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Originally published:

June 2021

Environmental Science: Nano 8(2021), 1934-1944

DOI: https://doi.org/10.1039/d1en00264c

ARTICLE

Dissolution-based uptake of CeO_2 nanoparticles by freshwater shrimp – A dual-radiolabelling study of the fate of anthropogenic cerium in water organisms

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Manufactured nanoparticles, such as CeO_2, give rise to novel risks when released into the environment. To assess these risks it is important to quantify the nanoparticle mass flows, as well as their speciation and the mechanisms of their transformation. We developed an innovative dual-radiolabelling strategy for CeO_2 nanoparticles using neutron activation and in-diffusion labelling to radiolabel CeO_2 nanoparticles with both Ce-141 and Ce-139. The different distribution of the radiolabels in the particles does not only allow easy dose determination in uptake studies but also enables us to track the uptake pathways of the anthropogenic cerium. By measuring the activity as well as the isotope ratio we tracked the uptake, transformation and excretion of CeO_2 nanoparticles in freshwater shrimp. We found that 99.99 % of the uptaken particles are excreted, leaving the gut with excrement. The remaining 0.01 % was internalized via a dissolution based pathway and accumulated in the hepatopancreas of the shrimp at a dose range of pg CeO_2 per shrimp. Most importantly, our results show that dissolution is not only coincidental but instrumental in the uptake of the cerium into the internal organs of the shrimp.

Introduction

The risks associated with manufactured nanoparticles (NPs) may greatly depend on the transformation of said particles. Chemical transformations, such as, redox reactions, surface passivation and dissolution, or physical transformations, such as homo and heteroaggregation, can have decisive influences on the behaviour of NPs in the environment.[1] One major transformation factor to be considered in risk assessment is the potential dissolution of the NPs. Fast dissolving NPs can be treated as dissolved species while persistent NPs can be treated as particles.[2,3] The scientific discussion on the exact definitions is still on-going (especially since other transformations can have a major impact on dissolution rates, for example in the case of sulfidation of Ag NPs)[4] but particles that are commonly considered fast-dissolving are e.g. Ag or ZnO, in contrast to TiO_2 which is considered persistent.[5,6] Slowly dissolving particles such as CeO_2 form a special case as transport and uptake can happen in particle form to deliver potentially toxic ions via a Trojan horse effect.[7,8] For CeO_2 in particular, there are conflicting results on the role of dissolution effects in uptake by organisms and the resulting toxicity.[9–11] Positive,[12,13] as well as, negative effects[14] have been reported for CeO_2 NP exposure to organisms. The main factor considered for potential toxic effects is the Ce^{3+}/Ce^{4+} ratio[15,16] of the particles and consequently leaching of ionic Ce^{3+} from the particles upon dissolution which may act as the toxic agent, mainly by inducing oxidative stress that can lead to cell death.[17–19] If, when, where, under which conditions and in what quantity CeO_2 NPs dissolve may be the main factor when it comes to judging their potential environmental effects.[1,20]

CeO_2 NPs today are already used or are widely researched for commercial applications such as fuel additives,[21] water remediation,[22] photo catalysts,[23] cosmetics[24] and surface coatings[25] and are measurable environmental contaminants in rain and river water at concentrations of about 1 and 100 ng/L, respectively.[26] One primary exposure pathway to the environment is the sedimentation of the particles from surface waters in the benthic zones of rivers and lakes where they can be taken up by water organisms that feed on the benthic periphyton.[27] This may result in trophic transfer along the food chain.[28]

Valuable mechanistic insights are typically gained by high exposure concentration experiments using XANES, EXAFS,

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Electronic Supplementary Information (ESI) available:
See DOI: 10.1039/x0xx00000x

and μXRF[29] that are often difficult to scale down to realistic environmental concentrations[30,31] while lower concentration distribution experiments give valuable insights in fate but the attribution of effects to specific molecular mechanisms, like dissolution inside organisms, becomes more fuzzy.[32,33] Both type of studies can only be realized with considerable experimental efforts in detection due to the complex media involved.[34] The radiolabelling of nanoparticles has the potential of providing an analysis technique for laboratory research that allows sensitive detection with unprecedented experimental ease[35–37] and has already proven to be a valuable tool for fate analysis of nanomaterials in organisms.[38–42] Employing smart radiolabelling strategies even has the potential of providing both, mechanistic insight at sub-acute toxicity concentrations.[10] Based on previous efforts we developed an isotopic dual-radiolabelling strategy to shed light on uptake pathways of anthropogenic cerium in organisms.[10,43,44] By employing two radiolabels we can develop a comprehensive picture of CeO_2 NP uptake in freshwater shrimp and show the importance of a dissolution-based uptake mechanism.

Experimental

Materials

CeO_2 NP reference material JRCNM02102a (formerly NM-212) was obtained from the JRC nanomaterial repository. A detailed characterisation report is available from the JRC.[45] In short, the primary particle size is around 16 nm,[46] with a hydrodynamic size of 221 nm and a zeta potential of around 42 mV when dispersed in DI water. The target lanthanum foil for the radionuclide production was procured from AlfaAesar. The fulvic acid used was isolated from bog water (Kleiner Kranichsee, Germany) following IHSS recommended procedures.[47] The acidities of the fulvic acid were measured by titration according to Stevensen[48] and elemental analysis was done using a Vario EL III element analyser (see ESI Tab. SI1 & SI2). All other chemicals were procured from Sigma Aldrich and used as is. Sera Shrimp Nature food pellets (protein: 44.0 %, fat: 8.9 %, fiber: 3.4 %, moisture: 5.6 %, ash: 11.3 %; additives: vit. A: 30 000 IU/kg, vit. D3: 1 500 IU/kg, vit. E (D, L-α-tocopheryl acetate): 60 mg/kg, vit. B1: 30 mg/kg, vit. B2: 90 mg/kg, stab. vit. C (L-ascorbyl monophosphate): 550 mg/kg) were obtained from a local aquarium shop.

Radiolabeling of CeO_2

Activation of CeO_2 30 mg of CeO_2 NPs were sealed in a quartz ampule and brought into the main channel of the TRIGA Mark II nuclear reactor at Jozef Stefan institute. They were irradiated with thermal neutrons at a neutron flux of $1 \cdot 10^{+13}$ for 63 h.[40] After a decay time of 29 d the ampule was broken and the [Ce-141]CeO_2 NPs taken up in DI water.

Production of Ce-139 radiotracer Using the in-house IBA Cyclone 18/9 cyclotron at HZDR Research Site Leipzig Ce-139 radiotracer was produced via 10.9 MeV proton irradiation of a lanthanum metal foil at a current of 22 μA for about 23 h yielding a current integral of 500 μAh.[49] The optimum proton energy, according to the reaction cross-section, was set by the design of the target (see ESI Fig. SI1 &SI2). The irradiated target was dissolved in conc. HNO_3 and the solution subsequently evaporated to dryness. The residue was dissolved again in 9 M HNO_3 and the produced cerium was oxidized by adding 0.05 M $Cr_2O_7^{2-}$. The separation of the Ce-139 from the lanthanum target material was achieved via extraction chromatography using UTEVA resin.[50] At 9 M HNO_3 the lanthanum is eluted while the Ce-139 remains on the column. The Ce-139 is eluted in a subsequent step with 0.1 M HNO_3. This was followed by another evaporation step. The Ce-139 was finally dissolved in 0.01 M HNO_3 to yield a non-carrier-added solution with 51.92 MBq/mL Ce-139.

Indiffusion labelling of CeO_2 In order to introduce Ce-139 into the activated [Ce-141]CeO_2 NPs, the radionuclide solution was added dropwise to the dry NP powder placed in a conic vial, which was placed into an aluminium heating block at 80°C (see ESI Fig. SI3). After each addition the solution was evaporated and a new drop was added. After a total of 4.7 MBq of Ce-139 was added the sample was tempered at 300°C for 7 h to facilitate diffusion of the Ce-139 into the surface. Afterwards, the [Ce-139/Ce-141]CeO_2 NPs were washed two times and taken up in DI water. NP properties were checked using a Malvern Zetasizer nano, a Perkin Elmer Lambda 365 UV/Vis spectrometer, a Jeol JSM IT200 SEM, and a conventional 200kV Jeol JEM-2100 TEM equipped with LaB6 electron source before and after the labelling steps.

Tab.1: Radiolabel half-lifes, γ-peaks, and abundancies.

Radioisotope	Half-life [d]	γ-peak [keV]
Ce-139	137.64	168.86 (80 %)
Ce-141	32.51	145.44 (48.4 %)

Dissolution experiments

Dissolution experiments were performed by placing 50 μg of radiolabelled [Ce-139/Ce-141]CeO_2 NPs on a 3 kDa centrifugal filter covering them with acidic leaching solution (1 mM HNO_3, pH 3). After a leaching step the samples were centrifuged and the Ce-139 and Ce-141 contents in the leachate were measured using gamma spectroscopy (AMETEK ORTEC: DSPEC-502, GEM-40190P, GammaVision) and the leaching solution was replaced. The dissolution rates were calculated based on the Ce-141 activity. Complementary control measurements using HR-ICP-MS (Thermo Fisher Scientific, ELEMENT XR) were performed on the initial batch of CeO_2 NPs and decayed [Ce-141]CeO_2 NP that underwent a non-radioactive in-diffusion labelling procedure.

Shrimps

Freshwater shrimp *Caridina pareparensis parvidentata* were obtained from a local aquarium supplier. Judging from the 1.8-2.3 cm size and slender built of the shrimp they were roughly around the same age, adult and all male.[51] Shrimp from the *Caridina* genus are widespread and abundant omnivorous freshwater shrimps (*Crustacea: Decapoda: Atyidae*) that feed on leaf litter, fine particulate organic matter, and periphyton in the benthic zones of rivers and lakes,[52,53] which are main sinks for manufactured nanoparticles from rain and river water.[54] They can play a major role in the diet of fish[55] and thus potentially act as one step in a food chain accumulation of manufactured nanoparticles.[56] They are known to react to environmental contaminants[57] and have been suggested as model organisms for toxicity testing.[58]

Uptake experiments

The shrimp were brought into the laboratory one week before the start of the experimental run to acclimate and were kept in aerated moderately hard standard freshwater (96 mg/L $NaHCO_3$, 60 mg/L $CaSO_4 \cdot 2\ H_2O$, 60 mg/L $MgSO_4$, 4 mg/L KCl, pH= 7.6) prepared according to EPA guideline EPA-821-R-02-013[59] and kept at 20°C under ambient light. The shrimp were not fed for 3 d before the experiments. In order to conduct the uptake experiments six shrimp in total were moved in pairs into 20 mL plastic vials filled with 10 mL of standard water (see ESI Fig. SI4). The vials were placed open onto an IKA Labortechnik HS250 basic horizontal shaker which moved the vials very slowly (30 rpm) in order to move the water surface and minimize risks of oxygen deficiency in the vials. For uptake, the radiolabelled CeO_2 NPs were dispersed in MilliQ water with 5 mg/L fulvic acid added for stabilization using a Bandelin Sonoplus HD3200 ultrasound homogenizer equipped with a BR30 resonance cup for 5 min at 90% energy input. This ensured a good dispersion (d_{Hydro} = 219 ± 3.8, ζ-pot. = -27.4 ± 1.5) and serves to facilitate uptake by mimicking the natural fine particulate organic matter diet.[28] Dispersion quality was checked using a Malvern Zetasizer nano. A waterstable, sinking Sera Shrimp Nature food pellet (84 mg) was then soaked in 50 µL of the dispersion for 10 min, absorbing it totally, before being added to the shrimp containers. Control experiments showed that >90% of the particles stay agglomerated with the food pellet and no dissolution of CeO_2 was measured in the moderately hard standard water (see ESI Fig. SI5 for more details on the food preparation). Our food preparation procedure thus simulates the built-up of an "eco-corona" by the CeO_2 NPs[60] and consequent heteroaggregation with larger organic matter particles and sedimentation into the benthic zone where shrimps feed. This provided a potential dose of about 59 ± 2 µg of CeO_2 per shrimp. The shrimps were left to consume the offered food for 16 h before being transferred to a fresh vial with fresh water. This was achieved by filtering and rinsing the shrimp over a plastic mesh with 100 µm mesh size. In the remainder of the experimental run the shrimp were fed roughly every week using about one third of a food pellet with no more CeO_2 NPs added. The shrimps were transferred into fresh water before any food addition and after the 16 h feeding steps. This yielded a 16 h step of combined feeding and excretion followed by on average 5 d of pure excretion for every preceding feeding step. In total the experimental run consisted of 30 d and six feeding events (including the initial CeO_2 NP containing exposure step). A detailed timeline of the experiment is shown in Fig. 2a. After every water transferal, the radionuclide content of the shrimp was determined by a measurement at a Perkin Elmer WIZARD 3" 1480 automatic gamma counter using a window of 125 – 155 keV for Ce-141 and 155 – 185 keV for Ce-139 according to their respective gamma peaks (see Tab. 1). Any overspill of the Ce-139 signal into the Ce-141 window was corrected using gamma spectroscopy data for calibration (see ESI Fig. SI6). Every measurement was then decay corrected to the same date for comparison. The remaining activity in the shrimp after the experiment was visualized using autoradiography. The sacrificed shrimp were placed on a BAS-MS phosphor storage screen for 3 d and the image was read out using an Amersham Typhoon biomolecular imager. Evaluation of the images was done using ImageJ.[61]

Results and discussion

Radiolabelling

Irradiation of CeO_2 NPs with neutrons at the JSI nuclear reactor triggered the nuclear reaction Ce-140(n,γ)Ce-141 to yield a Ce-141 activity concentration of 0.255 MBq/mg. In side reactions also Ce-143 and Ce-139 were formed at activity concentrations of 5.7 kBq/mg and 0.88 kBq/mg. The former was let decay with a half-life of 33 h while the latter was only formed two orders of magnitude less than the Ce-141, thus not interfering with the further labelling strategy (see ESI Fig. SI7). Ce-139 was produced at the HZDR cyclotron with a yield of 0.1 MBq/µAh. The radiochemical yield of the tracer separation was 96 %. The radiolabelling yield of the in-diffusion step was 99 % thereby successfully generating dual-labelled [Ce-139/Ce-141]CeO_2 NPs (see ESI Fig. SI8). The properties of the radiolabelled nanoparticles were not changed significantly from the original batch. Zeta potential in water and UV/Vis spectra suggest no major changes in Ce^{3+}/Ce^{4+} ratio[62,63] and DLS, SEM and TEM give no hint at major changes in particle sizes and morphology. Additionally no differences in dissolution rates of the original CeO_2 NPs and particles that underwent the labelling procedure are observed (Tab. 2 and ESI Fig SI9-12).

Tab. 2: Properties of used nanoparticles.

	CeO_2	[Ce-141]CeO_2	[Ce-139/Ce-141]CeO_2
DLS size [nm][a]	221 ± 4	222 ± 3	220 ± 4

Zeta Pot. [mV][a]	43.8 ± 1.2	43.2 ± 1.4	44.4 ± 0.9
Bandgap [eV][b]	3.2	3.1	3.3
Specific activity [MBq/mg(CeO_2)]	-	0.255	0.272 Ce-139 0.107 Ce-141
Dissolution rate [ng/mg·d] [c]	54.1 ± 2.8	n.d	52.6 ± 4.7

[a] measured at 100 mg/L in DI water

[b] obtained from Tauc plot of UV/Vis data[62] (see ESI Fig. SI9) measured in DI water at 4 mg/mL

[c] in 1 mM HNO_3 determined by ICP MS after 5 d of dissolution

Dissolution of CeO_2 NPs

A controlled dissolution study in 1 mM HNO_3 solution (pH 3) reveals that the two different radiolabels are distributed differently in the particles. Upon dissolution we see a large release of Ce-139 indicating a localisation of the in-diffused radionuclide close to the particle surface. It was previously shown that about 50% of the Ce-139 is located within the first atomic layer.[10] In contrast, the Ce-141 can be expected to be equally distributed within the particles yielding a much smaller release rate in the first stages of dissolution than the Ce-139. This leads to a very characteristic development of the Ce-139/Ce-141 ratio that can be detected in the leachate (Fig. 1).

Therefore, we can use the specific gamma peaks of the two radioisotopes (Tab. 1) to track the uptake of the CeO_2 NPs including dissolution processes. The Ce-141 signal can be directly translated into the CeO_2 mass using the specific activity (Tab. 2) while the Ce-139/Ce-141 ratio allows us inference of NP transformation along the uptake pathway. A growth of the ratio during the experiments indicates that dissolution of the CeO_2 NPs played a significant role on the uptake pathway.

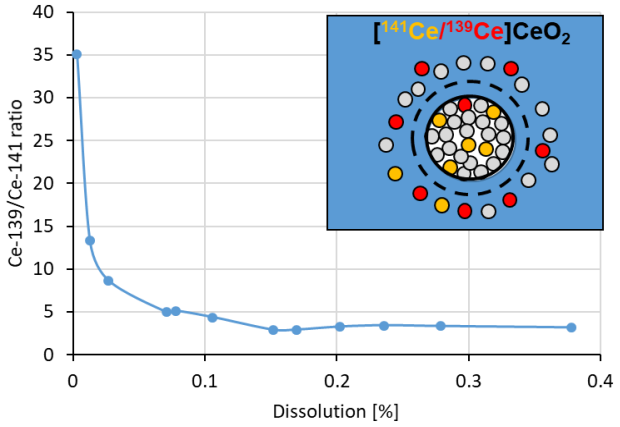

Fig. 1: Development of Ce-139/Ce-141 ratio in leachate of dissolution experiments, in dependence of dissolution percentage, as established by Ce-141 gamma spectroscopy measurement.

Uptake and Excretion of CeO_2 by shrimp

The value of the Ce-141 activity measurements can be directly translated into the taken up amount of CeO_2 NPs using the specific activity. This allows us to easily establish unambiguous doses. The offered amount of CeO_2 was 59 ± 2 µg per shrimp. After the 16 h feeding step only 4% of this amount remained in the shrimp yielding a dose of 2.5 ± 0.1 µg per shrimp (see Tab. 3, Fig. 2a and Fig. SI13 for detailed doses). Note that this does not equate to the total amount of CeO_2 NPs consumed since part of the taken up CeO_2 already got excreted within this period. Nevertheless we set this as the 100% reference point for any measurements of the remaining cerium discussed below. Although no standardized toxicity tests were performed we can report that no obvious signs of acute toxicity could be observed. All the shrimps remained alive during the experiment and no diminished mobility and reactions were observed. Food intake was not refused during the experiment and moulting events of two shrimps during the experimental run hint at largely normal life functions. However, chronic toxicity markers such as moulting frequency, growth performance, reproduction rates and food utilization indices were not measured.

Tab. 3: CeO_2 NP doses in our experimental run as calculated using the Ce-141 activity.

Dose	Offered dose per shrimp	Content per shrimp	Content per dry weight	Content per wet weight [a]
Start of experiment[b]	59 ± 2 µg	2.5 ± 0.1 µg	0.41 ± 0.07 µg/mg	0.081 ± 0.003 µg/mg
End of experiment	-	380 ± 173 pg	62.0 ± 28.2 pg/mg	12.4 ± 5.6 pg/mg

[a] estimated considering a dry weight of 20%[64]

[b] refers to day 0 for offered dose, and day 1 for shrimp CeO_2 content (see Fig 2.a)

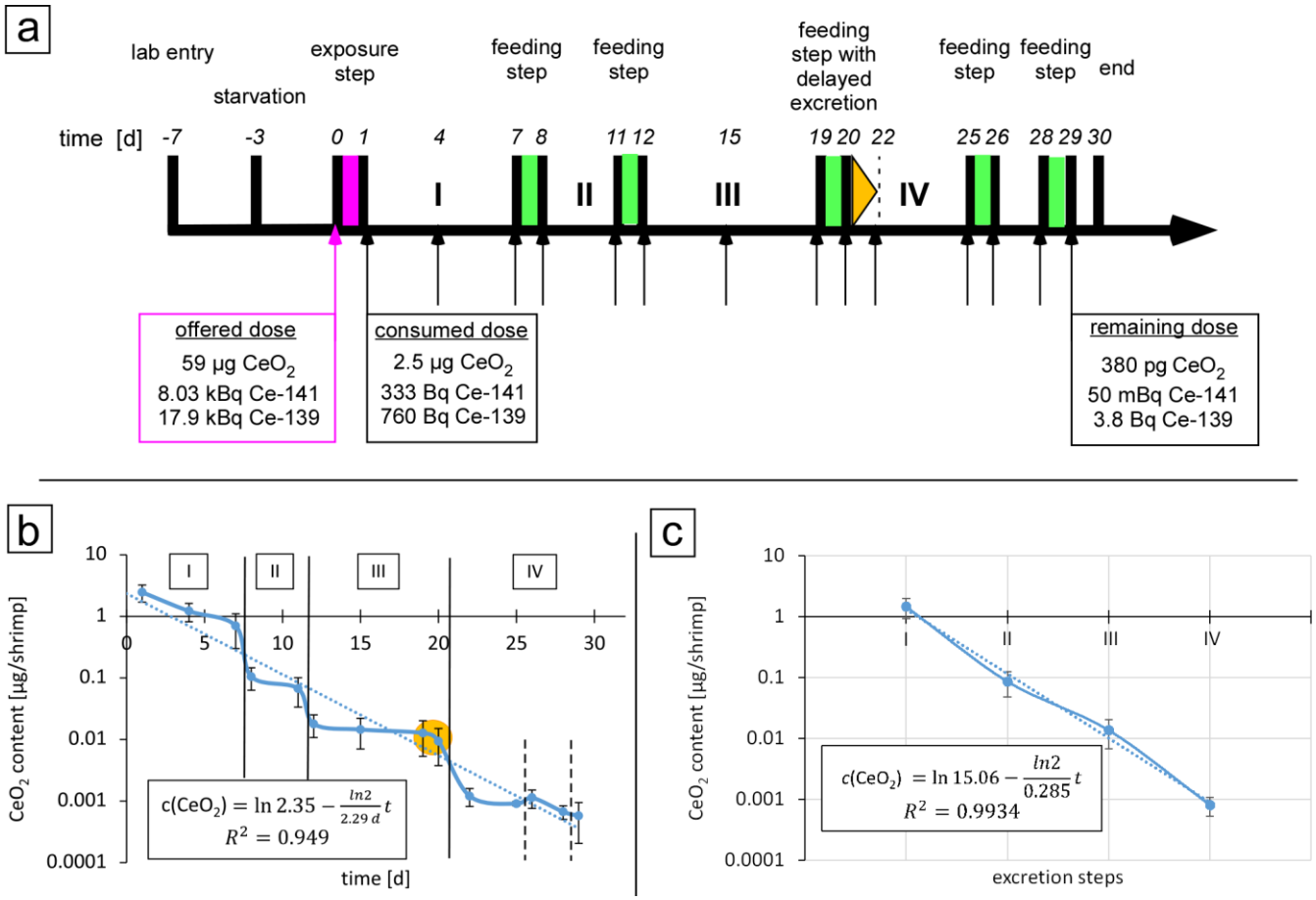

Fig. 2: (a) Experimental run of CeO_2 NP uptake experiment from lab entry to the end of the experimental run over four excretion periods (I-IV). The day of exposure is set as the zero point. The overnight exposure step with CeO_2 NP is indicated in pink, any subsequent feeding steps without CeO_2 are indicated in green. One moulting related delayed excretion is indicated in orange. Any activity measurements taken are indicated by arrows. For selected measurements the CeO_2 content, Ce-141 activity, and Ce-139 activity per shrimp are given (see ESI for more detailed data). (b) Excretion of CeO_2 NPs from shrimp with time. Two feeding steps and one delayed excretion event (solid lines) divide the experiment into four excretion periods (I-IV). The moulting occurrence is indicated in orange. Additionally, two feeding events are indicated in excretion period four (dashed lines) with no further excretion happening. (c) Excretion of CeO_2 NPs from shrimp in dependence of excretion periods I-IV.

The excretion of CeO_2 from the shrimp follows an exponential decay curve with a half-life of 2.3 d (see Fig. 2b). At the end of the 30 d experimental run only 380 ± 173 pg CeO_2 remain per shrimp, i.e. 99.985 ± 0.007 % of the initially taken up amount was eliminated from the shrimp with only 0.015 ± 0.007 % remaining.

The excretion generally follows a scheme consisting of a larger excretion in the hours right after feeding and a much smaller, if any, excretion in the remaining time till the shrimp were fed again. This is consistent with an elimination of the CeO_2 NPs from the tract with the remains of digested food which happens over a period of several hours after feeding.[65] Consequently, the first two feeding events can be clearly identified as steps in the activity diagrams. The third feeding shows a delayed effect due to two moulting events during which feeding and excretion was halted.[66] This occurrence allowed us to exclude CeO_2 NP sorption to the shrimp surface or cerium accumulation in the exoskeleton as a major contribution to our activity signal.

If we combine the measured values between feeding events into four excretion periods with one average value each, the exponential elimination curve of the CeO_2 content becomes even clearer (see Fig. 2c). The half-life of the CeO_2 NPs is about 0.3 excretion steps so that within one excretion step already about 91% of the taken up CeO_2 NPs leave the organism. If we extrapolate the excretion function to the zero point we can estimate that the consumed amount of CeO_2 NPs is about 15 µg per shrimp equating to 25 % of the offered dose. Taking into account that the bulk of the excretion takes place already during the 16 h feeding step we can estimate a biological half-life of about 5 h, which is in line with previous studies of the digestive kinetics of similar shrimp which report peak excretion around 5-8 h after feeding but continuing up to 20 h.[65] In nature, under constant grazing, digestion and excretion conditions, the elimination of up taken particles may even be faster than observed in our experiment, however with strong variations between different species and depending on the specifics of the food consumed.[67] However, the retention of 9 % of the particles after each excretion step may cause a slow

accumulation in a scenario of constant influx of new particles.[68]

The fourth excretion period identified above is actually comprised of two feeding events which however do not lead to a further reduction of CeO_2 content. Thus, overall, we can summarize the uptake and excretion of CeO_2 found in our experiment as a roughly 20 d elimination period during which the excretion follows an exponential decay function followed by an apparent stop of any further cerium elimination with about 0.1 ‰ remaining at the end of our experiment.

Fate of CeO_2 in shrimp

Our dual radiolabelling approach allows us to gain much further insight into the uptake and retention mechanisms. A closer look at the elimination curves of the different cerium nuclides reveals differences between the Ce-141 and Ce-139 elimination from the shrimp (see Fig. 3). We can see that both nuclides follow the same trend for the first three excretion periods. In stark contrast to that, no further excretion of Ce-139 is seen between the third and fourth excretion period with the Ce-139 activity remaining stable over the rest of the experiment, despite two further feeding events during period four. At the end of our experiment about 0.15 ‰ of the initially taken up Ce-141 remains within the shrimp in contrast to 0.5 % for the Ce-139. This is reflected accordingly in the Ce-139/Ce-141 ratio which remains stable during the first three excretion periods but jumps an order of magnitude with the beginning of the fourth excretion period.

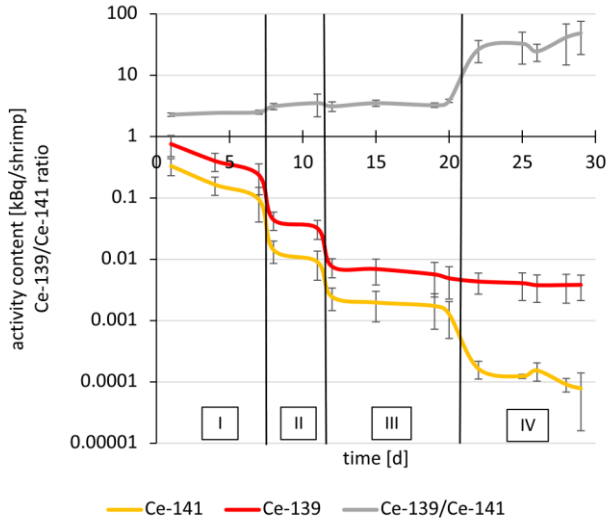

Fig. 3: Excretion of radionuclide activity of Ce-141 and Ce-139 and development of Ce-139/Ce-141 ratio inside the shrimp over four excretion periods I-IV.

As such a change in Ce-139/Ce-141 ratio is a result of a preferential dissolution of the surface near Ce-139 radiolabel in contrast to the volume based Ce-141 radiolabel, this leads us to conclude that between the third and fourth excretion period the vast majority of nanoparticles are eliminated from the organism via the feed stream while the remaining cerium took a dissolution-based uptake pathway into the organism and cannot be readily removed. This translates into a dissolution of about 0.15 ‰ of the CeO_2 within the digestive tract of the shrimp. This dissolution may be mediated mainly by the shrimp gut microbiome and digestive agents. For example, lactate producing bacteria have been found an important part of *caridina* gut microbiomes[69] and lactate has been shown to be a strong dissolving agent for CeO_2 NPs.[70] But also other food components may have an effect on the dissolution rate in the digestive tract of the shrimp. Most importantly the presence of complexing and reducing agents, e.g. vitamine C, promoting dissolution and, in contrast, phosphates or sulfides which inhibit dissolution by surface passivation and precipitation of Ce^{3+} species.[71–75] Our observations indicate that the gut microbiome may be the first point of action with regard to the bactericidal effects of dissolved Ce^{3+} [16] as it reacts sensitively to environmental contaminants.[76] While acutely toxic effects towards the shrimp seem to be unlikely at low cerium concentrations sub-lethal chronic exposure of the gut microbiome may affect food utilization indices and growth performance in the long run.[77] Consequently, the composition of the gut microbiome may be an important controlling factor for either the level of dissolution and/or subsequent chronic toxic effects.

So far all the measurements described have been non-destructively performed on the live shrimps. The radiolabelling allowed us to do a detailed kinetic excretion study with minimal experimental effort and animal sacrifice (in a conventional study for each data point several animals would have to be sacrificed, increasing the overall need of test animals in the first place). The very low radiation levels at the end of the experiment – far below any legal free limit - would also have allowed to take the shrimp out of the laboratory to live happily ever after in an aquarium.

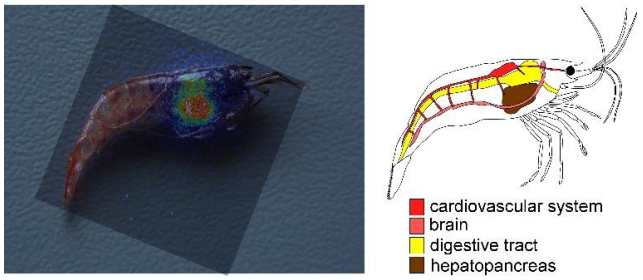

Fig. 4: Overlay of autoradiogram of activity distribution with photo of shrimp (left) and scheme of shrimp anatomy (right).

However, further insight can be gained upon sacrificing the shrimp at the end of the experiment. Autoradiographic analysis of the distribution of the activity in the shrimp reveal a consistent pattern for all the shrimp. The activity is primarily located in the lower cephalothorax of the shrimp (see Fig. 4). A cross-referencing with general shrimp anatomy[78,79] allows us to identify the hepatopancreas as the main accumulator of the remaining cerium. The hepatopancreas is the main digestive gland of crustaceans.

While there are strong variations on where metal contaminants may end up in crustaceans depending on the metal and uptake pathway,[80–82] the hepatopancreas is considered to be the major invertebrate organ that sequesters and detoxifies dietary or water-borne metals.[79,83] It is here were the enzymatic digestion beginning in the stomach continues and absorption of fluid nutrients occurs that are pressed out from the solids in the stomach.[67,79] Larger inert particles (> 100 nm) are typically barred from entering the hepatopancreas by the pyloric filters which are rather uniform in all decapods, and are transported through the mid- and hindgut for excretion although some digesta may stay in the hindgut only being voided when a subsequent meal is ingested.[79,84] This seems to be largely the fate of the CeO_2 NPs which while having a primary particle size of about 16 nm form agglomerates with hydrodynamic diameters of 220 nm when stabilized in aqueous media and due to our experimental procedure are heteroaggregated with the solid food components. Should they enter the hepatopancreas our results indicate that they are subsequently also largely removed from it, only leaving their dissolved remnants behind. Any dissolved cerium absorbed in the midgut will reach the hepatopancreas via the blood stream for sequestration.[83] It is difficult to estimate what the cerium accumulation in the hepatopancreas may entail, as there is conflicting data on the effects of CeO_2 NPs on water organisms.[9,12,14] However, any effect will be heavily dependent on the actual cerium species present and their concentrations. Our experiments suggest an exposure of the gut microbiome primarily to CeO_2 nanoparticles while the internal organs may be mainly exposed to dissolved cerium species or secondary precipitates. Literature data on dissolution-based uptake of CeO_2 into organisms suggest that depending on the chemical environment the cerium may be present mainly as carboxylates, $Ce(PO_4)_3$ precipitates or reformed CeO_2 NPs.[85,86] So the overall effect of CeO_2 on shrimp can be twofold, an alteration of the gut microbiome upon chronic exposure to CeO_2 NPs and dissolved cerium species, which can influence the shrimp's susceptibility to disease[77,87,88] and a damaging of hepatopancreatic function due to chronically increased oxidative stress due to cerium accumulation in the form of dissolved Ce^{3+} species or secondary precipitates.[89,90] A triple burden of gut microbiome-caused disease/parasite susceptibility, chronic hepatopancreatic oxidative stress in combination with other common bacterial disease stressors, such as acute hepatopancreatic necrosis disease could also have wider implications for shrimp farming endevours.[91] However, the stop of excretion of the cerium that took a dissolution-based pathway into the organism found in our experiment may not be the final outcome, as biological half-times for other metals in shrimp are known to be longer than the duration of our experiment (≈ 80 d for Cs from Baltic prawn)[92]. The general picture of a selective uptake of dissolved cerium into the body with an almost total elimination of the original particulate CeO_2 from the gut via the excrements may also be altered in the case of smaller particles. For example, studies indicate that CeO_2 NPs < 10 nm are able to translocate over the gut wall in freshwater worm.[93] However, to what extent a dissolution-based pathway may be responsible for the observed translocation was not addressed. Consequently, whether an accumulation of CeO_2 NPs in the gut and/or internal organs and/or dissolved species and secondary precipitates in the internal organs occurs will greatly depend on the influx of new CeO_2 NPs and their specific properties. These may be heavily controlled by usage patterns of CeO_2 NPs,[94] their release pathway[95] and their transformation on their way into[96] and in the environment[97] and weather effects controlling the influx patterns.[98] In interactions with the individual gut microbiomes this produces a complex picture of potential CeO_2 NP ecotoxicity. Reliable dose data as well as knowledge on the relevant uptake pathways is needed to shed light into these complex patterns and to allow a comprehensive understanding of CeO_2 NP uptake into organisms and an assessment of the consequences and risks thereof.

Conclusions

We developed an innovative dual-radiolabelling strategy for CeO_2 NPs that let us perform detailed kinetic uptake and excretion studies with easily established dose measurements. Using our dual-labelled [Ce-139/Ce-141]CeO_2 we could establish a biological half-life of CeO_2 NPs in freshwater shrimp on the hour scale as the particles are excreted with the feed stream. 0.15 ‰ of the particles took a dissolution-based pathway to translocate over the gut wall with the cerium getting accumulated in the *hepatopancreas* of the shrimp and not being excreted during our 30 d experiment. Our data shows that the dissolution is not only coincidental but instrumental in the uptake of CeO_2 into the shrimp, i.e. it was a prerequisite for cerium uptake into the internal organs of the shrimp under our experimental conditions. At constant CeO_2 influx accumulation effects could happen, with the gut microbiome being exposed primarily to CeO_2 NPs while the internal organs, most prominently the *hepatopancreas*, are mainly exposed to dissolved cerium and secondary precipitates. Resulting alterations in the gut-microbiome and chronical hepatopancreatic oxidative stress may lead to long-term consequences below acute toxicity.

Author Contributions

SS: conceptualization, supervision, investigation, formal analysis, visualization, resources, funding acquisition, project administration, writing – original draft, review and editing; IR: investigation, formal analysis, writing – review and editing; SU: investigation, formal analysis, writing – review and editing; SD: investigation, formal analysis, writing – review and editing; RP: resources, methodology, writing – review and editing; TR: resources, methodology, writing – review and editing; AM: investigation, resources, methodology; AL: resources,

methodology, funding acquisition, project administration, writing – review and editing; KF: investigation, formal analysis, resources, methodology, writing – review and editing; MS: funding acquisition, investigation, methodology, project administration, resources, writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We acknowledge financial support by the Helmholtz-Association, grant PIE-0007 (CROSSING). Also, funding by the Slovenian Research Agency research programme P2-0075 is acknowledged.

Notes and references

[1] G.V. Lowry, K.B. Gregory, S.C. Apte and J.R. Lead, Transformations of Nanomaterials in the Environment, *Environ. Sci. Technol.,* 2012, **46**, 6893.

[2] J.H. Arts, M. Hadi, M.A. Irfan, A.M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U.G. Sauer, D. Warheit, K. Wiench, W. Wohlleben and R. Landsiedel, A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping)., *Regul Toxicol Pharmacol,* 2015, **71**, S1.

[3] N.E. Landvik, V. Skaug, B. Mohr, J. Verbeek and S. Zienolddiny, Criteria for grouping of manufactured nanomaterials to facilitate hazard and risk assessment, a systematic review of expert opinions., *Regul Toxicol Pharmacol,* 2018, **95**, 270.

[4] C. Levard, E.M. Hotze, B.P. Colman, A.L. Dale, L. Truong, X.Y. Yang, A.J. Bone, G.E. Brown, R.L. Tanguay, R.T. Di Giulio, E.S. Bernhardt, J.N. Meyer, M.R. Wiesner and G.V. Lowry, Sulfidation of silver nanoparticles: natural antidote to their toxicity., *Environ Sci Technol,* 2013, **47**, 13440.

[5] V. Kononenko, D.B. Warheit and D. Drobne, Grouping of Poorly Soluble Low (Cyto)Toxic Particles: Example with 15 Selected Nanoparticles and A549 Human Lung Cells., *Nanomaterials (Basel),* 2019, **9**,

[6] W.J.G.M. Peijnenburg, E. Ruggiero, M. Boyles, F. Murphy, V. Stone, D.A. Elam, K. Werle and W. Wohlleben, A Method to Assess the Relevance of Nanomaterial Dissolution During Reactivity Testing., *Materials (Basel),* 2020, **13**,

[7] R. Ortega, C. Bresson, C. Darolles, C. Gautier, S. Roudeau, L. Perrin, M. Janin, M. Floriani, V. Aloin, A. Carmona and V. Malard, Low-solubility particles and a Trojan-horse type mechanism of toxicity: the case of cobalt oxide on human lung cells., *Part Fibre Toxicol,* 2014, **11**, 14.

[8] I.L. Hsiao, Y.K. Hsieh, C.F. Wang, I.C. Chen and Y.J. Huang, Trojan-horse mechanism in the cellular uptake of silver nanoparticles verified by direct intra- and extracellular silver speciation analysis., *Environ Sci Technol,* 2015, **49**, 3813.

[9] M. Sendra, P.M. Yeste, I. Moreno-Garrido, J.M. Gatica and J. Blasco, CeO2 NPs, toxic or protective to phytoplankton? Charge of nanoparticles and cell wall as factors which cause changes in cell complexity, *Sci Total Environ,* 2017, **590**, 304.

[10] S. Schymura, T. Fricke, H. Hildebrand and K. Franke, Elucidating the Role of Dissolution in CeO2 Nanoparticle Plant Uptake by Smart Radiolabelling, *Angew Chem Int Ed Engl,* 2017, **56**, 7411.

[11] P. Zhang, Y. Ma, S. Liu, G. Wang, J. Zhang, X. He, J. Zhang, Y. Rui and Z. Zhang, Phytotoxicity, uptake and transformation of nano-CeO2 in sand cultured romaine lettuce, *Environ Pollut,* 2017, **220**, 1400.

[12] F. Qin, T. Shen, H. Yang, J. Qian, D. Zou, J. Li, H. Liu, Y. Zhang and X. Song, Dietary nano cerium oxide promotes growth, relieves ammonia nitrogen stress, and improves immunity in crab (Eriocheir sinensis), *Fish & Shellfish Immunology,* 2019, **92**, 367.

[13] I. Celardo, M. De Nicola, C. Mandoli, J.Z. Pedersen, E. Traversa and L. Ghibelli, Ce^{3}+ ions determine redox-dependent anti-apoptotic effect of cerium oxide nanoparticles., *ACS Nano,* 2011, **5**, 4537.

[14] E. Artells, J. Issartel, M. Auffan, D. Borschneck, A. Thill, M. Tella, L. Brousset, J. Rose, J.Y. Bottero and A. Thiéry, Exposure to cerium dioxide nanoparticles differently affect swimming performance and survival in two daphnid species., *PLoS One,* 2013, **8**, e71260.

[15] G. Pulido-Reyes, I. Rodea-Palomares, S. Das, T.S. Sakthivel, F. Leganes, R. Rosal, S. Seal and F. Fernández-Piñas, Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states., *Sci Rep,* 2015, **5**, 15613.

[16] K.M. Kumar, M. Mahendhiran, M.C. Diaz, N. Hernandez-Como, A. Hernandez-Eligio, G. Torres-Torres, S. Godavarthi and L.M. Gomez, Green synthesis of Ce 3+ rich CeO2 nanoparticles and its antimicrobial studies, *Materials Letters,* 2018, **214**, 15.

[17] G. Pagano, M. Guida, A. Siciliano, R. Oral, F. Koçbaş, A. Palumbo, I. Castellano, O. Migliaccio, P.J. Thomas and M. Trifuoggi, Comparative toxicities of selected rare earth elements: Sea urchin embryogenesis and fertilization damage with redox and cytogenetic effects., *Environ Res,* 2016, **147**, 453.

[18] Y. Ma, J. Wang, C. Peng, Y. Ding, X. He, P. Zhang, N. Li, T. Lan, D. Wang, Z. Zhang, F. Sun, H. Liao and Z. Zhang, Toxicity of cerium and thorium on Daphnia magna., *Ecotoxicol Environ Saf,* 2016, **134P1**, 226.

[19] P. Zhang, Y. Ma, Z. Zhang, X. He, Y. Li, J. Zhang, L. Zheng and Y. Zhao, Species-specific toxicity of ceria nanoparticles to Lactuca plants., *Nanotoxicology,* 2015, **9**, 1.

[20] N.B. Turan, H.S.E. Erkan, GO and M.S. Bilgil, Nanoparticles in the aquatic environment: Usage, properties, transformation and toxicity—A review, *Process Safety and Environmental Protection,* 2019, **130**, 238.

[21] M. Auffan, M. Tella, W. Liu, A. Pariat, M. Cabié, D. Borschneck, B. Angeletti, G. Landrot, C. Mouneyrac, L. Giambérini and J. Rose, Structural and physical–chemical behavior of a CeO2 nanoparticle based diesel additive during combustion and environmental release, *Environ. Sci.: Nano,* 2017, **4**, 1974.

[22] R. Fiorenza, S.A. Balsamo, L. D'urso, S. Sciré, M.V. Brundo, R. Pecoraro, E.M. Scalisi, V. Privitera and G. Impellizzeri, CeO2 for Water Remediation: Comparison of Various Advanced Oxidation Processes, *Catalysts,* 2020, **10**, 446.

[23] S.B. Khan, M. Faisal, M.M. Rahman and A. Jamal, Exploration of CeO_2 nanoparticles as a chemi-sensor and photo-catalyst for environmental applications., *Sci Total Environ,* 2011, **409**, 2987.

[24] A. Zenerino, T. Boutard, C. Bignon, S. Amigoni, D. Josse, T. Devers and F. Guittard, New CeO2 nanoparticles-based topical formulations for the skin protection against organophosphates, *Toxicol Rep,* 2015, **2**, 1007.

[25] Y. Lu, S. Xiao, R. Gao, J. Li and Q. Sun, Improved weathering performance and wettability of wood protected by CeO2 coating deposited onto the surface, *Holzforschung,* 2014, **68**, 345.

[26] I. Jreije, A. Azimzada, M. Hadioui and K.J. Wilkinson, Measurement of CeO2 Nanoparticles in Natural Waters Using a High Sensitivity, Single Particle ICP-MS, *Molecules,* 2020, **25**,

[27] N.K. Geitner, J.L. Cooper, A. Avellan, B.T. Castellon, B.G. Perrotta, N. Bossa, M. Simonin, S.M. Anderson, S. Inoue, M.F. Hochella, C.J. Richardson, E.S. Bernhardt, G.V. Lowry, P.L. Ferguson, C.W. Matson, R.S. King, J.M. Unrine, M.R. Wiesner and H. Hsu-Kim, Size-Based Differential Transport, Uptake, and Mass Distribution of Ceria (CeO) Nanoparticles in Wetland Mesocosms, *Environ. Sci. Technol.,* 2018, **52**, 9768.

[28] J.R. Conway, S.K. Hanna, H.S. Lenihan and A.A. Keller, Effects and implications of trophic transfer and accumulation of CeO2 nanoparticles in a marine mussel., *Environ Sci Technol,* 2014, **48**, 1517.

[29] J. Lv, P. Christie and S. Zhang, Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges, *Environ. Sci.: Nano,* 2019, **6**, 41.

[30] H.F. Krug, Nanosafety research--are we on the right track, *Angew Chem Int Ed Engl,* 2014, **53**, 12304.

[31] B. Collin, M. Auffan, A.C. Johnson, I. Kaur, A.A. Keller, A. Lazareva, J.R. Lead, X. Ma, R.C. Merrifield, C. Svendsen, J.C. White and J.M. Unrine, Environmental release, fate and ecotoxicological effects of manufactured ceria nanomaterials, *Environ. Sci.: Nano,* 2014, **1**, 533.

[32] A. Bour, F. Mouchet, S. Cadarsi, J. Silvestre, E. Chauvet, J.-M. Bonzom, C. Pagnout, H. Clivot, L. Gauthier and E. Pinelli, Impact of CeO nanoparticles on the functions of freshwater ecosystems: a microcosm study, *Environ. Sci.: Nano,* 2016, **3**, 830.

[33] A. Bour, F. Mouchet, S. Cadarsi, J. Silvestre, L. Verneuil, D. Baqué, E. Chauvet, J.-M. Bonzom, C. Pagnout, H. Clivot, I. Fourquaux, M. Tella, M. Auffan, L. Gauthier and E. Pinelli, Toxicity of CeO nanoparticles on a freshwater experimental trophic chain: A study in environmentally relevant conditions through the use of mesocosms, *Nanotoxicology,* 2016, 1.

[34] H. Hildebrand, S. Schymura, K. Franke and C. Fischer, Analysis of studies and research projects regarding the detection of nanomaterials in different environmental compartments and deduction of need for action regarding method development, *UBA Texte,* 2019, **133**,

[35] A.R. Deline and J.A. Nason, Evaluation of labeling methods used for investigating the environmental behavior and toxicity of metal oxide nanoparticles, *Environ. Sci.: Nano,* 2019, **6**, 1043.

[36] Y. Yin, Z. Tan, L. Hu, S. Yu, J. Liu and G. Jiang, Isotope Tracers To Study the Environmental Fate and Bioaccumulation of Metal-Containing Engineered Nanoparticles: Techniques and Applications, *Chem. Rev.,* 2017, **117**, 4462.

[37] N. Gibson, U. Holzwarth, K. Abbas, F. Simonelli, J. Kozempel, I. Cydzik, G. Cotogno, A. Bulgheroni, D. Gilliland, J. Ponti, F. Franchini, P. Marmorato, H. Stamm, W. Kreyling, A. Wenk, M. Semmler-Behnke, S. Buono, L. Maciocco and N. Burgio, Radiolabelling of engineered nanoparticles for in vitro and in vivo tracing applications using cyclotron accelerators., *Arch Toxicol,* 2011, **85**, 751.

[38] W.G. Kreyling, U. Holzwarth, N. Haberl, J. Kozempel, A. Wenk, S. Hirn, C. Schleh, M. Schäffler, J. Lipka, M. Semmler-Behnke and N. Gibson, Quantitative biokinetics of titanium dioxide nanoparticles after intratracheal instillation in rats: Part 3, *Nanotoxicology,* 2017, **11**, 454.

[39] V. Lobaz, R. Konefał, J. Pánek, M. Vlk, J. Kozempel, M. Petřík, Z. Novy, S. Gurská, P. Znojek, P. Štěpánek and M. Hrubý, In Situ In Vivo radiolabeling of polymer-coated hydroxyapatite nanoparticles to track their biodistribution in mice., *Colloids Surf B Biointerfaces,* 2019, **179**, 143.

[40] D.H. Oughton, T. Hertel-Aas, E. Pellicer, E. Mendoza and E. Jautris Joner, Neutron Activation of Engineered Nanoparticles as a Tool for Tacing Their Environmental Fate and Uptake in Organisms, *Environ Tox Chem,* 2008, **27**, 1883.

[41] M. Kleiven, B.O. Rosseland, H.C. Teien, E.J. Joner and D. Helen Oughton, Route of exposure has a major impact on uptake of silver nanoparticles in Atlantic salmon (Salmo salar)., *Environ Toxicol Chem,* 2018, **37**, 2895.

[42] M. Al-Sid-cheikh, C. Rouleau, D. Bussolaro, C.A. Oliveira Ribeiro and E. Pelletier, Tissue Distribution of Radiolabeled Ag Nanoparticles in Fish: Arctic Charr, *Environ. Sci. Technol.,* 2019, **53**, 12043.

[43] F. Simonelli, P. Marmorato, K. Abbas, J. Ponti, J. Kozempel, U. Holzwarth, F. Franchini and F. Rossi, Cyclotron production of radioactive CeO(2) nanoparticles and their application for in vitro uptake studies., *IEEE Trans Nanobioscience,* 2011, **10**, 44.

[44] H. Hildebrand, S. Schymura, U. Holzwarth, N. Gibson, M. Dalmiglio and K. Franke, Strategies for radiolabeling of commercial TiO2 nanopowder as a tool for sensitive nanoparticle detection in complex matrices, *J Nanopart Res,* 2015, **17**,

[45] Singh C., S. Friedrichs, G. Ceccone, N. Gibson, K. Alstrup Jensen, M. Levin, H. Goenaga Infante, D. Carlander and K. Rasmussen *Cerium Dioxide, NM-211, NM-212, NM-213. Characterisation and test item preparation*. European Commision, Luxembourg, 2014.

[46] P. Rosenkranz, M.L. Fernández-Cruz, E. Conde, M.B. Ramírez-Fernández, J.C. Flores, M. Fernández and J.M. Navas, Effects of cerium oxide nanoparticles to fish and mammalian cell lines: An assessment of cytotoxicity and methodology., *Toxicol In Vitro,* 2012, **26**, 888.

[47] E.M. Thurman and R.L. Malcolm, Preparative Isolation of Aquatic Humic Substances, *Environ Sci Technol,* 1981, **15**, 463.

[48] Stevenson F.J. *Humus chemistry: genesis, composition, reactions*. John Wiley and Sons, Inc, New York, 1994.

[49] C. Vermeulen, G.F. Steyn, F.M. Nortier, F. Szelecsényi, Z. Kovács and S.M. Qaim, Production of 139Ce by proton-induced reactions on 141Pr and natLa, *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* 2007, **255**, 331.

[50] E.P. Horwitz, M.L. Dietz, R. Chiarizia, H. Diamond, A.M. Essling and D. Graczyk, Separation and preconcentration of uranium from acidic media by extraction chromatography, *Anal Chim Acta,* 1992, **266**, 25.

[51] Y. Cai and P.K.L. Ng, The freshwater shrimps of the genera Caridina and Parisia from karst caves of Sulawesi Selatan, Indonesia, with descriptions of three new species (Crustacea: Decapoda: Caridea: Atyidae), *Journal of Natural History,* 2009, **43**, 1093.

[52] R.S.W. Yam and D. Dudgeon, Stable isotope investigation of food use by Caridina spp. (Decapoda:Atyidae) in Hong Kong streams, *Journal of the North American Benthological Society,* 2005, **24**, 68.

[53] D. Dwiyanto, A. Farajallah and A. Annawaty, Notes on the distribution of, an endemic freshwater shrimp from Sulawesi, Indonesia, *BIO Web Conf.,* 2020, **19**, 00001.

[54] M. Tella, M. Auffan, L. Brousset, E. Morel, O. Proux, C. Chanéac, B. Angeletti, C. Pailles, E. Artells, C. Santaella, J. Rose, A. Thiéry and J.-Y. Bottero, Chronic dosing of a simulated pond ecosystem in indoor aquatic mesocosms: fate and transport of CeO nanoparticles, *Environ. Sci.: Nano,* 2015, **2**, 653.

[55] Y.L. Budeba and I.G. Cowx, The role of the freshwater shrimp (Roux) in the diet of the major commercial fish species in Lake Victoria, Tanzania, *Aquatic Ecosystem Health & Management,* 2007, **10**, 368.

[56] Y. Dai, Z. Wang, L. Zhang, Z. Jiang, S. Pu, Q. Fan, J. Zhao and B. Xing, Transfer and transformation of CeO NPs along a terrestrial trophic food chain, *Environ. Sci.: Nano,* 2020, **7**, 588.

[57] P.K. Mensah, W.J. Muller and C.G. Palmer, Using growth measures in the freshwater shrimp Caridina nilotica as biomarkers of Roundup® pollution of South African freshwater systems, *Physics and Chemistry of the Earth, Parts A/B/C,* 2012, **50-52**, 262.

[58] D. Sucahyo, N.M. Van Straalen, A. Krave and C.A. Van Gestel, Acute toxicity of pesticides to the tropical freshwater shrimp Caridina laevis., *Ecotoxicol Environ Saf,* 2008, **69**, 421.

[59] *EPA-821-R-02-013: Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Environmental Protection Agency, 2002.

[60] F. Nasser, J. Constantinou and I. Lynch, Nanomaterials in the Environment Acquire an "Eco–Corona" Impacting their Toxicity to —a Call for Updating Toxicity Testing Policies, *Proteomics,* 2020, **20**, 1800412.

[61] C.A. Schneider, W.S. Rasband and K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis., *Nat Methods,* 2012, **9**, 671.

[62] S.V.N.T. Kuchibhatla, A.S. Karakoti, D.R. Baer, S. Samudrala, M.H. Engelhard, J.E. Amonette, S. Thevuthasan and S. Seal, Influence of Aging and Environment on Nanoparticle Chemistry: Implication to Confinement Effects in Nanoceria, *J. Phys. Chem. C,* 2012, **116**, 14108.

[63] X. Wu, C.W. Neil, D. Kim, H. Jung and Y.-S. Jun, Co-effects of UV/H O and natural organic matter on the surface chemistry of cerium oxide nanoparticles, *Environ. Sci.: Nano,* 2018, **5**, 2382.

[64] P.T. Akonor, H. Ofori, N.T. Dziedzoave and N.K. Kortei, Drying Characteristics and Physical and Nutritional Properties of Shrimp Meat as Affected by Different Traditional Drying Techniques., *Int J Food Sci,* 2016, **2016**, 7879097.

[65] W. Dall, The functional anatomy of the digestive tract of a shrimp Metapenaeus bennettae Racek & Dall (Crustacea : Decapoda : Penaeidae), *Aust. J. Zool.,* 1967, **15**, 699.

[66] R.N. Lipcius and W.F. Herrnkind, Molt cycle alterations in behavior, feeding and diel rhythms of a decapod crustacean, the spiny lobster Panulirus argus, *Mar. Biol.,* 1982, **68**, 241.

[67] I.J. Mcgaw and D.L. Curtis, A review of gastric processing in decapod crustaceans, *J Comp Physiol B,* 2013, **183**, 443.

[68] X. Zhao, M. Yu, D. Xu, A. Liu, X. Hou, F. Hao, Y. Long, Q. Zhou and G. Jiang, Distribution, Bioaccumulation, Trophic Transfer, and Influences of CeO2 Nanoparticles in a Constructed Aquatic Food Web, *Environ Sci Technol,* 2017, **51**, 5205.

[69] Y. Zhao, C. Duan, X. Zhang, H. Chen, H. Ren, Y. Yin and L. Ye, Insights into the Gut Microbiota of Freshwater Shrimp and Its Associations with the Surrounding Microbiota and Environmental Factors, *Journal of Microbiology and Biotechnology,* 2018, **28**, 946.

[70] E.A. Grulke, M.J. Beck, R.A. Yokel, J.M. Unrine, U.M. Graham and M.L. Hancock, Surface-controlled dissolution rates: a case study of nanoceria in carboxylic acid solutions., *Environ Sci Nano,* 2019, **6**, 1478.

[71] J.T. Dahle, K. Livi and Y. Arai, Effects of pH and phosphate on CeO2 nanoparticle dissolution., *Chemosphere,* 2015, **119**, 1365.

[72] T.V. Plakhova, A.Y. Romanchuk, S.N. Yakunin, T. Dumas, S. Demir, S. Wang, S.G. Minasian, D.K. Shuh, T. Tyliszczak, A.A. Shiryaev, A.V. Egorov, V.K. Ivanov and S.N. Kalmykov, Solubility of Nanocrystalline Cerium Dioxide: Experimental Data and Thermodynamic Modeling, *J. Phys. Chem. C,* 2016, **120**, 22615.

[73] I. Römer, S.M. Briffa, Y. Arroyo Rojas Dasilva, D. Hapiuk, V. Trouillet, R.E. Palmer and E. Valsami-Jones, Impact of particle size, oxidation state and capping agent of different cerium dioxide nanoparticles on the phosphate-induced transformations at different pH and concentration., *PLoS One,* 2019, **14**, e0217483.

[74] F. Schwabe, R. Schulin, P. Rupper, A. Rotzetter, W. Stark and B. Nowack, Dissolution and transformation of cerium oxide nanoparticles in plant growth media, *J Nanopart Res,* 2014, **16**, 377.

[75] G. You, Y. Xu, P. Wang, C. Wang, J. Chen, J. Hou, L. Miao, Y. Gao and Y. Li, Deciphering the effects of CeO2 nanoparticles on Escherichia coli in the presence of ferrous and sulfide ions: Physicochemical transformation-induced toxicity and detoxification mechanisms, *Journal of Hazardous Materials,* 2021, **413**, 125300.

[76] L. Evariste, M. Barret, A. Mottier, F. Mouchet, L. Gauthier and E. Pinelli, Gut microbiota of aquatic organisms: A key endpoint for ecotoxicological studies., *Environ Pollut,* 2019, **248**, 989.

[77] C.C. Holt, D. Bass, G.D. Stentiford and M. Van Der Giezen, Understanding the role of the shrimp gut microbiome in health and disease., *J Invertebr Pathol,* 2020, 107387.

[78] B. Felgenhauer, Internal Anatomy of the Decapoda: An Overview, *Microscopic Anatomy of Invertebrates,* 1992, **10**, 45.

[79] G. Vogt, Functional cytology of the hepatopancreas of decapod crustaceans., *J Morphol,* 2019, **280**, 1405.

[80] M. Canli and R.W. Furness, Toxicity of heavy metals dissolved in sea water and influences of sex and size on metal accumulation and tissue distribution in the norway lobster Nephrops norvegicus, *Marine Environmental Research,* 1993, **36**, 217.

[81] T. Cresswell, S.L. Simpson, D. Mazumder, P.D. Callaghan and A.P. Nguyen, Bioaccumulation Kinetics and Organ Distribution of Cadmium and Zinc in the Freshwater Decapod Crustacean, *Environ. Sci. Technol.,* 2015, **49**, 1182.

[82] A. Kouba, M. Buřič and P. Kozák, Bioaccumulation and Effects of Heavy Metals in Crayfish: A Review, *Water Air Soil Pollut,* 2010, **211**, 5.

[83] G.A. Ahearn, P.K. Mandal and A. Mandal, Mechanisms of heavy-metal sequestration and detoxification in crustaceans: a review, *J Comp Physiol B,* 2004, **174**,

[84] S.P. Hopkin and J.A. Nott, Studies on the digestive cycle of the shore crab (L.) with special reference to the b cells in the hepatopancreas, *J. Mar. Biol. Ass.,* 1980, **60**, 891.

[85] F. Schwabe, S. Tanner, R. Schulin, A. Rotzetter, W. Stark, A. Von Quadt and B. Nowack, Dissolved cerium contributes to uptake of Ce in the presence of differently sized CeO2-nanoparticles by three crop plants., *Metallomics,* 2015, **7**, 466.
[86] P. Zhang, Y. Ma, C. Xie, Z. Guo, X. He, E. Valsami-Jones, I. Lynch, W. Luo, L. Zheng and Z. Zhang, Plant species-dependent transformation and translocation of ceria nanoparticles, *Environ. Sci.: Nano,* 2019, **6**, 60.
[87] T.T.G. Nguyen, T.C. Nguyen, M. Leelakriangsak, T.T. Pham, Q.H. Pham, C. Lueangthuwapranit and V.D. Nguyen, Promotion of Lactobacillus plantarum on growth and resistance against acute hepatopancreatic necrosis disease pathogens in white-leg shrimp (Litopenaeus vannamei), *Thai J Vet Med,* 2018, **48**, 19.
[88] J. Xiong, Progress in the gut microbiota in exploring shrimp disease pathogenesis and incidence, *Appl Microbiol Biotechnol,* 2018, **102**, 7343.
[89] S.Y. Han, M.Q. Wang, B.J. Wang, M. Liu, K.Y. Jiang and L. Wang, A comparative study on oxidative stress response in the hepatopancreas and midgut of the white shrimp Litopenaeus vannamei under gradual changes to low or high pH environment., *Fish & Shellfish Immunol,* 2018, **76**, 27.
[90] K. Wei and J. Yang, Oxidative damage of hepatopancreas induced by pollution depresses humoral immunity response in the freshwater crayfish Procambarus clarkii, *Fish & Shellfish Immunology,* 2015, **43**, 510.
[91] K.F.J. Tang and M.G. Bondad-Reantaso, Impacts of acute hepatopancreatic necrosis disease on commercial shrimp aquaculture, *Rev. Sci. Tech. OIE,* 2019, **38**, 477.
[92] N. Sezer, M. Belivermiş, Ö. Kılıç, S. Topcuoğlu and Y. Çotuk, Biokinetics of radiocesium in shrimp (Palaemon adspersus): seawater and food exposures., *J Environ Radioact,* 2014, **132**, 15.
[93] R.K. Cross, C.R. Tyler and T.S. Galloway, The fate of cerium oxide nanoparticles in sediments and their routes of uptake in a freshwater worm., *Nanotoxicology,* 2019, **13**, 894.
[94] F. Gottschalk, T. Sun and B. Nowack, Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies, *Environmental Pollution,* 2013, **181**, 287.
[95] T. Lange, P. Schneider, S. Schymura and K. Franke, The Fate of Anthropogenic Nanoparticles, nTiO2 and nCeO2, in Waste Water Treatment, *Water,* 2020, **12**, 2509.
[96] A. Gogos, J. Wielinski, A. Voegelin, H. Emerich and R. Kaegi, Transformation of cerium dioxide nanoparticles during sewage sludge incineration, *Environ. Sci.: Nano,* 2019, **6**, 1765.
[97] C. Gagnon, B. A, T. P, P. M and G. F, Fate of Cerium Oxide Nanoparticles in Natural Waters and Immunotoxicity in Exposed Rainbow Trout, *J Nanomed Nanotechnol,* 2018, **09**,
[98] J.H. Kang, Y.S. Lee, S.J. Ki, Y.G. Lee, S.M. Cha, K.H. Cho and J.H. Kim, Characteristics of wet and dry weather heavy metal discharges in the Yeongsan Watershed, Korea., *Sci Total Environ,* 2009, **407**, 3482.