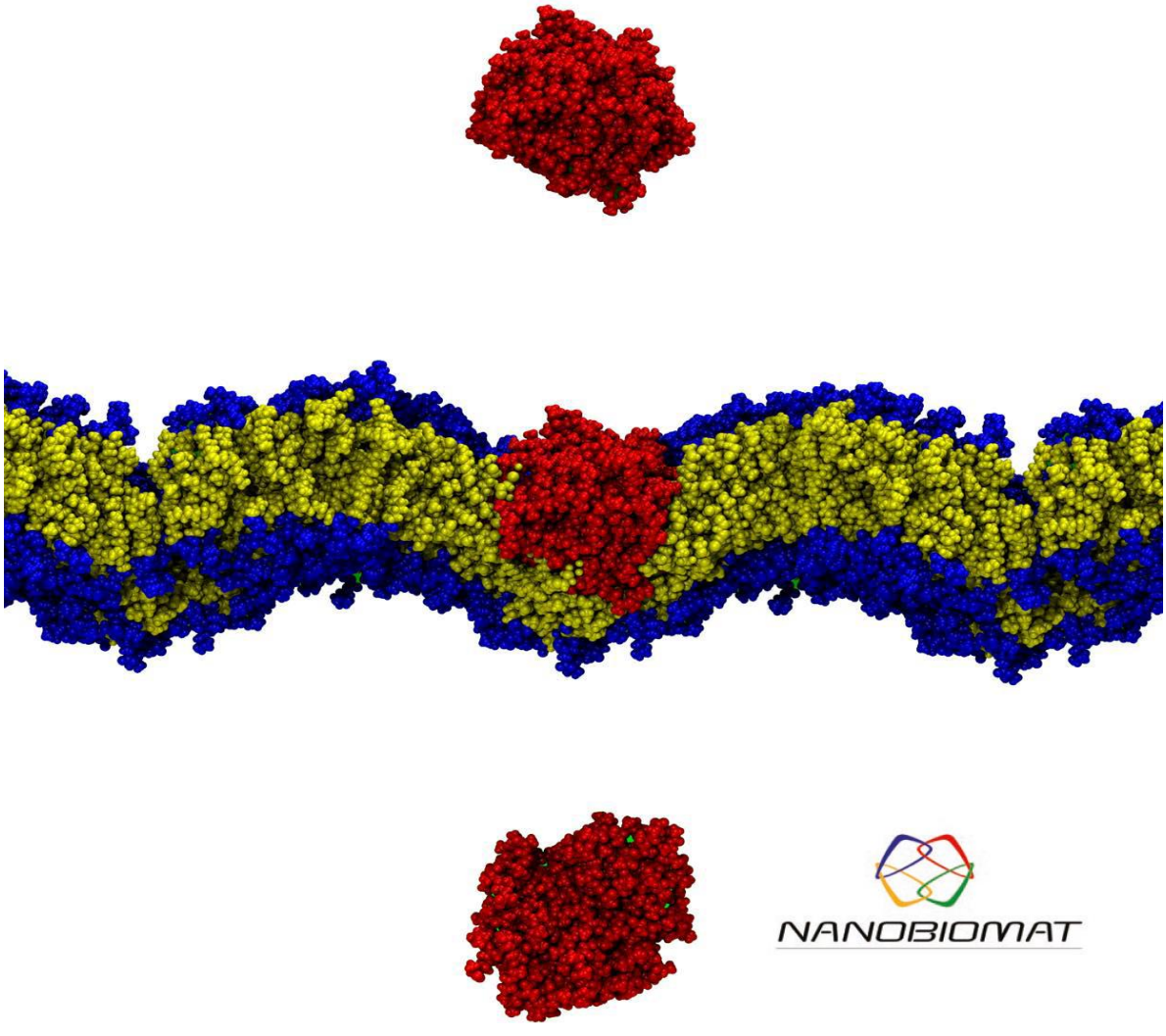
GoNanoBioMat

Knowledge Base

Environmental risks of polymeric nanobiomaterials



NANOBIOMAT

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Contents

Foreword

Nanomedicine is the use of nanotechnology in the medical field, which can be applied to pharmaceuticals and medical devices, as well as to imaging and diagnostics. One of the hot topics in nanomedicine is the use of nanobiomaterials for drug delivery (nanocarriers). In medicine, a nanobiomaterial is a nanoscale material (up to 1000 nm) able to elicit an appropriate host response in a specific application. On the one hand, using nanobiomaterials as drug carriers has various expected advantages compared to the use of their bulk material: 1) capacity to influence the permeability of biological barriers, 2) increased drug efficacy, 3) reduction of side effects, 4) targeted drug delivery, and 5) decreased drug doses. On the other hand, the nanoscale brings new challenges to product design, handling and manufacturing. For small and medium-sized enterprises (SMEs) in particular, this is complex, costly and requires the combination of knowledge from several different fields (chemistry, biology, medicine and pharmaceuticals). Innovative SMEs are recognized as important economic and social actors in Switzerland and Europe, but nano-specific challenges may prove to be too high a hurdle for them.

In this complex environment, the GoNanoBioMat project aims to support SMEs in Switzerland and Europe in their decision making regarding the development and production of polymeric nanobiomaterials for drug delivery. To achieve this, the consortium designed a Safe-by-Design (SbD) concept for supporting and guiding SMEs through the early phase of research and development of polymeric nanobiomaterials for drug delivery, and to enable SME to consider various aspects such as safe material design, human health and environmental risks, manufacturing, storage and transport, and the regulatory framework related to the topic at hand.

The project's main outcomes are the following: 1) a verified knowledge-base (built on peer-reviewed scientific publications); 2) guidelines for implementing the SbD approach for medicinal nanocarriers; and 3) an in-depth investigation of three selected materials, which are chitosan, polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) with regard to their application to drug delivery.

The Knowledge base elaborated within ProSafe transnational call consists of three knowledge base reports:

1. Polymeric nanobiomaterials for drug delivery
2. Human health risks of polymeric nanobiomaterials
3. Environmental risks of polymeric nanobiomaterials

These reviews are the underlining knowledge found in the "Guidelines for implementing a Safe-by-Design approach for medicinal polymeric nanocarriers". The guidelines' goals are to support informed decision making in the field of polymeric nanobiomaterials for drug delivery, improve and facilitate the communication within the different companies in the value chain and also between industry and the regulatory authorities (develop a common language), prevent misguided investments, and finally, enable SMEs to deliver high-quality products.

Executive summary

Nanobiomaterials (NBMs) have recently gained great recognition for their uses in medical applications. From there, they are likely to find their way into the environment either after use or in case of accidents. Therefore, in this report we aimed to evaluate the ecotoxicity and environmental risk of five polymeric NBMs (chitosan, polyacrylonitrile (PAN), poly(lactic-co-glycolic acid) (PLGA), Polyhydroxyalkanoates (PHA), and Poly(lactic acid) (PLA)) and one inorganic NBM (Hydroxylapatite (HAP)).

For an environmental risk assessment, first the predicted environmental concentration (PEC) needs to be known. This is done in an exposure assessment. As most of the NBMs are still in the development stage and not yet on the market, often the only way to do this is through exposure modelling. Exposure modelling has been done for several engineered nanomaterials but is yet to be done for NBMs.

The second step in an environmental risk assessment is the hazard assessment. The goal of the hazard assessment is to derive the predicted no effect concentration (PNEC) of the material in several environmental compartments. Thus, toxicity data was collected for these six materials. Only freshwater and soil data was found and only for chitosan, PAN and HAP. Therefore, no PNEC could be calculated for PLGA, PHA and PLA. In total, 231 data points were collected from 18 different sources. If only one ecotoxicological endpoint was available for a certain NBM, then an assessment factor of 1000 was applied on the lowest EC50 or other endpoint and this value was taken as the PNEC. If several endpoints were available, then the collected data points were converted to no-observed effect concentrations (NOECs) by use of two assessment factors and their probabilistic species sensitivity distributions (pSSDs) were calculated. Probabilistic pSSDs were used to include all the uncertainty associated with the data points found in literature. The PNEC was then calculated as the 5^{th} percentile of the pSSD. For HAP and PAN in soil only one data point was found and for PAN in freshwater only two data points were collected, so no pSSD was calculated for them. In studies were it was specially mentioned that the particles were "nanoparticles", they were characterized as such. If nothing was mentioned, they were characterized as "Non-Nano". During the preparation of chitosan, it is dissolved in a medium, thus also the term dissolved was used for non-nano chitosan. For chitosan and nano chitosan a pSSD was calculated by removing all pathogenic bacteria and fungi from the original data as chitosan is known to have antimicrobial activities. These pSSDs were termed chitosan (env) and nano chitosan (env), respectively. The REACH guideline specifies that confidence can be associated if at least 10 NOECs from 8 taxonomic groups were used. This was only the case for chitosan, so the other pSSDs and their derived PNECs should be treated with caution.

The calculated median PNECs for freshwater spanned more than 8 orders of magnitude. The most sensitive (toxic) NBM in freshwater is chitosan (env) with a mean PNEC of 8 µg/l. Slightly

less sensitive are chitosan and nano-chitosan with a mean PNEC of 35 and 47 µg/l, respectively. Even less sensitive is nano-chitosan (env) with a mean PNEC of 150 µg/l. The least sensitive are HAP with a median PNEC of 17,000 µg/l and PAN with a median PNEC of 3,000,000 µg/l. For soil, the least sensitive NBM is HAP with a mean PNEC of 0.3 mg/kg, followed by PAN and chitosan with a mean PNEC of 33 mg/kg and 120 mg/kg, respectively. It needs to be noted that even the most sensitive of the selected NBMs, chitosan (env), is less toxic than the engineered nanomaterials fullerenes, nano-ZnO and nano-Ag, the antibiotics estrogen, doxycycline and amoxicillin, the heavy metals Cu, Pb, Cd and Hg and the organic pollutants triclosan, dibutylphtalate (DBP) and dichlorvos in freshwater. In summary, chitosan nanoparticles are the NBM of highest concern regarding freshwater, while PAN and HAP do not represent significant toxicity.

In a complete environmental risk assessment, the risk that the NBMs may pose for a specific compartment is calculated by dividing the predicted environmental concentration (PEC) of that compartment derived in the exposure assessment by the predicted no effect concentration (PNEC) of that compartment derived from the pSSD in the hazard assessment. The risk is then considered as non-negligible when the ratio PEC/PNEC is above 1. At the moment, risk assessments for NBMs are highly problematic as only limited environmental hazard data and no exposure data is available. Due to these limitations, we could not investigate the risk but only the hazard of the previously mentioned NBMs. Hopefully, in the future more and better data will become available in order to fill these knowledge gaps.

Introduction

During the past decade, nanobiomaterials (NBMs) have become increasingly important for the use in biomedical engineering and pharmaceutics (X. Li et al., 2015). Apart from the technical performance of a product, the important question now is which NBMs should be implemented in pharmaceutical products in order to reduce the risk for the environment and human health (Som et al., 2013). The chemical, physical and biological characteristics of nanomaterials are considerably different from the properties of the microscale particles due to the higher surface area over volume ratio (Christenson et al., 2007; Guisbiers et al., 2012; Mahmoudi et al., 2012). Thus, available experience with inorganic and organic chemicals regarding human health and environmental safety may not be relevant to nanomaterials (Klaine et al., 2012). Some NBMs may affect the environment less severely than they affect human health, whereas the case may be reversed for others (Som et al., 2013). These uncertainties regarding the safety of NBMs could hold back their future market growth. Therefore, it is important to evaluate the risk of these materials as they may exhibit adverse effects towards human health or the environment (Bonakdar & Mashinchian, 2015; Christenson et al., 2007).

This report describes the current knowledge and knowledge gaps on environmental risks of five polymeric NBMs (chitosan, polyacrylonitrile (PAN), poly(lactic-co-glycolic acid) (PLGA), Polyhydroxyalkanoates (PHA), and Poly(lactic acid) (PLA)) and one inorganic NBM (Hydroxylapatite (HAP)). The first part describes the prevailing way of calculating the predicted environmental concentration (PEC) of nanomaterials through environmental exposure modelling. The second part derives the predicted no effect concentration (PNEC) of the above mentioned NBMs in freshwater and soil based on ecotox studies from literature data. The last part describes the current approach for the combination of the exposure and hazard assessment into a risk assessment including the state of the art of risk assessment for polymeric NBMs.

Exposure Assessment

For most NBMs, either there have been no studies done yet to evaluate their concentrations in the environment, or the NBMs are still in the development phase and thus not yet on the market. Therefore, the only way to estimate the prospective environmental concentrations is through exposure modelling (Gottschalk et al., 2009).

Pharmacokinetic Modelling

In a first step, the behavior of the NBMs inside the human body needs to be evaluated. How a particle interacts with the human body is fundamental for the long-term fate and the commercial viability, specifically whether the particles or their byproducts are subject to bioaccumulation within cells or organs (Carlander et al., 2016; D. Li et al., 2016; Mahmoudi et al., 2012). Additionally, the same unique physical and chemical properties of a nanoparticle that may be beneficial for human health may also be associated with potentially deleterious effects on human health (X. Li et al., 2015). Several processes need to be evaluated such as the absorption of the NBMs after administration, the distribution throughout the body including the accumulation of NBMs in tissues and organs, the metabolism into different metabolites, and excretion of the NBMs and their transformation products either through urine or feces (Moss & Siccardi, 2014).

Environmental Modelling

During the past decade, the presence of pharmaceuticals in the environment has gained increasing attention and many studies have evaluated the flows and associated environmental risks of pharmaceuticals in the environment (Küster & Adler, 2014). Unfortunately, not much is known yet regarding NBMs in the environment and their flow. However, for NBMs a lot can be learned from pharmaceuticals regarding their flows to the environment as they are expected to behave similar to pharmaceuticals. Like pharmaceuticals, NBMs are excreted in urine and feces and so enter the sewage system. From there, pharmaceuticals usually reach the waste water treatment plants where they are partially removed before being discharged into surface waters. Through surface waters, the pharmaceuticals are further distributed through the biosphere and can reach different compartments such as soil, ground water, ocean, soil as well as the atmosphere (Chèvre et al., 2013). Pharmaceuticals have been found worldwide in surface water, groundwater, tap/drinking water, manure, and soil (Umwelt Bundesamt, 2018). Therefore, if NBMs and their degradation products behave the same way as pharmaceuticals, there is an urgent need to quantify their flows to and in the environment.

Material Flow Analysis

Material flow analysis (MFA) is an established method to study materials or energy flows into, throughout and out of a system (Brunner & Rechberger, 2004). Based on the flows in the environment calculated through MFA, the predicted environmental concentration (PEC) in

technical and environmental compartments can be calculated. This is done by estimating the total input flows into the compartments and then dividing the amounts remaining in each compartment by the volume of the respective compartment (Sun et al., 2014).

Multi-compartment models described by different boxes are most commonly used to model the fate of nanomaterials. Production, use (application in the human body), environmental compartments (such as air, soil, and water), but also technical compartments (such as production plants, waste water treatment plants, landfills, waste incineration plants, etc.) are modelled. Based on a material flow analysis which covers the whole life cycle of a studied nanomaterial, the flows from the source to the natural compartments were organisms are exposed to the NBMs can be evaluated (Gottschalk et al., 2010). The figure below shows a very simplified scheme of how the flows for a NBM could look like.

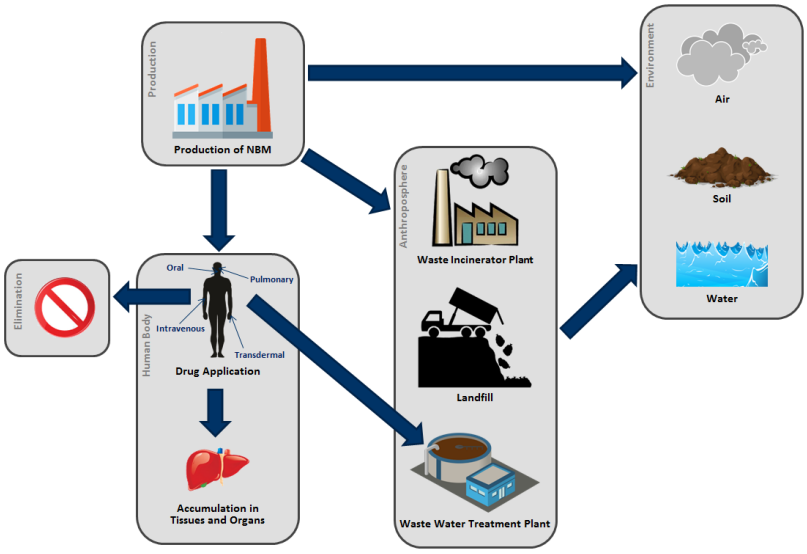

Figure 1: Simplified flow scheme of NBMs throughout their life cycle

Often, there is a large uncertainty and variability in the data or no data is available at all. Therefore a probabilistic approach is frequently used in which a probability distribution is created for all system parameters (Mahapatra et al., 2015). Those input distributions may be constructed based on empirical data, expert judgement or a combination of these sources. The mass balance and multi-compartmental approach of an MFA allows one to treat all parameters throughout the model as probability distributions (Gottschalk et al., 2009). This includes input and output flows, transfer coefficients and environmental concentrations. This way, uncertainties are considered at every point of the system (Gottschalk et al., 2010). Additionally, it needs to be acknowledged that when NBMs are applied inside the body, they are not all immediately excreted again but rather are released over a time span of month or even years. Thus dynamic models have sometimes been used to assess the past, present and future flows of a material

relying on knowledge of how the system behaves and to represent time-dependent residence times (Bornhöft et al., 2016; Sun et al., 2017). In these models the release in one period depends on the inflows of several previous periods and the delay characteristics of the stock (Bornhöft et al., 2016). If these two methods are combined, a dynamic probabilistic MFA results, which provides information about the behavior of the system as a function of time while also representing all uncertain system parameters as probability distributions (Sun et al., 2017).

State of the Art

MFA has extensively been used to predict the flows of engineered nanomaterials (ENM) to the environment (Gottschalk et al., 2009, 2010; Mueller & Nowack, 2008; Sun et al., 2014, 2017; Wang, Deng, et al., 2016; Wang, Kalinina, et al., 2016). Also the flows of pharmaceuticals have been quantified using this method (Chèvre et al., 2013). In this study, the flows of four different pharmaceuticals from hospitals in Lausanne were investigated into Lake Geneva.

One modeling study about gold-nanoparticles used as NBM is available, even though it is never mentioned in the article itself that nano-gold is an NBM in the considered application (Mahapatra et al., 2015). The authors evaluated the environmental exposure of gold-nanoparticles from medical applications in the United States and the United Kingdom. They used a probabilistic material flow analysis to calculate the environmental concentrations of selected medical applications. The model inputs were based on use and consumption of the medical applications. In previous studies, the input used to be calculated based on manufacturing and processing amounts, so this has been a novel approach. Assuming a worst case scenario, the flow of nano-gold was then traced through the sewage treatment, septic tanks, or hazardous or non-hazardous waste to soil, air, surface water and sediment. Based on these flows, the predicted environmental concentrations could be calculated and were then compared to a probabilistic species sensitivity distribution to estimate environmental risks. The study found that the majority of the nano-gold either stays in the body and ends up in the crematorium or burial, or flows through the environmental compartments until being deposited in the soil (Mahapatra et al., 2015). The risk assessment showed that even under the high-release scenario no risk for organisms in freshwater and soils is expected.

No study modelling the flows of polymeric NBMs in the body or the environment has been found. Therefore, there is an urgent need to close this gap and evaluate their flows in the environment.

Hazard Assessment

Hazards can be identified by the predicted no effect concentration (PNEC), which is the threshold of not having adverse effects on the ecosystem (Wang, Kalinina, et al., 2016). The following chapter describes in detail the calculation of the PNEC for the selected NBMs in freshwater and soil. For the other compartments, it was not possible to derive a PNEC as no data was found in literature.

Methods

Data collection

The current work contains environmental hazard literature review for five selected polymeric and one inorganic NBM. Specifically, ecotoxicological endpoints for chitosan, polyacrylonitrile (PAN), poly(lactic-co-glycolic acid) (PLGA), Polyhydroxyalkanoates (PHA), Poly(lactic acid) (PLA) and hydroxyapatite (HAP) were either obtained or estimated.[1]

The data searching process was based on certain criteria. For publications, only papers with impact factors higher than 2 in the year 2016 from 2000 to October 2017 from Google Scholar were considered. This was the major source of ecotoxicity studies for the different materials. Additionally, Material Safety Data Sheets from relevant companies were also used. However, they only contributed to about 0.8% (2 data points) of the total data collection. For each selected material, the keywords for searching literature were set as for example "toxicity chitosan", "toxicity chitosan nanoparticles", "ecological effect chitosan", and "ecological effect chitosan nanoparticles", etc. Around 18 to 20 relevant keywords were used for the literature search for each studied material, and the first 10 pages containing 10 publications each were viewed for each search to be rescreened later. Therefore, there were in total approximately 8000 publications reviewed. However, only less than 150 papers included ecotoxicological studies (approx. 1.9%), and 18 were eventually used for the hazard assessment (approx. 0.2%) as the others did not comply with the criteria of exclusion mentioned below.

For the ecological effects the studied materials have on certain organisms, only effects on survival, growth, reproduction, hatching and changes in significant metabolic processes (such as photosynthesis) were considered (Coll et al., 2016). Within one study, only data for one major effect was collected. Minor effects like changes in behavior, coloring, mild biochemical adjustments, or enzyme regulations were excluded. Moreover, only studies on living organisms exposed to the selected two compartments (freshwater and soil) were used as ecotoxicity data for sediment and marine compartments were unavailable. Additionally, chronic endpoints were preferred over acute if both were available in the same study. Cytotoxicity studies on animal/human tissues cells (e.g. monocytes, macrophages, fibroblastic, and osteosarcoma cell

[1] Data collection for PHA and PLA were not completed

etc.) were not used. In studies where even the highest exposure concentration showed no adverse effect on the test organism, this value was used as the Highest Observed No Effect Concentration (HONEC) for the calculations. When different particle sources, particle sizes, or culture conditions etc. were tested in the same study, all the different endpoints were taken. Therefore, the data presented later is not restricted to a specific nanomaterial form or particle property (e.g. specific surface coating or surface charge), but rather considers a range of possible biological nanomaterials characteristics and thus making the model more applicable to the wide range of NBMs.

Data processing

In most cases, chronic No Observed Effect Concentration (NOEC) values, which are needed for the derivation of the PNEC value, were not available. Thus each of the ecotoxicological endpoint was transformed by two different assessment factors (AFs) based on the REACH guidance (ECHA, 2008) in order to derive the PNEC. This method helps to overcome challenges associated with the variability and uncertainty of endpoint concentrations, which are mostly due to diverse or unspecified experimental conditions and the characteristics of nanoparticles (Coll et al., 2016).

Literature data is often presented either as EC_x (concentration affecting x% of organisms), IC_x (x% growth inhibition), LC_x (concentration lethal to x% of a population), minimum inhibitory concentration (MIC), or no observed effect concentration (NOEC) (Gottschalk & Nowack, 2013). The first assessment factor (AF_{NOEC}) is used for extrapolating the observed effect into standardized no observed effect concentrations as shown in Table 1.

Table 1: Assessment factor for extrapolation from observed effect into no effect concentration (AF_{NOEC})

Observed ecotoxicological endpoint	*Assessment factor to derive no effect concentration*
$LC/EC/IC_{25-50}$	10
$LC/EC/IC_{10-20}$, MIC[1], LOEC[2]	2
HONEC, NOEC	1

[1] The minimum inhibitory concentration, which stands for the lowest concentration where an effect has been observed
[2] The lowest observed effect concentration, which is the lowest tested concentration that is significantly different from the control

The second assessment factor (AF_{time}) accounts for the extrapolation of short-term (acute) to long-term (chronic) effects. As shown in Table 2, short-term studies receive higher assessment factors than long-term ones. The REACH guidance requires the categorization into short-term/long-term based on the species or taxonomic group (ECHA, 2008). The factors used were defined as recommended in the study by Gottschalk et al. (2013) and are shown in Table 3. For unicellular organisms, bacteria and fungi, 24 hours were used as a minimum time threshold for the consideration as a chronic effect, since under favorable conditions some of those organisms can multiply within hours or less. Furthermore, results with vertebrates and invertebrates were defined as chronic only if the test duration was 21 days or more. Lastly, for fish up to 7 days of exposure was considered short-term (up to 14 days as prolonged acute exposure).

Table 2: Assessment factor for extrapolation of short-term to long-term effects concentrations (AF_{time})

Categorization of studies	*Assessment factor to derive long-term effect concentration*
Short-term studies	10
Long-term studies	1

Table 3: Assessment factor for extrapolation of short-term to long-term effect concentrations for different test organisms (species and taxonimic groups)

Taxonomic group	*Exposure time [d]*	*AF2*[1]
Unicellular	<1	10
	≥1	1
Bacteria	<1	10
	≥1	1
Algae	<3	10
	≥3	1
Vertebrate & Invertebrate	<21	10
	≥21	1
Fish	<7	10
	≥7	1
Fungi	<1	10
	≥1	1

[1] Assessment factors that were applied to each of the collected ecotoxicological endpoint concentrations are given in Appendix Table A1-A6

Data evaluation

There were 231 data points (from 18 papers) collected in total from the literature search (state November 2017). The majority of the toxicological studies for the selected NBMs focused on the freshwater compartment (169 data points, 73%) followed by the soil compartment (62 data points, 27%). All details are displayed in Appendix Table A1-A6.

Modeling of Probabilistic Species Sensitivity Distributions (pSSDs)

The collected endpoints were converted into PNECs based on two approaches. If only one ecotoxicological endpoint was available for a certain NBM, then an assessment factor of 1000 was applied on the lowest EC50 or other endpoint (if no other was available) as suggested by the REACH guideline (ECHA, 2008).

If several endpoints were available across multiple species, a species sensitivity distribution (SSD) was constructed. The PNEC was then estimated based on the novel approach by Adam et al (2018). This means, that the pSSDs were built in three steps. In a first step, the ecotoxicological values were converted into probability distributions of NOECs. This was done by combining three triangular probability distributions (Figure 2A). First, a range of variability was associated with the experimental measurement: The mode of the distribution was the experimentally measured

value and a coefficient of variation of ±30% was applied to find to minimum and maximum of the distribution representing the inter-laboratory variability. For the two assessment factors, their modes corresponded to the defined values and a coefficient of variation of ±50% was applied to obtain the minima and maxima of each distribution. The probability distribution of the measurement was then divided by the product of the two assessment factors to get the distribution of each NOEC (Adam et al., 2018).

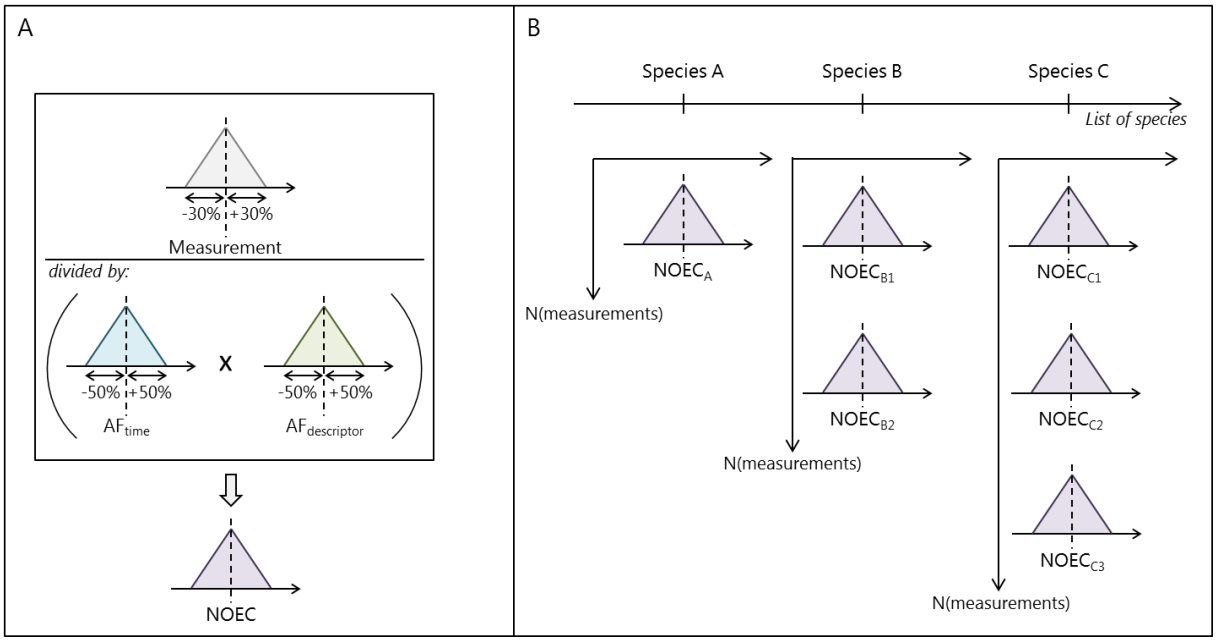

Figure 2: Conversion of measured values to NOEC distributions (Adam et al., 2018)

Next, a matrix including all NOECs of all species was created. The type of probability distribution depended on the number of NOECs available for each species. If only one NOEC was available, the triangular distribution was kept. If two NOECs were available, a trapezoidal distribution was built with the modes, minimum and maximum as those of the NOEC probability distribution. If three or more NOECs were available, uniform distributions were built between each NOEC and triangular distributions were added at the maximum and minimum of the minimal/maximal NOECs of the species (Adam et al., 2018).

In a last step, the pSSDs were extracted. Each simulation was extracted as one pSSD. Then, as recommended by the REACH guideline R.10 (ECHA, 2008), the PNEC was calculated as the fifth percentile of the pSSDs (HC_5, hazardous concentration for 5% of species). This resulted in a probability distribution of the PNEC (Adam et al., 2018). The calculations were done in R (R Development Core Team 2008).

Results

Summary of data collection

Overall, there were 231 data points collected through literature search. The summarized results are shown in Table 4. Most ecotoxicological experiments useful for our hazard assessment were conducted on freshwater organisms, while there were only a few studies on soil organism. No ecotoxicological studies were found for other environmental compartments (i.e. sediment, marine, and air) or technical systems (i.e. treated sludge, waste water treatment, incineration, landfill). Besides the number of endpoints, species and taxonomic groups, also the number of endpoints on environmental organisms (later called "env") was recorded. These endpoints exclude all pathogenic bacteria and fungi. Additionally, in studies were it was specially mentioned that the particles were "nanoparticles", they were characterized as such. If nothing was mentioned, they were characterized as "Non-Nano". During the preparation of chitosan, it is dissolved in a medium (e.g. acetic acid, HOAc, or gelation with TTP). Therefore, also the term dissolved was used for non-nano chitosan. No studies were found for pure PLGA, PLA or PHA complying with the data searching criteria specified above. Therefore, no PNEC could be calculated for these materials.

Table 4: Summary of ecotoxicological endpoints used for the selected NBMs

NBMs	*Compartment*	*# Endpoints*	*# Species*	*# Taxonomic Groups*	*# Endpoints on Env. Organisms*[1]
Nano-Chitosan	Freshwater	16	7	3	4
	Soil	0	0	0	0
Non-Nano Chitosan	Freshwater	138	25	5	5
	Soil	60	8	2	0
Nano-HAP	Freshwater	13	6	3	8
	Soil	1	1	1	0
Non-Nano HAP	Freshwater	0	0	0	0
	Soil	0	0	0	0
PAN Nanofibers	Freshwater	2	1	1	0
	Soil	1	1	1	0
Non-Nano PAN	Freshwater	0	0	0	0
	Soil	0	0	0	0
Nano-PLGA	Freshwater	0	0	0	0
	Soil	0	0	0	0
Non-Nano PLGA	Freshwater	0	0	0	0
	Soil	0	0	0	0
Nano-PLA	Freshwater	0	0	0	0
	Soil	0	0	0	0
Non-Nano PLA	Freshwater	0	0	0	0
	Soil	0	0	0	0
Nano-PHA	Freshwater	0	0	0	0
	Soil	0	0	0	0
Non-Nano PHA	Freshwater	0	0	0	0
	Soil	0	0	0	0

[1] Environmental organism stands for all species except for pathogenic bacteria and fungi

In general, there were only a limited number of studies found for the selected nanomaterials for soil. Besides, ecotoxicological data for dissolved and/or bulk forms of HAP, PAN and PLGA were not available. A possible reason for this could be that the majority of toxicological studies of those materials were done before the literature publication time frame defined in the data searching criteria (i.e. before the year 2000). The literature search was also started for the materials PLA and PHA, but the data searching criteria described in "Data collection" were not followed strictly. As no studies were found, the search was stopped without being completely confident that there exists no ecotoxicity study PLA and PHA.

Due to the biological application of the studied materials (e.g. wound dressings, tissue scaffolds and bone engineering), their antimicrobial (antibacterial/fungicidal) properties were of more interest. Thus these studies did not consider the potential hazard to environmental organisms. This is especially the case for chitosan and HAP. As a result, the majority of ecotoxicological studies focused on microorganism, and therefore not enough taxonomic groups were found for every selected material. This is not in accordance with REACH guidance (ECHA, 2008), which states that for the freshwater compartment, confidence can be associated with a PNEC derived by statistical extrapolation from a database containing at least 10 NOECs (preferably more than 15) for different species covering at least 8 taxonomic groups. Furthermore, due to their biodegradability, the amounts of residues from polymeric NBMs (such as PAN and PLGA) released to the ecosystem are substantially reduced. Therefore, most toxicological studies of these materials showed more concern for human risks (*in vivo*) than for ecological hazard. Most animal tests were done with mice, rats, rabbits etc. for oral or pulmonary exposure and only a handful to obtain ecotoxicological data. Another possible reason for lacking data is that for the selected polymeric nanomaterials, their ecotoxicity was usually not studied alone, but used as coating/embedding/encapsulating/doping materials together with other more toxic nanomaterials, and/or in modified structures with novel properties. Therefore, it was difficult to obtain ecotoxicological data of the "pure" selected NBMs. Sometimes this data was only available as the blank/control group in ecotoxicological experiment that tested another substance.

Special care was taken to discard experiments for HAP that used heavy metal doped hydroxyapatite nanoparticles as this increases the toxicity to the test organisms, which leads to an overestimation of the toxicity.

Nanoparticle characterization

The nanoparticle size distributions of nano chitosan and HAP used in freshwater studies are shown in Figure 3. Additionally, it is shown for how many of the data points the diameter was available and for how many no diameter was recorded in the paper (called "NA" in figure). For chitosan and HAP nanoparticles, the mean diameters ranged from 0 to 350 nm. For nano

chitosan, the diameter was only recorded for 88% of the data points whereas for HAP, the mean diameter was available for all the data points. The morphologies of all the applied nanomaterials are recorded in the appendix in Table A7. The shapes of the nanoparticles varied greatly for different material in different studies. For chitosan, most nanoparticles are round or oval shaped, and some agglomerated clusters; for HAP nanoparticles, most are rod shaped; and for PAN, they are nanofibers loaded with other materials. Therefore, a wide range of morphologies of NBMs was involved in the ecotoxicological studies leading to more general results for the calculated pSSD.

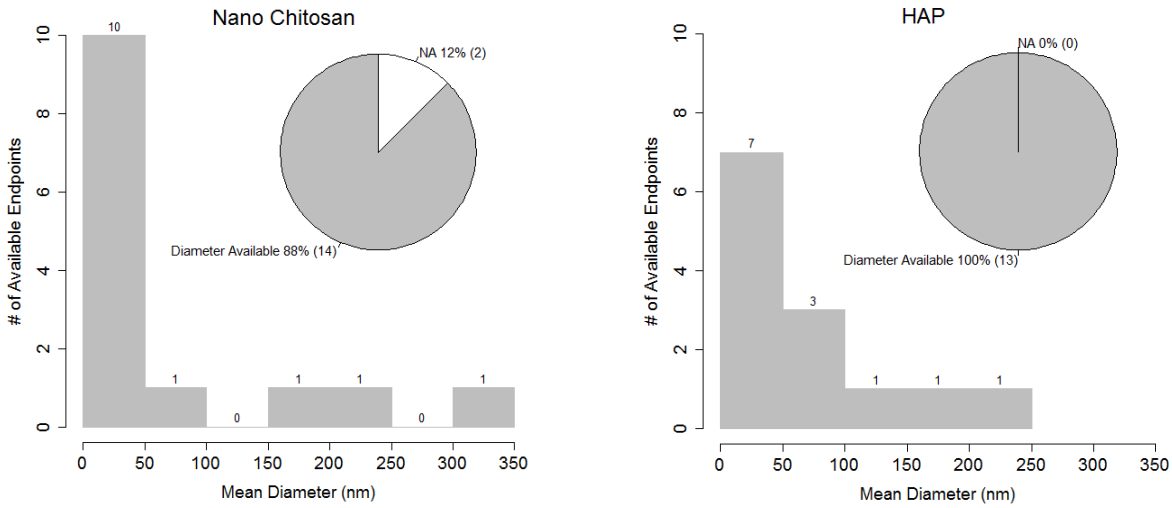

Figure 3: Summary of size distributions of chitosan and HAP used in freshwater studies

Probabilistic Species Sensitivity Distributions (pSSDs)

The pSSDs for chitosan, nano-chitosan, HAP, and PAN are shown in Figure 4 for freshwater and in Figure 6 for soil. For chitosan, the nano and dissolved form is shown for freshwater but only the dissolved form for soil as no data was found for the nano form. In red the single runs for the pSSDs are shown and in black the mean of all these individual pSSDs. The individual NOECs derived from the different endpoints were grouped together by species and are shown in blue. A species' NOECs often varied greatly between different studies. For many species, the range of individual NOEC values spans many orders of magnitude. For example, the NOECs for *E. coli, S. aureus* and *S. typhi* for chitosan in freshwater ranges over three orders of magnitude (see Figure 4A). This can be attributed to a number of uncertainties, such as different nanoparticles properties, different experimental conditions, etc.

For soil organisms, much fewer studies providing usable endpoints were found, especially for HAP and PAN nanoparticles, where only one endpoint was found for each nanoparticle. Therefore, no pSSD was calculated for them. For the ecotoxicity of dissolved and nano chitosan in freshwater, Figure 5 was generated where all pathogenic bacteria and fungi are removed from

the original pSSD in Figure 4A and B, they were then called Chitosan (env) and Nano Chitosan (env). The reason behind this is that a majority of studies focused on the antimicrobial properties of dissolved chitosan/nano-chitosan instead of their ecological impacts, and thus most of the test organisms were pathogenic bacteria and fungi. By removing these data points, the ecotoxicity for all other environmental organisms are displayed in a more explicit way as studies with the purpose to kill bacteria or fungi were excluded and only studies with the purpose to investigate potential hazardous effects on environmentally "valuable" organisms were considered. Figure 7 shows the pSSDs of all materials in freshwater.

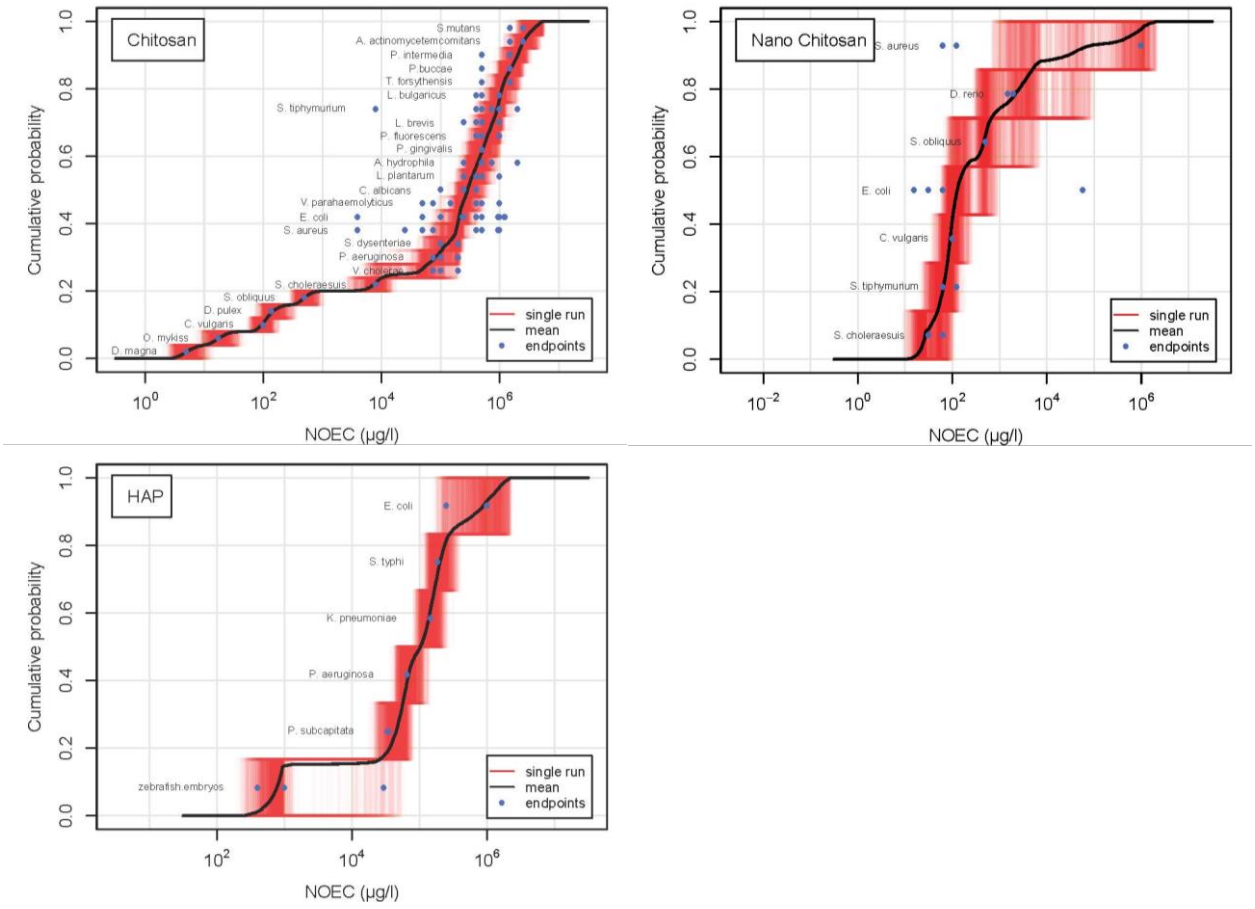

Figure 4: Probabilistic species sensitivity distributions (pSSDs) of chitosan, chitosan nanoparticles, and HAP nanoparticles in freshwater

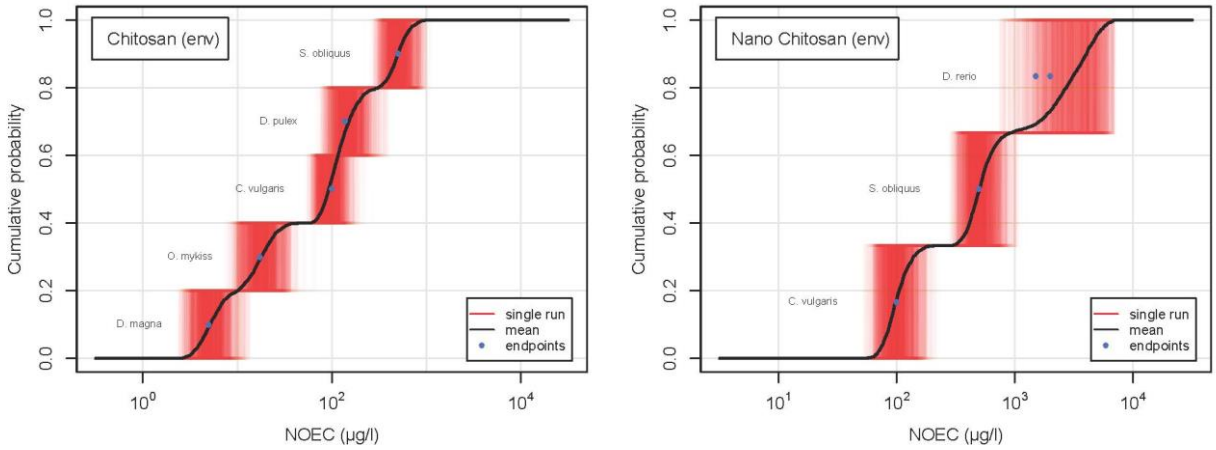

Figure 5: Probabilistic species sensitivity distributions (pSSDs) of chitosan and chitosan nanoparticles in freshwater by removing all pathogenic bacteria and fungi in the collected data

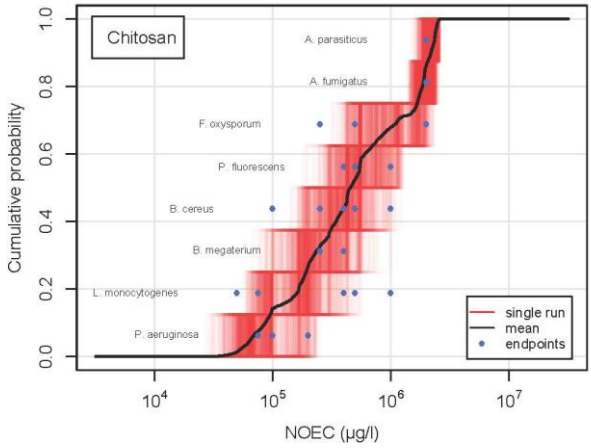

Figure 6: Probabilistic species sensitivity distributions (pSSDs) of chitosan in soil

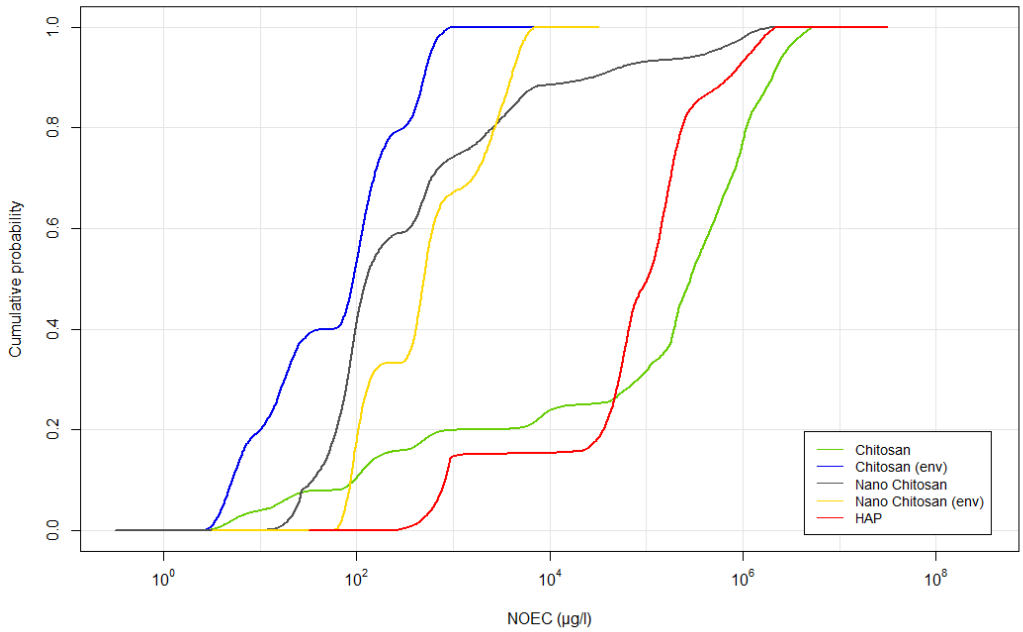

Figure 7: Cumulative probabilistic species sensitivity distributions (pSSDs) of chitosans and HAP in freshwater

Predicted No-Effect Concentration (PNEC) Distribution

The PNEC distribution for each NBM was derived by calculating the 5th percentile of each single pSSD run. The distribution is different for each nanomaterial and is shown for freshwater in the appendix in Figure A1. Based on these distributions, the median, mean and mode can be calculated. They are shown for each NBM including their nano and environmental form (if available) in Table 5 for freshwater and Table 6 for soil. Additionally, the minimum, maximum and the 25th and 75th percentile are also listed. If only one endpoint was available, the calculated PNEC was listed as mean value (i.e. PAN in freshwater, and HAP and PAN in soil).

From Table 5 and Table 6 it can be suggested that the PNEC for freshwater and soil increases from most sensitive (toxic) to least sensitive in the following order:

Freshwater: Chitosan (env) < Chitosan ~ Nano Chitosan < Nano Chitosan (env) << HAP << PAN

Soil: HAP < PAN < Chitosan

These orders indicate that PAN nanofibers have among the highest predicted no-effect concentration in both compartments and are therefore expected to be the least toxic and have the lowest impact on the environment of the investigated NBMs. It is also interesting to note that HAP has a high PNEC in freshwater but the lowest PNEC among the investigated materials

in soil. This shows that the toxicity of materials is very much dependent on the environmental compartment they are in.

Table 5: Median, mean, mode, minimum, maximum, 25th and 75th quartile from predicted no-effect concentrations (PNEC) distribution in freshwater

[µg/L]	*Chitosan*	*Chitosan (env)*[1]	*NanoChitosan*	*NanoChitosan (env)*	*HAP*	*PAN*
Median	34	8	43	140	$1.4*10^4$	NA[2]
Mean	35	8	47	150	$1.7*10^4$	$3.0*10^6$
Mode	32	7	34	140	$1.4*10^4$	NA
Min	19	4	13	90	$5.2*10^3$	NA
25%	30	7	33	130	$1.1*10^4$	NA
75%	39	9	58	160	$1.8*10^4$	NA
Max	66	23	136	230	$6.0*10^4$	NA

[1] env: PNEC values for environmental species by removing all pathogenic bacteria and fungi from original pSSD curves

[2] no distribution as only one endpoint was found

Table 6: Median, mean, mode, minimum, maximum, 25th and 75th quartile from predicted no-effect concentrations (PNEC) distribution in soil

[mg/kg]	*Chitosan*	*HAP*	*PAN*
Median	110	NA[1]	NA[1]
Mean	120	0.3	33
Mode	640	NA	NA
Min	36	NA	NA
25%	70	NA	NA
75%	150	NA	NA
Max	390	NA	NA

[1] no distribution as only one endpoint was found

Discussion

Figure 8 compares the PNEC value in the freshwater compartment for the studied NBMs (red dots) and for several common pollutants (brown dots for ENMs, green for pharmaceuticals and blue for other pollutants). The details are shown in Appendix Table A8. Generally, chitosan has a relatively high toxicity in freshwater, while PAN and HAP can be treated as almost non-toxic. However, it needs to be noted that even the most sensitive (toxic) of the four selected NBMs, chitosan (env), is less toxic than the engineered nanomaterials fullerenes, nano-ZnO and nano-Ag, the antibiotics estrogen, doxycycline and amoxicillin, the heavy metals Cu, Pb, Cd and Hg

and the organic pollutants triclosan, dibutylphtalate (DBP) and dichlorvos. So in summary, chitosan nanoparticles are the NBM of highest concern, while PAN and HAP do not represent significant toxicity for the environment and should be of lower concern regarding their release and exposure in freshwater.

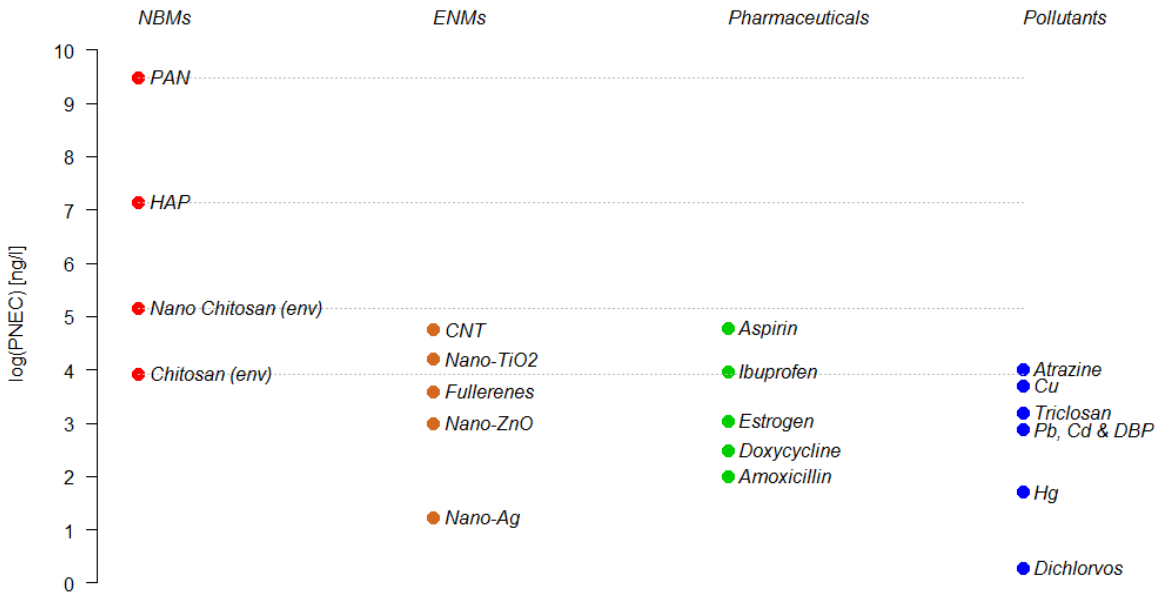

Figure 8: Predicted no-effect concentrations (PNEC) of studied nanobiomaterials (NBMs), engineered nanomaterials (ENMs), pharmaceuticals and some popular pollutants in freshwater

There are several reasons for the lack of data for some NBMs in certain environmental compartments, besides the ones highlighted in "Summary of data collection". For PAN, most of toxicity studies focused on animal tests, in vitro cytotoxicity and human risks assessment. Additionally, the ecological endpoints for a number of studies on nano-PAN membrane and PAN nanofibers were not available because the exact concentrations used were not mentioned in the study. PLGA is regarded as one of the most successfully used biodegradable nanosystem for the development of nanomedicines as it undergoes hydrolysis in the body to produce the biodegradable metabolites lactic acid and glycolic acid. Because these two monomers are endogenous and easily metabolized by the body via the Krebs cycle, a minimal systemic toxicity is associated with the use of PLGA for drug delivery or biomaterial applications (Kumari et al., 2010). As a result, PLGA is approved by the US FDA and European Medicine Agency (EMA) for various drug delivery systems in humans (Danhier et al., 2012). Besides, ecotoxicological effect studies for PLGA were rarely found based on the literature search criteria, due to their biodegradability.

As for the shortage of data in the soil compartment, one of the most important reasons were that some ecotoxicological studies were discarded because soil biota were studied in aqueous suspensions and these testing conditions were deemed inappropriate for risk assessment

purposes according to the evaluation criteria (Coll et al., 2016). Furthermore, it should not be forgotten that journals often discard studies that show no effect on the studied organism (Krug, 2014). This triggers possible bias or overestimation of the environmental effects (i.e. ecotoxicity), especially for the relatively less toxic PAN nanofibers and PLGA nanoparticles.

Nanoparticles' size distribution is another problem of concern. Conventionally, nanoparticles are defined as particles between 1 and 100 nm in size with a surrounding interfacial layer (European Commission, 2011). The term "nanoparticles" however, was also used here for some materials whose sizes were larger than 100 nm in the original studies. This contradiction should be noticed when making policies or guidelines for such "nanoparticles". However, in the medical field, particles with sizes up to 1000 nm may be considered as nanoparticles, thus all sizes examined here fall into this range. Moreover, the number of available information on nanoparticle size distributions was very limited, in some studies even missing completely (as shown in Figure 3). In other studies, the selected nanomaterials, particularly PAN and PLGA nanofibers/ nanoparticles, were studied in combination with other materials (e.g. coating, embedding, encapsulating, doping, or loading) rather than the pure materials alone.

Risk Assessment

The risk of a particular NBM is determined by its potential hazard and by the extent the material will come in contact with an organism (Mueller & Nowack, 2008). So risk is a function of hazard and exposure, or in short: if there is no hazard or no exposure, there will be no risk (Som et al., 2013).

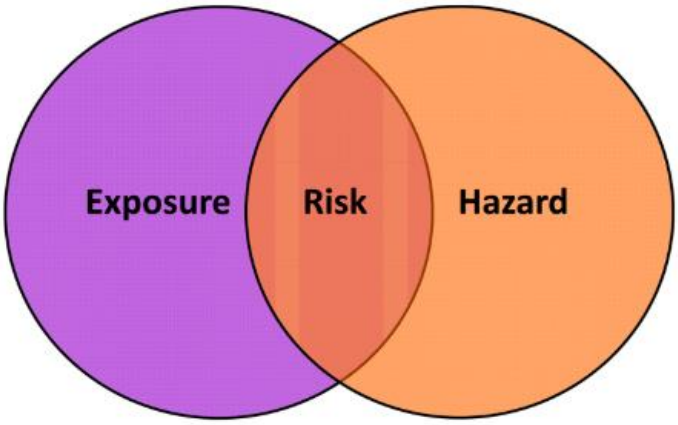

Figure 9: Correlation of Exposure, Hazard and Risk (Som et al., 2013)

The risk that the NBMs may pose for a specific compartment is calculated by dividing the predicted environmental concentration (PEC) of that compartment derived in the exposure assessment by the predicted no effect concentration (PNEC) of that compartment derived from the 5^{th} percentile of the pSSD in the hazard assessment. This way, a risk quotient for different environmental compartments (such as freshwater, soil, air, sediment, etc.) and technical systems (i.e. waste water treatment, incineration, landfill), can be calculated. The risk is then considered as non-negligible when the ratio PEC/PNEC is above 1 (Chèvre et al., 2013). The calculation of this risk quotient involves a safety factor of 1000. Therefore, a risk quotient above one does not constitute an immediate risk but is an indication that further data are needed (Gottschalk et al., 2013).

At the moment, there have been no risk assessments conducted for none of the NBMs, chitosan, HAP, PAN, PLA, PHA and PLGA, investigated here. This is mainly due to extensive uncertainties, which may be the result of different reasons, such as lack of data on potential human health and environmental hazards and exposure, contradictory experimental data, uncertainty regarding the physicochemical properties of the NBM that may be responsible for any specific toxicity or hazard, uncertainty about the dose metric for the NBM, or a lack of data on toxicokinetics of the material (Som et al., 2013).

Additionally, often the only data available is for a generic NBM and not a specific material (i.e. disregarding size, surface charge/cover, etc.). All these properties influence the behavior of the

material and thereby also the risk they pose (Som et al., 2013). On top of that, NBMs have been found to undergo transformation both inside the human body as well as in the environment. Therefore, their fate changes even more, leading to even greater uncertainty (Mahapatra et al., 2015).

So for NBMs, risk assessments are currently highly problematic as there is only limited environmental hazard data, and no exposure or human health data. Hopefully, in the future more and better data will become available in order to fill these knowledge gaps. The Biorima project as part of the European Horizon 2020 framework aims to develop an integrated risk framework for NBMs used in advanced therapy medicinal products and medical devices. Thus, more data on risk exposure, hazard and risk assessment for different NBMs are expected to be available by the end of the project (Website: https://www.biorima.eu/).

References

Adam, V., Wigger, H., Wenger-Kawecki, D., Hauser, M., & Nowack, B. (2018). Harmonized an reliable probabilistic species sensitivity distribution approach for transparent PNEC estimation (paper in preparation).

Bonakdar, S., & Mashinchian, O. (2015). Toxicology of Nanobiomaterials. *Stem Cell Nanoengineering*, 171–184. https://doi.org/10.1002/9781118540640.ch10

Bornhöft, N. A., Sun, T. Y., Hilty, L. M., & Nowack, B. (2016). A dynamic probabilistic material flow modeling method. *Environmental Modelling & Software*, *76*, 69–80. https://doi.org/10.1016/j.envsoft.2015.11.012

Brunner, P. H., & Rechberger, H. (2004). *Practical Handbook of Material Flow Analysis*. CRC Press LLC, Boca Raton, Florida. https://doi.org/10.1002/pssb.201300062

Carlander, U., Li, D., Jolliet, O., Emond, C., & Johanson, G. (2016). Toward a general physiologically-based pharmacokinetic model for intravenously injected nanoparticles. *International Journal of Nanomedicine*, *11*, 625–640. https://doi.org/10.2147/IJN.S94370

Chèvre, N., Coutu, S., Margot, J., Wynn, H. K., Bader, H. P., Scheidegger, R., & Rossi, L. (2013). Substance flow analysis as a tool for mitigating the impact of pharmaceuticals on the aquatic system. *Water Research*, *47*(9), 2995–3005. https://doi.org/10.1016/j.watres.2013.03.004

Christenson, E. M., Anseth, K. S., van den Beucken, J. J. J. P., Chan, C. K., Ercan, B., Jansen, J. A., … Mikos, A. G. (2007). Nanobiomaterial Application in Orthopedics. *Journal of Orthopedic Researyh*, (January), 11–22. https://doi.org/10.1002/jor

Coll, C., Notter, D., Gottschalk, F., Sun, T., Som, C., & Nowack, B. (2016). Probabilistic environmental risk assessment of five nanomaterials (nano-TiO2, nano-Ag, nano-ZnO, CNT, and fullerenes). *Nanotoxicology*, *10*(4), 436–444. https://doi.org/10.3109/17435390.2015.1073812

Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., & Préat, V. (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release*, *161*(2), 505–522. https://doi.org/10.1016/j.jconrel.2012.01.043

ECHA. (2008). Guidance on information requirements and chemical safety assessment Chapter R . 10 : Characterisation of dose [concentration] -response for environment, (May), 1–65.

European Commission. (2011). Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). *Official Journal of the European Union*, *L275/38*(June 2010), 38–40.

Gottschalk, F., Kost, E., & Nowack, B. (2013). Engineered nanomaterials in water and soils: A risk quantification based on probabilistic exposure and effect modeling. *Environmental Toxicology and Chemistry*, *32*(6), 1278–1287. https://doi.org/10.1002/etc.2177

Gottschalk, F., & Nowack, B. (2013). A probabilistic method for species sensitivity distributions taking into account the inherent uncertainty and variability of effects to estimate environmental risk. *Integrated Environmental Assessment and Management*, *9*(1), 79–86. https://doi.org/10.1002/ieam.1334

Gottschalk, F., Scholz, R. W., & Nowack, B. (2010). Probabilistic material flow modeling for assessing the environmental exposure to compounds: Methodology and an application to engineered nano-TiO2 particles. *Environmental Modelling and Software*, *25*(3), 320–332. https://doi.org/10.1016/j.envsoft.2009.08.011

Gottschalk, F., Sondere, T., Schols, R., & Nowack, B. (2009). Modeled Environmental Concentrations of Engineered Nanomaterials for Different Regions. *Environmental Science & Technology*, *43*(24), 9216–9222. https://doi.org/10.1021/es9015553

Guisbiers, G., Mejía-Rosales, S., & Leonard Deepak, F. (2012). Nanomaterial properties: Size and shape dependencies. *Journal of Nanomaterials*, *2012*, 2012–2014. https://doi.org/10.1155/2012/180976

Klaine, S. J., Koelmans, A. A., Horne, N., Carley, S., Handy, R. D., Kapustka, L., … von der Kammer, F. (2012). Paradigms to assess the environmental impact of manufactured nanomaterials. *Environmental Toxicology and Chemistry*, *31*(1), 3–14. https://doi.org/10.1002/etc.733

Krug, H. F. (2014). Nanosafety research-are we on the right track? *Angewandte Chemie - International Edition*, *53*(46), 12304–12319. https://doi.org/10.1002/anie.201403367

Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*, *75*(1), 1–18. https://doi.org/10.1016/j.colsurfb.2009.09.001

Küster, A., & Adler, N. (2014). Pharmaceuticals in the environment: scientific evidence of risks and its regulation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1656), 20130587–20130587. https://doi.org/10.1098/rstb.2013.0587

Li, D., Morishita, M., Wagner, J. G., Fatouraie, M., Wooldridge, M., Eagle, W. E., … Jolliet, O. (2016). In vivo biodistribution and physiologically based pharmacokinetic modeling of inhaled fresh and aged cerium oxide nanoparticles in rats. *Particle and Fibre Toxicology*, *13*(1). https://doi.org/10.1186/s12989-016-0156-2

Li, X., Lee, S. C., Zhang, S., & Akasaka, T. (2015). Biocompatibility and Toxicity of Nanobiomaterials 2014. *Journal of Nanomaterials*, *2015*, 2012–2014. https://doi.org/10.1155/2015/259264

Mahapatra, I., Sun, T. Y., Clark, J. R. A., Dobson, P. J., Hungerbuehler, K., Owen, R., … Lead, J. (2015). Probabilistic modelling of prospective environmental concentrations of gold nanoparticles from medical applications as a basis for risk assessment. *Journal of Nanobiotechnology*, *13*(1), 1–14. https://doi.org/10.1186/s12951-015-0150-0

Mahmoudi, M., Hofmann, H., Rothen-Rutishauser, B., & Petri-Fink, A. (2012). Assessing the in vitro and in vivo toxicity of superparamagnetic iron oxide nanoparticles. *Chemical Reviews*, *112*(4), 2323–2338. https://doi.org/10.1021/cr2002596

Moss, D. M., & Siccardi, M. (2014). Optimizing nanomedicine pharmacokinetics using physiologically based pharmacokinetics modelling. *British Journal of Pharmacology*, *171*(17), 3963–3979. https://doi.org/10.1111/bph.12604

Mueller, N. C., & Nowack, B. (2008). Exposure modelling of engineered nanoparticles in the environment. *Environmental Science & Technology*, *42*(12), 44447–44453. https://doi.org/10.1021/es7029637

Som, C., Nowack, B., Krug, H. F., & Wick, P. (2013). Toward the development of decision supporting tools that can be used for safe production and use of nanomaterials. *Accounts of Chemical Research*, *46*(3), 863–872. https://doi.org/10.1021/ar3000458

Sun, T. Y., Gottschalk, F., Hungerbühler, K., & Nowack, B. (2014). Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environmental Pollution*, *185*, 69–76. https://doi.org/10.1016/j.envpol.2013.10.004

Sun, T. Y., Mitrano, D. M., Bornhöft, N. A., Scheringer, M., Hungerbühler, K., & Nowack, B. (2017). Envisioning Nano Release Dynamics in a Changing World: Using Dynamic Probabilistic Modeling to Assess Future Environmental Emissions of Engineered Nanomaterials. *Environmental Science and Technology*, *51*(5), 2854–2863. https://doi.org/10.1021/acs.est.6b05702

Umwelt Bundesamt. (2018). Database - Pharmaceuticals in the Environment. Retrieved from https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-0

Wang, Y., Deng, L., Caballero-Guzman, A., & Nowack, B. (2016). Are engineered nano iron oxide particles safe? an

environmental risk assessment by probabilistic exposure, effects and risk modeling. *Nanotoxicology*, *10*(10), 1545–1554. https://doi.org/10.1080/17435390.2016.1242798

Wang, Y., Kalinina, A., Sun, T., & Nowack, B. (2016). Probabilistic modeling of the flows and environmental risks of nano-silica. *Science of the Total Environment*, *545–546*, 67–76. https://doi.org/10.1016/j.scitotenv.2015.12.100

Appendix

Table A1: Data for freshwater toxicity of chitosan

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/L) (nano-sized)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentration/AF
Costa et al (2012)	bacterium	P. gingivalis	MIC	1,000,000	72	1	2	500,000
Costa et al (2012)	bacterium	P. gingivalis	MIC	1,000,000	72	1	2	500,000
Costa et al (2012)	bacterium	T. forsythensis	MIC	1,000,000	72	1	2	500,000
Costa et al (2012)	bacterium	T. forsythensis	MIC	3,000,000	72	1	2	1,500,000
Costa et al (2012)	bacterium	P.buccae	MIC	3,000,000	72	1	2	1,500,000
Costa et al (2012)	bacterium	P.buccae	MIC	1,000,000	72	1	2	500,000
Costa et al (2012)	bacterium	A. actinomycetemcomitans	MIC	5,000,000	72	1	2	2,500,000
Costa et al (2012)	bacterium	A. actinomycetemcomitans	MIC	3,000,000	72	1	2	1,500,000
Costa et al (2012)	bacterium	P. intermedia	MIC	1,000,000	72	1	2	500,000
Costa et al (2012)	bacterium	P. intermedia	MIC	3,000,000	72	1	2	1,500,000
Tsai et al (2002)	bacterium	E. coli	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	E. coli	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	E. coli	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	E. coli	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	E. coli	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	E. coli	MIC	200,000	48	1	2	100,000
Du et al (2008)	bacterium	E. coli	MIC	468,000	24	1	2	234,000
No et al (2002)	bacterium	E. coli	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	E. coli	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	E. coli	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	E. coli	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	E. coli	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	E. coli	HONEC	1,000,000	72	1	1	1,000,000
Du et al (2008)	bacterium	E. coli	MIC	**117,000**	24	1	2	58,500
Fernandes et al (2008)	bacterium	E. coli	MIC	1,900,000	24	1	2	950,000
Fernandes et al (2008)	bacterium	E. coli	MIC	2,400,000	24	1	2	1,200,000
Fernandes et al (2008)	bacterium	E. coli	MIC	2,500,000	24	1	2	1,250,000
Qi et al (2004)	bacterium	E. coli	MIC	**125**	24	1	2	62.50
Qi et al (2004)	bacterium	E. coli	MIC	8,000	24	1	2	4,000
Qi et al (2004)	bacterium	E. coli	MIC	**62.50**	24	1	2	31.250
Qi et al (2004)	bacterium	E. coli	MIC	**62.50**	24	1	2	31.250
Qi et al (2004)	bacterium	E. coli	MIC	8,000	24	1	2	4,000
Qi et al (2004)	bacterium	E. coli	MIC	**31.25**	24	1	2	15.6250
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
No et al (2002)	bacterium	P. fluorescens	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	P. fluorescens	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	P. fluorescens	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. fluorescens	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. fluorescens	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. fluorescens	MIC	1,000,000	72	1	2	500,000
Qi et al (2004)	bacterium	S. choleraesuis	MIC	**125**	24	1	2	62.50
Qi et al (2004)	bacterium	S. choleraesuis	MIC	16,000	24	1	2	8,000
Qi et al (2004)	bacterium	S. choleraesuis	MIC	**62.50**	24	1	2	31.250
No et al (2002)	bacterium	S. tiphymurium	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	S. tiphymurium	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	S. tiphymurium	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	S. tiphymurium	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	S. tiphymurium	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	S. tiphymurium	HONEC	1,000,000	72	1	1	1,000,000
Tsai et al (2002)	bacterium	S. tiphymurium	MIC	1,500,000	48	1	2	750,000
Tsai et al (2002)	bacterium	S. tiphymurium	MIC	1,500,000	48	1	2	750,000
Tsai et al (2002)	bacterium	S. tiphymurium	MIC	1,500,000	48	1	2	750,000
Tsai et al (2002)	bacterium	S. tiphymurium	MIC	1,500,000	48	1	2	750,000

Table A1: Data for freshwater toxicity of chitosan (cont.)

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/L) (nano-sized)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentration/AF
Tsai et al (2002)	bacterium	S. tiphymurium	HONEC	2,000,000	48	1	1	2,000,000
Tsai et al (2002)	bacterium	S. tiphymurium	HONEC	2,000,000	48	1	1	2,000,000
Qi et al (2004)	bacterium	S. tiphymurium	MIC	**125**	24	1	2	63
Qi et al (2004)	bacterium	S. tiphymurium	MIC	16,000	24	1	2	8,000
Qi et al (2004)	bacterium	S. tiphymurium	MIC	**250**	24	1	2	125.00
Tsai et al (2002)	bacterium	S. dysenteriae	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	A. hydrophila	HONEC	500,000	48	1	1	500,000
Tsai et al (2002)	bacterium	A. hydrophila	HONEC	500,000	48	1	1	500,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	1,000,000	48	1	2	500,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	1,000,000	48	1	2	500,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	1,000,000	48	1	2	500,000
Tsai et al (2002)	bacterium	A. hydrophila	MIC	1,500,000	48	1	2	750,000
Tsai et al (2002)	bacterium	A. hydrophila	HONEC	2,000,000	48	1	1	2,000,000
Tsai et al (2002)	bacterium	S. dysenteriae	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	S. dysenteriae	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	S. dysenteriae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	S. dysenteriae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	S. dysenteriae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	S. dysenteriae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	V. cholerae	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	V. cholerae	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	V. cholerae	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	V. cholerae	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	V. cholerae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	V. cholerae	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	HONEC	150,000	48	1	1	150,000
Tsai et al (2002)	bacterium	V. parahaemolyticus	HONEC	150,000	48	1	1	150,000
No et al (2002)	bacterium	V. parahaemolyticus	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	V. parahaemolyticus	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	V. parahaemolyticus	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	V. parahaemolyticus	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	V. parahaemolyticus	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	V. parahaemolyticus	HONEC	1,000,000	72	1	1	1,000,000
Costa et al (2012)	bacterium	S. mutans	MIC	3,000,000	72	1	2	1,500,000
Costa et al (2012)	bacterium	S. mutans	MIC	5,000,000	72	1	2	2,500,000
Tsai et al (2002)	bacterium	S. aureus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	S. aureus	MIC	50,000	48	1	2	25,000
Tsai et al (2002)	bacterium	S. aureus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	S. aureus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	S. aureus	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	S. aureus	MIC	150,000	48	1	2	75,000
No et al (2002)	bacterium	S. aureus	MIC	1,000,000	48	1	2	500,000
No et al (2002)	bacterium	S. aureus	HONEC	1,000,000	48	1	1	1,000,000
No et al (2002)	bacterium	S. aureus	MIC	800,000	48	1	2	400,000
No et al (2002)	bacterium	S. aureus	MIC	800,000	48	1	2	400,000
No et al (2002)	bacterium	S. aureus	MIC	800,000	48	1	2	400,000
No et al (2002)	bacterium	S. aureus	HONEC	1,000,000	48	1	1	1,000,000
Fernandes et al (2008)	bacterium	S. aureus	MIC	1,900,000	24	1	2	950,000
Fernandes et al (2008)	bacterium	S. aureus	MIC	1,000,000	24	1	2	500,000
Fernandes et al (2008)	bacterium	S. aureus	MIC	1,000,000	24	1	2	500,000
Qi et al (2004)	bacterium	S. aureus	MIC	**250**	24	1	2	125.0
Qi et al (2004)	bacterium	S. aureus	MIC	**125**	24	1	2	62.5
Qi et al (2004)	bacterium	S. aureus	MIC	8,000	24	1	2	4,000
Sadeghi et al (2008)	bacterium	S. aureus	MIC	1,000,000	24	1	2	500,000

Table A1: Data for freshwater toxicity of chitosan (cont.)

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/L) (nano-sized)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentration/AF
Sadeghi et al (2008)	bacterium	S. aureus	MIC	**2,000,000**	24	1	2	1,000,000
No et al (2002)	bacterium	L. plantarum	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. plantarum	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. plantarum	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	L. plantarum	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	L. plantarum	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	L. plantarum	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	L. bulgaricus	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. bulgaricus	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. bulgaricus	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. bulgaricus	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. bulgaricus	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	L. bulgaricus	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	L. brevis	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. brevis	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	L. brevis	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. brevis	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	L. brevis	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. brevis	MIC	800,000	72	1	2	400,000
Tsai et al (2002)	fungi	C. albicans	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	fungi	C. albicans	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	fungi	C. albicans	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	fungi	C. albicans	MIC	800,000	48	1	2	400,000
Tsai et al (2002)	fungi	C. albicans	MIC	800,000	48	1	2	400,000
Tsai et al (2002)	fungi	C. albicans	MIC	800,000	48	1	2	400,000
Hu et al (2011)	fish	D. rerio	LOEC	**40,000**	96	10	2	2,000
Hu et al (2011)	fish	D. rerio	LOEC	**30,000**	96	10	2	1,500
Sigma-Aldrich (2016)	fish	O. mykiss	LC50	1,730	96	10	10	17.3
Wen et al (2010)	algae	C. vulgaris	EC27	1,000	168	1	10	100
Wen et al (2010)	algae	C. vulgaris	EC25	**1,000**	168	1	10	100
Wen et al (2010)	algae	S. obliquus	EC15	1,000	168	1	2	500
Wen et al (2010)	algae	S. obliquus	EC17	**1,000**	168	1	2	500
Rizzo et al (2008)	invertebrate	D. magna	EC40	500	24	10	10	5.00
Sigma-Aldrich (2016)	invertebrate	D. pulex	EC50	13,690	48	10	10	136.9

Table A2: Data for freshwater toxicity of HAP

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/L)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
Zhao et al (2013)	fish	zebrafish embryos	HONEC	300,000	80	10	1	30,000
Zhao et al (2013)	fish	zebrafish embryos	HONEC	300,000	80	10	1	30,000
Pujari-P. et al (2017)	fish	zebrafish embryos	EC50	100,000	120	10	10	1,000
Pujari-P. et al (2017)	fish	zebrafish embryos	EC50	100,000	72	10	10	1,000
Pujari-P. et al (2017)	fish	zebrafish embryos	EC50	100,000	72	10	10	1,000
Pujari-P. et al (2017)	fish	zebrafish embryos	EC50	40,000	24	10	10	400
Li et al (2010)	bacterium	E.coli	LC50	10,000,000	24	1	10	1,000,000
Baskar et al (2016)	bacterium	E.coli	MIC	500,000	24	1	2	250,000
Baskar et al (2016)	bacterium	P. aeruginosa	MIC	131,700	24	1	2	65,850
Baskar et al (2016)	bacterium	K. pneumoniae	MIC	292,800	24	1	2	146,400
Baskar et al (2016)	bacterium	S. typhi	MIC	370,700	24	1	2	185,350
Pereira et al (2017)	algae	P. subcapitata	IC50	340,000	72	1	10	34,000
Pereira et al (2017)	algae	P. subcapitata	IC50	350,000	72	1	10	35,000

Table A3: Data for freshwater toxicity of PAN

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/L)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
He et al 2016	bacterium	E.coli	EC50	3,000,000,000	24	1	10	300,000,000
Shi et al 2011	bacterium	E.coli	HONEC	32,500,000	18	10	1	3,250,000

Table A4: Data for soil toxicity of chitosan

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/kg)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	P. aeruginosa	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
Tsai et al (2002)	bacterium	P. aeruginosa	HONEC	200,000	48	1	1	200,000
No et al (2002)	bacterium	P. aeruginosa	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	P. aeruginosa	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	P. aeruginosa	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. aeruginosa	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. aeruginosa	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	P. aeruginosa	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	B. cereus	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	B. cereus	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	B. cereus	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	B. cereus	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	B. cereus	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	B. cereus	HONEC	1,000,000	72	1	1	1,000,000
Tsai et al (2002)	bacterium	B. cereus	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	B. cereus	MIC	200,000	48	1	2	100,000
Tsai et al (2002)	bacterium	B. cereus	MIC	1,000,000	48	1	2	500,000
Tsai et al (2002)	bacterium	B. cereus	MIC	500,000	48	1	2	250,000
Tsai et al (2002)	bacterium	B. cereus	MIC	1,000,000	48	1	2	500,000
Tsai et al (2002)	bacterium	B. cereus	MIC	1,000,000	48	1	2	500,000
No et al (2002)	bacterium	B. megaterium	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	B. megaterium	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	B. megaterium	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	B. megaterium	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	B. megaterium	MIC	500,000	72	1	2	250,000
No et al (2002)	bacterium	B. megaterium	MIC	800,000	72	1	2	400,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	100,000	48	1	2	50,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	150,000	48	1	2	75,000
Tsai et al (2002)	bacterium	L. monocytogenes	MIC	150,000	48	1	2	75,000
No et al (2002)	bacterium	L. monocytogenes	MIC	1,000,000	72	1	2	500,000
No et al (2002)	bacterium	L. monocytogenes	HONEC	1,000,000	72	1	1	1,000,000
No et al (2002)	bacterium	L. monocytogenes	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. monocytogenes	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. monocytogenes	MIC	800,000	72	1	2	400,000
No et al (2002)	bacterium	L. monocytogenes	MIC	1,000,000	72	1	2	500,000
Tsai et al (2002)	fungi	F. oxysporum	MIC	500,000	168	1	2	250,000
Tsai et al (2002)	fungi	F. oxysporum	MIC	500,000	168	1	2	250,000
Tsai et al (2002)	fungi	F. oxysporum	MIC	1,000,000	168	1	2	500,000
Tsai et al (2002)	fungi	F. oxysporum	MIC	500,000	168	1	2	250,000
Tsai et al (2002)	fungi	F. oxysporum	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	F. oxysporum	HONEC	2,000,000	168	1	1	2,000,000

Table A4: Data for soil toxicity of chitosan (cont.)

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/kg)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. fumigatus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000
Tsai et al (2002)	fungi	A. parasiticus	HONEC	2,000,000	168	1	1	2,000,000

Table A5: Data for soil toxicity of HAP

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/kg)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
Baskar et al (2016)	bacterium	K. pneumoniae	MIC	292,800	24	1	2	146,400

Table A6: Data for soil toxicity of PAN

Reference	Taxonomy	Test organisms	Ecotoxical endpoint	Concentration (µg/kg)	Exposure time (h)	AF-time	AF-NOEC	species sensitivity (µg/L) = concentratioNAF
Shi et al (2011)	bacterium	B. cereus	HONEC	32,500,000	18	10	1	7,552,000

Table A7: Summary of nanoparticle size and characterization in collected ecotoxicological data points

Nano Particle	*Particle Size (nm)*	*# data points*	*Morphology/Characterization*[1]	*Reference*
Chitosan	40	15	Agglomerated nanoparticles, shaped like snowflakes	(Qi et al., 2004)
Chitosan	54	2	Primary nanoparticles[2], perfect spherical shape	(Du et al., 2008)
Chitosan	200	1	Primary nanoparticles, round shape	(Hu et al., 2011)
Chitosan	235	1	Primary nanoparticles, round to oval in shape	(Sadeghi et al., 2008)
Chitosan	340	1	Primary nanoparticles, round shape	(Hu et al., 2011)
HAP	11	1	Agglomerated nanoparticles, rod-shaped	(Pereira et al., 2017)
HAP	14	5	Primary nanoparticles, spherical	(Baskar et al., 2017)
HAP	15	1	Primary nanoparticles, dots	(Pujari-Palmer et al., 2017)
HAP	19	1	Agglomerated nanoparticles, rod-shaped	(Pereira et al., 2017)
HAP	60	1	Primary nanoparticles, fibers	(Pujari-Palmer et al., 2017)
HAP	70	1	Primary nanoparticles, elongated spheroid	(Li et al., 2010)
HAP	75	1	Primary nanoparticles, sheets	(Pujari-Palmer et al., 2017)
HAP	150	1	Primary nanoparticles, rod-shaped	(Zhao et al., 2013)
HAP	200	1	Primary nanoparticles, long rods	(Pujari-Palmer et al., 2017)
HAP	230	1	Primary nanoparticles, needle-shaped	(Zhao et al., 2013)
PAN	221	1	La_2O_3 nanoparticle-doped PAN nanofibers[3]	(He et al., 2016)
PAN	400	2	Ag/PAN hybrid nanofibers[3]	(Shi et al., 2011)

[1] Forms and morphologies shown in TEM/AFM images
[2] The status of nanoparticles in the solution is dispersive
[3] Size distribution/particle characterization only available for certain forms of nanoparticles/nanofibers

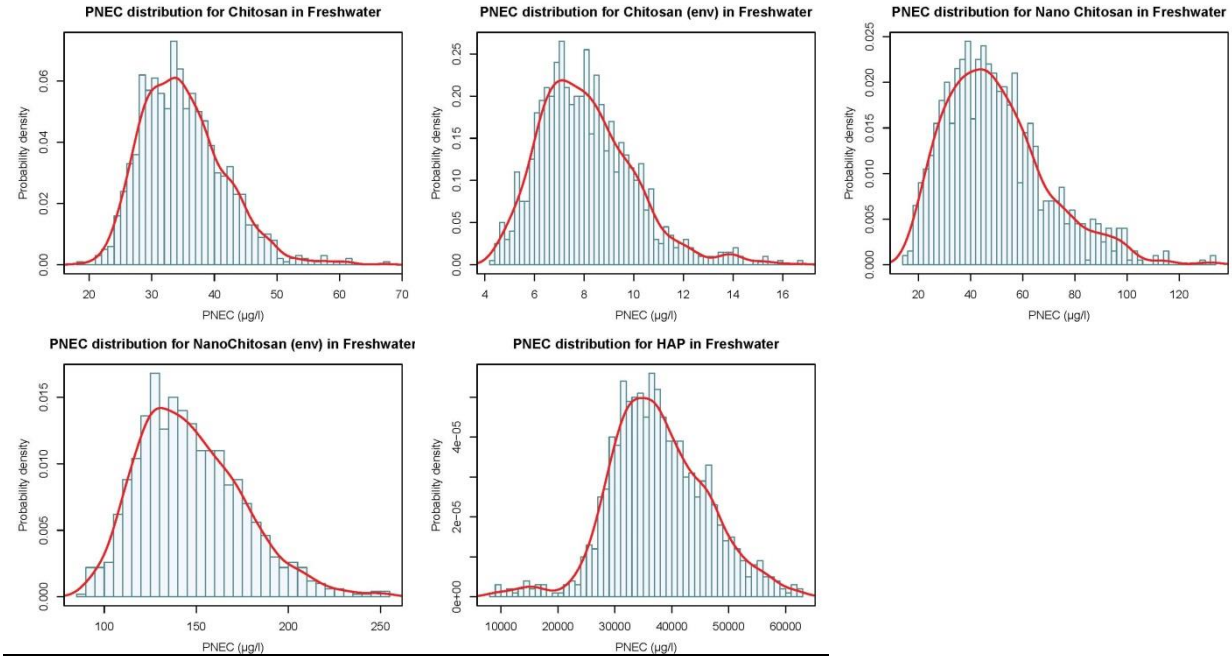

Figure A1: Predicted no-effect concentration (PNEC) distribution for chitosan, chitosan (env), nano chitosan, nano chitosan (env), HAP, and PAN in freshwater

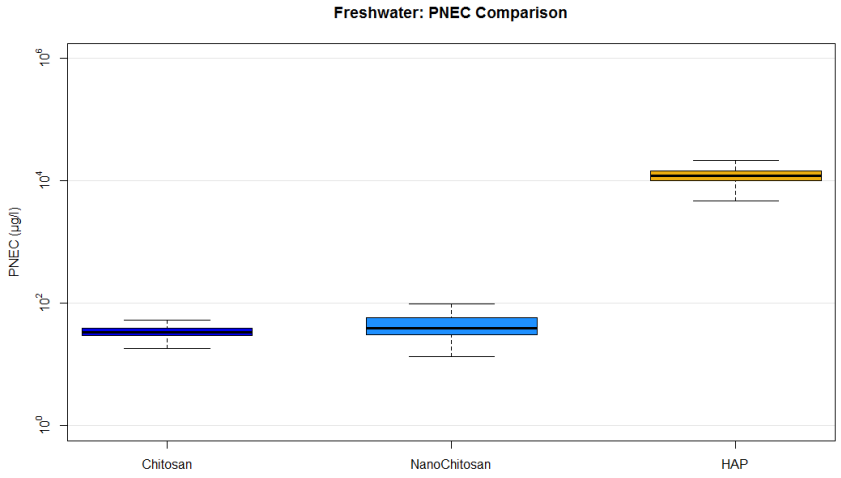

Figure A2: Comparison of PNEC distributions for chitosan, nano chitosan, PAN and HAP

Table A8: Predicted no-effect concentrations (PNECs) of studied nanobiomaterials and other pollutants in freshwater

Material	*PNEC (ng/L)*	*Category*	*Reference*
Chitosan	35,000	NanoBioMaterial	calculated
Chitosan (env)	8100	NanoBioMaterial	calculated
Nano Chitosan	47,000	NanoBioMaterial	calculated
Nano Chitosan (env)	150,000	NanoBioMaterial	calculated
HAP	$3.8 * 10^{7}$	NanoBioMaterial	calculated
PAN	$2.6 * 10^{11}$	NanoBioMaterial	calculated
CNT	55,600	ENMs	(Coll et al., 2016)
Nano-TiO_2	15,700	ENMs	(Coll et al., 2016)
Fullerenes	3840	ENMs	(Coll et al., 2016)
Nano-ZnO	1000	ENMs	(Coll et al., 2016)
Nano-Ag	17	ENMs	(Coll et al., 2016)
Aspirin	61,000	Pharmaceuticals	(Jones et al., 2002)
Ibuprofen	9060	Pharmaceuticals	(Jones et al., 2002)
Estrogen	1060	Hormones	(Stuer-Lauridsen et al., 2000)
Doxycycline	300	Antibiotics	(Kümmerer & Henninger, 2003)
Amoxicillin	100	Antibiotics	(Kümmerer & Henninger, 2003)
Atrazine	100,000	Pesticides/POPs	(Sangchan et al., 2014)
Cu	50,000	Heavy metal	(Wang et al., 2010)
Triclosan	1550	Antimicrobial agent	(Capdevielle et al., 2008)
Pb & Cd	1000	Heavy metal	(Wang et al., 2010)
DBP	740	Plasticizer	(Slobodnik et al., 2012)
Hg	50	Heavy metal	(Wang et al., 2010)
Dichlorvos	1.9	Pesticides/POPs	(Sangchan et al., 2014)

References Appendix

Baskar, K., Anusuya, T., & Devanand Venkatasubbu, G. (2017). Mechanistic investigation on microbial toxicity of nano hydroxyapatite on implant associated pathogens. *Materials Science and Engineering C*, *73*, 8–14. https://doi.org/10.1016/j.msec.2016.12.060

Capdevielle, M., Van Egmond, R., Whelan, M., Versteeg, D., Hofmann-Kamensky, M., Inauen, J., … Woltering, D. (2008). Consideration of exposure and species sensitivity of triclosan in the freshwater environment. *Integrated Environmental Assessment and Management*, *4*(1), 15–23. https://doi.org/10.1897/IEAM_2007-022.1

Coll, C., Notter, D., Gottschalk, F., Sun, T., Som, C., & Nowack, B. (2016). Probabilistic environmental risk assessment of five nanomaterials (nano-TiO2, nano-Ag, nano-ZnO, CNT, and fullerenes). *Nanotoxicology*, *10*(4), 436–444. https://doi.org/10.3109/17435390.2015.1073812

Costa, E. M., Silva, S., Pina, C., Tavaria, F. K., & Pintado, M. M. (2012). Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens. *Anaerobe*, *18*(3), 305–309. https://doi.org/10.1016/j.anaerobe.2012.04.009

Du, W. L., Xu, Y. L., Xu, Z. R., & Fan, C. L. (2008). Preparation, characterization and antibacterial properties against E. coli K88 of chitosan nanoparticle loaded copper ions. *Nanotechnology*, *19*(8), 0–5. https://doi.org/10.1088/0957-4484/19/8/085707

Fernandes, J. C., Tavaria, F. K., Soares, J. C., Ramos, Ó. S., João Monteiro, M., Pintado, M. E., & Xavier Malcata, F. (2008). Antimicrobial effects of chitosans and chitooligosaccharides, upon Staphylococcus aureus and Escherichia coli, in food model systems. *Food Microbiology*, *25*(7), 922–928. https://doi.org/10.1016/j.fm.2008.05.003

He, J., Wang, W., Shi, W., & Cui, F. (2016). La2O3nanoparticle/polyacrylonitrile nanofibers for bacterial inactivation based on phosphate control. *RSC Advances*, *6*(101), 99353–99360. https://doi.org/10.1039/c6ra22374e

Hu, Y.-L., Qi, W., Han, F., Shao, J.-Z., & Gao, J.-Q. (2011). Toxicity evaluation of biodegradable chitosan nanoparticles using a zebrafish embryo model. *International Journal of Nanomedicine*, *6*, 3351–3359. https://doi.org/10.2147/IJN.S25853

Jones, O. A. H., Voulvoulis, N., & Lester, J. N. (2002). Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water Research*, *36*(20), 5013–5022. https://doi.org/10.1016/S0043-1354(02)00227-0

Kümmerer, K., & Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clinical Microbiology and Infection*, *9*(12), 1203–1214. https://doi.org/10.1111/j.1469-0691.2003.00739.x

Li, Y., Ho, J., & Ooi, C. P. (2010). Antibacterial efficacy and cytotoxicity studies of copper (II) and titanium (IV) substituted hydroxyapatite nanoparticles. *Materials Science and Engineering C*, *30*(8), 1137–1144. https://doi.org/10.1016/j.msec.2010.06.011

No, H. ., Park, N. ., Lee, S. ., & Meyers, S. (2002). Antibacterial activity of chitosans and chitosanoligomers with different molecular weights. *Int. J.FoodMicrobiol*, *74*, 65–72.

Pereira, F. F., Paris, E. C., Bresolin, J. D., Foschini, M. M., Ferreira, M. D., & Corrêa, D. S. (2017). Investigation of nanotoxicological effects of nanostructured hydroxyapatite to microalgae Pseudokirchneriella subcapitata. *Ecotoxicology and Environmental Safety*, *144*(June), 138–147. https://doi.org/10.1016/j.ecoenv.2017.06.008

Pujari-Palmer, S., Lu, X., & Ott, M. K. (2017). The Influence of Hydroxyapatite Nanoparticle Morphology on Embryonic Development in a Zebrafish Exposure Model. *Nanomaterials*, *7*(4), 89. https://doi.org/10.3390/nano7040089

Qi, L., Xu, Z., Jiang, X., Hu, C., & Zou, X. (2004). Preparation and antibacterial activity of chitosan nanoparticles.

Carbohydrate Research, *339*(16), 2693–2700. https://doi.org/10.1016/j.carres.2004.09.007

Rizzo, L., Di Gennaro, A., Gallo, M., & Belgiorno, V. (2008). Coagulation/chlorination of surface water: A comparison between chitosan and metal salts. *Separation and Purification Technology*, *62*(1), 79–85. https://doi.org/10.1016/j.seppur.2007.12.020

Sadeghi, A. M. M., Dorkoosh, F. A., Avadi, M. R., Saadat, P., Rafiee-Tehrani, M., & Junginger, H. E. (2008). Preparation, characterization and antibacterial activities of chitosan, N-trimethyl chitosan (TMC) and N-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. *International Journal of Pharmaceutics*, *355*(1–2), 299–306. https://doi.org/10.1016/j.ijpharm.2007.11.052

Sangchan, W., Bannwarth, M., Ingwersen, J., Hugenschmidt, C., Schwadorf, K., Thavornyutikarn, P., … Streck, T. (2014). Monitoring and risk assessment of pesticides in a tropical river of an agricultural watershed in northern Thailand. *Environmental Monitoring and Assessment*, *186*(2), 1083–1099. https://doi.org/10.1007/s10661-013-3440-8

Shi, Q., Vitchuli, N., Nowak, J., Caldwell, J. M., Breidt, F., Bourham, M., … McCord, M. (2011). Durable antibacterial Ag/polyacrylonitrile (Ag/PAN) hybrid nanofibers prepared by atmospheric plasma treatment and electrospinning. *European Polymer Journal*, *47*(7), 1402–1409. https://doi.org/10.1016/j.eurpolymj.2011.04.002

Sigma-Aldrich. (2016). Safety Data Sheet, 1–7.

Slobodnik, J., Mrafkova, L., Carere, M., Ferrara, F., Pennelli, B., Schüürmann, G., & von der Ohe, P. C. (2012). Identification of river basin specific pollutants and derivation of environmental quality standards: A case study in the Slovak Republic. *TrAC - Trends in Analytical Chemistry*, *41*, 133–145. https://doi.org/10.1016/j.trac.2012.08.008

Stuer-Lauridsen, F., Birkved, M., Hansen, L. P., Holten Lützhøft, H.-C., & Halling-Sørensen, B. (2000). Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere*, *40*(7), 783–793. https://doi.org/10.1016/S0045-6535(99)00453-1

Tsai, G. J., Su, W. H., Chen, H. C., & Pan, C. L. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fisheries Science*, *68*(1), 170–177. https://doi.org/10.1046/j.1444-2906.2002.00404.x

Wang, J., Chen, S., & Xia, T. (2010). Environmental risk assessment of heavy metals in Bohai Sea, North China. *Procedia Environmental Sciences*, *2*, 1632–1642. https://doi.org/10.1016/j.proenv.2010.10.174

Zhao, X., Ong, K. J., Ede, J. D., Stafford, J. L., Ng, K. W., Goss, G. G., & Loo, S. C. J. (2013). Evaluating the toxicity of hydroxyapatite nanoparticles in catfish cells and zebrafish embryos. *Small*, *9*(9–10), 1734–1741. https://doi.org/10.1002/smll.201200639