag, TiO2, CeO2, nZVI, ZnO, CuO, and QDs. Only a few studies have addressed biodegradation of carbon-based ENMs. However, based on available information limited mineralisation seems to occur and the process is very dependent on the specific test conditions. For carbon-based ENMs (e.g., CNT and CB) biodegradation is therefore expected to play a minor role in their fate and behaviour in the environment. For all ENMs biological degradation of organic surface coatings may occur and has to be considered when relevant.

Biodegradation is a biological process that involves the decomposition of an organic substance by microorganisms. This implies that this process is not relevant for inorganic ENMs. Only few studies have addressed the biodgradation of carbon-containing ENMs such as CNTs and $C_{60}$. These ENMs are generally considered resilient to biotic degradation by microorganisms as a result of their closed-cage structure. Carbon ENMs may however be transformed by abiotic processes (Hou & Jafvert, 2008), e.g. by formation of carbonyl and C-O surface groups (Hartmann et al., 2011) by which the closed-cage carbon structure can become more susceptible to biological degradation.

In a study by Hartmann et al. (2011) the ready biodegradability of aged $C_{60}$ suspensions was investigated in an OECD guideline test. Aging of the $C_{60}$ suspensions in sunlight had resulted in a transformation into more water-dispersible forms. However, the carbon cage structure was found not to be susceptible to biological degradation by activated sludge microorganisms. From this test it was concluded that the aged suspensions of $C_{60}$ were not readily biodegradable following the OECD standard test guideline for biodegradability (Hartmann et al. 2011).

In other non-guideline studies carbon-based ENMs have been observed to undergo biological degradation, however. For instance, it has been observed that fullerol ($C_{60}(OH)_{19-27}$) can be oxidised to $CO_2$ by white rot basidiomycete fungi (Schreiner, Filley et al. 2009). It was observed that $^{13}C$ from labelled fullerols was incorporated into the fungi biomass. In another study carboxylated SWCNT was found to be degraded when incubated with horseradish peroxidase and $H_2O_2$, whereas pristine SWCNT did not degrade (Allen, Kotchey et al. 2009).The difference in degradation of carboxylated versus pristine SWCNT was hypothesised to be due to strong adsorption of horseradish peroxidase to the carboxylated form but not to the hydrophobic pristine form. In a recent study the biological mineralisation of $^{14}C$ labelled MWCNT was studied using a mixed bacterial culture. After a 7-day incubation period at 39 degrees Celsius 2-7% mineralisation was observed (Zhang et al., 2013). Although the above mentioned studies indicate a potential for degradation of carbon-based ENMs by biological processes their relevance under environmentally relevant conditions remain to be established.

Finally, if ENMs have organic surface coatings biological degradation of this coating can have great consequences for their behaviour. For example it was found by Kirschling et al. (2011) that a covalently bound macromolecular stabilizer (end-grafted polyethylene oxide chains) bound to a cross-linked polyethylene oxide ENM core was bioavailable to microorganisms (A mixed culture of PEO-degrading bacteria). Biological degradation of surface coatings should be considered when estimating or modelling their environmental fate.

### 2.7.2 Bio-modification

#### Summary

Bio-modification is defined as a biologically mediated transformation process. This includes processes that occur after uptake into organisms and processes that are ’indirectly’ mediated by an organism, for example by release exudates that bind to the ENMs and change their properties. The focus of this section is on modifications resulting from the uptake of the ENM into organisms.

Studies of metal oxide and metal hydroxide ENM modifications by plants indicate a general process of ENM adsorption to the roots followed by dissolution and complexation. This process may be promoted by reducing substances released from the roots (e.g., ascorbic acids) and organic acids (e.g., citric acids). In the aquatic environment two different biological transformation processes are described: removal of CNT surface coating, as a result of ingestion by crustacean Daphnia magna, and changes in ENM size and shape of bovine serum albumin (BSA) stabilized gold nanoparticles when interacting with filter-feeding bivalves. It is highlighted in several papers that ENM bio-modifications and their role in ENM fate and transport are still poorly understood.

Biological modifications may be relevant for all ENMs as e.g. the state of agglomeration or dissolution may change as a result of uptake in plants or other organisms. However, based on current knowledge and in comparison to other transformation processes, bio-modifications are considered to have less importance for ENM fate and behaviour.

Bio-modification refers to the processes resulting in changes of properties of the ENM due to the interaction with living organisms. This may take place upon ingestion (or other routes of uptake), where the internal conditions of the organism lead to transformations of the ENM properties. It may also more indirectly be the case when organisms, such as algae, release exudates that bind to the ENMs and change their properties (see section 2.6.1). Finally some studies demonstrate the biosynthesis of various ENMs i.e. biologically mediated conversion of metal salts into metal ENMs (e.g. Karmakar et al., 2010).

Several studies have investigated the modifications of ENMs as a result of plant uptake. For example, it was observed by Zhang et al. (2012) that $CeO_2$, which are generally considered stable under normal environmental conditions, were biotransformed in cucumber plants. It was hypothesized that $CeO_2$ ENMs were first adsorbed onto the root surface and then partly dissolved. It is believed that reducing substances released from the roots (e.g., ascorbic acids) contributes to this process and that organic acids (e.g., citric acids) promote $CeO_2$ dissolution. Upon dissolution the Ce(III) ions precipitated in intracellular spaces and on root surfaces with phosphate or formed carboxyl compounds complexes. Similarly, $La_2O_3$ ENMs (as well as bulk $La_2O_3$) were transformed into intracellular needle-like clusters in cucumber roots. Organic acid root exudates are thought to play a role in this transformation as *in vitro* experiments demonstrated enhanced dissolution of $La_2O_3$ by acetic acid (Ma et al., 2011). Also, Parsons et al (2010) observed biomodification of

$Ni(OH)_2$ ENMs by mesquite plants (*Prosopis sp.*). The results suggested that the transformation process involved sorption of the $Ni(OH)_2$ NPs onto the root followed dissolution and complexation of Ni ions by organic acid ligands. Dissolution was observed to be more noticeable for the smaller and citrate coated nanoparticles. Biomodifications of $CeO_2$ and ZnO ENMs in soy were investigated by Hernandez-Viezcas et al (2013). ZnO NPs were observed within the plant tissues, but spectroscopy data showed O-bound Zn, in a form resembling Zn-citrate, indicating the presence of Zn complex. Ce was found to remain mostly as $CeO_2$ NPs (Ce in oxidation state IV) within the soybean plant, with a minor part biotransformed to Ce(III).

The transformation of ENMs by aquatic animals has also been the subject of several studies. It was found by Roberts et al (2007) that *Daphnia magna* was able to ingest and modify lysophophatidylcholine coated single-walled CNTs. The CNTs were ingested through normal feeding behaviour and the lysophophatidylcholine coating was used as a food source. This changed the dispersability of the CNTs which was likely to be caused by the removal of the CNT lipid coating. Hull et al (2011) investigated biomodifications of two types of bovine serum albumin (BSA) stabilized gold nanoparticles by filter-feeding bivalves. It was observed that 46 nm BSA stabilised gold NPs in bivalve faeces changed in appeared. After exposure the particles were much less spherical and uniform in size compared to prior to exposure. This is thought to be caused by dissolution or mechanical alteration during digestion and excretion. Smaller-sized 15 nm BSA stabilised gold NP formed dense clusters but dense retained their initial spherical shape and uniform size Hull et al (2011). Although some information is available on ENM biotransformation by aquatic animals, their role in ENM fate and transport is still poorly understood (Montes et al, 2012). This is also highlighted as a knowledge gap by Kirschling et al (2011) stating that at present "little is known about biologically mediated coating removal and transformation mechanisms for engineered nanoparticles".

*Methods to measure biomodifications*

The appropriate method to detect and measure biological modifications of ENMs by plants and animals obviously depend on which ENM property has been altered by the transformation process. If the ENM changes in size and shape some electron microscopy techniques might be relevant. If it is e.g. the state of agglomeration that is changed then other methods might be more appropriate.

Although not specific to ENMs, a variety of surface-sensitive spectroscopic methods can be applied for the characterisation of biological transformations of minerals. An overview of these methods (with a specific focus on microbial transformation processes) can be found in Geesey et al (2002). These techniques allows for a surface chemical analyses at micrometer and nanometer scales.

Some techniques identify and map the distribution of elements on a surface (EDS and soft X-ray TEM), while other techniques can provide information on the oxidation state of an element (XPS, EELS and X-PEEM). XANES and EXAFS can give information on the identity and orientation of neighbouring atoms. Some of these techniques (X-PEEM, RS, EELS, XANES and EXAFS) can even be applied to wet samples, making them suitable for charactering biological reactions (including mineral redox reactions and the associated adsorption and precipitation processes). Some of these techniques have also been applied to characterise the biological modifications of ENMs. Near edge X-ray absorption fine structure (XANES) has been used to investigate the forms of Ce in cucumber plants exposed to $CeO_2$ ENMs Zhang et al. (2012). Synchrotron μ-XRF and μ-XANES analyses were used to determine the forms of Ce and Zn in $CeO_2$ and ZnO ENM-treated soybean plants by Hernandez-Viezcas et al 2013. $Ni(OH)_2$ in mesquite plants was investigated by EXAFS where the spectra indicated that in roots, whereas in shoots the data suggests a partial conversion of the Ni(OH)2 ENMs into an organic acid complex (Parsons et al (2010)). Furthermore, the EXAFS data indicated a complete biotransformation of the Ni in the leaves (Parsons et al 2010). Thus, for some types of ENMs and certain ENM transformations such techniques can be applied to investigate biologically mediated changes to ENM surface properties as well as dissolution and complexation processes.

Environmental fate and behaviour of nanomaterials

# 3. Significance of identified processes for selected ENMs in environmental compartments

As it is the case for modelling of the environmental fate and behaviour of “conventional chemicals”, the most important transformation processes for ENMs, described in Chapter 2, need to be identified for individual ENMs. Such identification has to include an assignment of the processes to different environmental compartments in order to become operational for the purpose of estimating e.g. the environmental exposure concentration. Therefore, Chapter 3 will first describe general fate and behaviour patterns for ENMs in relevant environmental compartments. This will be followed by short individual profiles for each of the case-study materials, with respect to which environmental processes are of the highest (and lowest) importance for describing their fate and behaviour in the environment. . The terms ‘low’, ‘medium’ and ‘high’ are used to describe how crucial the specific process is in a model prediction of the fate of the ENM in question. A process of ‘low’ significance is considered to have a low impact on the fate modelling of that specific ENM and omitting the process will therefore not result in a large error. In the same way a process of ‘high’ significance is vital to accurately model the fate and behaviour of the ENM in question.

**It must be emphasized that the following review of transformation processes relate to the un-coated, non-functionalized form of the ENMs only.** Therefore, the influence of engineered surface modifications of the ENMs or presence of stabilizing agents is not included in the descriptions below. It is recognised that surface coatings will potentially play a major role in the ENM fate and behaviour. For surface coated materials their environmental fate and behaviour cannot be predicted based on core material properties and a case-by-case evaluation is required, taking into account the coating properties. Where surface coatings have been found to be of particular importance to transformation processes this is mentioned in the text below.

## 3.1 General relevance of environmental transformation processes of ENMs in different environmental compartments

Based on the descriptions in Chapter 2 and the overview provided in the ENRHES review (Stone et al., 2010), Table 3.1 provides a suggested ranking of the general relevance of each transformation process for modelling of the fate and behaviour of ENMs in different environmental compartments. It should be noted that this ranking is not limited to the ENMs selected for this study but describe the relevance of these processes to ENMs in general.

**TABLE 3.1.**

GENERAL RELEVANCE OF ENVIRONMENTAL TRANSFORMATION PROCESSES OF ENMS IN DIFFERENT ENVIRONMENTAL COMPARTMENTS. THE EVALUATIONS HAVE BEEN MADE BASED ON AN EXPERT EVALUATION TAKING INTO ACCOUNT THE REVIEW OF THE CURRENT STATE-OF-KNOWLEDGE ON PROCESSES AS PRESENTED IN THIS REPORT.

| Environmental transformation processes | | Compartments | | | |
|---|---|---|---|---|---|
| | | Air | Water | Sediment | Soil |
| **Chemical and photochemical processes** | Photochemical reactions | ++ | + | - | - |
| | Redox reactions | - | ++* | ++* | +* |
| | Dissolution/speciation | - | -/++* | -/++* | -/++* |
| **Physical processes** | Aggregation/agglomeration | + | ++ | + | + |
| | Sedimentation/deposition | + | ++ | - | - |
| **Interactions with surfaces / substances** | NOM adsorption | - | + | ++ | + |
| | Sorption to other surfaces | - | + | ++ | ++ |
| **Biologically mediated processes** | Biodegradation | - | -/++* | -/++* | -/++* |
| | Biomodification | - | -/+ | -/+ | -/+ |

++: Highly relevant for inclusion in ENM fate modelling;
+: Relevant for inclusion in ENM fate modelling;
-: Low/no relevance for inclusion in ENM fate modelling.
*: Highly dependent on the ENM chemical composition.

### 3.1.1 Processes of relevance for environmental fate and behaviour of nanomaterials in water

Since most of the descriptions of transformation processes in Chapter 2 are concentrated on the water phase, the following description will only briefly summarise the main reasons for the relative ranking shown in Table 3.1. For further details the reader is referred to Chapter 2.

For almost all processes in water the general water chemistry plays a major role for the extent and rate at which they participate in transforming the ENMs. In general the pH of the water is of high importance for processes like redox reactions, dissolution, sorption, and agglomeration/aggregation. If the pH is close to the so-called “point of zero charge”, the zeta-potential (see Chapter 2) approaches zero and the particles will not be stable in suspension. This will typically mean that an agglomeration process, leading to sedimentation of larger agglomerates, has begun. Also the ionic strength has a huge influence of processes like dissolution, speciation, aggregation and sorption. The higher ionic strength of salt water compared to freshwater may for example generally lead to higher agglomeration which in turn can be an onset to limited dissolution and increased sedimentation. However, not only the magnitude of the ionic strength will influence agglomeration, but also the identity of the individual ions contributing to the ionic strength has been shown to influence agglomeration. For example, the presence of divalent cations like $Ca^{2+}$ and $Mg^{2+}$ will have a stronger impact on agglomeration than presence of $Na^{+}$ and $K^{+}$. Also the identity of anions present in the water may influence the transformation processes dependent on the ENM in question. For silver nanoparticles the presence or absence of chloride will be determining for the formation of complexes and precipitates in water.

Finally, the presence (quantity as well as quality) of NOM may have a high impact on the transformation processes governing the fate and behaviour of ENMs in water. All processes listed in Table 3.1 may be influenced by the presence of NOM since this may lead to a “natural coating” of the particles released. Hereby the pristine particle properties change and more stable suspensions with a lower degree of agglomeration may be formed. This may lead to agglomeration, less or slower dissolution, higher sorption potential or a larger degree of biomodification.

For many ENMs photochemical reactions and redox reactions may be significant processes for transforming the released particles in the water compartment. The potential and magnitude hereof is however very dependent on the chemical composition of the ENM in question.

The processes of aggregation/agglomeration and sedimentation are of very high relevance for most ENMs in water. For some ENMs (e.g., Ag and ZnO) also dissolution must be included whereas this is not relevant under realistic environmental conditions for others (e.g., CNT and $TiO_2$). Sorption to other surfaces is relevant to consider for most ENMs as well as interaction with NOM (see above). In general biodegradation must be considered for fate modelling, but for most ENMs no degradation data (quantitatively or qualitatively) exist today. For many ENMs it is however irrelevant to include biodegradation as a transformation process, e.g. for elements (like silver and carbon) and metaloxides (like $TiO_2$ and $CeO_2$).

### 3.1.2 Processes of relevance for environmental fate and behaviour of nanomaterials in soil and sediment

As described in the previous section, the aggregation/agglomeration behaviour of most ENMs may lead to sedimentation and hereby a build-up of ENMs in sediments. The same holds true for ENMs released to soils in aqueous suspensions, which could include land application of suspended biosolids on agricultural soil.

While the process description for the water phase can be claimed to have gained consensus in the scientific community, this is not the situation for the range of processes and possible transformations in soils and sediments. For instance, for silver nanoparticles, which is one of the most studied ENMs in soils and sediments, SCENIHR, (2014) concluded that: “Soil conditions are complex and variable; therefore, generic predictions on the environmental fate of silver are extremely difficult to make.”

The ranking of fate processes in Table 3.1 has been made mainly by considering the transformation processes relevant for the water compartment and including some soil and sediment specific conditions. It should be noted that within the soil and sediment matrices sedimentation will by definition not occur. Secondly, it is expected that ENMs will adhere to solids depending on the range of parameters discussed above for the water compartment, but the presence of available solid surfaces makes sorption even more important than for the water compartment. Thirdly, though soils and sediments are described together in this section, it is important to remember that sediments will most often contain much more natural organic matter than soil and that the redox conditions of the two compartments are very different. In contrast to soils, there is no oxygen present beyond the upper few millimetres in sediments. These differences in NOM content and redox conditions may result in very different transformation patterns of ENMs in these two compartments. Therefore, the relevance of including NOM interaction and redox conditions in environmental fate modelling have been rated higher for the sediment compartment than for the soil compartment in Table 3.1.

### 3.1.3 Processes of relevance for environmental fate and behaviour of nanomaterials in air

Even though the fate processes for ultrafine particles in air are well described, there are still some major issues to be addressed with regards to disclosing the processes governing behaviour, transport and fate of air-borne ENMs (Stone et al., 2010). For example, particles in the lower end of the size range of 1-100 nm will be governed by other transport processes than those in the higher end (Mädler and Friedlander, 2007). The particle diffusion in air is governed by Brownian motion (see Chapter 2 for a description of Brownian motion), and the rate of diffusion is inversely proportional to particle diameter. Particles with high diffusion coefficients (such as those on the nanoscale) therefore have high mobility and will mix rapidly in aerosol systems (Aitken et. al., 2004).

This increased particle mobility in air at the nano-scale is important for the transformation processes since the rate of agglomeration is governed primarily by particle mobility and number concentration, both of which increase as particle size decreases (Aitken et. al., 2004). Thus, ‘aerosolised’ nanoparticles may agglomerate rapidly, even at a low mass concentration.

In Chapter 2, the term “sedimentation” was used to describe the settling of particles, but for air it is more common to use the term “deposition” for this process (Aitken et. al., 2004). The principles of “sedimentation” and “deposition” are however identical. As described in Chapter 2, the deposition of particles is dependent on the gravitational settling velocity, which is proportional to the diameter of the particle. As a consequence hereof, smaller nanoparticle in air will deposit at a much slower rate than larger particles. Agglomeration will therefore significantly increase the deposition of ENMs.

ENMs suspended in air will most likely be exposed to sunlight, and especially to UV wavelengths of light, to a much larger degree than for the other environmental compartments. This increases the possibilities for photochemical transformations depending on the ENM in question, as outlined in section 3.2.

Therefore, the processes of the highest relevance to include in the air compartment of a fate model for ENMs are photochemical reactions, agglomeration and deposition. The other processes listed in Table 3 are evaluated to be of much less relevance or even to be irrelevant.

## 3.2 Material specific assessments of most important processes for ENM environmental fate modelling

In this chapter we provide an overview of the importance of the environmental processed for the fate and behaviour of nine selected ENMs: Ag, TiO2, ZnO, Carbon Nanotubes, CuO, nZVI, CeO2, Carbon Black and Quantum Dots. Based on the review of chemical, physical and biologically mediated transformation processes as well as ENM interactions with other substances and surfaces the relevance of these processes are evaluated for each of the materials.

For each material an overview table is provided that is intended to give a quick overview of which processes would be the most important with regards to determining and predicting their environmental fate. . The terms ‘low’, ‘medium’ and ‘high’ are used to describe how crucial the specific process is in a model prediction of the fate of the ENM in question.A process of ‘low’ significance is considered to have a low impact on the fate modelling of that specific ENM and omitting the process will therefore not result in a large error. In the same way a process of ‘high’ significance is vital to accurately model the fate and behaviour of the ENM in question. This may feed into further developments of environmental fate models.

**It has to be noted that the assessments presented in these tables are not absolute but are relative and based on an evaluation of the available information from the scientific literature up until January 2014. It can be expected that these evaluations may change in the future as new data emerge.**

### 3.2.1 Relative importance of environmental processes for Ag NPs

TABLE 3.2.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF AG NPS

| **Known major uses:** Textiles, washing powders, deodorants, electronics, household appliances, cosmetics, medical devices, water disinfectants, and room sprays. | | | | |
|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | |
| | | Low | Medium | High |
| **Chemical and photochemical processes** | *Photochemical* | | ● | |
| | *Redox* | | | ● |
| | *Dissolution* | | | ● |
| **Physical processes** | *Aggregation / Agglomeration* | | | ● |
| | *Sedimentation* | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | ● |
| | *Sorption onto other surfaces/ retention in soil* | | ● | |
| **Biologically mediated processes** | *Biodegradation* | ● | | |
| | *Biomodification* | ● | | |

The review by Stone et al. (2010) of the scientific literature until 2009 concluded that the environmental fate and behaviour of Ag ENMs was relatively unknown. Since then the transformation of Ag-NPs has been described in a number of detailed studies (see review by Levard et al. (2012)). Based on this it can be concluded that 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. Though silver is known to be unstable in the presence of light, 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.  The process of dissolution of nano-Ag and the kinetics hereof are issues that many current research projects are focused at, but the factors governing this remain to be worked out. A number of models have been proposed and most of these assume that dissolution rate increases with decreasing particle diameter. Some also suggest that nano-Ag dissolution is more dependent on the initial Ag-NPs size than on the aggregation occurring in the media (Kent & Vikesland 2012). 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 (Levard et al. 2012). The silver sulphides are usually considered as not bioavailable, though recent studies suggests that some unexplained mechanisms may result in uptake of nano-Ag transformed to sulphide compounds (Lowry et al. 2012a). Sulphide is abundant under reduced redox conditions (anaerobic conditions) and abundantly available in wastewater treatment plants and soils as well as in both freshwater and saltwater sediments.

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 it is generally the case for ENMs, aggregation and agglomeration plays a major role. However, as dissolution is considered another very important process for Ag NPs the processes of agglomeration and aggregation will ‘compete’ with ENM dissolution. The manufactured Ag NPs consist of an inner core of metallic silver and usually an organic coating is used to stabilize Ag NPs against aggregation (Levard et al. 2012). Uncoated Ag NPs may be electrostatically stable because of the presence of negative charges on their surface due to hydroxide, oxide or sulphide groups (Levard et al. 2012). However, the aggregation of Ag NPs is influenced by general water chemistry as well as the pH, and the amount of dissolved organic carbon, suspended particulate matter, and natural organic matter in the water bodies (Gao et al., 2009).

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 highly influence the behaviour of the core nano-Ag.

Biomodification of Ag ENMs is not very well investigated but may include dissolution and changes in agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis. Potential coatings may be altered by biomodification processes. However, the evaluations presented here are for the uncoated form of the ENM.

In general the high influence of surface coating on the fate and behaviour of Ag NPs in the environment require further studies. Also, it is not well investigated how the medium, in which Ag ENMs are dispersed, influences the above mentioned transformation processes.

Environmental fate and behaviour of nanomaterials

### 3.2.2 Relative importance of environmental processes for $TiO_2$ NPs

**TABLE 3.3.**

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF $TIO_2$ NPS

| **Known major uses:** Paints, sunscreens, self-cleaning surface treatment, water treatment, personal care products, solar cells | | | | | | |
|---|---|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | | | |
| | | Low | | Medium | | High |
| **Chemical and photochemical processes** | *Photochemical* | | | ● | | |
| | *Redox* | ● | | | | |
| | *Dissolution* | ● | | | | |
| **Physical processes** | *Aggregation / Agglomeration* | | | | | ● |
| | *Sedimentation* | | | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | | ● | |
| | *Sorption onto other surfaces/ retention in soil* | | | | ● | |
| **Biologically mediated processes** | *Biodegradation* | ● | | | | |
| | *Biomodification* | | ● | | | |

$TiO_2$ is one of the more studied ENMs regarding environmental fate. Several processes affect the fate of these 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. Comparisons of $TiO_2$ aggregation behaviour in natural waters- described as typical to Nordic conditions, have been made by Sillanpää et al. (2011), showing a formation of larger aggregates in brackish water compared to fresh water. The difference was attributed to the higher ionic strength of the brackish water. The difference of aggregation in fresh waters with different compositions was linked to presence of organic substances and pH (Sillanpää et al., 2011). Another study applied multi-dimensional testing to investigate the effects water chemistry of TiO2 aggregation in natural waters. This study confirmed the importance of organic matter, pH, and ionic strength but also presented in more details the influence of certain mono- and divalent ions (Ottofulling et al 2011), with divalent ions causing more aggregation. Aggregation can lead to sedimentation and may also include hetero-aggregation, which is the adsorption of $TiO_2$ to particulates present in the water. Therefore, the processes of aggregation and sedimentation are considered of high importance for $TiO_2$ ENMs.

$TiO_2$ has been shown to display high photocatalytic activity under UV illumination. Traditionally the anatase form of $TiO_2$ has been considered to be a better photocatalyst compared to the rutile form. However, recent studies point to the fact that when synthesized using low-temperature methods, the rutile form can also exhibit photocatalytic activity for degradation of organic pollutants - even superior to that of the anatase $TiO_2$ (Luan, Jing et al. 2012). For metal oxide ENMs (including $TiO_2$), photoactivation has been found to alter binding to dissolved organic matter. This may in turn influence their environmental behaviour. Photochemical transformation processes are hence considered to be of medium importance. From a chemical point of view, $TiO_2$ is considered less likely to participate in electron transfer or uptake and unlikely to dissolve to any significant extend under environmental conditions. Hence redox reactions and dissolution processes are considered to be of low importance.

Model-based estimates for environmental concentrations of $TiO_2$ ENMs have been reviewed as part of the ITS-Nano project (ITS-Nano, 2013), based on different modelling approaches. For example, Gottschalk et al. (2009) and Gottschalk et al. (2010) applied a probabilistic material flow analysis (PMFA) method to calculate, from a whole life cycle perspective, nanoparticle concentrations in all environmental compartments. These models vary also in spatial boundary (US, Switzerland, Europe, Switzerland local etc.). Predicted concentration ranges are in the ng/L to µg/L range for surface waters and µg/kg to mg/kg range for sediments (ITS-Nano, 2013). Although the estimated values vary greatly, due to limited data availability for model parameters, they all point to the fact that sediment concentrations will be higher compared to surface water concentrations, which again highlights sedimentation as being of high importance for $TiO_2$ ENMs.

If entering the soil compartment, Fang et al. (2013) found that ionic strength and pH will be determining factors for $TiO_2$ adsorption to soil particles and its transport in saturated and unsaturated granular media. Micron sized aggregates were observed to form rapidly at higher ionic strengths (50 mM NaCl). However, $TiO_2$ agglomerates were found to be highly mobile in both saturated and unsaturated conditions as long as the IS was < 50 mM NaCl and > pH 7. Nonetheless, it is considered of high importance to consider adsorption/retention in soil is environmental fate modelling for $TiO_2$ NPs.

As $TiO_2$ is an inorganic material the process of biodegradation is considered not to be relevant. Biomodifications may include changes in agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis. Potential coatings may be altered by biomodification processes. However, the evaluations presented here are for the uncoated form of the ENM.

### 3.2.3 Relative importance of environmental processes for ZnO NPs

TABLE 3.4.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF ZNO NPS

| **Known major uses:** cosmetics, sunscreens, paints, anticorrosion agents | | | | | | |
|---|---|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | | | |
| | | Low | | Medium | | High |
| **Chemical and photochemical processes** | *Photochemical* | | ● | | | |
| | *Redox* | | ● | | | |
| | *Dissolution* | | | | | ● |
| **Physical processes** | *Aggregation / Agglomeration* | | | | ● | |
| | *Sedimentation* | | | | ● | |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | | ● | |
| | *Sorption onto other surfaces /retention in soil* | | | ● | | |
| **Biologically mediated processes** | *Biodegradation* | ● | | | | |
| | *Biomodification* | | ● | | | |

ZnO is one of the most produced ENMs based on production volumes (Piccinno et al., 2012). Studies have investigated the environmental fate and behaviour. ZnO NPs have been shown to exert photo catalytic behaviour. However, compared to $TiO_2$ the photocatalytic activity is limited (Valenzuela et al., 2002). Thus, it was evaluated that the importance of photochemical transformations is low.

Rapid dissolution of ZnO NP has been shown (Kasemets et al., 2009, Bian et al., 2011) but concurrently aggregation/agglomeration (Keller et al., 2010) will control the resulting equilibrium concentration of the suspension depending on the size of the ZnO NP (David et al., 2012). As dissolution is considered a very important process for ZnO NPs the processes of agglomeration and aggregation will ‘compete’ with ENM dissolution. Additionally, sedimentation of larger aggregates/agglomerates will still be important. The importance of dissolution, aggregation/agglomeration and sedimentation was evaluated high and high/medium, respectively.

NOM has been shown to adsorb to ZnO NP (Keller et al., 2010; Zhang et al., 2009). For high pH (9 and 11) the addition of NOM showed an increase in dissolution (Bian et al., 2011). This could be due to steric stabilization keeping the ZnO NP small and thus increasing the rate of dissolution as described above. However, the processes of stabilization and dissolution of ZnO NP under the influence of NOM is still not completely understood. Hence, the process of NOM adsorption was evaluated high/medium.

Studies showed high sorption/retention of ZnO NP in e.g. soil column (Jiang et al. 2013). Furthermore, it would be suggested that high aggregation/agglomeration would naturally reduce the pore space size resulting in high retention of larger aggregates/agglomerates.

Voegelin et al. (2011) studied complexation of bulk ZnO in four contaminated soils over a wide range of pH, clay content and organic matter. They found that the most typical complex of Zn resulting from ZnO being Zn-layered double hydroxides over all the tested soils. Similar inner sphere complexes were found by Scheckel et al. (2010) to be stable for 12 months after spiking a kaolin suspension with ZnO NP. Consequently, 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 to be of low relevance. Biomodifications are not very well investigated but may include dissolution and changes in agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis. Potential coatings may be altered by biomodification processes. However, the evaluations presented here are for the uncoated form of the ENM.

In general the high influence of surface coating on the fate and behaviour of ZnO NPs in the environment require further studies. Also, it is not well investigated how the medium, in which ZnO is dispersed, influences the above mentioned transformation processes.

### 3.2.4 Relative importance of environmental processes for Carbon Nanotubes (CNTs)

TABLE 3.5.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF CNTS

| **Known major uses:** Reinforced composites, conductive materials, hydrogen storage media, drug delivery vessels, sensors and sorbents | | | | |
|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | |
| | | Low | Medium | High |
| **Chemical and photochemical processes** | *Photochemical* | | ● | |
| | *Redox* | ● | | |
| | *Dissolution* | ● | | |
| **Physical processes** | *Aggregation / Agglomeration* | | | ● |
| | *Sedimentation* | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | ● |
| | *Sorption onto other surfaces/ retention in soil* | | | ● |
| **Biologically mediated processes** | *Biodegradation* | ● | | |
| | *Biomodification* | | ● | |

It should be noted that the term carbon nanotubes (CNTs) cover a broad range of fibrous carbon containing nanoparticles for which their length, diameter, entanglement, and surface modification may show high impacts on their fate and behaviour in natural media.

CNTs are capable of absorbing sunlight (Chen, Jafvert 2010) and photochemical surface modifications may also occur (Savage, Bhattacharya et al. 2003) and photochemical transformation is hence evaluated a relevant process influencing their fate in the environment. On the other hand CNTs are considered to be stable, non-soluble, and not to participate in redox reactions under normal environmental conditions. However, as it is the case for other ENMs, not only their surface properties but also the environmental compartment they are released into play a major role in their ultimate fate and behaviour (Jackson et al., 2013). 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 (Jackson et al. 2013). 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 (Jackson et al., 2013). The pristine and potentially modified surface properties also highly influence the adsorption capacity of CNTs as well as CNTs ability to sorb to other surfaces. However, in general the influence of both of these interactions on the overall fate and behaviour of CNT in the environment is evaluated to be high.

CNTs are generally considered resilient to biotic degradation by microorganisms as a result of their closed-cage structure. While the pristine CNTs is considered to be non-degradable in the environment they may be transformed by abiotic processes (Hou & Jafvert, 2008), e.g. by formation of carbonyl and C-O surface groups (Hartmann et al., 2011) by which the closed-cage carbon structure can become more susceptible to biological degradation. In the overall picture, the inclusion of biodegradation data in environmental fate modelling is believed to be of minor importance based on an evaluation of CNTs as highly persistent under most environmentally realistic conditions (see review Hansen et al., 2013).

Biomodifications are not very well investigated but may include changes in agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis.

Potential coatings may be altered by biomodification processes. For example, biomodification of coated CNTs by Daphnia magna was reported by Roberts et al. (2007). They found that upon ingestion as the Daphnia stripped off the coating from the particle surface thereby decreasing the solubility of the CNTs. However, the evaluations presented here are for the uncoated form of the ENM.

### 3.2.5 Relative importance of environmental processes for CuO NPs

TABLE 3.6.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF CUO NPS

| **Known major uses:** Solar cell technology, photovoltaic applications, gas sensors, combustion catalyst, glass, ceramic resistors, semiconductor applications, antifouling paints | | | | | | |
|---|---|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | | | |
| | | Low | | Medium | | High |
| **Chemical and photochemical processes** | *Photochemical* | | ● | | | |
| | *Redox* | | ● | | | |
| | *Dissolution* | | | | ● | |
| **Physical processes** | *Aggregation / Agglomeration* | | | | | ● |
| | *Sedimentation* | | | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | | ● | |
| | *Sorption onto other surfaces/ retention in soil* | | | ● | | |
| **Biologically mediated processes** | *Biodegradation* | ● | | | | |
| | *Biomodification* | | ● | | | |

CuO is a metal oxide ENM with photo-catalytic properties. For another photo-catalytic metal oxide ($TiO_2$) it was found that its binding to dissolved organic matter can be altered by photo-activation (Carp et al., 2004). Although the photo-catalytically active of CuO is lower than that of $TiO_2$ (Miyauchi et al., 2002; Yabe & Sato, 2003), this may still be a relevant process for CuO ENM. However, in the overall picture, the importance of photochemical transformations is considered to be low. Based on chemical considerations the importance of redox reactions is considered to be low.

Dissolution is known to be relevant for CuO NPs. Release of Cu-ions from CuO NPs has for example been measured by Aruoja et al. (2009) demonstrating that the 25% of copper CuO NPs was bioavailable (and hence in a dissolved form) compared to and only 0.18% of copper from CuO in bulk form. This is however in contrast to another study finding comparable dissolution of nano and bulk sized CuO (Mortimer et al., 2010). It was also shown by Lin et al. (2013) that CuO NPs (partly) dissolve in aquatic media – however to a lesser extend compared to e.g. ZnO NPs. As dissolution is hence considered a significant process for CuO NPs the processes of agglomeration and aggregation will to some extend 'compete' with ENM dissolution.

Little is known about sorption/retention of CuO NP in e.g. soil column. However, this process is considered to be relevant and also to be influenced by the agglomeration/aggregation behaviour of the CuO NPs. 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. Biomodifications are not very well investigated and the medium importance assigned to this process may require further studies. Potential biomodifications may include changes in dissolution and agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis.

### 3.2.6 Relative importance of environmental processes for Nano Zero Valent Iron (nZVI)

TABLE 3.7.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF NZVI

| | | | | | | |
|---|---|---|---|---|---|---|
| **Known major uses:** Soil and groundwater remediation, wastewater treatment. | | | | | | |
| | | **Importance of environmental processes in fate modelling** | | | | |
| | | Low | | Medium | | High |
| **Chemical and photochemical processes** | *Photochemical* | ● | | | | |
| | *Redox* | | | | | ● |
| | *Dissolution* | ● | | | | |
| **Physical processes** | *Aggregation / Agglomeration* | | | | | ● |
| | *Sedimentation* | | | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | | ● | |
| | *Sorption onto other surfaces/ retention in soil* | | | | | ● |
| **Biologically mediated processes** | *Biodegradation* | ● | | | | |
| | *Biomodification* | | ● | | | |

The use of nano zero-valent iron (nZVI) in groundwater remediation has created a great demand for information on the environmental fate and behaviour of nZVI. Due to this primary application of the particles, they are not only known to be found in the environment, but are also expected to be reactive.

Through oxidation the 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 (Li et al., 2006). 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 (Phenrat et al., 2007; Yin et al., 2012). This is confirmed by Adeleye et al. (2013) who studied the fate and persistence of commercial nZVI. They concluded that in water nZVI will likely end up in sediment due to aggregation-induced sedimentation and the insolubility of the produced iron oxide. Hence, the importance of agglomeration is considered as high whereas the importance of solubility is considered to be low.

However, surface modifications are known to increase the stability of the suspension, resulting in greater mobility (Hwang et al., 2014; Petersen et al., 2012). A similar influence on the mobility of nZVI is observed in the presence and sorption of NOM (Johnson et al., 2009). Adeleye et al. (2013)

also found NOM to reduce the reactivity which could increase the lifespan of nZVI. Sorption of pollutants to nZVI is part of the remediation process (Su & Puls, 2008) and as such, sorption to other chemicals and surfaces is relevant for nZVI. For these reasons physical processes and interactions with surfaces and substances like NOM are considered to be of high importance.

As nZVI is an inorganic material the process of biodegradation is considered not to be relevant.

Potential biomodifications may include changes in agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis.

### 3.2.7 Relative importance of environmental processes for $CeO_2$ NPs

TABLE 3.8.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF $CEO_2$ NPS

| **Known major uses:** Diesel fuel additive (catalyst), catalyst support, water cleaning processes, Plastic colouring, fuel cells, UV absorbent, superconductors, infrared filters | | | | | | |
|---|---|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | | | |
| | | Low | | Medium | | High |
| **Chemical and photochemical processes** | *Photochemical* | | ● | | | |
| | *Redox* | ● | | | | |
| | *Dissolution* | | ● | | | |
| **Physical processes** | *Aggregation / Agglomeration* | | | | | ● |
| | *Sedimentation* | | | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | | ● | |
| | *Sorption onto other surfaces/ retention in soil* | | | | ● | |
| **Biologically mediated processes** | *Biodegradation* | ● | | | | |
| | *Biomodification* | | ● | | | |

$CeO_2$ is a metal oxide ENM with photo-catalytic properties. For another photo-catalytic metal oxide ($TiO_2$) ENMs attachment to dissolved organic matter can be altered by photo-activation (Carp et al., 2004). Although the photo-catalytically active of $CeO_2$is lower than that of $TiO_2$ (Miyauchi et al., 2002; Yabe & Sato, 2003), this may still be a relevant process for $CeO_2$ ENMs. The importance of photochemical transformations is therefore considered to be low/medium. Based on chemical considerations the importance of redox reactions is considered to be low.

$CeO_2$ is an inert material and not likely to dissolve to any relevant extend under normal conditions. The importance of dissolution for environmental fate and behaviour or $CeO_2$ ENMs is hence considered to be low.

It has been found that $CeO_2$ NPs aggregate in soil and are retained in the soil column. Addition of phosphate decreased the retention of $CeO_2$ NP in soils by limiting electrostatic forces thus decreasing the sorption of $CeO_2$ NP onto natural colloids. On the other hand higher soil pH was found to cause a higher retention (Cornelis et al. 2011). An increase in attachment efficiency has also been also observed with increasing ionic strength (Li et al., 2011). On this basis the importance of sorption and retention for $CeO_2$ is evaluated to be relatively high.

As $CeO_2$ is an inorganic material the process of biodegradation is considered not to be relevant. Potential biomodifications may include changes in dissolution and agglomeration state as a result of organism uptake. However, compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate modelling. For modelling of specific environments this may be evaluated on a case by case basis.

### 3.2.8 Relative importance of environmental processes for Carbon Black (CB)

TABLE 3.9.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF CB

| **Known major uses:** Car tyres, plastics, pigment in paints, rubber gaskets, concrete repair and sealants, fibreglass insulation, shoe polish, laserjet printer toners, inkjet printer cartridges, electronic sealants, and diaper ointment | | | | |
|---|---|---|---|---|
| | | **Importance of environmental processes in fate modelling** | | |
| | | Low | Medium | High |
| **Chemical and photochemical processes** | *Photochemical* | ● | | |
| | *Redox* | ● | | |
| | *Dissolution* | ● | | |
| **Physical processes** | *Aggregation / Agglomeration* | | | ● |
| | *Sedimentation* | | | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | | | ● |
| | *Sorption onto other surfaces/ retention in soil* | | | ● |
| **Biologically mediated processes** | *Biodegradation* | ● | | |
| | *Biomodification* | ● | | |

Carbon black (CB) may be described as elemental carbon in the form of an extremely fine black powder consisting of near-spherical colloidal particles and particle aggregates. According to OECD (2006) CB is “a generic term for a high purity elemental form of carbon consisting of near spherical colloidal primary particles (10-500 nm in diameter) fused into aggregates of such particles (80-810 nm in diameter) during a totally enclosed production process. The aggregates are tightly bound, forming the primary, dispersible unit of carbon black, and rapidly form agglomerates in the reactor.” It should thus be noted that the term carbon black (CB) covers a broad range of carbon-containing nanoparticles for which the actual chemical structures, diameters, aspect ratios and surface modifications are unknown. As these parameters are expected to govern the fate and behaviour of CB in the environment, the ranking above is based on an overall evaluation of CB as population of particles consisting of elemental carbon (in accordance with OECD, 2006).

As carbon black has no functional groups it is insoluble in water and cannot be further degraded by light or by photo-degradation in air or in surface water (OECD, 2006). As further stated by OECD (2006), parameters like water solubility, octanol/water partition coefficient, dissociation constant or adsorption/desorption cannot be analytically measured, however, the deposition in soil or sediments is considered to be most relevant compartment for carbon black 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.

With regards to biodegradation, the OECD (2006) states that “As an inorganic compound with the chemical structure "C", carbon black will not be further biodegraded by microorganisms”. Potential biomodifications may include changes in agglomeration state as a result of organism uptake. Through our literature study, no studies of biomodification of CB have been identified. Compared to other transformation processes, biomodification (of the uncoated form of the ENM) is likely to account only for a smaller part of the total transformations and is therefore evaluated as having relatively low importance in environmental fate. For modelling of specific environments this may be evaluated on a case by case basis.

### 3.2.9 Relative importance of environmental processes for Quantum Dots (QDs)

TABLE 3.10.

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF QDS

| | | |
|---|---|---|
| **CAS no.** Various<br>**Known major uses:** Semiconductors transistors, solar cells, light emitting devices (e.g . LEDs), diode lasers and medical imaging devices | | |
| | | **Importance of environmental processes in fate modelling**<br>Low Medium High |
| **Chemical and photochemical processes** | *Photochemical* | **Case by case evaluation** |
| | *Redox* | **Case by case evaluation** |
| | *Dissolution* | **Case by case evaluation** |
| **Physical processes** | *Aggregation / Agglomeration* | ● |
| | *Sedimentation* | ● |
| **Interactions with surfaces / substances** | *NOM adsorption* | ● |
| | *Sorption onto other surfaces/ retention in soil* | ● |
| **Biologically mediated processes** | *Biodegradation* | **Case by case evaluation** |
| | *Biomodification* | **Case by case evaluation** |

In an OECD WPNM background paper (OECD, 2012) QDs are defined as “extremely small particles of semiconductor materials with customizable electrical and optical features”...()...” often comprised from cadmium and selenium”. However, other definitions of QDs exist. QDs have also been described as “1 nm structures made of materials such as silicon, capable of confining a single electron, or a few thousand, whose energy states can be controlled by applying a given voltage” (Allianz, 2005).

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 e.g. 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 (Lukanov et al., 2004)

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.

### 3.2.10 Overview of relative importance of environmental processes for ENMs with focus on nine case-study materials

On the basis of sections 3.2.1 – 3.2.9 the key transformation processes for ENMs is summarised in Table 3.11, showing the relative importance of transformation processes for modelling of environmental fate of ENMs with a specific focus on 9 case-study materials.

**TABLE 3.11.**

RELATIVE IMPORTANCE OF TRANSFORMATION PROCESSES FOR MODELLING OF ENVIRONMENTAL FATE OF ENMS SELECTED FOR THIS STUDY. IT MUST BE EMPHASIZED THAT THIS EVALUATION RELATES TO THE UN-COATED, NON-FUNCTIONALIZED FORM OF THE ENMS ONLY. IT IS RECOGNISED THAT SURFACE COATINGS WILL POTENTIALLY PLAY A MAJOR ROLE IN THE ENM FATE AND BEHAVIOUR. FOR SURFACE COATED MATERIALS THEIR ENVIRONMENTAL FATE AND BEHAVIOUR CANNOT BE PREDICTED BASED ON CORE MATERIAL PROPERTIES AND A CASE-BY-CASE EVALUATION IS REQUIRED, TAKING INTO ACCOUNT THE COATING PROPERTIES. (AG: SILVER, TIO2: TITANIUM DIOXIDE, ZNO: ZINC OXIDE, CNT: CARBON NANOTUBES, CUO: COPPER OXIDE, NZVI: NANO ZERO VALENT IRON, CEO2: CERIUM OXIDE, CB: CARBON BLACK, QD: QUANTUM DOT)

| | **Process** | **Importance of the environmental process in fate modelling** | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Low* | | *Medium* | | | *High* |
| **(Photo) chemical** | *Photochemical* | nZVI, CB | ZnO, CuO | Ag, CeO2 | TiO2, CNT | | |
| | *Redox* | TiO2, CNT, CeO2, CB | ZnO, CuO | | | | Ag, nZVI |
| | *Dissolution* | TiO2, CNT, nZVI, CB | CeO2 | | | CuO | Ag, ZnO |
| **Physical** | *Aggregation / Agglomeration* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB, |
| | *Sedimentation* | | | | | Ag, ZnO | TiO2, CNT, CuO, nZVI, CeO2, CB |
| **Interactions with surfaces/ substances** | *NOM adsorption* | | | | | Ag, TiO2, ZnO, CuO, nZVI, CeO2 | CNT, CB |
| | *Sorption onto other surfaces/ retention in soil* | | | | Ag, ZnO, CuO | TiO2, CeO2 | CNT, nZVI, CB |
| **Biologically mediated** | *Biodegradation* | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | | |
| | *Bio-modification* | | Ag, TiO2, ZnO, CuO, nZVI, CeO2, CB | CNT | | | |

# 4. Identification of data and knowledge gaps

The literature review completed in Chapters 2 and 3 revealed a number of gaps in the current knowledge when it comes to describing the most important transformation processes that govern the fate and behaviour of ENMs in environmental matrices. Furthermore, some gaps relevant for making valid qualitative and quantitative estimations of the environmental fate and behaviour became evident. In this chapter these gaps will be outlined by dividing them in: 1) Gaps related to the specific environmental fate processes, 2) gaps related to the ENMs characterization and measuring methods, and 3) gaps related to access to information and data. It should be mentioned that the relative importance of these knowledge gaps for the further development and improvement of environmental fate modelling for ENMs depends on the specific modelling approach in question. For chemical fate models gaps related to knowledge on environmental processes are of great importance, whereas mass-flow based models depend more on the knowledge related to data availability and accessibility.

## 4.1 Gaps related to specific environmental transformation processes of ENMs

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. Although, it is well understood that ENMs in an environmental matrix do not form a static system, the complexity of understanding and describing the dynamic and interacting transformation processes is staggering. For the key environmental transformation processes identified in this report a number of knowledge gaps was identified as outlined in the following sections. These knowledge gaps are highly important in the context of chemical fate models.

### 4.1.1 Chemical / photochemical transformation processes

It was found that photochemical transformations were mostly relevant for CNT due to photo induced oxidation. However, not much is known about photochemical degradation, transformation or surface modifications of ENM. Another major knowledge gap is to which extent and in which cases the generation of reactive oxygen species can cause a transformation and/or degradation of the ENM or components of the ENM (including organic coating material). Here it is important to note that for all the selected case-study ENMs surface coating is relevant and the presence of surface coatings may alter the photochemical transformation potential of the ENM.

Altered binding of $TiO_2$ to organic matter due to photo-activation is described in the literature. This could also be relevant for ZnO, $CeO_2$ and CuO but has not been studied yet. Also, different forms of silver are known to be prone to photochemical transformations; however for AgNPs the literature on this topic is sparse.

### 4.1.2 Dissolution/ precipitation/speciation processes

Dissolution was considered to be of high importance for a number of ENMs within the scope of this report such as ZnO, Ag, CuO, and QD (depending on specific chemical composition). The general hypothesis mentioned in Chapter 2 is "...that for metal-based ENMs with a slow dissolution rate, benthic (sediment) organisms will have a higher exposure to metal ions than pelagic (water column) organisms, if the ENMs will distribute mainly into the sediment is the longer-term". This hypothesis has yet to be tested. However, it is well known that the solubility product of an ENM may be different from that of the bulk material and that solubility will depend on media composition (e.g. ionic strength, ligands, pH, and temperature) as well as ENMs properties including particle size, state of aggregation, particle coating, water chemistry and presence of natural organic matter. A number of models have been developed to describe this dissolution process, but currently no model takes into account all parameters believed to play a role. Tests to measure dissolution kinetics of ENMs have been proposed as the way forward, taking into account the non-equilibrium behaviour of ENM dissolution. Until now it appears that only very few studies have focussed at this kind of dynamic testing. This process may be influenced by aggregation behaviour as well as the presence of natural organic matter, but also the role and kinetics of these influencing parameters need to be studied in much more detail.

### 4.1.3 Agglomeration/aggregation processes

Aggregation and agglomeration was considered to be relevant for all the selected ENMs. It was found that aggregation and agglomeration may result from different combinations of particle properties and environmental conditions, and that these processes are likely to occur after release of ENMs from products, as well as during emission and/or residence in the environment. It has been highlighted that it is of vital importance to know the rate of growth of particle aggregates as a function of time when it comes to exposure assessment. Models have been developed for predicting the aggregate size distribution under different thermal and shear conditions, but the literature review revealed a lack of studies focused at these kinetic changes for ENM in environmentally relevant media. Furthermore, a number of studies indicate that humic acid and natural organic matter stabilise various types of ENMs, but there are exceptions to this rule and only a limited number of studies have investigated whether and to which extend other factors than humic acid affect ENM agglomeration/aggregation behaviour.

While the DLVO-theory (for explanation see section 2.5.1) would be the obvious theoretical framework to incorporate aggregation/agglomeration in environmental exposure models, the literature review in Chapter 2 revealed a growing body of literature suggesting that the DLVO-theory may have to be adapted to reflect the aggregation behaviour of ENMs in natural media. The limitations of the DLVO theory are among other factors linked to the novel surface chemistries and novel shapes of ENMs (Lowry et al., 2010; Hotze et al., 2010). In fact, the DLVO theory has never been successfully applied to complex matrices, such as the environmental matrices, but should possibly rather be regarded an approach to explain behavioural trends of particles in the environment (Lowry et al., 2010).

### 4.1.4 Biological transformation processes

Only very few studies have provided information on biodegradation that is useful for inclusion in models for predicting the environmental fate of carbon-containing ENMs. The carbon containing case-study materials in this report, CNT and carbon black, are believed to be persistent, but for other types of ENMs, e.g. hydroxylated fullerenes, biological degradation may reduce their residence time in the environment. However, this remains to be studied.

A few studies have investigated the modifications of ENMs as a result of plant uptake and it has been hypothesized that, for instance, CeO2 ENMs are first adsorbed onto the root surface assisted by reducing substances released from the roots (e.g., ascorbic acids) and then partly dissolved by organic acids (e.g., citric acids). It is, however, not possible to project how bio-modification of ENMs in plants varies between ENMs of different composition, sizes, shapes and coatings. Very little information is available on biotransformation of ENMs by aquatic organisms. However, it has been clearly demonstrated that surface modified ENMs can e.g. be transformed by filter feeders that use the coating as a food source. Only coated carbon nanotubes and gold nanoparticles have been investigated so far. Based on the currently limited information it is not possible to project how bio-modification of ENMs in aquatic organisms will vary between ENMs of different sizes, shapes and coatings.

It is almost certain that bio-modification will take place under environmental conditions, but little is known about the bio-modifications and the removal/transformation mechanisms involved. Even less is known on how bio-modification influences the ENM fate and transport of different ENMs.

### 4.1.5 Sedimentation, adsorption and desorption processes

Sedimentation of ENMs in surface waters may be a dominant transport process for ENMs to the sediments, but the extent to which this occurs for different chemical compositions, sizes, and shapes of ENMs is generally unknown. Furthermore, there is a need for determining the role and extent of sorption as a distribution process for ENMs in solid-water matrices. This is not a trivial matter since it involves a range of interacting processes and a full description hereof is highly complex. For instance, a stabilizing effect of NOM on ENM by adhering to the surface has been hypothesized, but which concentrations and types of NOM that cause stabilization, and which cause destabilization, and how that varies between different ENMs, remains to be understoo0d.

### 4.1.6 Gaps related to the ENM characterization and measuring methods

The advancement of the current understanding of ENM fate and behaviour is hampered or limited by the lack of appropriate ENM characterization and measuring methods. For instance, as mentioned for the process of dissolution in Chapter 2, there is a potential size-dependent solubility of nano-sized particles, but there are serious technical problems that need to be solved in order to distinguish between the truly dissolved fraction and the dispersed ENMs. Also, dissolution kinetics for the release of dissolved species from the solid ENMs may be of specific relevance for ENMs. The dissolution rates for certain ENMs will be a determining factor in their ultimate environmental fate and for evaluating their influence on the organisms inhabiting specific environmental compartments. Hence, methods to determine dissolution kinetics for relevant time frames are highly needed. Furthermore, the current OECD guidelines for the testing of ENM recommends that the redox potential is measured using a potentiometer (high impedance voltmeter) with an oxidation-reduction potential (ORP) electrode. However, Tantra et al., (2012) note that this technique has several limitations and further evaluation and development of appropriate methods to measure redox potential of ENMs is needed.

As described throughout this report it is essential to be able to assess the aggregation/ agglomeration behaviour of ENMs in each environmental compartment. A number of different methods exist to determine agglomeration of ENMs, but although these are often used in combination there are several technical limitations to their applicability under real world conditions. Among these are artefacts induced by sample preparation (e.g., if dilution is needed to reach the correct measuring range, the dilution may alter the agglomeration of ENMs compared to the original sample) as well as problems in measuring and quantifying sizes and size distributions in polydisperse samples. While most of the studies reviewed agree that agglomeration and aggregation of nanoparticles is one of the most important, if not the most important, environmental transformation process for ENM, it is also widely acknowledged that the possibilities for analytically quantifying these *in situ* (in real environmental matrices) are limited. Similarly, the OECD test guidelines for sorption (OECD 106) that have been developed to assess the behaviour of compounds in association with a soil-water interface, lack a direct link to environmental conditions and their applicability to ENMs remains to be tested.

## 4.2 Gaps related to access to information and data

The behaviour of ENMs in complex matrices is not well-understood at present, but models and frameworks to describe the fate and distribution of ENMs have been emerging within the last five years. Some of these incorporate classical knowledge of colloid science (Arvidsson et al., 2011) whereas others apply principles used for chemical fate modelling and material flow modelling (Mueller and Nowack, 2008; Blaser et al. 2008; Gottschalk et al., 2009: 2010a; 2010b; Praetorius et al. 2012). Mass-flow based models face knowledge gaps related to data availability and accessibility. However, common for all of these 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 - both intentionally and non-intentionally, from point sources and from diffuse sources. As pointed out by Stone et al. (2010) characterisation of sources and release mechanisms for nanomaterials into the environment is furthermore critically needed including the development of metrological strategies for monitoring transport and fate of nanomaterials within different environmental compartments.

## 4.3 Implications of the identified gaps

The identified knowledge gaps put some profound limitations on our overall ability to understand the role and significance of the interacting environmental transformation processes of ENMs. This poses a serious challenge when models for estimating the environmental fate and distribution of ENMs have to be formulated and applied. Thus, there is a significant gap between the present state-of-science and the increased demand for reliable environmental concentrations for environmental risk assessment of ENM. In an ideal situation, environmental fate models for ENMs should be based on descriptions of all environmental processes that take into account the specific properties of the ENMs. Back in 2010, Stone et al. (2010) argued that our current abilities to model the environmental transformation processes of ENMs were primitive. Today, very few models, if any, have attempted to build models for ENMs similar to what is currently used in the EUSES model for organic chemicals, i.e. a combination of mass flow analyses and environmental process descriptions.

Current models and frameworks for environmental exposure estimation have indeed become more sophisticated during the last five years. However, to improve their reliability improved incorporation of information on environmental transformation processes of ENMs may be required. In the short term this involves informed choices on the basis of existing knowledge during model formulation. The information presented in this report is intended to feed into such model developments. In the longer term, efforts are needed to address knowledge gaps in relation to photoinduced transformation (including degradation and/or surface modifications), the kinetics of dissolution and agglomeration, influence of NOM on transformation processes, adaptations of the DVLO theory to ENMs in environmental matrices, and the role and extent of sorption as a distribution process for ENMs in solid-water matrices.

In addition, until now the focus of environmental fate modelling has very much been on non-functionalized/uncoated ENMs. This may reflect the general focus of research within the field of ENMs environmental fate and behaviour, but it ignores the fact that most applications of ENMs require surface functionalization. This has previously been identified as having an important impact on subsequent transport and fate of ENMs in the environment as stated by Stone et al. (2010), stating that: “considerable further work is therefore required to develop a full understanding of the effect different functionalization have on the transport and fate of nanomaterials in air, water and soil systems“.

The discrepancy found between the current state-of-science and the urgent need to further develop and validate available environmental fate models/approaches may falsely lead to the conclusion that exposure modelling is impossible. However, as reported in a review by Gottschalk et al. (2013), some modelled results already exist for most environmental compartments giving indications of potential ranges of environmental concentrations. Also, initial measurements are emerging that detect and quantify ENMs (based on techniques including filtration, microscopic, chromatographic, spectroscopic and others). Hence, although model parameters currently used are partly based assumptions/analogies and partly on analytical data derived under laboratory conditions, environmental modelling ENM exposure has moved beyond the stage of complete lack of quantitative evidence.

For a further model development improvements on descriptions of ENM behaviour and fate processes is suggested based on the findings of this report. This includes quantification of aggregation/agglomeration (see tables above) and subsequent sedimentation. This is highly relevant for ENMs like $TiO_2$, Ag, ZnO, Cu, $CeO_2$, and CNT. To some extent also the new insights into dissolution (Ag, ZnO) and NOM adsorption ($TiO_2$, Ag, ZnO, $CeO_2$, CNT, CB) and redox reactions (Ag, nZVI) may be integrated in exposure models. However, quantitative data for these processes are still largely lacking. Whenever possible improvements should also be sought for quantification of ENM fate and behaviour in soils and possible insights on sorption and retention data may be gained from the studies review in this report. This may impact the exposure evaluations of ENMs like, $TiO_2$, $CeO_2$, nZVI, CNT, and CB.

## 4.4 Prioritisation of the identified gaps

By comparing the identified knowledge gaps related to specific environmental transformation processes (sections 4.1) with the relative importance of these processes for environmental fate modelling of ENMs (Table 3.9) 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

In addition, knowledge gaps regarding ENM characterization and measuring methods must be prioritized. These analytical methods are key to the establishment of model input parameters as well as model validation by *in-situ* measurements in the environment.

Finally, for the purpose of mass-flow based models, data has to be availability and accessibility regarding. Priority empirical and experimental data-gaps include e.g.: data on ENM production quantities, specific uses and use concentrations, as well as quantitative data on release during production, transport, use and disposal.

# 5. Conclusion

In this report the current knowledge on key environmental transformation processes for ENMs was reviewed for ENMs in general and applied to nine case-study materials. For each of these materials different processes were evaluated to contribute to a different degree to the expected outcome if a modelling approach is chosen to predict the environmental fate of ENMs. For each material a specific overview processes of low, medium, and high importance is listed in this report and summarised in Table 3.9. Below the general patterns that emerged from this analysis are outlined:

- The overall comparison of the relative importance of key transformation processes for environmental fate modelling of ENMs showed thati) agglomeration / aggregation, ii) sedimentation, iii) NOM adsorption and iiii) sorption to other surfaces / retention in soil were the processes of the highest importance for all ENMs regardless of chemical composition.

- Bio-modifications of core-materials are considered to be of relatively low relevance for inorganic ENMs, but may be of relevance for CNTs if studies demonstrate that these processes are possible under environmental conditions.

- Some transformation processes are highly material-specific. For example the transformation of Ag, ZnO and CuO is evaluated to be highly influenced by dissolution. For Ag and nZVI also redox processes are of high importance. TiO2 and CNTs (and to a lesser extent Ag and CeO2) may undergo some photo-chemically induced transformations, which are considered of medium importance in the modelling of their environmental fate.

- Due to their varying chemical compositions and the possibility of ‘tuning’ the specific properties of Quantum Dots (QDs), their ability to take part in environmental transformation processes cannot be generalised but will have to be evaluated case-by-case.

- The presence of surface coatings has not been taken into account in this evaluation. It is crucial to consider that surface coating may be the determining factor for ENM fate and behaviour while the core material itself is of less significance.

- Given the significance of the transformation processes described in this report mass flow (multimedia) models will have to incorporate mechanistic descriptions of environmental fate and behaviour parameters for ENMs.

By comparing the identified current knowledge gaps to the relative importance of the transformation processes reviewed in this report the following knowledge gaps needs to be addressed in the development of environmental fate modelling and exposure assessment of ENMs:

- Knowledge gaps related to environmental transformation processes of ENMs, including i) kinetic changes in aggregation/agglomeration including rate of growth, ii) possible adaptation of the DLVO theory, iii) the role of chemical compositions, sizes, and shapes on ENM sedimentation in surface waters, iv) parameters influencing the stabilising (or de- stabilising) effects of NOM on ENM suspensions, the role and v) extent of sorption as a distribution process for ENMs in solid-water matrices, vi) models to describe dissolution processes, and vii) development of appropriate tests to measure dissolution kinetics

- Knowledge gaps regarding ENM characterization and measuring methods are explored and minimised. These analytical methods are key to the establishment of model input parameters as well as model validation by in situ measurements in the environment.

- Empirical and experimental data-gaps should be closed e.g. for data on ENM production quantities, specific uses and use concentrations, as well as quantitative data on release during production, transport, use and disposal.

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# 6. Abbreviations and acronyms

| | |
|---|---|
| AFM | Atomic Force microscopy |
| Ag | Silver |
| Ag NP | Silver nanoparticle |
| Ag+ | Silver ion |
| $Ag^0$ | Metallic silver |
| $Ag^2S$ | Silver sulfide |
| ANUC | Analytical ultracentrifugation |
| BSA | Bovine serum albumin |
| BSA | Bovine serum albumin |
| $C_{60}$ | Carbon fullerene |
| CB | Carbon black |
| Cd | Cadmium |
| CdS/ZnS<br>CdSe/ZnS<br>CdSe/CdS | Core/Shell Nanocrystals |
| Ce | Cerium |
| CeO2 | Cerium oxide |
| CNT | Carbon nanotube |
| Cu | Copper |
| CuO | Copper oxide |
| DLS | Dynamic light scattering |
| DLVO Theory | A theory that describes particle agglomeration. Briefly it states that if the sum of repulsive forces on a particle is smaller than the sum of attractive van der Waals forces, the particles will aggregate. The theory is named after Deryaguin, Landau, Verwey and Overbeek who are the scientists that first introduced this theory. |
| DOC | Dissolved organic carbon |
| DSMC | Direct simulation monte carlo |
| ECHA | European Chemicals Agency |
| EDC | Electronic double layer |
| EELS | Electron energy loss spectroscopy |
| EM | Electron microscopy |
| ENHRES | Engineered Nanoparticles – Review of Health and Environmental Safety |
| ENM | Engineered nanomaterial |
| EUSES | The European Union System for the evaluation of substances |
| EXAFS | Extended X-ray absorption fine structure |
| FFF | Field Flow Fractionation |
| HA | Humic acid |
| Ksp | Solubility product |
| $La2O_3$ | Lanthanum oxide |
| $Me_0$ | Metals in their elemental state |
| MWCNT | Multi walled carbon nanotube |

| | |
|---|---|
| NanoDEN | Nanomaterials – Occurrence and effects in the Danish Environment |
| NM | Nanomaterial |
| NOM | Natural organic matter |
| NP | Nanoparticle |
| nZVI | Nano zero valent iron |
| OECD WPMN | OECD Working Party on Manufactured Nanomaterials |
| ORP | Oxidation-reduction potential |
| PAH | Polycyclic aromatic hydrocarbon |
| PEC | Predicted environmental concentration |
| PEG | Polyethylene glycol |
| PMFA | Probabilistic material flow analysis |
| PVP | Polyvinylpyrrolidone |
| QD | Quantum dot |
| ROS | Reactive oxygen species |
| SANS | Small angle neutron scattering |
| SDS | Sodium dodecyl sulphate |
| SEM | Scanning electron microscopy |
| $SiO_2$ | Silicon dioxide |
| SRFA | Suwannee River Fulvic acid |
| SRHA | Suwannee River humic acid |
| SRNOM | Suwannee River natural organic matter |
| STEM | Scanning transmission electron microscopy |
| STM | Scanning tunnelling M |
| SWCNT | Single walled carbon nanotube |
| TEM | Transmission electron microscopy |
| TiO2 | Titanium dioxide |
| TOC | Total organic carbon |
| UV | Ultra violet |
| XANES | X-ray absorption near-edge structural |
| X-PEEM | X-ray photoelectron emission microscope |
| XPS | X-ray photoelectron spectroscopy |
| XRD | X-ray diffraction |
| Zn | Zink |
| ZnO | Zinc oxide |
| ZVI | Zero valent iron |

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Yoon TH, Johnson SB, Brown GE (2005) Adsorption of organic matter at mineral/water interfaces. IV. Adsorption of humic substances at boehmite/water interfaces and impact on boehmite dissolution. Langmuir, 21:5002-5012

Zhang, Y., Chen, Y., Westerhoff, P., & Crittenden, J. (2009). Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles. Water Research, 43(17), 4249-4257.

Zhang, P., Ma, Y., Zhang, Z., He, X., Zhang, J., Guo, Z., ... & Chai, Z. (2012). Biotransformation of ceria nanoparticles in cucumber plants. ACS Nano, 6(11), 9943-9950.

Zhang L, Petersen EJ, Habteselassie MY, Mao L, Huang Q (2013) Degradation of multiwall carbon nanotubes by bacteria. Environmental Pollution, 181:335-339

Zhou, D., Bennett, S. W., & Keller, A. A. (2012). Increased mobility of metal oxide nanoparticles due to photo and thermal induced disagglomeration. PlOS ONE, 7(5), e37363.

**Ref for literature search is listed in Appendix 1 & 2.**

# Appendix 1: List of literature used for "backwards searching"

Aitken et al. 2011. Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH (RIP-oN 3). RNC/RIP-oN3/FPR/1/FINAL 07 July 2011 (http://ec.europa.eu/environment/chemicals/nanotech/pdf/report_ripon3.pdf)

Arvidson R, Molander S, Sandén BA, Hassellöv M (2011) Challenges in Exposure Modelling of Nanoparticles in Aquatic Environments, Human and Ecological Risk Assessment: An International Journal, 17:1, 245-262

Benn, T. and Westerhoff, P. "Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics" (2008b) Environmental Science and Technology, 42 (18), pp 7025–7026

Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuehler, K (2008) Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, Science of the Total Environment 390, 396-409

Boxall, A.B. A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., Watts, C. 2008. Current And Future Predicted Environmental Exposure To Engineered Nanoparticles. York: Central Science Laboratory.

Ganzleben & Hansen

Gottschalk F, Scholz RW, Nowack B, (2010) Probabilistic material flow modelling for assessing the environmental exposure to compounds: methodology and an application to engineered nano-TiO2 particles, Environmental Modelling & Software 25: 320-332

Hansen et al. (2011). Report from NanoImpactNet workshop on ENP environmental fate and behaviour, in Bilthoven, NL. NanoImpactNet Deliverable, Lausanne, CH.

Kaegi R, Ulrich A, Sinnet B, Vonbank R, Wichser A, Zuleeg S, Simmler H, Brunner S, Vonmont H, Burkhardt M, Boller M. "Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment" (2008) Environmental Pollution, 156(2) p233-9

Mueller NC, Nowack B. "Exposure modeling of engineered nanoparticles in the environment" (2008) Environmental Science and Technology 42, No.12, p4447–53

Nowack, B., Ranville, J. F. Diamond, Stephen; Gallego-Urrea, Julian A.; Metcalfe, Chris; Rose, Jerome; Horne, Nina; Koelmans, Albert A.; Klaine, Stephen J (2012) Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environmental Toxicology and Chemistry — 2012, Volume 31, Issue 1, Sp. Iss. SI, pp. 50-59

Praetorius A, Scheringer M and Hungerbühler K (2012) Development of Environmental Fate Models for Engineered Nanoparticles—A Case Study of TiO2 Nanoparticles in the Rhine River, Environmental Science & Technology 46 (12): 6705-6713

Quik JTK, Vonk AI, Hansen SF, Baun A, Van De Meent D (2011) How to assess exposure of aquatic organisms to manufactured nanoparticles? Environment International 37(6): 1068-77

Robichaud CO, Uyar AE, Darby MR, Zucker LG and Wiesner MR, 2009, Estimates of upper bounds and trends in nano-TiO2 production as a basis for exposure assessment, Environ. Sci. Technol. 43: 4227-4233

Stone, V., Nowack, B., Baun, A., van den Brink, N., von der Kammer, F., Dusinska, M., Handy, R., Hankin, S., Hassellöv, M., Joner, E., Fernandes, T.F. (2010). Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterization. Sci. Tot. Environ., 408 (7), 1745-1754

Stone, V, Hankin, S, Aitken, R, Baun, A, Christensen, F, Fernandes, T, Hansen, SF, Hartmann, NB, Hutchison, G, Johnston, H, Micheletti, C, Peters, S, Ross, B, Sokull-Klüttgen, B, Stark, D & Tran, L 2010, Engineered nanoparticles: Review of health and environmental safety, Final report of FP7 Coordination and Support Action. Grant Agreement number: 218433, http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

Som, C., Wick, P., Krug, H., Nowack, B. 2011. Environmental and health effects of nanomaterials in nanotextiles and façade coatings. Environment International 37: 1131–1142

# Appendix 2: Literature search strategy and search outputs

The starting point for this literature review was the final report of the ENHRES project (Stone et al. 2010). The state-of-knowledge described in the ENHRES final report has been updated on the bases of scientific papers published in the period 2009-2013 through a  literature review using the following databases (in a ranked order) for a first round of searches:

1. ICON EHS database hosted by Rice University (http://icon.rice.edu/research.cfm)
2. ISI Web of Science
3. NHECD hosted by JRC (http://nhecd.jrc.ec.europa.eu/)
4. OECD WPNM database

The search was limited to include papers published after 2009. Based on initial searches, ISI Web of Science was found to give the most comprehensive records and a refined search strategy was therefore implemented in this database.

The literature searches in ISI Web of Science were carried out with the search terms outlined below in combination:

1. The list of processes described above in an truncated form (e.g. photochem*, aggre*, desorp*) to expand word endings
2. Combinations of more general search terms, limiting the searches to records relevant for fate and behavior of ENMs in the environment: e.g.  nano*; environ*;  fate; behav*

For each process an initial ‘broad’ search was carried out as a first step (“*process_truncation**” AND “nano*” AND “environ*”) and the search was subsequently adjusted to be more specific or more broad depending on the number of records from this initial search.

In case of an extensive number of records in this initial search, two independent searches with the addition of the terms “fate” and “behav*”, respectively, was used to narrow the search outcome. In the case of oxidation the search was combined with “NOT Topic=(oxide)” to remove irrelevant records. In case of low number of records in the initial search the search was broadened by removing “environ*”. The truncation of the process names and asterisks were used to make all endings of the word accessible in the search. The results of these searches are listed in the following and form what can be considered a “gross list” of publications relevant for the selected processes. This list was then checked for duplicates and manually revised to contain articles with relevance for the NanoDEN project in terms of processes and selected ENMs.

A summary of the number of publications per process on the “gross list” and the refined “net list” is shown in Table A.2.1 below. To narrow the “gross list” down to the “net list”, titles of all papers were reviewed in order to identify papers which obviously were not relevant for the topic of ENM fate and behaviour in the environment. All papers in the “net list” were included as background for the review of processes provided in Chapter 3 of this report. All lists from the literature search are available in the form of links to RefShare.

**Table A.2.1.** Number of scientific papers for each environmental behaviour/distribution process resulting from searches in ISO Web of Science using the search terms listed in Appendix 2. The "gross list" refers to all hits returned and the "net list" to the number of papers identified as relevant after reviewing papers on the "gross list".

| **Environmental process** | **Number of papers** | |
|---|---|---|
| | **"Gross list"** | **"Net list"** |
| 1. (Photo)chemical transformation | | |
| 1.1.a Photocatalytic degradation | 208 | 34 |
| 1.1.b Oxidation (chemical) | 705 | 55 |
| 1.1.c Reduction (chemical) | 518 | 28 |
| 1.2.a Speciation/complexation | 365 | 26 |
| 1.2.b Dissolution | 494 | 74 |
| 1.2.c Precipitation | 135 | 13 |
| 2. Physical transformations | | |
| 2.1.a Agglomeration | 612 | 57 |
| 2.1.b Aggregation (homo-aggregation) | 2676 | 160 |
| 2.2.a Sedimentation | 350 | 35 |
| 3. Interactions with other substances & surfaces | | |
| 3.1.a Adsorption / 'hetero-aggregation' | 774 | 66 |
| 3.1.b Desorption | 155 | 17 |
| 3.1.c Adsorption of macromolecules | 216 | 75 |
| 4. Biological transformations | | |
| 4.1.a Biologically mediated processes | 78 | 20 |

Detailed information on specific search terms and search outputs are listed below.

The result of the initial search is displayed as the number in parentheses in the first line. The searches were then narrowed down or broadened and the results were included in the **'gross list'** (first link). This list was then manually revised to contain articles with relevance for the NanoDEN project in terms of processes and compounds (the '**net list'**, second link).

**Search strategy for Photocatalytic degradation (75 articles):**
Topic=(photochem*) AND Topic=(nano*) AND Topic=(environ*)
Topic=(photochem*) AND Topic=(nano*) AND Topic=(environ*) AND Topic=(degr*)
Search strategy for Photocatalytic degradation (148 articles):
Topic=(photochem*) AND Topic=(nano*) AND Topic=(degr*)
Combined for photocatalytic degradation (208 articles)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FPhotochemical%20activity
Revised articles (34 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FPhotochemical%20activity%2F%2F%2FPhotochemical%20activity%20revised

**Search strategy for oxidation (6014 articles):**
Topic=(nano*) AND Topic=(oxi*) AND Topic=(environ*) NOT Topic=(oxide)
Search strategy for oxidation (271 articles)
Topic=(nano*) AND Topic=(oxi*) AND Topic=(environ*) AND Topic=(fate)
Search strategy for oxidation (466 articles)
Topic=(nano*) AND Topic=(oxi*) AND Topic=(environ*) AND Topic=(behav*) NOT Topic=(oxide)
Search strategy for oxidation (22 articles)
Topic=(nano*) AND Topic=(oxi*) AND Topic=(environ*) AND Topic=(behav*) NOT Topic=(oxide) AND Topic=(fate)
Combined for oxidation (705 articles) (The search with results of 6014 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FOxidation
Revised articles (55 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FOxidation%2F%2F%2FOxidation%20revised

**Search strategy for reduction (6718 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(reduct*)
Search strategy for reduction (78 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(reduct*) AND Topic=(fate)
Search strategy for reduction (497 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(reduct*) AND Topic=(behav*)
Combined for reduction (518 articles) (the search with results of 6718 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FReduction
Revised articles (28 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FReduction%2F%2F%2FReduction%20revised

**Search strategy for dissolution (6247 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(diss*)
Search strategy for dissolution (138 articles):
Topic=(nano*) AND Topic=(environ*) AND Topic=(diss*) AND Topic=(fate)
Search strategy for dissolution (494 articles):
Topic=(nano*) AND Topic=(environ*) AND Topic=(diss*) AND Topic=(behav*)
Combined for dissolution (494 articles) (The search with results of 6247 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FDissolution
Revised articles (74 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FDissolution%2F%2F%2FDissolution%20revised

**Search strategy for precipitation (1701 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(precipi*)
Search strategy for precipitation (31 articles):
Topic=(nano*) AND Topic=(environ*) AND Topic=(precipi*) AND Topic=(fate)
Search strategy for precipitation (177 articles):
Topic=(nano*) AND Topic=(environ*) AND Topic=(precipi*) AND Topic=(behav*)
Combined for precipitation (135 articles) (The search with results of 1701 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FPrecipitation
Revised articles (13 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FPrecipitation%2F%2F%2FPrecipitation%20revised

**Search strategy for Speciation – complexation (1782 articles – 411 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*)
Search strategy for speciation – complexation ( 38 - 21 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*) AND Topic=(fate)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*) AND Topic=(fate)
Search strategy for speciation – complexation (237 - 105 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*) AND Topic=(behav*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*) AND Topic=(behav*)
Combined for speciation - complexation (365 articles) (The search with results of 1782 and 411 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSpeciation%20-%20Complexation
Revised articles (26 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSpeciation%20-%20Complexation%2F%2F%2FSpeciation%20-%20Complexation%20revised
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**Search strategy for adsorption (6106 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*)
Search strategy for adsorption (149 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*) AND Topic=(fate)
Search strategy for adsorption (682 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*) AND Topic=(behav*)
Combined for adsorption (774 articles) (The search with results of 6106 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAdsorption
Revised articles (66 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAdsorption%2F%2F%2FAdsorption%20revised

**Search strategy for desorption (880 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*)
Search strategy for desorption (25 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*) AND Topic=(fate)
Search strategy for desorption (142 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*) AND Topic=(behav*)
Combined for desorption (155 articles) (The search with results of 880 articles was not used)

http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FDesorption
Revised articles (17 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FDesorption%2F%2F%2FDesorption%20revised

**Search strategy for combustion (738 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*)
Search strategy for combustion (6 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*) AND Topic=(fate)
Search strategy for combustion (77 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*) AND Topic=(behav*)
Combined for combustion (80 articles) (The search with results of 738 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FCombustion
Revised articles (18 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FCombustion%2F%2F%2FCombustion%20revised

**Search strategy for biotransformation (78 articles)**
Topic=(nano*) AND Topic=(environ*) AND Topic=(biotran*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(biomodi*)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FBiotransformation
Revised articles (20 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FBiotransformation%2F%2F%2FBiotransformation%20revised

**Search strategy for agglomeration (612 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*)
Search strategy for agglomeration (27 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*) AND Topic=(fate)
Search strategy for agglomeration (85 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*) AND Topic=(behav*)
Combined for agglomeration (103 articles) (The search with results of 612 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAgglomeration

**Search strategy for aggregation (2676 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*)
Search strategy for aggregation (186 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*) AND Topic=(fate)
Search strategy for aggregation (550 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*) AND Topic=(behav*)
Combined for aggregation (598 articles) (The search with results of 2676 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAggregation

**Search strategy for sedimentation (357 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*)
Search strategy for sedimentation (34 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*) AND Topic=(fate)

Search strategy for sedimentation (46 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*) AND Topic=(behav*)
Combined for sedimentation (350 articles)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSedimentation
Revised articles (35 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSedimentation%2F%2F%2FSedimentation%20revised

**Search strategy for Speciation – complexation (1782 articles – 411 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*)
Search strategy for speciation – complexation ( 38 - 21 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*) AND Topic=(fate)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*) AND Topic=(fate)
Search strategy for speciation – complexation (237 - 105 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(complexa*) AND Topic=(behav*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(specia*) AND Topic=(behav*)
Combined for speciation - complexation (365 articles) (The search with results of 1782 and 411 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSpeciation%20-%20Complexation

**Search strategy for adsorption (6106 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*)
Search strategy for adsorption (149 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*) AND Topic=(fate)
Search strategy for adsorption (682 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(adsorp*) AND Topic=(behav*)
Combined for adsorption (774 articles) (The search with results of 6106 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAdsorption

**Search strategy for desorption (880 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*)
Search strategy for desorption (25 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*) AND Topic=(fate)
Search strategy for desorption (142 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(desorp*) AND Topic=(behav*)
Combined for desorption (155 articles) (The search with results of 880 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FDesorption

**Search strategy for combustion (738 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*)
Search strategy for combustion (6 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*) AND Topic=(fate)
Search strategy for combustion (77 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(combus*) AND Topic=(behav*)
Combined for combustion (80 articles) (The search with results of 738 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FCombustion

**Search strategy for biotransformation (78 articles)**
Topic=(nano*) AND Topic=(environ*) AND Topic=(biotran*)
Topic=(nano*) AND Topic=(environ*) AND Topic=(biomodi*)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FBiotransformation

**Search strategy for agglomeration (612 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*)
Search strategy for agglomeration (27 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*) AND Topic=(fate)
Search strategy for agglomeration (85 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(agglo*) AND Topic=(behav*)
Combined for agglomeration (103 articles) (The search with results of 612 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAgglomeration

**Search strategy for aggregation (2676 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*)
Search strategy for aggregation (186 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*) AND Topic=(fate)
Search strategy for aggregation (550 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(aggre*) AND Topic=(behav*)
Combined for aggregation (598 articles) (The search with results of 2676 articles was not used)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FAggregation

**Search strategy for sedimentation (357 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*)
Search strategy for sedimentation (34 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*) AND Topic=(fate)
Search strategy for sedimentation (46 articles)
Topic=(nano*) AND Topic=(environ*) AND Topic=(sedimenta*) AND Topic=(behav*)
Combined for sedimentation (350 articles)
http://www.refworks.com/refshare2?site=039271152590400000/65841362477434120/nanoDEN%2F%2F%2FSedimentation

**Search strategy for macrocolecules (777 articles):**
Topic=(nano*) AND Topic=(environ*) AND Topic=(macromolecule*) → 646 articles
Topic=((nano*) AND (environ*) AND ((macromolecule*) OR (humic*) OR (NOM))) AND Topic=((behav*) OR (fate)) → 216 articles → 216 articles
http://www.refworks.com.globalproxy.cvt.dk/refshare2?site=039271152590400000/70801377779398192/NanoDEN%20Macromolecules
Revised articles ( 75 articles) accessible through:
http://www.refworks.com/refshare2?site=039271152590400000/70801377779398192/NanoDEN%20Macromolecules%2F%2F%2FSelected

## Environmental fate and behaviour of nanomaterials

Rapporten gennemgår den eksisterende viden om de processer der er bestemmende for nanomaterialers miljøskæbne og peger på de største videnhuller.

In the current report, the existing knowledge on the fate of nanomaterials in the environment is reviewed and the major knowledge gaps are identified.

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