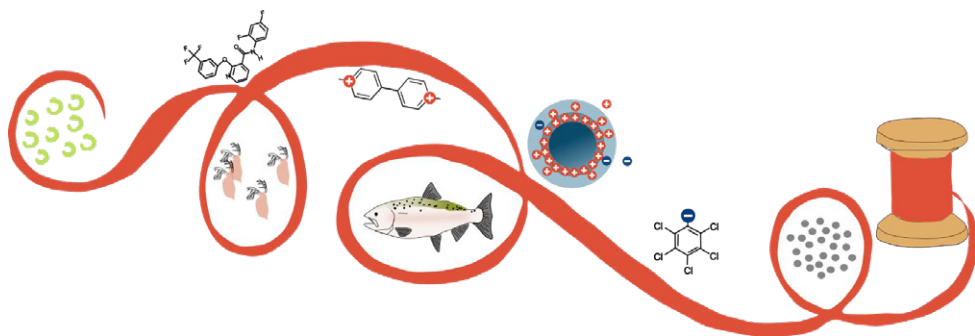# PH.D. THESIS

The aquatic ecotoxicity of manufactured silica nanomaterials and their interactions with organic pollutants

Frida Book

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GOTHENBURG

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To my family and all the people who are fighting and have fought in the history for girls and women's right to education

Abstract

Manufactured silica nanomaterials are one of the nanomaterials consumed in the highest volumes (more than 4 000 000 tons/year/globally) and are used in a wide range of products and industries such as food, cosmetics, coatings, paints, textiles, concrete and the paper and pulp industry. They also provide promising properties that help solving societal challenges, such as water remediation, by binding contaminants. However, their large and wide use leads to an inevitable release into surface waters, which raises concerns of potential environmental impacts, because of their small size, reactive surfaces and the risk of facilitating biological uptake of other co-occurring chemicals ("trojan horse effect"). Therefore, this thesis systematically investigates the aquatic ecotoxicity of manufactured silica nanomaterials alone and in combination with toxic organic chemicals. The thesis is based on an in-depth ecotoxicological evaluation of nine silica nanomaterials with different size, charge, surface modification and shape in experiments with bacteria (*Pseudomonas putida*), algae (*Raphidocelis subcapitata*), crustacean (*Daphnia magna*) and fish gill cells (*Oncorhynchus mykiss*). These data are then complemented with data from other scientific publications in a systematic review in the ecotoxicology of silica nanomaterials, in order to derive the maximum acceptable environment concentration in the aquatic environment (PNEC, the predicted no effect concentration). Finally, this thesis examines the ability of silica materials to adsorb organic pollutants with different charges: paraquat (cationic), hexadecylpyridinium (cationic), pentachlorophenol (anionic), diflufenican (neutral) and whether this interaction alters the ecotoxicity of exposed organisms and cells.

The results show that impacts are a result of surface area, surface chemistry and exposed organism/cell type. Silica nanomaterial that is sterically stabilized with glycerol propyl tails is benign in all assays showing no signs of toxic action. This is likely due to a steric hindrance that prevents contact between the material and the cells/species. Weakly anionic (non-modified) and strongly anionic (aluminium-modified) silica are toxic to fish gill cells with EC50 values between 12 and 93 mg/L. This toxicity in fish cells depends on the total surface area of the nanomaterial that is covered with deprotonated silanol groups which binds to and interrupts membrane proteins function. As a consequence, if concentrations are expressed as surface area instead of mass, the toxicity of different nanomaterials becomes quite similar, with EC50 values differing not more than by a mere factor of 1.4 (1.8-2.5 m^2/L). In contrast to experiments with fish cells, strongly anionic

silica is not toxic to algae at concentrations up to 500 mg/L, likely due to the presence of a cell wall, which hampers nanomaterial-cell interactions. However, cationic and non-modified silica nanomaterials cause an inhibition of algal growth, EC50 values of 124 mg/L and 200 mg/L, respectively. This effect is likely caused by an adsorption of the material to the algae, shading them and thereby impacting the photosynthetic production of physiological energy.

The results from the exposures to mixtures of silica nanomaterial and organic pollutants show that strongly anionic nanomaterials bind cationic paraquat and thereby reduce paraquat toxicity to algal cells. In addition, the cationic material can bind and reduce toxicity of pentachlorophenol in algae, which is likely pH and phosphate dependant. Experiments with fish cells indicate that the anionic NMs bind the cationic hexadecylpyridinium, but do not reduce the toxicity in exposed fish cells. Instead, the observed effects correspond well with the effects predicted with the two concepts independent action and concentration addition. In general, the mixture experiments indicate that silica nanomaterials possess promising properties to bind and remove certain pollutants in water. However, the use of silica nanomaterials for such purpose in industrial applications requires additional research on the water types that can be cleaned (industrial wastewater, household wastewater, raw drinking water, surface waters etc.), life-cycle based assessments of costs and benefits, and a comparison with other materials for water treatment.

Sammanfattning

Syntetiserade nanomaterial bestående av kiseldioxid används i ett brett utbud av produkter och industrier så som livsmedel, kosmetika, elektronik, färger, textilier, betong och pappersindustrin. Nanomaterialet har egenskaper som gör att det på ett effektivt sätt skulle kunna rena förorenat vatten genom att binda till sig giftiga kemikalier, men deras användning i produkter leder också till oundvikliga utsläpp till vatten, vilket ger upphov till oro för miljöpåverkan. Deras potentiella toxiska effekt tillskrivs ofta deras små storlek (nanostorlek), reaktiva yta och att de kanske kan underlätta upptaget av andra samtidigt närvarande potentiellt farliga ämnen (Trojansk häst-effekt). Syftet med avhandlingen är därför att systematiskt undersöka hur giftiga dessa nanomaterial av kiseldioxid är för vattenlevande organismer och dess effekter i blandningar med andra (kända giftiga) organiska föroreningar.

Avhandlingen omfattar tester med totalt nio kiselprodukter med olika storlek, form, laddning och ytkemi i experiment med bakterier, alger, kräftdjur och fiskceller. Resultaten kompletteras också med data från andra vetenskapliga publikationer i en systematisk översiktsanalys för att beräkna vilka koncentrationer i vattenmiljön som inte förväntas medföra några negativa effekter. Vidare undersöker avhandlingen nanomaterialets förmåga att binda till sig organiska föroreningar (bekämpningsmedel) med olika laddningar: parakvat (positiv), hexadecylpyridinium (positiv), pentaklorfenol (negativ) och diflufenikan (oladdad) och om förmågan att binda till sig föroreningarna förändrar giftigheten i de exponerade organismerna och cellerna.

Resultaten visar att effekterna på organismerna/cellerna är beroende av materialets ytarea samt ytkemi. Kiseldioxidnanomaterial som är ytbehandlade med hydrofila (vattenälskande) glycerol-propyl molekyler var ofarliga och visade inga tecken på giftighet. Detta beror sannolikt på att ytkemin förhindrar att materialet och organismerna kommer i kontakt med varandra. Svagt negativt laddade material samt starkt negativt laddade material gav upphov till en 50 % minskning av cellviabiliteten hos gälceller från fisk mellan koncentrationerna 12 och 93 mg/L (EC50 värden). Denna effekt beror på den totala ytan hos nanomaterialet som bär på ytmolekyler som binder till och stör funktionen hos proteiner i cellmembranet. Därav skiljer sig EC50 värdena endast med en faktor 1.4 (1.8-2.5 m^2/L) när koncentrationerna uttrycks i ytarea istället för massa. I motsats till experiment med fiskceller visar de starkt negativt laddade materialen inga effekter hos alger vid koncentrationer upp till 500 mg/L. Detta beror troligen

på att alger har en cellvägg som hämmar interaktioner mellan nanomaterialet och cellerna. Istället hämmar de svagt negativt och positivt laddade kiseldioxidmaterialen tillväxten hos alger med EC50 värden på 124 mg/L respektive 200 mg/L. Denna effekt orsakas sannolikt av att dessa material binder till utsidan på algerna och skuggar dem, vilket påverkar den fotosyntetiska produktionen av fysiologisk energi.

Resultaten från exponeringen för blandningarna av nanomaterial och organiska föroreningar visar att starkt negativt laddade material binder positivt laddad parakvat och på så vis minskar biotillgängligheten och giftigheten i algerna. Det positiva kiseldioxidmaterialet binder negativt laddad pentaklorfenol, men den bindningsförmågan verkar vara beroende av pH och fosfathalt i vattenmiljön. Experimenten indikerar att nanomaterialen skulle kunna avlägsna vissa föroreningar i vatten. Användningen av dessa nanomaterial för vattenrening kräver dock ytterligare forskning kopplat till vilka typer av vatten som kan renas (till exempel industriellt avloppsvatten, hushållsavloppsvatten, obehandlat dricksvatten och ytvatten), livscykelanalyser, nyttokostnadsanalyser och jämförelser med andra material som används eller planeras att användas inom vattenrening.

List of publications

I. Ecotoxicity screening of seven different types of commercial silica nanoparticles using cellular and organismic assays: Importance of surface and size
    Frida Book, Mikael T. Ekvall, Michael Persson, Sara Lönnerud, Tobias Lammel, Joachim Sturve, Thomas Backhaus. (2019) *NanoImpact*, 13, 100-111. Doi: 10.1016/j.impact.2019.01.001

II. Aquatic ecotoxicity of manufactured silica nanoparticles: A systematic review and meta-analysis
    Frida Book and Thomas Backhaus. (2021) *Science of The Total Environment,* 806, 150893. Doi: 10.1016/j.scitotenv.2021.150893

III. Colloidal silica nanomaterials reduce the toxicity of pesticides to algae, depending on charge and surface area
    Frida Book, Michael Persson, Eric Carmona, Thomas Backhaus, Tobias Lammel. (2022) *Environmental Science. Nano*. Accepted for publication with revisions.

IV. Co-exposure of colloidal silica and pesticides to the rainbow trout gill cell line RTgill-W1
    Frida Book, Francis Okoroigbo, Thomas Backhaus, Tobias Lammel (2022). Submitted for publication.

Publications not included in the thesis

- Teaching practices in science education related to chemical usage, their hazards and risks
  Bethanie Carney Almroth, Noomi Asker, Giedrė Ašmonaitė, Lina Birgersson, **Frida Book**, Tobias Lammel, Joachim Sturve. *Integrated Environmental Assessment and Management*. (2021). 17 (2), 482-483. Doi: 10.1002/ieam.4386

- Assessing the effects of textile leachates in fish using multiple testing methods: from gene expression to behaviour
  Bethanie Carney Almroth, Josefine Cartine, Christina Jönander, Max Karlsson, Julie Langlois, Matilda Lindström, Jakob Lundin, Nina Melander, Argus Pesqueda, Ida Rahmqvist, Juliette Renaux, Josefin Roos, Francis Spilsbury, Joel Svalin, Hanne Vestlund, Liqian Zhao, Noomi Asker, Giedrė Ašmonaitė, Lina Birgersson, Tahereh Boloori, **Frida Book**, Tobias Lammel, Joachim Sturve. (2021). *Ecotoxicology and Environmental Safety,* 207, 111523. Doi: 10.1016/j.ecoenv.2020.111523

Table of contents

List of abbreviations

AF = Assessment factor

AB = AlamarBlue

CA = Concentration addition

DLS = Dynamic light scattering

ECx = Effect concentration causing x % effect

HC05 = Hazard concentration putting 5 % of species at risk

IA = Independent action

ICx = Concentration causing x % inhibition

LC-MS = Liquid chromatography- mass spectrometry

LCx = Concentration causing x % lethality

LDV = Laser doppler velocimetry

LOEC = Lowest observed effect concentration

MoA = Mode of action

MWCT = Multi-walled carbon nanotubes

NanoCRED = Nano criteria for reporting and evaluating ecotoxicity data

NM = Nanomaterial

NOEC = No observed effect concentration

OECD = Organisation for economic co-operation and development

PALS = Phase analysis light scattering

PEC = Predicted environmental concentration

PNEC = Predicted no effect concentration

SbD = Safety-by-design

SEM = Scanning electron microscopy

SSD = Species sensitivity distribution

ZP = Zeta-potential

1 Introduction

Nanotechnology is identified as a key enabling technology for sustainable competiveness and growth (European Commission, 2012) and has expanded exponentially during the twentieth and twenty-first centuries (Evans *et al.*, 2021). Current and potential applications cover broad areas such as textiles, renewable energy, food, agriculture, health care, biomedicine and remediation, with the potential to solve major societal challenges such as clean water supply, energy efficiency and new cancer treatments (Tsuzuki, 2009; van Wezel *et al.*, 2018; Khan, Saeed and Khan, 2019; Zhu *et al.*, 2019) (Figure 1). According to the Nanodatabase, there are more than 5 000 commercial products that contain NMs (www.Nanodatabase) (Hansen, Hansen and Nielsen, 2020) and the global market of nanomaterials is estimated to be worth 8.5 billion U.S dollars and is expected to increase with 13 % per year from 2020-2027 (Pandey and Jain, 2020).

Figure 1. Current and potential applications of nanomaterials. Adapted from Tsuzuki (2009).
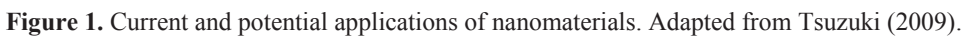
A NM is as material that has at least one of its dimension in the nanoscale 1-100 nm (Boverhof *et al.*, 2015). The unique properties of nanomaterials (NMs) are owed to their small size, leading to high surface area and surface energy per mass (Evans *et al.*, 2021). These properties also make them reactive since more molecules are exposed to the surroundings, as compared to when imbedded in the bulk material (Kolasinski, 2019). A simple illustration that shows the vast increase in surface area-to-volume for NMs is the cube example (National Nanotechnology Initiative, 2022) (Figure 2). If a 1 cm^3 cube is split into 1 nm^3 cubes the surface area increases 10 000 000 fold (from 6 cm^2 to 60 000 000 cm^2).
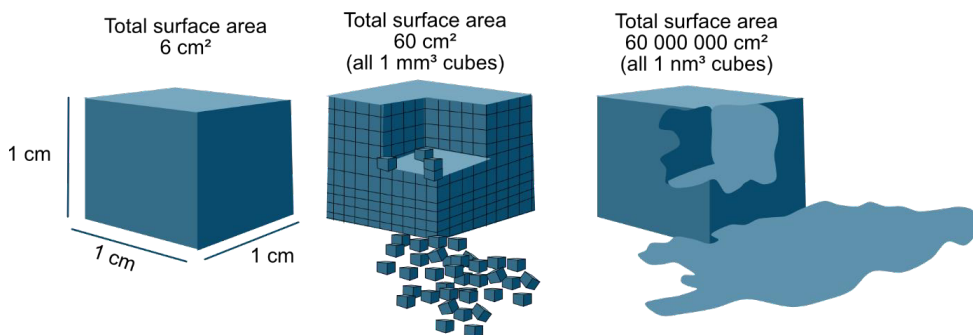
Figure 2. Graphical illustration of the increased surface area when splitting a 1 cm^3 cube into 1 nm^3 cubes. Adapted from the National Nanotechnology Initiative (2022).

Nanotechnology also enables customizing NMs with different physico-chemical properties such as shape, surface charge, surface functionalization and composition, depending on the specific application. For example, NMs can be spherical or rod-shaped, amorphous or crystalline, inorganic or organic, hollow or dense, cationic or anionic, hydrophilic or hydrophobic, aggregated or appear as single entities etc. (Figure 3) (Hassellöv and Kaegi, 2009).

Figure 3. Schematic picture of possible physical-chemical properties of nanomaterials with regard to concentration, shape, size, size distribution, structure, composition, surface area, surface functionality, surface charge and agglomeration state. Adapted from Hasellöv och Kaegi (2009).

1.1 Concerns related with the use of manufactured nanomaterials

NMs can have beneficial effects in several different fields (as mentioned above), but the rapid development of the nanotechnologies and the possibilities they possess 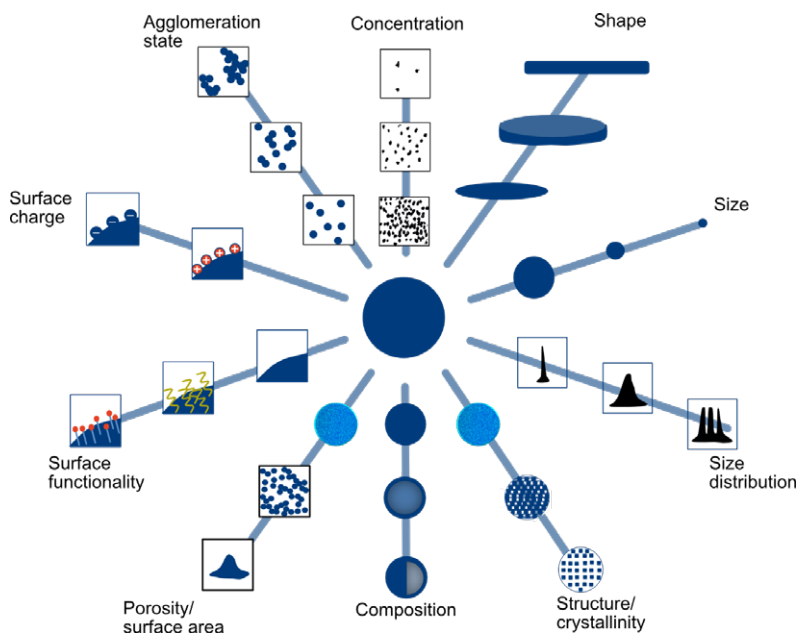have also led to an increased concern regarding human and environmental safety. This stems from the fact that NMs are in the same size as many of the vital subcellular components of biological life such as DNA, proteins and membranes (Handy *et al.*, 2008; Kasemo *et al.*, 2013) (Figure 4). Manufactured NMs might therefore interfere with biological processes, which could lead to toxic impacts on human and environmental health (Handy *et al.*, 2008). History also reveals lessons not to be repeated, as in the development of the nano-sized crystalline asbestos, which provided good properties for the construction industry, but if inhaled could cause silicosis (a long-term lung disease) and lung cancer (Napierska *et al.*, 2010; Murugadoss *et al.*, 2017). The human and environmental concern raised is also well reflected by the enormous amount of effort and money put into safety research, on an European and global level (EU Nanosafety Cluster, 2016; Rasmussen *et al.*, 2016).
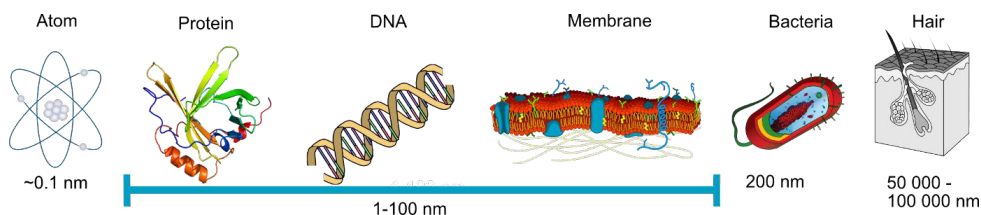
Figure 4. Illustration of different biological entities within the nanoscale 1-100 nm in comparison to the size of an atom (0.1 nm), bacteria (200 nm) and thickness of a human hair (50 000-100 000 nm).

1.1.1 Safety-by-design

The concept of "Saftey-by-Design" (SbD) was suggested as a consequence to the potential adverse effects on human health and the environment by newly developed NMs (Schmutz *et al.*, 2020). The purpose of the SbD concept is to gain knowledge of which properties that could make a NM more or less safe. This knowledge can then be used during the innovation and design of new nano-enabled products, in order to reduce potential risks (Kraegeloh *et al.*, 2018). For example, if experiments show that a specific surface modification is less hazardous than other modifications while being equally efficient to use in a certain product it should be a first-hand choice to choose the more benign modification. Unfortunately, the current nano(eco)toxiclogical testing of NMs cannot keep pace with the speed of which nanotechnology is developing, so far, the testing is complex, slow and of high costs (Fadeel *et al.*, 2018; Afantitis *et al.*, 2020). This calls for experiments with NM libraries with systematically varying physico-chemical properties, which enable a mechanistic understanding of the drivers of manufactured NM toxicity(Fadeel *et al.*, 2018).

1.2 Silica nanomaterials

Silica NMs consist of three dimensional networks of tetrahedral units consisting of silicon and oxygen (SiO_4). These units can be connected in a regular network (crystalline silica) or an irregular network (amorphous silica) (Fruijtier-Pölloth, 2012) (Figure 5). Silica NMs found in the environment can be of both natural and manufactured origin. Examples of natural origin are weathering from rocks, suspended particles in water and diatom frustules (Dürr *et al.*, 2011; Liesegang *et al.*, 2017; Tramontano *et al.*, 2020; Cheng *et al.*, 2021).
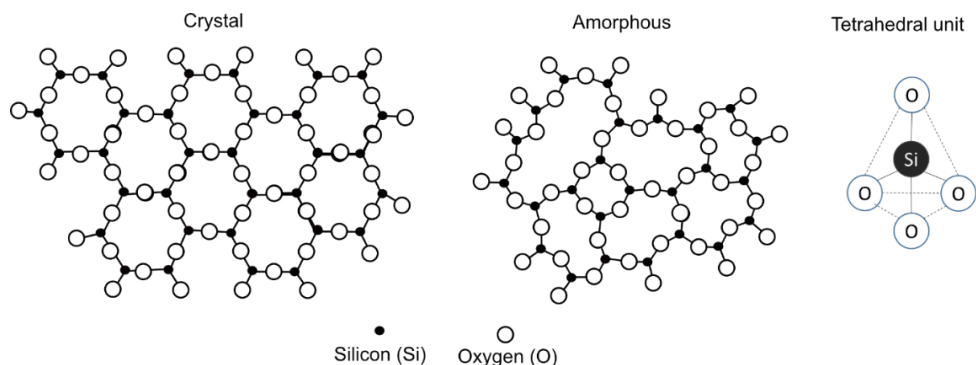
Figure 5. Crystalline (regular) and amorphous (irregular) structures of tetrahedral units consisting of silicon and oxygen.

1.2.1 Manufactured silica nanomaterials

Manufactured silica NMs is one of the NMs produced in the largest volumes with a consumption volume of more than 4 000 000 tonnes per year globally (IHS, 2017). More than 1 000 000 tons per year might be manufactured or imported to the European Union alone (ECHA, 2020). Silica NMs are used in numerous products and industries such as paints, coatings, cosmetics, food, textiles, tyres, concrete, biocides and the pulp and paper industry (Yamashita *et al.*, 2011; Kaiser, Zuin and Wick, 2013; Products, 2014). They are also extensively studied in the biomedicine field for drug delivery, where hollow mesoporous silica is often used which can be loaded with drugs that are released at the target site (Kwon *et al.*, 2013; Vallet-Regí *et al.*, 2018; Gao *et al.*, 2020).

Silica NMs can be manufactured through various synthesis processes often originating from a solution with silicic acid (Napierska *et al.*, 2010). When the silicic acid reaches a sufficient high concentration, the silicic acid monomers start to polymerize and particles take form. The final silica product is then a result of the specific synthesize route, which can result in amorphous (e.g. precipitated, colloidal, mesoporous, fumed) or crystalline (e.g. quartz and porosil) forms (Figure 6). Due to the different synthesis methods, these products will have different surface chemistries. Colloidal and precipitated silica are produced under wet and low temperature conditions, which leads to a high number of silanol groups at the surface. In contrast, pyrogenic silica is synthesized at high temperature and dry conditions, which results in surface dehydroxylation and formation of hydrophobic siloxane bonds (Napierska et al 2010, Zhang et al 2012). In addition, these bare silica surfaces can further be modified with either

inorganic or organic atoms or molecules to obtain enhanced properties for specific applications (Figure 1). The sizes of manufactured silica NMs differ depending on synthesis process. Fumed and precipitated silica consist mostly of small aggregates with dimensions larger than 100 nm, mesoporous silica is a pore-structured material with pore diameters of 2-50 nm and colloidal silica comprises of particles in suspensions usually between 5-100 nm (in diameter)
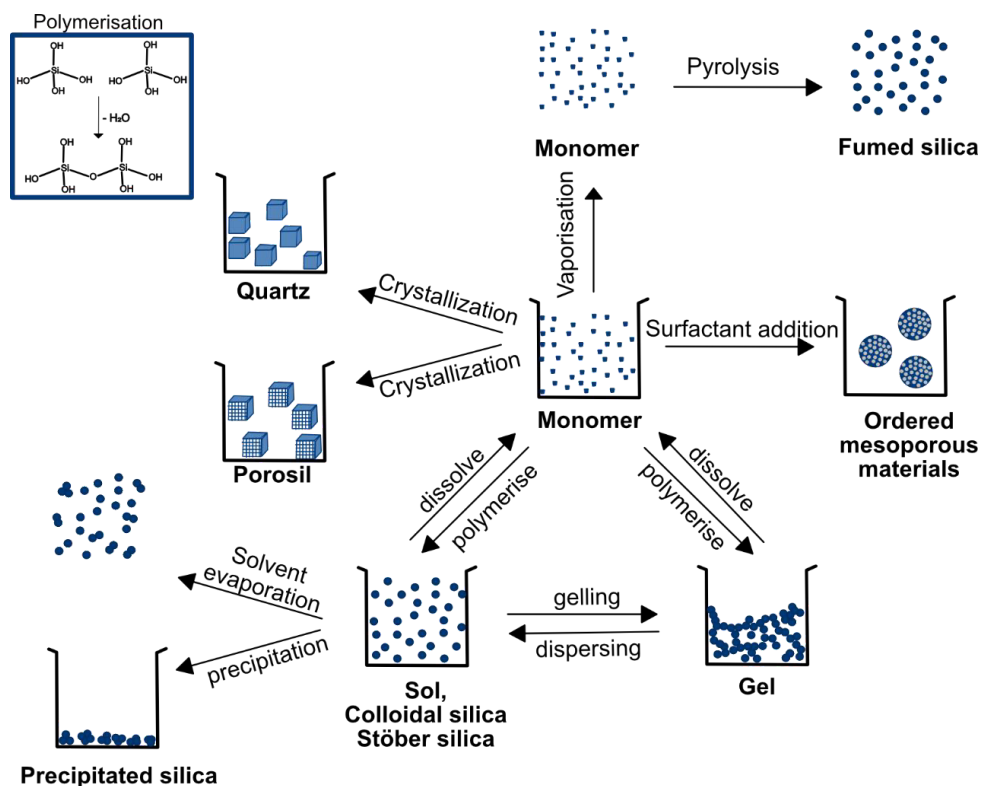
Figure 6. Graphical scheme depicting silica synthesis processes and their different end-products. Adapted from Napierska *et al* (2010).

1.2.2 Manufactured silica nanomaterials in the aquatic environment

The large and wide use of manufactured silica NMs will lead to an inevitable release (during production, use and waste disposal) into the aquatic environment (Y. Wang *et al.*, 2016; Wang and Nowack, 2018). Today, there is no method to measure the concentration of manufactured silica NMs in waters since the concentration of the manufactured cannot be separated from natural occurring

silica (Yang *et al.*, 2019). Exposure estimates are therefore based on predictions by computational models. Estimated concentrations in surface waters in Europe are reported to be between 0.1 to 2.6 µg/L (Y. Wang *et al.*, 2016; Wang and Nowack, 2018). These values are based on production volumes between 17.5-836 467 tons/year which might be an underestimation in view of the >1 000 000 tons/year that is reported to ECHA (ECHA, 2020) and the consumption volume of more than 4 000 000 tons/year/globally (IHS, 2017). These estimates also do not account for typical environmental fate processes, such as NM sedimentation, dissolution or any transformation (Arvidsson *et al.*, 2011), all of which leads to further uncertainties of reported values.

1.2.2.1 Aquatic ecotoxicity

The number of ecotoxicological studies with silica NMs has increased exponentially during the two last decades (Ale *et al.*, 2021). However, the perception of the number of relevant studies differs between authors. A review by Fruijtier-Pölloth (2012) claims that there is an extensive amount of data available, while the summary report from the organisation for economic co-operation and development (OECD) Working party on manufactured nanomaterials (OECD, 2016) and the review by Ale *et al.*, (2021) both argue that there are only a few relevant publications available and that more research is needed.

The effect concentrations for silica NMs vary greatly both within and between aquatic species. Studies with fish report malformation and mortality in zebrafish (*Danio Rerio*) embryos at 10-25 mg/L (Lee *et al.*, 2011; Duan *et al.*, 2013) while other studies report no toxicity in zebrafish embryos at concentrations up to 1000 mg/L (George *et al.*, 2011; Christen and Fent, 2012). Studies on the crustacean *Daphnia magna* report 10-50 % mortality at 1-2 mg/L (Lee, Kim and Choi, 2009; NanoReg, 2016), while another study reports 2.5 % mortality at 10 000 mg/L (OECD, 2016). Exposures to green algae *Raphidocelis subcapitata* show 20 % growth inhibition at 16.3 mg/L (Van Hoecke *et al.*, 2008) but also no growth inhibition up to 1000 mg/L (Casado, Macken and Byrne, 2013). Exposures to the bacteria species *Escherichia coli* report 15 % growth inhibition at 500 mg/L (Adams, Lyon and Alvarez, 2006) while another study with the same species reports 58 % growth inhibition already at 20 mg/L (Jiang, Mashayekhi and Xing, 2009).

The large variability in reported effect concentrations in combination with a lack of reported physico-chemical properties makes it difficult to draw conclusions on the NM properties that might drive ecotoxicity. This applies to not only research

with silica NMs, but for the nanoecotoxicological research as a whole. Specifically, reports and papers from the first ~15 years of research (2000-2015) (Krug, 2014; Kühnel and Nickel, 2014). Toxicity in general may depend on different NM properties including shape, size, surface area and surface chemistry as well as abiotic and biotic factors in the experimental set-up such as pH, ionic strength and natural organic matter (NOM), since these can alter NM behaviour (Kühnel and Nickel, 2014; Fadeel *et al.*, 2018; Ekvall *et al.*, 2021). For example, high ionic strength in the media often increases NM agglomeration, which in turn increase size and decreases surface area (Pfeiffer *et al.*, 2013). Therefore, characterization of the test material is important. The pristine material should be characterized as well as the NMs in the biotest system (Kühnel and Nickel, 2014; Hartmann *et al.*, 2017; OECD, 2020).

Despite the large variability and difficulties in comparing studies, ecotoxicological research with silica NMs reveals some common features. For example, silica NMs have a higher ecotoxicity per mass in comparison to the bulk material (Van Hoecke *et al.*, 2008; Wei *et al.*, 2010a; Ye *et al.*, 2013; Yang *et al.*, 2014; Katsumiti *et al.*, 2015). The mode of action (MoA) seems to stem from the deprotonated silanol groups at the material surface that bind to and interrupt proteins in the cell membrane (Fruijtier-Pölloth, 2012). Moreover, they frequently adsorb onto the surface of various species groups, for example bacteria (Jiang, Mashayekhi and Xing, 2009), algae (Wei *et al.*, 2010a; Yu *et al.*, 2018), crustacean (Yang *et al.*, 2014), fish chorion, gill (Fent *et al.*, 2010; Lee *et al.*, 2011; Xu *et al.*, 2012; Ye *et al.*, 2013; Lacave *et al.*, 2016; Pham *et al.*, 2016) and impacts are often suggested to originate from this adsorption, such as shading and flocculation for algae (Jiang, Mashayekhi and Xing, 2009; Van Hoecke *et al.*, 2011), blocking of gas exchange in the fish chorion (Fent *et al.*, 2010) or alterations in gill ionoregulation (Krishna Priya *et al.*, 2015).

1.3 Mixture exposures
When manufactured NMs (including silica) are released into the aquatic environment (e.g. rivers and lakes) they will co-occur with other chemicals/chemical mixtures (Hartmann and Baun, 2010; Naasz, Altenburger and Kühnel, 2018). This leads to an increased concern for mixture effects and whether the NM can act as carrier for pollutants leading to enhanced toxicity (Martín-de-Lucía *et al.*, 2017; Naasz, Altenburger and Kühnel, 2018; Martinez *et al.*, 2022). Scientific evidence for exposure to NM-mixtures reflect six different exposure

scenarios based on how the bioavailability of the chemical changes when a NM is present due to adsorption/desorption and uptake/no uptake (Naasz, Altenburger and Kühnel, 2018) (Figure 7). The different scenarios are termed Trojan horse (+), Trojan horse (-), surface enrichment, retention, inertism and coalism. Trojan horse (+) is the phenomena where the NM acts as a carrier of chemicals leading to an increased bioconcentration and toxicity. In contrast, the trojan horse (-) leads to a decrease in toxicity due to no desorption after uptake. Surface enrichment modifies the bioavailability of the chemical, for example by increased local concentration of the chemical near the organism. Retention is a prevention of exposure due to strong chemical adsorption in combination with no uptake of the NM. Inertism and coalism correspond to situations where no chemical adsorption occurs, but where bioconcentrations could increase due to physical damage caused by the NM (coalism) or due to co-transport (inertism).
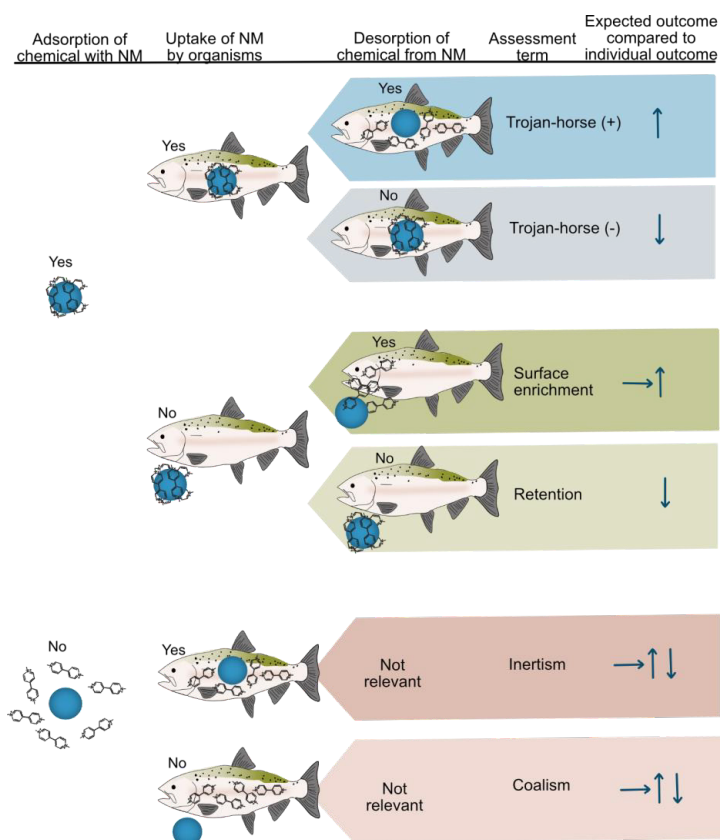

Figure 7. Suggested classifications of NM-chemical mixtures and their effects. Adapted from Naasz, Altenburger and Kühnel (2018).

Ecotoxicological effects from exposures to nano-mixtures have gained increased attention during the last years (Trinh and Kim, 2021; Martinez *et al.*, 2022). According to the review by Trinh and Kim, (2021), the majority of studies (128 out of 183) have investigated binary mixtures comprised of the NM TiO_2, C_{60}, multi-walled carbon nanotubes (MWCT), ZnO or Ag together with a heavy metal or organic chemical. Mixture studies with silica NMs approximately 1 % of all studies (Trinh and Kim, 2021). Mixture exposures with silica NMs often result in a decrease in toxicity of the other chemical(s). Srikanth *et al.*, (2015) observed decreased oxidative stress in eel brain exposed to 50 µg/L mercury in the presence of 2.5 mg/L silica coated iron NMs functionalized with dithiocarbamate. Martín-de-Lucía *et al.*, (2017) investigated the toxicity of a wastewater treatment plant effluent to bioluminescent cyanobacteria in the presence of silica NMs (1-500 mg/L). The silica NM (which was non-toxic to the bacteria) was able to reduce the toxicity of the wastewater significantly due to adsorption of wastewater pollutants, which lowered their bioavailability. Moreover, Cui *et al.*, (2017) report a decreased toxicity of Cd contaminated soil in rice cells in co-exposure with silica NMs. The silica NMs increased the dry weights of grains and shoots and at the same time decreased the total Cd content in grains and shoots.

1.3.1 Predicting mixture effects

The mathematical models used for predicting effects of chemical mixtures are concentration addition (CA) and independent action (IA), which are based on the concept that all mixture components are contributing to the overall toxicity either by acting similarly (CA) or dissimilarly (IA) (OECD, 2018; Rudén *et al.*, 2019). These concepts assume that the compounds of the mixture do not interact and affect each other's uptake, transport, metabolism or excretion and therefore do not account for synergism or antagonism (Cedergreen, Svendsen and Backhaus, 2013). NMs have a large specific surface area and energy, and thus high adsorption capability for molecular (i.e., non-particulate) chemicals (Figure 7) and could possibly cause synergistic or antagonistic effects (Martinez *et al.*, 2022). The concepts CA and IA might therefore not always work as prediction models for NM-mixtures (Martinez *et al.*, 2022). The review by Trinh and Kim (2021) report that merely 9 out of 183 studies that investigated effects of NM-mixtures have applied CA and IA models, which are too few to draw any general conclusions regarding the applicability of CA and IA for mixtures containing NMs. Moreover, none of these studies used a silica NM, which show that they are unexplored in research investigating ways to predict effects from NM-mixtures.

1.4 Silica nanomaterials as adsorbents for pollutants in water

One promising application for NMs is the remediation of environmental pollutions in water by adsorption (Yunus *et al.*, 2012; Adeleye *et al.*, 2016; Mauter *et al.*, 2018; Iravani, 2020). The adsorption efficiency is owed to their large specific surface area and surface reactivity (Auffan *et al.*, 2011; Guerra *et al.*, 2018). In addition, the surface can also be modulated, in order to alter the adsorption efficiency and for example to be selective (Yunus *et al.*, 2012). Studies show that NMs have the potential to remove a broad range of pollutants with diverse physico-chemical properties such as Per- and Polyfluoroalkyl Substances (PFAS), heavy metals, chlorinated solvents, pharmaceuticals and pesticides (Santhosh *et al.*, 2016; Cai *et al.*, 2018; Ganie *et al.*, 2021) through intermolecular binding forces such as hydrogen bonding, electrostatic interaction, covalent bonding and hydrophobic forces (Hao *et al.*, 2017; Sadegh *et al.*, 2017; Otalvaro and Brigante, 2018).

Silica NM is one type of NM that is suitable for use as an adsorbent for water pollutants due to their tunable chemical structure and shape, amendable surface, thermal stability and mechanical strength (Brigante and Schulz, 2011; Zhang, Li and Yu, 2016; Cao *et al.*, 2018) and have been shown to bind heavy metals and pesticides in water (Brigante and Schulz, 2011; Cao *et al.*, 2018; Lu and Astruc, 2018). They are also used as binders and flocculation agent in paper and ceramic productions to bind oppositely charged debris (Roberts and Griffin, 1992; Persson *et al.*, 2011). Silica NM can further be synthesized with a core made of iron, which enables a quick removal of from pollutants from the water phase by applying a magnetic field. In return, the silica shell prevents the iron from rusting (Adeleye *et al.*, 2016).

2 Aims and specific objectives

The overall aims of this thesis are to 1) assess the aquatic ecotoxicity of manufactured silica NMs and 2) investigate the use of silica NM as adsorbents for organic water pollutants through electrostatic interaction, in an ecotoxicological context. This resulted in five specific objectives as follows:

1. Characterize the toxicity of non-modified silica NMs to different species and across trophic levels (**paper I and II**)
2. Investigate the importance of surface chemistry as a drivers for toxicity (**paper I, III and IV**)
3. Investigate the importance of particle size, particle number and surface area as relevant descriptors for ecotoxicity and exposure (**paper I, III and IV**)
4. Estimate the maximum acceptable environment concentration (PNEC) for silica nanomaterials in the freshwater aquatic environment based on a SSD (**paper II**)
5. Characterize the ability of differently charged silica NMs to adsorb organic pollutants (**paper III and IV**).
6. Assess the aquatic ecotoxicity of mixtures comprising of silica NMs and organic pollutants (**paper III and IV**)

3 Material and methods

3.1 Selection of the silica nanomaterial test library

The test library of manufactured silica NMs was chosen based on three criteria, in order to meet the overall aims of the thesis. The criteria are as follows:

- Manufactured silica NMs with the same surface chemistry, but different size
- Manufactured silica NMs with the same surface chemistry, but different shape
- Manufactured silica NMs with similar size and shape, but different surface chemistry

These criteria resulted in a selection of nine different colloidal silica NMs with different sizes, shapes and surface chemistries (Table 1). All samples were supplied from the company Nouryon (formerly known as AkzoNobel) and belong to the Levasil® product line which are widespreadly used in products such as paints, surface polishing materials, catalysts, materaials coatings, cement and paper. Five out of nine products contain silica that was non-modified, weakly anionic, spherical and had diameters between 17 nm and 88 nm. Four materials have modified surface chemistries, either with aluminium or silane glycerol-propyl tails (Figure 8). The aluminium modification changes the surface charge to become cationic or increased anionic, depending if the aluminium is incorporated into the surface (with sodium aluminate) or attached onto the surface (with aluminium chlorohydrate). These aluminium modified surface charges are utilized within the paper and pulp industry, in order to bind oppositely charged debris in the papermaking production.
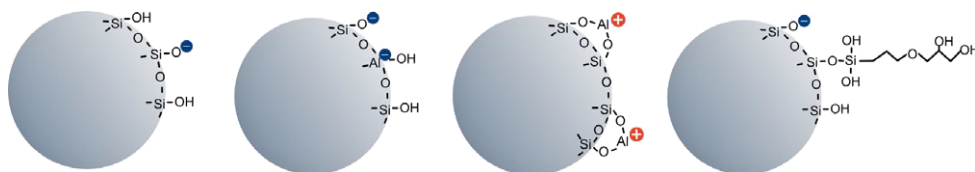

Figure 8. The four different surface chemistries used in the study. Non-modified with a weakly anionic charge (left), aluminium-modified with a strong anionic charge (middle left), aluminium-modified with a cationic charge (middle right) and modified with glycerol-propyl tails (right). Information on the surface chemistries was retrieved from Nouryon.

The silane modified silica is produced by letting (3-Glycidloxypropyl)triethoxysilane react with silanol groups on the silica surface. This results in a sterically stabilized material due to the forming of glycerol-propyl tails. Approximately 1.4 of a total 4.6 silanol per nm^2 are bonded, which results in a 30 % surface coverage and enhanced hydrophilicity (due to the glycerol-propyl tails). This modification is used in paints and coating formulations due to anti-soiling properties, since dirt is less prone to attach to hydrophilic surfaces. All materials were sampled directly within the product line, prior to the step where organic biocides are added, in order to avoid false positive results. The vast majority of the products are spherical except for one aluminium modified (strongly anionic) NM that is elongated (5 nm particles attached in a chain). The specific surface area of the elongated material ($1100 \ m^2/g$) is three times higher than the SSA of corresponding spherical material ($360 \ m^2/g$).

Table 1. List of the stock silica nanomaterials used in the thesis with information provided by the supplier. The nanomaterials are presented with product name, shape, mean average size, measured specific surface area (SSA), silica content, pH, surface chemistry, charge and relative charge density.

Product name	Shape	Average particle size (nm)[a]	Measured SSA (m^2/g)	Silica (wt%)	pH	Surface chemistry	Charge
Levasil® CS30-236	Spherical	17	360[b]	30	8-11	Non-modified	Weakly anionic
Levasil® CS25-436	Spherical	20	360[c]	25	5-11	Aluminium-modified	Strongly anionic
Levasil® CC301	Spherical	18	360[c]	28		Silane modified	Weakly anionic
Levasil® CS40-222	Spherical	21	220[b]	40	8-11	Non-modified	Weakly anionic
Levasil® CS40-213	Spherical	30	130[b]	40	8-11	Non-modified	Weakly anionic

Levasil®CS50-34P	Spherical	66	50[b]	50	8-11	Non-modified	Weakly anionic
Levasil®CS50-33P	Spherical	88	30[b]	50	8-11	Non-modified	Weakly anionic
Levasil®RD 2180	Elongated	5 (particles attached in a chain)	1100[c]	6.5	5-11	Aluminium-modified	Strongly anionic
Levasil®CS30-516P	Spherical	35	160[c]	25	2-5	Aluminium-modified	Cationic

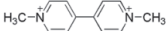[a] Number average measured with electrospray differential mobility analyser (ES-DMA) (Johnson *et al.*, 2008).
[b] SSA measured by Sears titration (Sears, 1956).
[c] SSA before surface modification with aluminium.
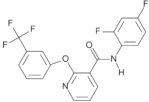
3.2 Selection of chemicals for investigating adsorption and co-exposures

Four different chemicals were selected for investigating NM adsorption capacity and ecotoxicological effects from co-exposures. These chemicals were chosen based on their known toxicity to aquatic biota and the possibility for electrostatic interaction with the charged silica. The final selection comprised of: 1) the cationic herbicide paraquat (PQ) (**paper III**), which is a photosystem I inhibitor, 2) the cationic surfactant hexadecylpyridinium (HDP) (**paper IV**), which exerts its toxic action by interrupting cell membranes, 3) the pesticide pentachlorophenol (PCP) (**paper III and IV**), an uncoupler of oxidative phosphorylation (weak acid) and 4) the neutrally charged herbicide diflufenican (**paper III**), which is a carotenoid biosynthesis inhibitor (DFF) (Table 2).

Table 2. List of organic chemicals used in the thesis. The chemicals are presented with name, CAS number, structure, pKa, net charge at pH 7, LogP and MoA. Charge at pH 7 was determined based on the chemical structure or the pKa.

Pesticide	CAS number	Structure[a]	pKa[b]	Net charge at pH 7	Log P	Mode of action[b]
Paraquat	4685-14-7		Not applicable	+2	-4.5[b]	Photosystem I (electron transport) inhibitor
Hexadecyl-pyridinium	6004-24-6		Not applicable	+1	1.71[c]	Interrupts membranes
Pentachloro phenol	87-86-5		4.7 (weak acid)	-1	3.3[b]	Uncoupler of oxidative phosphorylation
Diflufenican	83164-33-4		Not applicable	0	4.2[b]	Inhibition of carotenoid biosynthesis (bleaching)

[a] Chemical structures were retrieved from Wikimedia commons website(*Wikimedia Commons*, 2021).
[b] pKa, LogP and mode of action were retrieved from the Pesticide Properties DataBase (PPDP)(AERU, 2021).
[c] LogP was retrieved from Pubchem (PubChem, 2022).

3.3 Nanomaterial characterization methods

NM characterization has come to play a major role in nanotoxicological research and should include characterisations of the NM before exposure and in the test media (OECD, 2012). During the thesis, different characterization methods/techniques were used, dynamic light scattering (DLS), a combination of laser doppler velocimetry (LDV) and phase analysis light scattering (PALS) (**paper I, III and IV**), scanning electron microscopy (SEM) (**paper III**) and transmission electron microscopy (TEM) **(paper I)**. DLS and a combination of LDV and PALS were used to measure NM size and charge (zeta-potential (ZP)), respectively. SEM and TEM were used to characterize NM size and shape. In addition, SEM was used to image NM-algae interaction at the algae surface. The following sections describes the basic theories behind the methods.

3.3.1 Dynamic light scattering

The DLS method measures size based on Brownian motion (random movement of particles suspended in a medium) where the movement speed is dependent on size. Smaller particles move rapidly so when a sample is exposed to a laser beam, smaller particles will scatter light in a different pattern than in comparison with larger particles. This relationship between size and light scattered patterns can be expressed mathematically and is further utilized by the instrument software to calculate the particle size distribution (PSD). Measurements give the distribution by intensity, but these data can be converted to volume distribution (according to the Mie theory) and number distribution (Malvern instruments Ltd, 2004).

3.3.2 Laser doppler velocimetry and phase light scattering

LDV and PALS were utilized for measuring ZP, which is the potential measured at the slipping plane, that is the interface that separates ion mobile fluid and ion fluid that remains attached and moves with the particle (Figure 9). The ZP gives an indication of the stability of the colloidal system through the ability of the particles to repel each other and not agglomerate (Malvern instruments Ltd, 2004). The measurements are performed in cuvettes with electrodes. When a potential is applied, the particles will be attracted to the electrode with opposite charge. A stable suspension (when the particles move quickly) results in high negative (-30 mV) or high positive (+30 mV) ZPs. The speed of the particles is measured by illuminating the sample with a laser beam. The fluctuation rate of the intensity signal of the scattered light (arising from illuminating the sample as the particles move toward the opposite electrode) is proportional to the velocity of the particles, which is used for calculating the ZP (Malvern instruments Ltd, 2004).
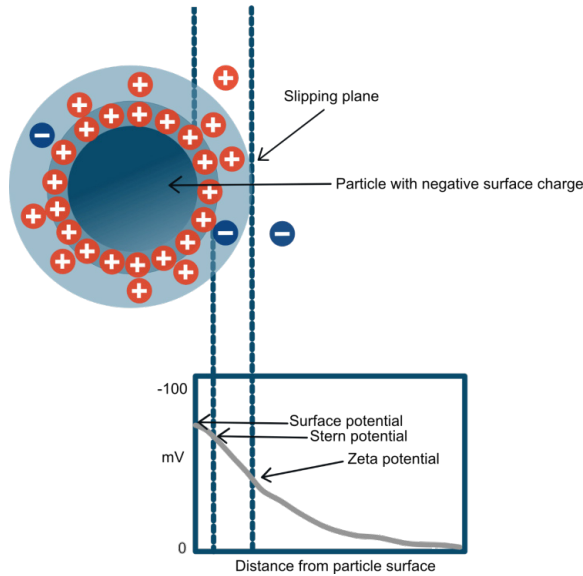
Figure 9. Potential difference in relation to distance from the charged surface of a particle suspended in a colloidal system. Adapted from Malvern instruments Ltd (2004).

3.3.3 Electron microscopy

SEM and TEM are high resolution imaging techniques used for studying a sample's features at or below nano-level by using a beam of electrons (Figure 10). In SEM, the focused beam scans over the specimen surface in a raster where each point in the raster corresponds to one pixel in the image. When the electron beam hits the specimen electrons are scattered back to detectors positioned above the sample (Figure 10). In this way, the technique images the sample's topography. Resolution is determined by factors such as the diameter of the electron probe as well as the atomic number of the elements in the specimen surface. Contrast is determined by atomic number and surface topography. For example, edges result in many scattered electrons which results in brighter pixels while flat areas result in low number of scattered electron and hence darker pixels (Hafner, 2007; Dehm, Howe and Zweck, 2012).

In TEM, the detector collects electrons transmitted through the whole image area simultaneously, and is positioned below the sample. Therefore, this technique often requires very thin samples (in the nanometre scale), in order for the electrons to be transmitted through the specimen. Information from the 3D sample thickness is then collapsed into a 2D projection. Contrast is determined by factors such as atomic number and sample thickness. For example regions with heavier atoms

will scatter electrons more (that is less electrons will reach the detector) resulting in darker pixels in comparison with regions containing lighter atoms (resulting in brighter pixels) (Williams and Carter, 2012).
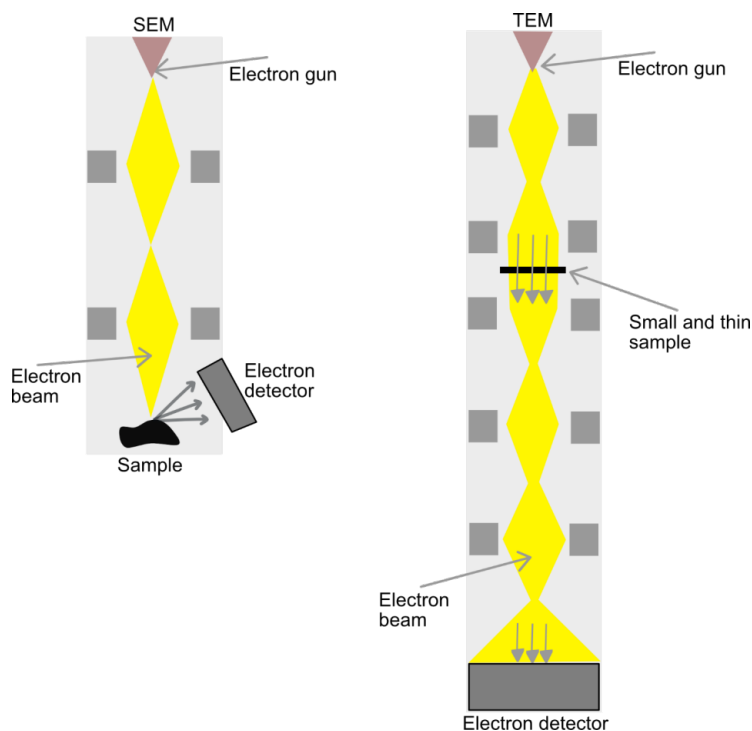
Figure 10. A simplified schematic picture of how scanning and transmission electron microscopy and works.

3.3 Ecotoxicological assays

The thesis comprised of laboratory experiments with four different test systems representing different levels in the aquatic food chain: bacteria (decomposers), algae (primary producers), zooplankton (secondary consumer) and fish (predator) (Figure 11). Exact species, their main function in the food web and the type of assay used are described in more detail below. Test concentration are based on previously used test concentrations in scientific literature.

3.3.1 Bacteria

Bacteria, *Pseudomonas putida,* is a gram-negative rod-shaped bacteria that is ubiquitous in waters and soils and important for nutrient cycles and biodegradation of organic matter (Matzke, Jurkschat and Backhaus, 2014; Fernández *et al.*, 2015). Experiments were performed according to the ISO growth inhibition assay ISO Guideline 10712 (ISO, 1995) (**Paper I**). *P. putida* were exposed to silica concentrations of 5-500 mg/L, for 16 h ±1 h and at the temperature 23 ± 1°C, where after growth was measured by optical density with a spectrophotometer at 700 nm.

3.3.2 Algae

Algae, *R. subcapitata*, is a freshwater green algae (formerly known as *Pseudokirchneriella subcapitata*) and plays an important role in ecosystems as primary producers forming the basis of the food chain by creating food through photosynthesis as well as being a main source of oxygen (Lin *et al.*, 2009). Algal cells were exposed for 72 h at a temperature of 22 ± 2°C according to the OECD Guideline 201 (OECD, 2011) (**Paper I and III**). After 72 h exposure, biomass is estimated by either counting the cells (with a flow cytometer) or by fluorometric measurements of chlorophyll content at the excitation/emission wavelengths 425nm/680nm. **Paper I** exposed *R. subcapita* to silica NM concentrations between 5-500 mg/L and measured biomass by cell counting. In **paper III**, algal cells were exposed to similar silica concentrations (1-500 mg/L), but biomass was instead estimated by measuring the chlorophyll content. The silica NMs in **paper II** were also tested for their toxicity when being in mixture with 4 µM PQ (EC80), 0.2 µM PCP (EC90) or 0.002 µM DFF (EC80).

3.3.3 Crustacean

Crustacean, *D. magna*, occurs in ponds and lakes and plays an important ecological role as a primary consumer of phytoplankton as well as a food source for secondary consumers (Miner *et al.*, 2012). The crustacean was exposed in accordance to the OECD Guideline 202 (OECD, 2004), for 24 h or 48 h at a temperature of 18-22°C ±1°C, where after mortality was measured by counting the number of immobilized organisms (**paper I**). *D. magna* were exposed to the silica concentrations 1000 mg/L and 10 000 mg/L.

3.3.4 Fish

Fish plays an important role in the aquatic food web such as regulators of lower trophic levels, but they are also one of the main source of food for humans. The fish cell line RTgill-W1, is a continuous cell line derived from the rainbow trout (*Oncorhynchus mykiss*) gill (Bols *et al.*, 1994) that can be used to 1) predict fish acute toxicity, 2) chemical screening before performing *in vivo* toxicity tests (to minimize animal testing) and 3) generate toxicity information for hazard assessment in combination with quantitative structure-activity relationship (OECD, 2021). After 24 h exposure, the cell viability is assessed by adding different fluorescent dyes. In the current thesis, two fluorescent dyes were used, AlamarBlue (AB) and 5-Carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM). AB measures metabolic activity at excitation/emission wavelengths 532 nm/590 nm and CFDA-AM measures membrane integrity at excitation/emission wavelengths 485 nm/535 nm (Bols *et al.*, 2005; Dayeh and Schirmer, 2005; OECD, 2021) (**paper I and IV**). **Paper I** exposed the fish cells to silica NM concentrations between 0.78-100 mg/L. **Paper IV** exposed the fish cells to silica NM (0.78-100 mg/L) and in mixtures with 0.25-8 µM PCP or 0.63-20 µM HDP (concentration ranges correspond to the concentration-response curves).
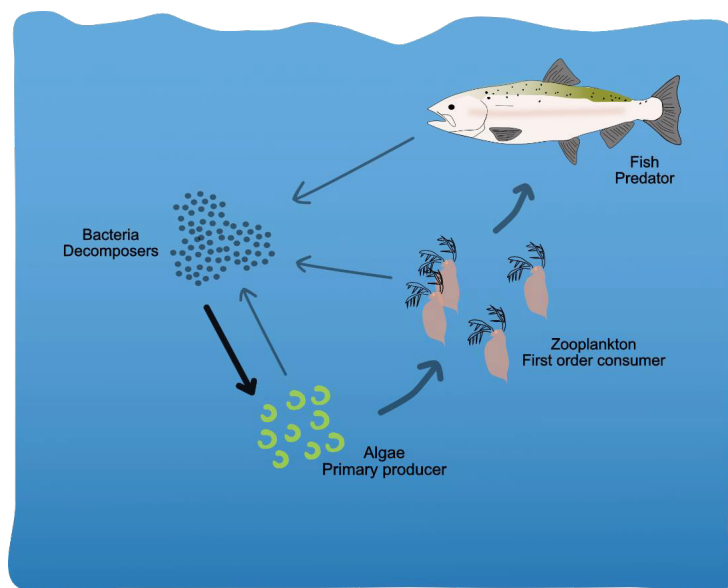

Figure 11. Species from four different levels in the aquatic food web included in the thesis, bacteria (decomposers), algae (primary producers), zooplankton (first order consumer) and fish (predator).

4.4 Systematic review

In addition to laboratory tests, sensitivity between species and trophic levels were assessed based on evidence collected from the open scientific literature by conducting a systematic review and meta-analysis (**paper II**) (Ganeshkumar and Gopalakrishnan, 2013; Moher *et al.*, 2015; Luo *et al.*, 2020; Lasserson, Thomas and Higgins, 2021). Literature search was performed in the United States Environmental Protection Agency ECOTOX database (https://cfpub.epa.gov/ecotox/) and Scopus (www.scopus.com) comprising the period 1st of January 2005 – 28th of June 2021. The literature search had no restrictions on ecotoxicological endpoints or species and covered guideline and non-guideline studies, acute and chronic assays as well as other reviews and meta-analysis. The total number of studies included peer-reviewed scientific articles, pre-prints, technical reports, reports from authorities and industries and PhD theses. Extracted data from the studies included information on test organism, test material, effect type and parameter (no observed effect concentration (NOEC), lowest observed effect concentration (LOEC), concentration causing x % effect (ECx), concentration causing x % inhibition (ICx) and concentration causing x % lethality (LCx)), but also information on specific observations on organism-particles interactions, nanomaterial behaviour and interference control. Study quality was assessed based on nano criteria for reporting and evaluating ecotoxicity data (nanoCRED) criteria and finally compiled into a SSD.

4.4.1. Quality assessment based on nanoCRED criteria

The nanoCRED criteria is a transparent framework to assess the regulatory adequacy of ecotoxicity data for NMs (Hartmann *et al.*, 2017), which was developed as a complement to the already established CRED framework for conventional soluble substances (Moermond *et al.*, 2016) and that is lacking nano-specific guidance. The nanoCRED criteria covers important information on for example test material, test setup and test conditions and is applicable on both guideline and non-guideline studies and takes into account challenges and characterization requirements for the ecotoxicological testing of NMs (Hartmann *et al.*, 2017). One specific challenge for silica NMs is the common addition of biocides in colloidal products that are added to prolong shelf-life and which can interfere with the hazard assessment (Bergna and Roberts, 2005; OECD, 2016).

4.4.2 Species sensitivity distribution

A species sensitivity distribution (SSD) is an widely used tool to study sensitivity between species to chemicals and to estimate thresholds concentrations considered safe in the context of regulatory chemical risk assessment (ECHA, 2008; Garner and Keller, 2014; Posthuma *et al.*, 2019). A SSD is a cumulative distribution that relates the concentration of a compound (x-axis) to the risk (y-axis). The input parameter is often an effect concentration such as the NOEC value and the output a HC05 value, which is the concentration putting 5 % of the species at risk. Hence, the HC05 is divided with an assessment factor (AF), in order to get the PNEC, i.e. the concentration of a substance (if below) that does not result in any unwanted adverse effects in the environment (ECHA, 2008). The PNEC is one of the corner stones in a chemical risk assessment where the PNEC value is compared to the predicted environmental concentration (PEC). A PEC/PNEC ratio of < 1 suggests there is no significant risk while a ratio ≥ 1 indicates that there is a risk and calls for risk reduction measures (Amiard-Triquet, Jean-Claude and Mouneyrac, 2015).

The thesis used chronic NOECs to establish the SSD. Acute data and ECx concentrations were therefore extrapolated into chronic NOECs with extrapolation factors that are dependent on test species and exposure duration (Warne *et al.*, 2015; Posthuma *et al.*, 2019) (Table 3). The limit tests, that is effect concentrations reported with the symbol ">", for example EC50>100 mg/L, was set to EC50=100 mg/L.

Table 3. Criteria for distinguishing between acute and chronic tests within each species groups.

Species group	Acute test	Chronic test
Algae	12 hours	>12 hours
Bacteria	12 hours	>12 hours
Unicellular animals	12-24 hours	>24 hours
Crustaceans	24-48 hours	>48 hours
Fish	4-7 days	>7 days
Mollusks, worms etc	2-7 days	>7 days

4.5 Approaches to study chemical adsorption onto silica nanomaterials

Chemical adsorption of organic chemicals onto the silica NM was studied with liquid chromatography- mass spectrometry (LC-MS) and/ or DLS. For analysis with LC-MS, samples were prepared as follows: 1) mixing the silica NM with the chemical, 2) ultracentrifuge the mixture (to let the NM sediment), 3) collect the supernatant, 4) determine organic chemical concentration in the supernatant with liquid chromatography- mass spectrometry LC-MS (**paper III**) (Figure 12). The concentration of the chemical in the supernatant was compared to the concentration in the controls (one containing only the chemical and one containing only the NM). This approach requires full sedimentation of the NM during ultracentrifugation in order to confirm that the chemical analysis only captures the free chemical. In the present thesis, the samples were ultracentrifuged for 2 h according to previous empirical data (Tsao, Chen and Wang, 2011). The supernatant was also measured with DLS in order to verify that no NM was left in the supernatant.
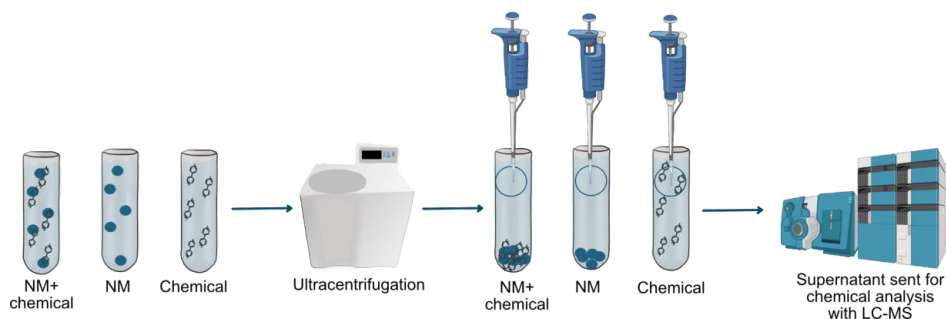
Figure 12. Flowchart of sample preparation before chemical analysis with LC-MS, in order to study the chemical binding of the silica NMs. Figures of the LC-MS and pipets were obtained with permission from BioRender (www.biorender.com).

The thesis also used a second (more simple) approach to study chemical adsorption onto the silica NM. This approach compared the DLS data of the NM alone with DLS data for the NM when being in mixture with the chemical (**paper III and IV**). The principle idea behind this approach is that the adsorbed chemical neutralizes the NM surface, which leads to lowered colloidal stability followed by agglomeration and flocculation (preceding sedimentation). This process is reflected by the DLS measurements as increased size and ZP closer to 0 mV (López-Maldonado *et al.*, 2014).

4.6 Approaches to predict mixture toxicity (IA and CA)

Observed effects from mixture exposures of silica NM and organic chemicals to fish cells (**paper IV**) were compared to predicted effects calculated by CA and IA. IA is based on the idea that the mixture components are dissimilarly acting (different MoA) and that the toxicity of each chemical is not influenced by the presence of the other chemical. IA is mathematically expressed as:

(1)
$$E_{Mix} = 1 - \prod_{i=1}^{n} [1 - E(c_i)]$$

where E_{Mix} is the total effect of the mixture, $E(c_i)$ is the effect caused by concentration c for compound i and n is the number of mixture compounds.

The second concept, concentration addition (CA) (Backhaus *et al.*, 2004), is based on the opposite idea, i.e. that the components constituting the mixture are similarly acting and differ only with respect to their individual potency. It is mathematically formulated as:

(2)
$$ECx_{Mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where ECx_{Mix} is the concentration of a mixture causing x % effect, ECx_i is the single substance concentration (for compound i) causing x % effect, p is the fraction of compound i in the mixture and n the number of mixture compounds.

5. Most significant findings

Objective 1: Characterize the toxicity of non-modified NMs to different species and across trophic levels (**paper I, II and III**)

Paper I compares the toxicity of five differently sized non-modified silica NMs (17, 21, 30, 66 and 88 nm) to bacteria (*P. putida*), algae (*R. subcapitata*), crustacean (*D. magna*) and fish gill cells. The paper demonstrates that silica NM is not toxic to *P. putida*, *R. subcapitata* and *D .magna* with reported EC50 values of >500 mg/L, >500 mg/L and >10 000 mg/L, respectively. However, these five NMs are toxic to the fish cells with reported EC50 values between 13-92 mg/L, Table 4.

Table 4. EC20 and EC50 values of silica NMs after exposure to bacteria, algae and fish cells. Concentrations are presented in mg/L.

Test organism	Silica nanomaterial						
	20 nm-aluminized	**17nm**	**18nm-silanized**	**21nm**	**30nm**	**66nm**	**88nm**
Bacteria							
EC20	>500	>500	>500	>500	>500	>500	>500
Algae							
EC20	>500	295	>500	>500	>500	>500	>500
EC50	>500	>500	>500	>500	>500	>500	>500
Daphnia							
EC50	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
Fish cells							
EC20	5	5	>100	6	6	31	35
EC50	15	13	>100	16	21	92	91

The relatively low toxicity to bacteria and algae shown in **paper I** also goes in line with the SSD reported in **paper II**, which is based on experimental data with non-modified silica NMs retrieved from the open scientific literature. Bacteria and algae dominate the upper part of the SSD curve while being absent in the lower part of the SSD (Figure 13). This low sensitivity of microorganisms (in **paper I and II)** can be explained by their cell wall acting as a protective barrier against cell uptake, as also suggested by other authors (Schirmer *et al.*, 2013; Newkirk *et al.*, 2021). Reported diameters of pores in algae cell walls are 5-20 nm (Smita *et al.*, 2012; Navarro *et al.*, 2015), which are smaller than several reported

diameters of silica NMs (Fruijtier-Pölloth, 2012). Instead, of passing through the cell wall, silica NMs seem to adsorb to the cell wall (Jiang, Mashayekhi and Xing, 2009; Wei *et al.*, 2010a; Yu *et al.*, 2018). It should be noted that cell counting in toxicity tests with algae (**paper I**) is less sensitive than measuring the chlorophyll content (**paper III**). 295 mg/L silica NM is required to cause 20 % effect if based on cell counts, but already 50 mg/L are sufficient to cause the same effect if based on chlorophyll content. This difference could be due to the so called "shading effect", that is the NM adsorbs to the algae cell wall and reduces the amount of light that reaches the cell as suggested by previous studies (Navarro *et al.*, 2008; Van Hoecke *et al.*, 2008; Wei *et al.*, 2010b; Manzo *et al.*, 2015). The adsorption of silica NM onto the algae cell wall was also confirmed by the SEM imaging in **paper III (**Figure 14).
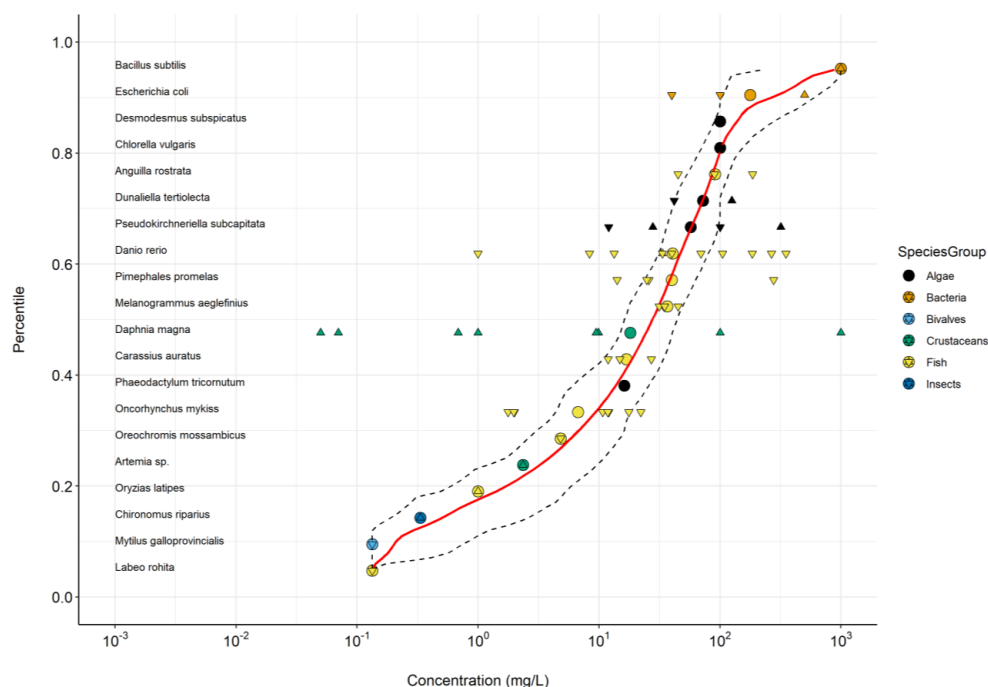
Figure 13. SSD based on all endpoints, limit tests excluded and only explicitly biocide-free material. Red solid line is the mean of 10 000 bootstrap, dashed black lines cover the 95% CI. Horizontal triangles represent individual NOECs for each species. Upwards triangles correspond to regulatory endpoints and downwards triangles other endpoints. Large coloured circles correspond the geometric means of the individual NOECs for each species.

Objective 2. Investigate the importance of surface chemistry as a driver for toxicity (**paper I, III and IV**)

Paper I compares the toxicity of three different silica NM surface chemistries: non-modified, sterically stabilized with silane-glycerol-propyl tails and aluminium-modified in exposure to bacteria, algae, daphnia and fish cells. In contrast to the other surface chemistries, the silanized material was not toxic in any of the assays with reported EC20 values of >500 mg/L for bacteria and algae, >100 mg/L for fish cells and >10 000 mg/L for daphnia (Table 4). Its benign properties are probably due to an increased steric hindrance that prevents contact between deprotonated silanol groups and the organism. The aluminium modified NM shows similar toxicity as the non-modified NM in tests with fish cells with EC50s of 13 and 15 mg/L, respectively. This likely stems from the fact that the aluminium is incorporated into the surface and does not significantly affect the amount of silanol groups on the NM surface. Fish cells were also exposed to a NM without silanol groups (**paper IV**), which was not toxic to the fish cells and could further strengthen the fact that it is the deprotonated silanol groups on the NM surface that are drivers for toxicity.

Paper III conducted an in-depth study of how different surface chemistries affect the interaction of silica NM with algae. SEM images show a clear difference in cell-NM interaction between non-modified (weakly anionic), aluminium modified (cationic) and aluminium modified (strongly anionic) NMs. Both the non-modified and the cationic NMs adsorb well to the algal cell surface and show a clear concentration dependent decrease in chlorophyll content of exposed algae. In contrast, the strongly anionic NMs do not adsorb on the surface and no effects were observed (Figure 14). These findings clearly show that the impact of surface chemistry is of importance and that it needs to be taken into considerations in ecotoxiclogical assays.
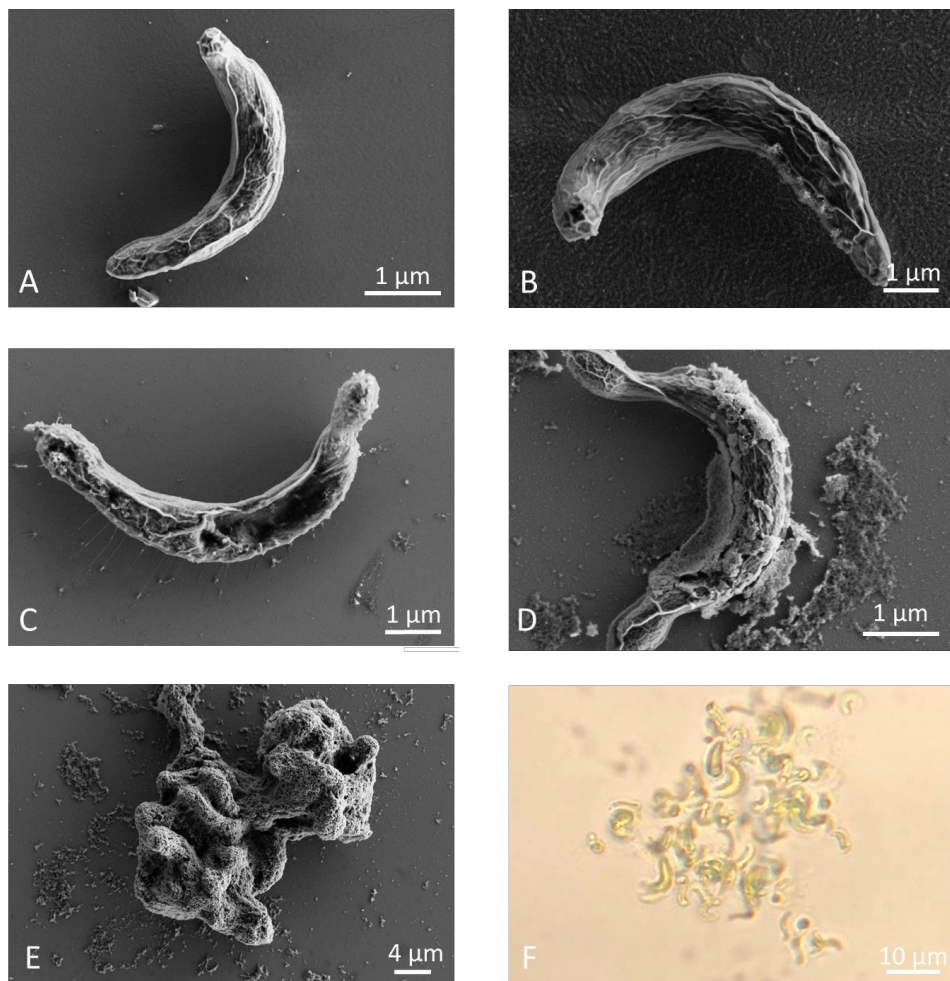
Figure 14. SEM images showing charge-dependant adsorption of NMs onto the algae the cell wall after 72 h exposure to 50 mg/L. Image A shows the unexposed algae (control) at a magnification of 26 000x, B depicts cells exposed to the strongly anionic NM (magnification 35 000x), C is algae exposed to the strongly anionic elongated NM (magnification 35 000x), D is algae exposed to the weakly anionic NM (magnification 26 000x) and E-F depicts algae exposed to the cationic NM after imaging with SEM (E) (magnification of 7000x) or a light microscope (F) (magnification of 40x).

Figure 15. Concentration-response relationships for the different silica nanomaterials (NMs), weakly anionic (A), cationic (B), strongly anionic (C) and strongly anionic elongated (D). The boxes show the inhibition at the tested concentration and the dotted line shows the fitted curve calculated from a two-parametric concentration-response model.
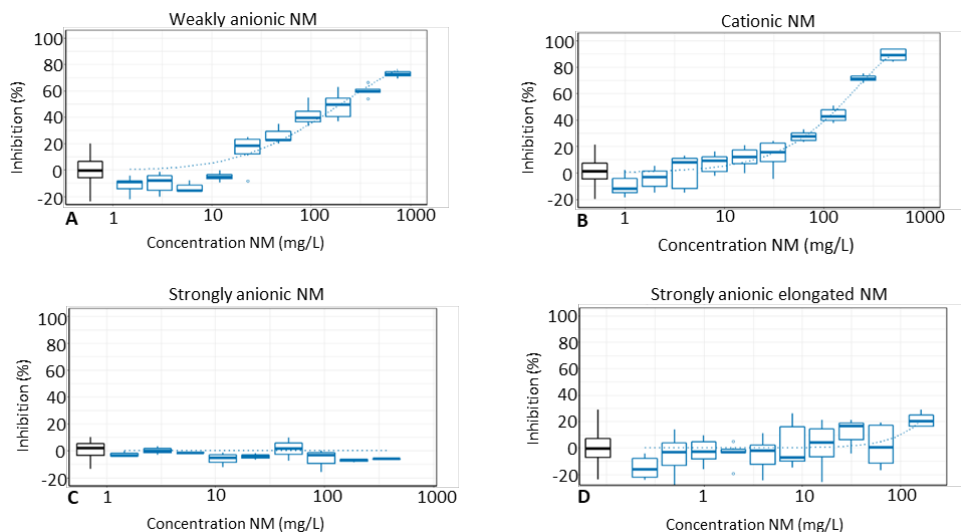
Objective 3. Investigate the importance of particle size, particle number and surface area as relevant descriptors for ecotoxicity and exposure (**paper I, III and IV**)

Paper I compares the three different exposure metrics mass, surface area and number of particles for fish cell data for the five different silica NMs with the same surface chemistry (non-modified). The results show that the same data set results in different concentration-response plots, depending on whether mass, surface area or particle number are used (Figure 16). Surface area is the only exposure metric that results in overlapping concentration-response curves for all NMs, with the largest and lowest EC50 values differing by a mere factor of 1.4. The corresponding factors for number of particles and mass are 32 and 7. The small difference in EC50s when using surface area as the exposure metric goes in line with the proposed MoA, that is increased surface area (due to smaller sized particles per mass) leads to an increased amount of deprotonated silanol groups, which increases the toxicity in exposed cells.

Paper III and IV compare the toxicity of spherical and elongated aluminized silica NMs, having different surface areas per mass (360 m^2/g and 1100 m^2/g,

respectively) to algae (**paper III**) and fish gill cells (**paper IV**). No difference in algal toxicity was observed between the two shapes (at the same mass concentrations) (Figure 15), which indicate that shape and indirectly number and surface area do not matter as long as the NMs are strongly anionic.

In exposure to the fish cells, the strongly anionic elongated NM is more toxic (half the EC50 value) than the strongly anionic spherical at the same mass concentration (**paper IV**). The higher toxicity of the elongated material (per mass) is likely due to the increased surface area and in line with the surface driven toxicity observed with the non-modified silica in **paper I**.

To conclude, surface area is the key metric for toxicity, provided that the surface chemistry allows for interactions with the exposed cells (non-modified and aluminized-anionic NMs in tests with fish cells and non-modified and aluminized-cationic NM in tests with algae).
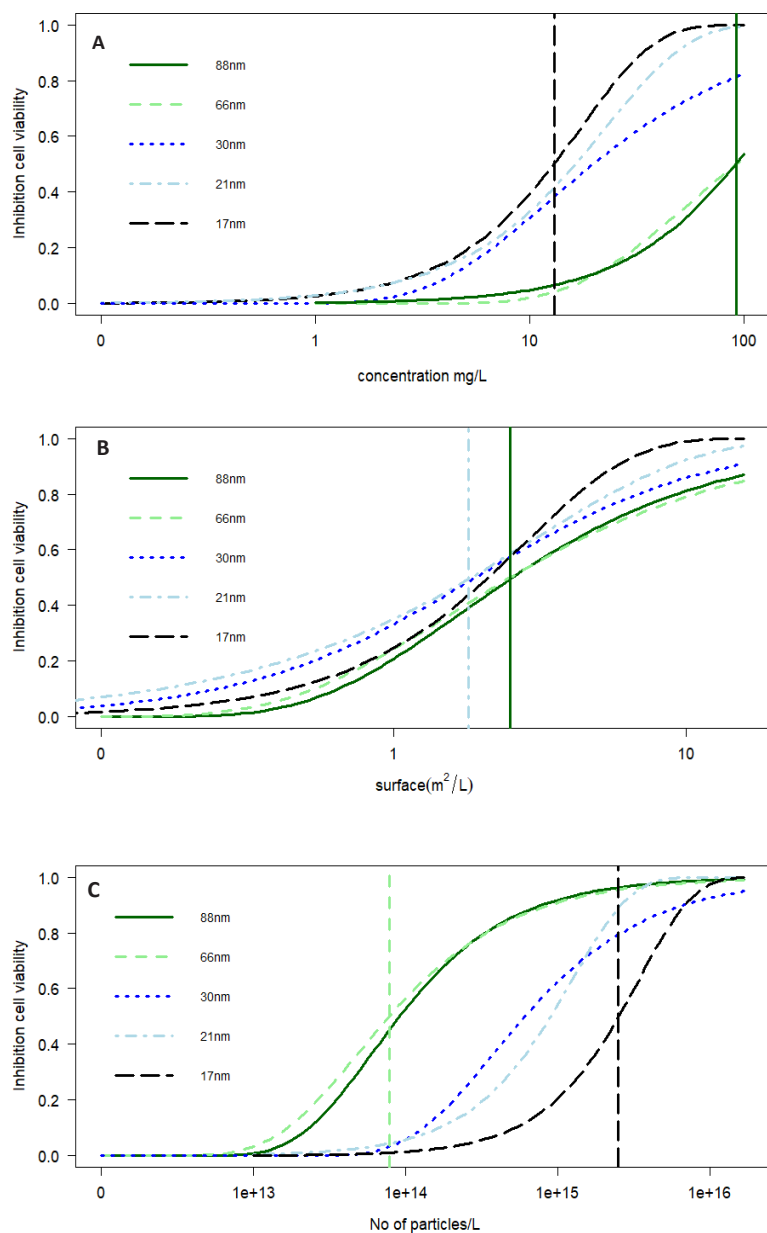
Figure 16. Concentration-response curves for five differently sized non-modified NMs in the fish cell assay using mass (A), surface area (B) and number of particles (C) exposure metrics. Different colours and line types differentiate the various NMs. Vertical lines show the highest and the lowest EC50 value and to which NM this E50 value corresponds (indicated by the line type).

Objective 4: Estimate the maximum acceptable environment concentration (PNEC) for silica nanomaterials in the freshwater aquatic environment based on a SSD (**paper II**)

Suggest the maximum acceptable environment concentration (PNEC) of silica nanomaterials in the aquatic environment based on SSD (**paper II**)

Paper II systematically assesses and compiles the scientific literature on the aquatic ecotoxicity of silica NM into SSDs. The HC05 is estimated at 130 µg/L covering 6 taxonomic groups and 20 species, which is further divided with an AF of 5 resulting in a PNEC of 30 µg/L. This PNEC is based exclusively on data with explicitly biocide-free materials (silica NM supplied as a powder or colloidal silica explicitly stated as biocide-free) and limit tests are excluded, as inclusion would lead to a toxicity overestimation. The PNEC is biased toward the pelagic freshwater environment as only 11 % and 3 % of the retrieved data are performed with marine and benthic species, respectively. This reflects the progress of the nano(eco)toxicological field in general (Selck *et al.*, 2016).

The PNEC reported in the present thesis is 11-250 times higher than the reported PEC values of 0.12-2.6 µg/L (Y. Wang *et al.*, 2016; Wang and Nowack, 2018) for European waters. This indicates only negligible risks for the freshwater environment at the moment, which, however, might change in the future when production and use volumes might increase.

Objective 5: Characterize the ability of differently charged silica NMs to adsorb organic pollutants (**paper III and IV**).

This following text discuss the results from tests with mixtures of NMs and organic chemicals without organisms or cells being present.

Paper III and IV investigate charged silica NMs' ability to adsorb differently charged organic chemicals. **Paper III** shows that all tested silica NMs (weakly anionic, strongly anionic spherical, strongly anionic elongated and cationic) are able to adsorb paraquat (PQ) in the descending order (when concentration is expressed as mass) strongly anionic elongated>strongly anionic>cationic>weakly anionic (Table 5). The strongly anionic elongated material is able to adsorb 93 % and 99.9 % of the PQ at a NM concentration of 0.4 and 16 mg/L, respectively. Also the strongly anionic (spherical) NM is able to efficiently remove PQ (99.5 % adsorption at a concentration of 10 mg/L). The adsorption to strongly anionic

NMs is also well reflected by the characterization data with zeta-potentials (ZPs) switching from negative to positive at several mixture concentrations (**paper III**). S1. A concentration of 10 mg/L of cationic and weakly anionic NM are able to adsorb 61 % and 18 % of the PQ, respectively. Interestingly, the cationic material is able to remove more PQ than the weakly anionic (which was not anticipated due to being both positively charged). This reason behind this could be that the cationic NM agglomeration and precipitation trap the chemical (Martinez *et al.*, 2022).

Paper III also shows that 50 mg/L of the cationic NM is able to adsorb 20 % pentachlorophenol (PCP) which is not observed for any of the other NMs (Table 5). However, this is only valid for the cationic NM-PCP mixture prepared with a pre-suspension at a 100 times stronger concentration. This adsorption is likely due to the acidic pH 4.1 in the pre-mixture (compared to pH 7.2 ± 0.5 in the final mixture added to the algae), which keeps the NM stable, i.e. ZP and sizes are similar to those in the original stock suspension from the supplier. Moreover, the 20 % adsorption correspond to the 20 % concentration of deprotonated PCP at pH 4.1 according to the pKa formula. The stability of the cationic NM in the pre-mixture could also be partly a result of the lower NM to phosphate ratio, since phosphate is known to bind to cationic silica NMs (Van Hoecke *et al.*, 2011) that could possibly affect colloidal stability. To conclude, the capacity for the cationic NM to adsorb anionic chemicals is likely pH and phosphate dependant.

In addition, **Paper III**, also investigates the adsorption of the neutral compound diflufenican (DFF). The results show that the strongly anionic and the cationic NMs are able to slightly remove DFF. The strongly anionic removed 12-16 % while the cationic removed 5-12 % (Table 5). Again, the aggregation/agglomeration and precipitation of the cationic NM could have trapped the DFF. Another explanation could be an adsorption driven by hydrophobic forces, since DFF is more hydrophobic (logP of 4.2) than PQ (logP of -4.5) and PCP (logP=1.71). According to the ZP measurements, the strongly anionic has ~58 % of its size population with a low ZP (-4 ± 5 mV) at 50 mg/L, similar to the cationic ZP of -1 ± 3 mV. This could be in favour for adsorption by hydrophobic forces.

Table 5. Concentration of the organic pollutant after ultracentrifugation in control and mixture with 0.4, 16, 10 and 50 mg/L silica NM in control.

		Weakly anionic		Cationic		Strongly anionic		Strongly anionic elongated		
Pestic ide	Control only the pesticide	10	50	10	50	10	50	0.4	16	50
PQ	100 (±5)	82 (±8)	33 (±3)	39 (±10)	10 (±5)	0.5 (±0.1)	0.4 (±0.2)	7 (±3)	0.1 (±0.2)	0.0 (±0)
PCP	100 (±0.5)	102 (±1)	107 (±4)	103 (±0.7) 101* (±0.8)	81 (±2) 100* (±1)	106 (±1)	106 (±7)			
DFF	100 (±7)	102 (±4)	116 (±2)	95 (±8)	88 (±4)	88 (±7)	84 (±3)			

*Final mixtures were diluted from a 100 times stronger pre-mixture.

Paper IV investigates the strongly anionic and the cationic NMs' ability to adsorb the cationic surfactant HDP or the anionic PCP with DLS measurements. According to the dynamic light scattering (DLS) data, the strongly anionic NMs most likely adsorb the cationic surfactant, which is reflected by the agglomeration and cationic ZP in NM-HDP mixtures. The HDP probably adsorb onto the strongly anionic surface with its cationic head group and with the hydrophobic tail turned to the water phase. Consequently, this leads to the agglomeration since the hydrophobic tails at the particles surface gets attracted to each other and agglomerate.

Objective 6: Assess the aquatic ecotoxicity of mixtures comprising of silica NMs and organic pollutants (**paper III and IV**)

Paper III shows that the NM-PQ mixtures with the strongly anionic spherical NM and the strongly anionic elongated NM eliminate the toxicity of PQ completely at 50 mg/L and 16 mg/L, respectively (Figure 17C-D), which goes well together with the adsorption measurements (Table 5). Interestingly, the corresponding surface areas at these two mass concentration are the same, that is

18 m^2/L (Table 6), which shows that surface area and not NM shape drives the toxicity reduction. In contrast, the weakly and the cationic NMs do not significantly reduce the PQ toxicity despite the fact that they are able to remove 18-67 % and 61-90 % of the PQ in the medium, respectively. This indicates that the algae compete with the NM for uptake and/or adsorption of the co-exposed chemical.
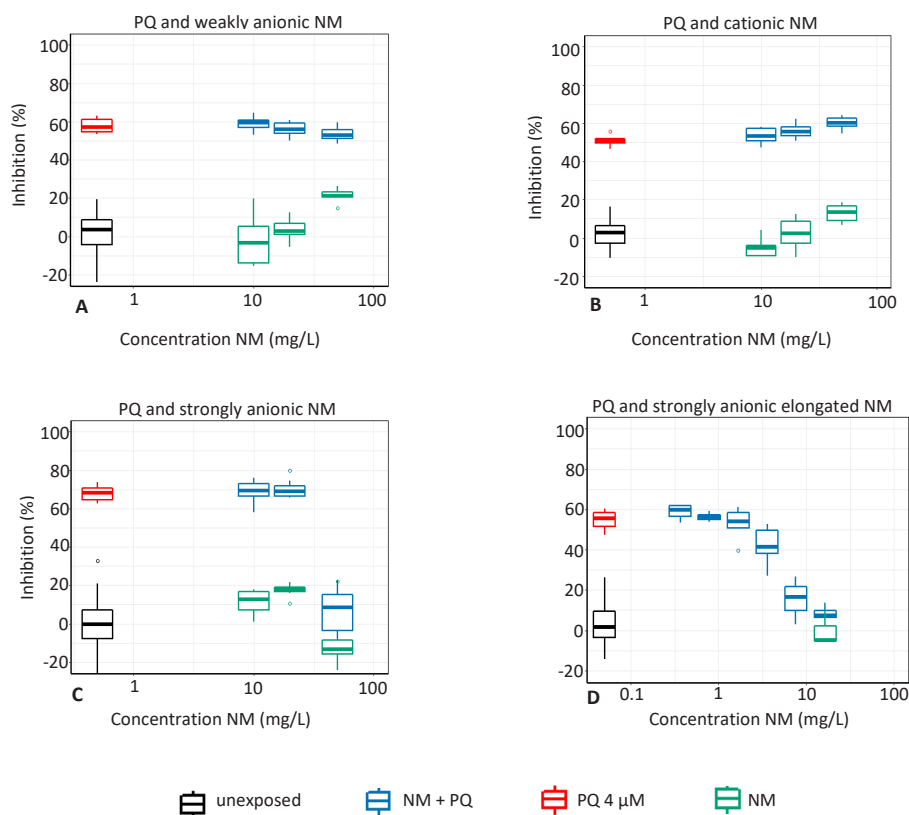
Figure 17. Effects of binary mixtures of PQ and silica NM with different surface chemistry on algae growth. The mixtures comprised of PQ (4 µM) in binary mixture with the weakly anionic NM (A), cationic NM (B), strongly anionic NM (C) and strongly anionic elongated NM (D) at 10, 20 and 50 mg/L (blue boxes). The black boxes represent the unexposed algae (control), the red box shows results from exposure to only PQ and the green boxes represent the inhibition after exposure to only the NM. The boxplots show the median, quartile 1 (Q1), quartile 3 (Q3) and the maximum (Q3 plus 1.5 times the interquartile range) and minimum (Q1 minus 1.5 times the interquartile range).

Table 6. Comparison of surface area (SSA) per mass test concentration between the strongly anionic spherical and elongated NMs. The SSAs are calculated from the surface area in the original product, Levasil CS25-436 (360 m^2/g) and Levasil RD 2180 (1100 m^2/g).

Mass concentration (mg/L)	SSA (m^2/L) Levasil CS25-436 (spherical)	SSA (m^2/L) Levasil RD 2180 (elongated)
50	18	55
20	7	22
16	6	18
8	3	9
4	1.4	4.4
2	0.7	2.2
1	0.4	1.1
0.4	0.1	0.4

Paper III also investigates the toxicity of co-exposures to NM and PCP. The results show that the cationic NM is the only material able to significantly reduce PCP toxicity by 30 % or more at all test concentrations (Figure 18C), as long as the mixture is prepared via a pre-mixture 100 times more concentrated than the final test concentrations. This is in accordance with the adsorption experiments, which show that only this mixture (prepared from a 100 times concentrated pre-mixture) removes PCP from the medium (Table 5) and that it is driven by electrostatic attraction. **Paper III**, also studies the effects of co-exposure to NM-mixtures with DFF (Figure 19). These results show that the cationic NM is the only material that reduces the DFF toxicity (10-20 % in a concentration dependant manner). This is in accordance with the adsorption experiments, which show that this material remove up to 12 % of the chemical in the medium.

Figure 18. Mixture exposures comprising PCP (0.2 μM) and the particles weakly anionic (A), strongly anionic (B), cationic (C) and cationic with no pre-mix (D) at 10, 20 and 50 mg/L (blue boxes). The black box is the non-treated algae (control), the red box is the inhibition with exposure to only the pesticide and the green box is the inhibition after exposure to only the NM. The boxplots show the median, quartile 1 (Q1), quartile 3 (Q3) and the maximum (Q3 plus 1.5 times the interquartile range) and minimum (Q1 minus 1.5 times the interquartile range).
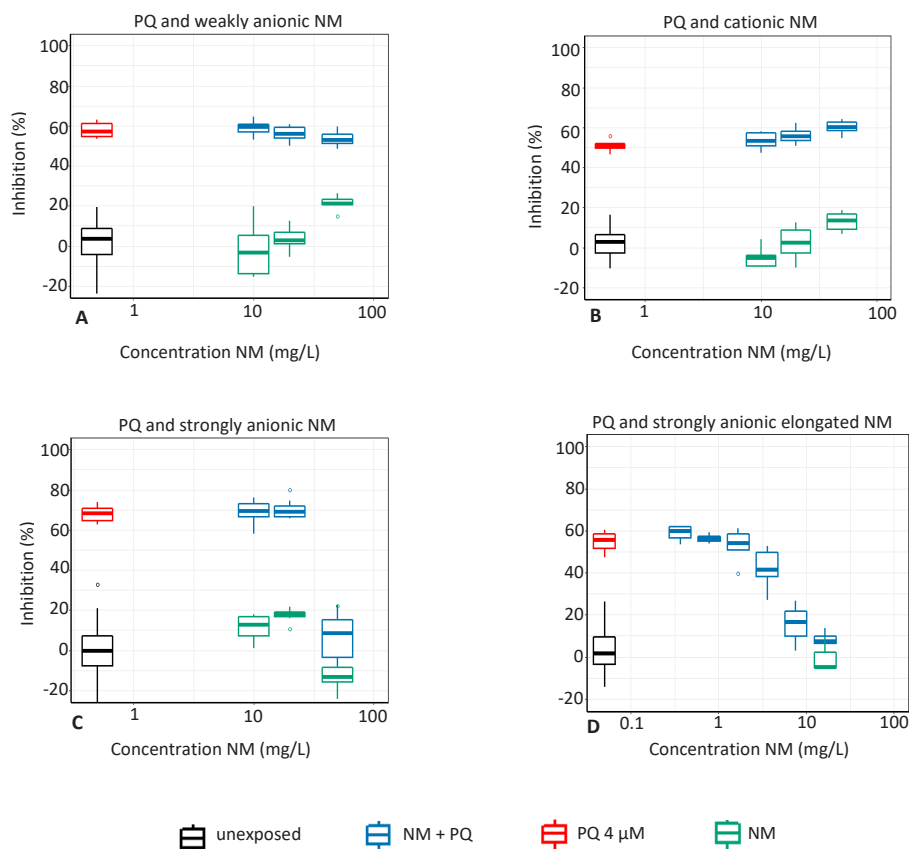
Figure 19. Mixture exposures comprising of DFF (0.002 μM) and the particles weakly anionic (A), strongly anionic (B), cationic (C) at 10, 20 and 50 mg/L (blue boxes). The black box is the non-treated algae, i.e. the control, the red box is the inhibition with exposure to only the pesticide and the green box is the inhibition after exposure to only particles. The boxplots show the median, quartile 1 (Q1), quartile 3 (Q3) and the maximum (Q3 plus 1.5 times the interquartile range) and minimum (Q1 minus 1.5 times the interquartile range).
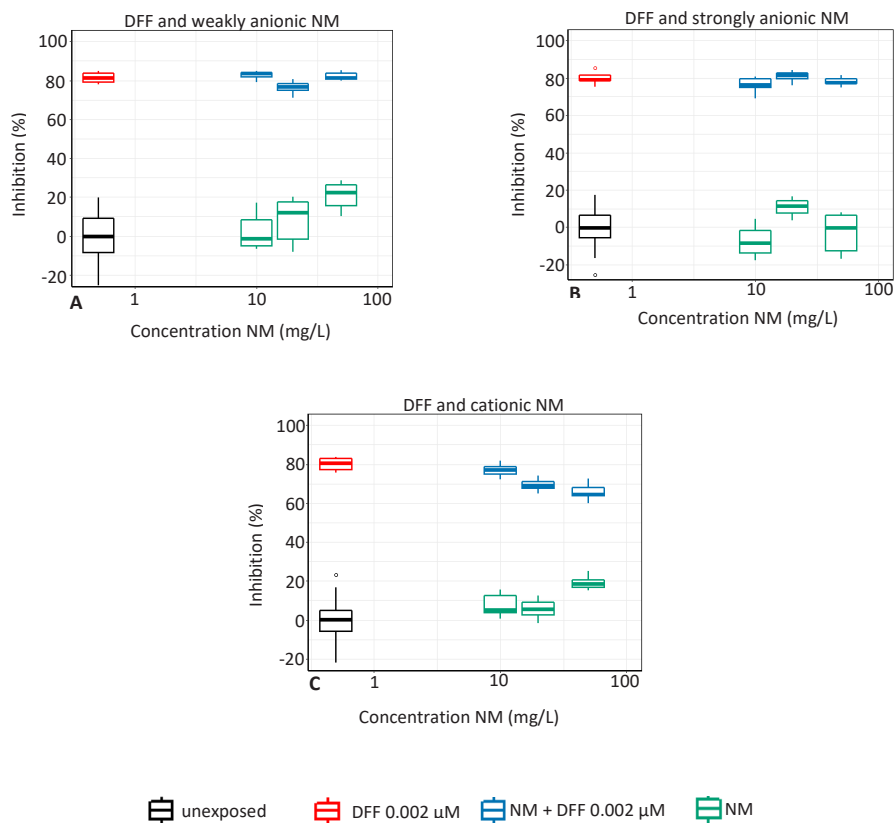
Paper IV exposes fish cells to binary mixtures of a strongly anionic NM or cationic NM with HDP or PCP. In addition, the observed effects are compared to predicted mixture effects CA and IA mixture concepts. The results indicate that the NM-mixtures have an additive effect in fish gills cells since the predictive effects are at or within a model deviation of 0.5-2 (Table 7), the suggested limit for synergistic or antagonistic effects (Belden, Gilliom and Lydy, 2007; Martin *et al.*, 2021). This is observed despite the fact that the strongly anionic NMs likely adsorb the cationic HDP, as reflected by the characterization measurements. However, the NM and the pollutant were added subsequently to the fish cells (with a ~15 min difference), which might be "too late" for the NM to alleviate the

toxicity of the chemical. For example, HDP is a disinfectant that has rapid effects in exposed cells and disinfect surfaces within minutes (Popkin *et al.*, 2017; Newkirk *et al.*, 2021). Therefore, effects are likely dependant on how NM-mixtures are prepared, that is mixed before or added subsequently to the test system.

Table 7. Predicted and observed EC50s for HDP and PCP in mixtures with 1 mg/L, 5 mg/L or 10 mg/L strongly anionic silica NMs (elongated and spherical) measured with CFDA-AM and AB. The model deviation ratio is presented in brackets.

Pestic ide	Assay	silica 1 mg/L			silica 5 mg/L			silica 10 mg/L		
		Strongly anionic elongated								
		obser ved	CA	IA	obser ved	CA	IA	obser ved	CA	IA
HDP	AB	3.5	2.9 (1.2)	3.1 (1.1)	0.8	0.9 (0.9)	1 (0.8)	0.1	0.1 (1.0)	0.1 (1.0)
HDP	CFDA	6.4	5.1 (1.3)	5.5 (1.2)	3.6	2.8 (1.3)	4 (0.9)	0.7	1.3 (0.5)	0.9 (0.8)
PCP	AB	2.7	2.4 (1.1)	2.8 (1.0)	0.9	0.7 (1.3)	1.3 (0.7)	0.2	0.1 (2.0)	0.1 (2.0)
PCP	CFDA	4.4	3.8 (1.2)	4.4 (1.0)	2.6	1.9 (1.4)	4.2 (0.6)	0.6	0.8 (0.8)	1.4 (0.4)
		Strongly anionic spherical								
HDP	AB	2.3	1.8 (1.3)	2.4 (1.0)	1.6	0.9 (1.8)	2 (0.8)	0.7	0.1 (7.0)	1.2 (0.6)
HDP	CFDA	3.6	3.5 (1.0)	3.6 (1.0)	4	2.9 (1.4)	3.5 (1.1)	2.6	2.1 (1.2)	3 (0.9)
PCP	AB	2.1	2.1 (1.0)	2.5 (0.8)	1.6	1.2 (1.3)	2.5 (0.6)	1.2	0.6 (2.0)	1.7 (0.7)
PCP	CFDA	2.8	1.6 (1.8)	2.4 (1.2)	3.1	1.2 (2.6)	2.5 (1.2)	3	1.0 (3.0)	2.4 (1.3)

6. Outlook

Clean water and life below water are two of the 17 sustainable goals adopted by all United Nations member states in 2015 with the aim to ensure availability and sustainable management of water and sanitation for all as well as conserve and sustainably use the oceans, seas and marine resources for sustainable development (UN, 2022). These goals play a central role in policymaking and form a basis for the European Green Deal, which includes the Chemical Strategy for Sustainability, the European Union's chemicals strategy for sustainability towards a toxic-free environment (European Commission, 2022) and the Zero Pollution Action Plan (SWD(2021) 140 final).

This thesis contributes to achieving both of these goals, approaching them from two perspectives. The first perspective is linked to the concern that nanomaterials may pose a risk to human and environmental health, as a consequence of their release into surface waters. The second perspective is based on the fact that nanomaterials can be used as a tool towards achieving these goals, by removing organic contaminants from water. In this way, they can be both, the solution or the source of pollution. The thesis combines these two perspectives by assessing the toxicity of silica nanomaterials alone as well as studying their adsorption capacity and combined toxicity to aquatic life.

6.1 Knowledge gaps

Based on the data retrieved in the thesis, the maximum acceptable environment concentration of manufactured silica NMs in the aquatic environment is estimated at 0.03 mg/L. However, the natural background concentration of particulate silica, in the size range of 1 nm – 1 μm (Chen, Wang and Lee, 2021), in river water is estimated to be in the 100 mg/L range (Y. Wang *et al.*, 2016). Despite this, none of the experiments retrieved from the scientific literature or the assays performed in the present study addresses this. Therefore, one cannot fully evaluate and assume how the toxicity of manufactured silica NMs differs from naturally occurring suspended silica, which are present at approximately three orders of magnitude higher concentrations. Aquatic organisms have different ways to protect themselves from their surroundings. For example algae and bacteria have cell walls that provides protection from suspended matter (Holzinger *et al.*, 2015), daphnids are filter feeders (thereby constantly exposed to suspended matters) and can reject particles that interfere with filtration or are noxious (Porter, Orcutt and Gerritsen, 1983). Fish gills are covered with a mucus layer that works as a

protective barrier and which increases when the fish is exposed to silt laden water (Shephard, 1993). Therefore, a control that takes into account the effects from the natural background of silica NM would have been useful.

In all the papers of this dissertation, manufactured silica NMs were added directly from stock suspensions to a standardized test system with short exposure durations (16-72 h). These systems are deliberately kept as simple as possible in order to maximize reproducibility and compliance with the demands for environmental risk assessment. However, this implies that the observed ecotoxicological effects need to be extrapolated to effects that might occur in the real aquatic environment, which is a far more complex system. For example, NM ageing and presence of natural organic matter impact the ecotoxicological profile, NMs become, in general less toxic due to changed surface chemistries (Arvidsson, Hansen and Baun, 2020; Ellis, Valsami-Jones and Lynch, 2020). The data from this thesis might therefore represent a realistic worst-case scenario, encountered e.g., next to emission sources (see also the discussion regarding NOM in the next section).

The impacts of silica NM in ecological contexts are still largely unknown. This involves considerations regarding food-chain transfer and indirect ecological effects (in particular top-down effects, as organisms higher up in the food chain seems to be most sensitive). As for environmental pollutants in general, knowledge gaps are massive with respect to tropical species and possible impacts in ecosystems from the Southern hemisphere.

The experiments with silica NM-mixtures in the present thesis show that manufactured silica NMs adsorb and lower the bioavailability of co-occurring organic chemicals. However, although these findings provide a solid starting point, it should be emphasized that the road from these findings to the use of silica NM for water purification application on an industrial scale application might still be somewhat long and winding. Major knowledge gaps that need to be closed still include: in which industrial contexts (sewage treatment plants, drinking water production facilities, remediation of contaminated sites) and for which water types (surface water, ground water, industrial water) can they be used? Are silica NMs less or more efficient than other nanomaterials (such as activated carbon, which treatment plants are already being upgraded with) for which types of pollutants? And last but not least: how high are the involved costs and what are the overall environmental impacts from a life-cycle perspective (including, for example,

considerations regarding the environmental impacts of producing silica NM, their recyclability etc)?

6.1.1 Impacts from natural organic materials

The review by Arvidsson *et al* (2020) indicates that the presence of natural organic matter reduces the ecotoxicity of NM's, due to changed surface chemistries (also called the "eco-corona") However, the review includes only one study with silica NM, which might be insufficient, given that the huge variability in surface chemistries used with commercial silica NM. Van Hoecke *et al* (2011) studied the impact of NOM on the toxicity of non-modified silica NM in exposure to *R. subcapitata* and found that the toxicity decreased (2-19 fold) with increasing concentration of NOM (1.3-9.1 mg C/L). The study could not identify the reason for the decreased toxicity and could not confirm whether NOM adsorb to the silica NM. It was suggested that the decreased toxicity could stem from NOM binding to the algae surface, which shields the algae from direct contact with the NM. The study by Pradhan *et al* 2017 investigated the adsorption of humic acid (HA) and its degradation product benzoic acid (DHBA), as typical NOM's, to the surface of silica NM and observed a weak adsorption due to strong (electrostatic) repulsion. This indicates that also the negatively charged fulvic acid (FA), which together with HA constitute half of the NOM present in natural environmental waters (Li *et al.*, 2013; Z. Wang *et al.*, 2016) are not attracted to negatively charged silica NM. At the same time it is well known that cationic pollutants, such as the paraquat studied in this thesis bind FA and HA by electrostatic forces (Iglesias *et al.*, 2009). It can therefore be hypothesized that adding NOM would be competing with the sorption of the co-exposed organic pollutants by silica NMs, leading to an additative but not antagonistic overall sorption of the co-exposed organic pollutants. This would obviously be an advantage for the application of silica NMs as sorbents for water purification. However, experimental verification of this hypothesis is currently lacking.

6.2 Considerations for future experiments

The highlighted knowledge gaps require increased environmental realism and a more application oriented evaluation of silica NMs in the future. This can be achieved by applying different approaches. Either, experiments could increase realism in a step-wise/bottom-up (controlled) way by investigating the impact of

one parameter at the time (such as pH, ionic strengths, NOM, NM surface modification, type of co-exposed pollutant(s) in standardized systems. This approach would require to analyse the resulting data from a holistic perspective in the end, especially in order to identify interactions between the different parameters. A complementary approach, which would further increase the environmental realism, but at the expense of reduced reproducibility), would be to use water taken directly from a relevant source (surface water, groundwater or wastewater etc.) for the assays.

In either case, the work conducted in this thesis clearly shows that it is crucial to document technical details (such as how the test mixtures are prepared and added to the test system), because this can and will significantly affect the outcome of the experiments.

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