Environmental
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Environmental significance

Our understanding of the effect of ultraviolet radiation (UV) on the breakdown of nanoplastic in nature is negligible. While the effects on larger plastics have been studied there is, to our knowledge, a current gap when it comes to nanoplastic (<100 nm) breakdown and how this UV mediated breakdown may affect the material and the potential toxicity of the material. We show how UV exposure affects polystyrene nanoparticles, resulting in particle breakdown and a reduced toxicity of an amine-modified particle that is toxic towards the freshwater zooplankter *Daphnia magna* in its pristine form. We also show that a complex toxic mixture of small molecules is released during the breakdown process. This study highlights potential outcomes of UV mediated nanoplastic breakdown.

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UV-B degradation affects nanoplastic toxicity and leads to release of small toxic substances

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Abstract

Fragmented micro- and nanoplastics are widespread pollutants with adverse effects on the environment. However, the breakdown process does not end with micro- and nanoplastics but is expected to continue until carbon dioxide has been formed. During this process the plastics will undergo chemical changes and small molecules may be released. We have broken down small amine-modified (∅53 nm) and carboxyl-modified (∅62 nm) polystyrene nanoparticles by UV-B irradiation during 100 days. We see a decreasing size and an oxidation of the nanoparticles over time. Simultaneously, the acute toxicity to zooplankton *Daphnia magna* decreases. The UV-B irradiation releases small, dissolved molecules that are toxic to *Daphnia magna*. The dissolved molecules include aminated alkyls, styrene remnants and

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secondary circularization products. The study show that UV-B radiation can change the original toxicity of nanoplastics and release new toxic substances.

Introduction

Micro- and nanoplastic particles are today ubiquitous and present in all compartments of the natural environment where they can be retained and taken up by organisms and induce ecotoxicological issues, both direct toxicological effect at species level and through cascading effects [1-3]. Nanoplastics are defined as plastic pieces that have been degraded in nature to a size below 100 nm [4] or 1 µm [5]. Nanoplastics are, due to their small size, difficult to isolate in nature but were first demonstrated in the Atlantic in 2017 [6]. Studies confirm the abundance of nanoplastics and suggest there is up to 0.5 mg/L nanoplastics in Swedish lakes close to cities [7]. The size distribution between micro- and nanoplastics has recently been measured in mussels with the interesting result that the mass (187 ng/mg) of the nanoplastic fraction (20-200nm) is similar to the mass (216 ng/mg) of the microplastic fraction (≥2.2 µm) [8]. In a comparison based on particle number or surface area, nanoplastics will dominate and may therefore have a greater impact on biological effects. For example, small carboxylated polystyrene nanoparticles inhibit plasma coagulation by binding to the active site in Factor12a int the coagulation cascade, whereas larger particles activate coagulation by providing a surface for the activation complex[9].

Polystyrene is the 6th most consumed plastics [10], but none or only small amounts of polystyrene nanoplastics were found in Swedish lakes [7]. However, in mussels, the ratio followed the expected pattern for polystyrene nanoplastics [8]. Polystyrene nanoplastics have also been found in soil [11]. The release of polystyrene nanoplastics after ultraviolet (UV) irradiation of polystyrene coffee cup lids was shown in 2016 [12], and from plastic sheets and boxes in 2023 [13, 14]. The release of micro- and nanoplastics from commercial micro-sized polystyrene beads after UV irradiation has been demonstrated [15, 16]. Furthermore, the release of nanoplastics from expanded polystyrene foam and coffee cup lids after being subjected to mechanical force was shown in 2019 [17] and, in 2021, from expanded polystyrene after mild mechanical forces mimicking the mechanical friction in the ocean [18]. The nanoplastics formed from the mechanical degradation were chemically transformed, as compared to the starting material, and a higher level of oxygen was found in the formed nanoparticles [17, 18]. Furthermore, styrene oligomers have been detected worldwide in sand and seawater at mean concentrations of 3679 ± 8199.2 ng/g and 5.1 ± 6.4 ng/L, respectively [19]. In the Pacific Ocean, the mean concentration of styrene oligomers has been measured to 1.48 µg/L and 1.32 µg/L in surface water and deep water, respectively [20]. The styrene oligomers are expected to originate from polystyrene indicating that the presence of styrene oligomers is a result of the degradation occurring in nature.

Many possible intermediate breakdown products can originate from polystyrene subjected to UV irradiation during the degradation process, but continued UV irradiation ultimately results in carbon dioxide [21]. The mechanisms behind UV mediated degradation of polystyrene have been studied for decades and are reviewed in for example Yousif and Haddard, 2013 [22], and later from a micro-/nanoplastics in the environment perspective by Andrady, et al., 2022 [23]. High intensity UV irradiation of carboxyl-modified polystyrene beads, 0.1 to 2.5 μm continued the degradation processes until the polystyrene was totally broken down to mostly carbon dioxide and other volatile compounds [24]. Volatile compounds such as benzene, toluene, styrene and phenol have been detected after UV irradiation [25]. In addition, cyclization products were found after long-term irradiation of polystyrene films [26]. Different additives and stabilizers will also affect the resulting degradation products [21, 22]. In conclusion, it seems likely that there is a complex mixture of various compounds released during the UV-mediated degradation of polystyrene.

An interesting aspect of micro- and especially nanoplastics is that the penetration depth of the UV radiation is much larger than the particle diameter [27], which also affects the energy absorption of the polystyrene. An effect of this may have been seen in the degradation of carboxyl-modified polystyrene microbeads as they were reported to start to degrade from the inside first and that decreasing diameter of the particles came later in the degradation process [24], whereas other studies have only reported a shrinking diameter [15, 28], suggesting that the particles are degraded from the outside. The detection of only surface oxidation following short-term irradiation of radiolabeled 230 nm polystyrene nanoparticles indicates that surface degradation is occurring. [29]. Higher UV intensity, longer irradiation time, and smaller size of the microbeads accelerated the release of dissolved organic matter [28]. The contents in the media, as e.g., dissolved organic matter [30], also affect the UV mediated degradation. The impact of UV irradiation on microplastics behaviour and effects on the environment has been reviewed (see e.g., Cheng et al. 2021[31]), and include effects on migration, release of additives, interactions with pollutants, and toxicity.

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Although there is a rapidly growing number of articles describing the UV mediated degradation of microplastics, particles with an original size smaller than 100 nm have, to our knowledge, not been studied. Furthermore, the link between toxicity and UV mediated degradation needs to be examined in more detail to predict potential environmental effects related to the degradation process. Small positively charged, amine-modified polystyrene nanoparticles are a good starting material to study. These particles have previously been shown to be acutely toxic to the freshwater zooplankton *Daphnia magna* [32]. However, in the same study it was shown that larger polystyrene particles, with similar or different surface modifications, were not acutely toxic [32]. Similar results were shown for amine-modified polystyrene nanoparticles ranging between 20 and 100 nm, where all particles were toxic but 20 and 40 nm exhibited stronger toxicity[33, 34]. A size dependent toxicity seems to be a general feature among plastic nanoparticles as reviewed by Pikuda et al, 2023 [35]. We have here studied the physical degradation of 53 nm amine-modified polystyrene nanoparticles and compared the degradation with 62 nm carboxyl-modified polystyrene nanoparticles. We have further followed the acute toxicity which was shown to decrease for the 53 nm particles after UV irradiation. However, also a dissolved fraction from the degradation products was shown to be toxic to *D. magna*.

Materials and methods

Model particles and UV-B lamp Two different polystyrene particles were bought from Bangs Laboratories Inc., USA. Particle 1 had a mean diameter of 53 nm and amine-modified surface (catalogue code: PA02N, Lot nr.: 15045). Particle 2 had a mean diameter of 62 nm and carboxyl-modified surface (catalogue code: PC02N, Lot nr.: 11652). Both particles were delivered in a colloidal suspension with a concentration of ≈10% solids (w/w) according to the manufacturer.

To remove additives, the particle solution was transferred to a dialyzation tube, (MilliPore MWCO 3000), and thoroughly dialyzed against MilliQ H_2O before further use.

The UV-B lamps, ExoTerra Reptile UV-B 200, 25W, were purchased from a local pet shop. The lamps emitted wavelength spectra, taken with an air-coupled Avantes Mini, Czerny-Turner Spectrometer with 2048 pixels, a 10 μm slit with a 1 ms integration time, can be found in Supplementary Information Figure S1.

UV-B treatment of particles The dialyzed 53 nm amine-modified particles were diluted 40x with MilliQ H_2O, to a final concentration of $\approx$ 0.25 % (w/w) or $\approx$ 2.5 mg/mL. 80 ml of the solution was transferred to Petri dishes, either made of quartz or glass, and placed in a fridge. Three UV-B lamps were fitted in a cardboard box to shield the surrounding environment from UV radiation, see Figure S2, so the lamps tips were 10 cm over the Petri dishes. When the three lamps were on the temperature in the cardboard box, inside the fridge on maximum cooling, was stable at 21 ± 1 °C.

The 62 nm carboxyl-modified particles were treated the same way except that they were diluted 2000x with MilliQ H_2O, to a final concentration of $\approx$ 0.005 % (w/w) or $\approx$ 0.05 mg/mL.

The quartz petri dish was chosen as the material allows for wavelengths in the UV range to pass through the material while the glass petri dish was chosen as a control since it was expected to filter out the UV wavelengths.

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DLS measurements The size distribution of the 53 nm amine-modified and 62 nm carboxyl-modified samples were analysed using a DynaPro Plate Reader II from Wyatt Technology, USA. Measurements were carried out in 96-well half width plates manufactured by Corning. Four 100 µL sub-samples of each treatment (UV-B exposed and un-exposed, respectively) were loaded into separate well. The measured value from each well is based on 10 consecutive measurements at 25 °C.

DSC measurements After the end of the exposure period the particles were diluted five times in MilliQ H_2O and 100 µL of each sample was applied to an 8-24% sucrose gradient capped with 0.5 mL of dodecane and analysed at 24000 rpm on a DC24000 disc centrifuge (CPS Instruments, USA).

Zeta potential measurements The zeta potentials were determined using a Zetasizer Ultra, Mavern Panalytical, United Kingdom. The software used was ZS XPLORER 2.0.1.1. Cuvettes, DTS1070, from Malvern Panalytical were used for all measurements. Each sample was measured three times with at least 30 runs for each measurement and the temperature was set to 25 ˚C. The analysis model was set to auto.

FTIR measurements A Spectrum Two FTIR equipped with a UATR Two unit from (PerkinElmer) was used to record the FTIR spectra. 6-10 µL of the sample was placed on the crystal of the UATR unit, and the H_2O was allowed to evaporate for 30-45 minutes before the spectrum was recorded. This process does not always work due to how the particles migrates during the drying, see Figure S8. The spectrum was processed using the PerkinElmer Spectrum IR application, version 10.7.2.1630. The data presented in this article has been corrected using Data Tune-up and Interactive Baseline Correction.

Acute toxicity tests An acute toxicity test on the UV-B exposed and UV-B shaded particle dispersions (i.e. 53 nm and 62 nm, see above) was performed using the freshwater zooplankter *D. magna*. One neonate was put in a 50 mL Falcon tube containing 40 mL of either UV-B exposed or UV-B shaded particle dispersed in tap water with a nanoplastic concentration of 5 mg/L. A control treatment containing tap water only was prepared and run alongside the particle exposures as a reference. Each treatment was replicated 15 times and all experimental groups were kept at 18 ˚C temperature with 16:8 hours light:dark cycle. The experiment lasted for 48 hours, and the *D. magna* were not fed during the experiment.

Particle separation and acute toxicity tests The 53 nm particles were separated from small molecules by applying 250 µL on a PD10 column (Cytoviva). An additional 750 µL H_2O was added before the samples were eluted in 1 mL steps with H_2O. Each fraction was analysed by measuring the absorbance at 230 nm. Fractions 3 to 5 containing the particles or particle remnants, and fractions 8-9 containing the smaller breakdown molecules were pooled. For the toxicity tests on *D. magna* 12 separations were made and pooled. The toxicity tests were carried out in 10 replicates in a total volume of 9 mL of which the collected fractions were

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1.5 mL and 7.5 mL ISO test water or 1.5 mL Milli-Q water and 7.5 mL ISO test water in the controls testing the 53 nm particle. The ISO test water used was prepared according to OECD 202 test guidelines [36]. The particle concentration was approximately 50 mg/L. The survival was checked regularly for 24 hours.

Detection of small molecules Fractions 10-11 from the separation were analysed by mass spectrometry (MS). MS spectra were acquired using a Autoflex Speed MALDI TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in positive reflector mode. The sample was acidified to a concentration of 0.5 % trifluoroacidic acid (TFA) before 1 µL sample was mixed with 0.5 µL Matrix solution, consisting of 5 mg/mL α-cyano-4-hydroxy cinnamic acid, 80% acetonitrile, 0.1% TFA, and added to a MALDI stainless steel plate. The spectrum was externally calibrated using Peptide calibration standard II (Bruker Daltonics) containing 9 internal standard peptides.

The fractions 8-9 were subjected to non-targeted analysis using an Agilent HPLC 1290 equipped with a Acquity HSS C18 column (3.0 x 100 mm, 1.8 µm), connected to a Bruker timsTOF Pro 2 via a 6-port valve which allowed to inject calibration solution in each injection and recalibrate mass and mobility for more accurate results. The calibration solution used was a mixture of Agilent ESI-Low concentration Mix for mobility and sodium formate for mass calibration. The calibration segment was 0.5 min and total analysis time 18 min. Acetonitrile (0.1% FA) and water (0.1% FA) were used as mobile phase with a flow of 0.4mL/min. The range of m/z was 20-1300, and the range of mobility was set according to that range to find small molecules. The sample was analysed in positive and negative mode together with an instrument blank, each in triplicates. The data obtained was later processed with Metaboscape (2022b) and using the NORMAN target list [37] to tentatively identify compounds. Thereafter, the data was further processed by an in-house R script to filter out false positives by using the repetitions and blanks. InChIKeys were used to predict mobility and MS/MS fragmentation. If the prediction was not good enough (>5% for mobility) features were deleted.

Statistical analysis Size differences between UV-B exposed and unexposed materials were evaluated using t-tests. *D. magna* survival in acute toxicity tests was evaluated using Kaplan-Meier survival analysis and the p-value was attained from the log-rank (Mantel-Cox) test. All statistical evaluations were performed using GraphPad Prism (version 8.4.3).

Results and discussion

UV-B exposure of 53 nm amine-modified polystyrene particles – size The overall aim of this study was to investigate if UV radiation affects the acute toxicity to *D. magna* exhibited by small, positively charged, amine-modified polystyrene nanoparticles. Two characteristics of these particles, their small size and positive charge, are thought to confer the toxicity. Both these characteristics can possibly be changed by UV-B exposure. Therefore, we exposed 53 nm amine-modified polystyrene particles to UV-B radiation in a controlled laboratory setup, see Figure S2. After 100 days of UV-B exposure, optical examination revealed striking differences between the sample and the control as the 53 nm particle dispersion has changed colour from white to brownish and from opaque to clear, compared to the UV-B

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shaded control, Figures S3 and S4. The particle size was characterized using DLS after the UV-B exposure. The DLS measurements show that UV-B exposure significantly decreases the size of the particles compared to unexposed particles (t=4.796, df=6, p=0.003), Figure 1 and Table 1. A decreasing size of micro-sized polystyrene particles after high intensity UV exposure has been demonstrated before [15]. The results confirm that in low intensity UV-B exposure the same processes can be seen, however, the time scale is much longer.

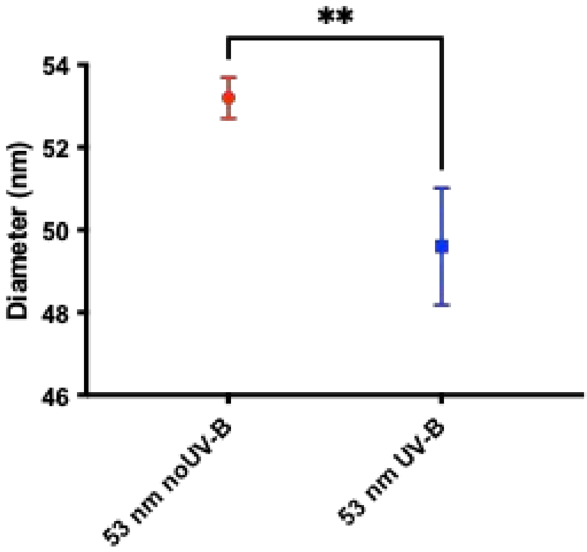

Figure 1. The size difference measured by DLS after 100 days UV-B exposure showing that the particle size significantly decreased after UV-B treatment compared to the control.

The size of the particles was further characterized with DSC, which, in contrast to DLS, shows that UV-B exposure increases the particle size and broadens the size distribution, Figure 2. The techniques differ, as DLS calculates the particle size based on Brownian motions and DSC from the sedimentation time through a sucrose gradient. Therefore, a possible interpretation of the contrasting results is that UV-B irradiation breaks up the polystyrene

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chain resulting in less compressed particles or particles with less material due to partial degradation in the entire particle [24]. Both cases would facilitate the exchange of water to sucrose in the internal of the particles, resulting in a higher density of the newly formed particles which could explain the observed results.

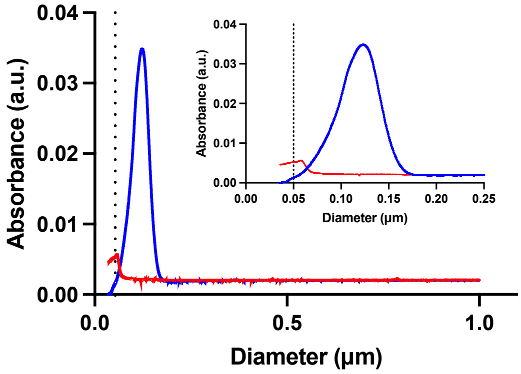

Figure 2. The size difference measured by DSC after 100 days UV-B exposure. UV-B treated particles are in blue and untreated in red. The dotted vertical line indicates the nominal size of the starting material, i.e. 53 nm. The inserted plot shows a zoom of the size range of 0-250 nm.

UV-B exposure of 53 nm amine-modified polystyrene particles – chemistry As mentioned above, a possible explanation for the acute toxicity exhibited by the small amine-modified polystyrene particles is their positive surface charge. However, the zeta potential did not significantly change after 100 days of UV-B exposure (Table 1, Figure S5). These results could lead to various interpretations, such as the possibility that most amine groups remain intact or that the chemical changes still resulted in positively charged surface groups. However, as thoroughly reviewed by S. Bhattacharjee, measuring the zeta potential of nanoparticles reveals very little about the actual surface charge of the nanoparticles[38].

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Table 1. Size and zeta potential of the particles.

Method	**DLS[1]** **Size (nm)**	**Zeta potential[2]** **(mV)**
Control 53 nm	53.2 ± 0.5	27.8 ± 1.3
UV-B 53 nm	49.6 ± 1.4	28.8 ± 0.6

[1] DynaPro

[2] Zetasizer Ultra

The UATR-FTIR spectra for the control (red) and UV-B-treated (blue) samples are shown in Figure 3. Four grey arrows indicate regions in the spectra exhibiting differences. Three of these, arrows 1, 2 and 4, areas are associated with oxygen-bounds. The carbonyl index is used to measure the chemical oxidations of polymers. Almond et al. extensively reviewed various methods for calculating the carbonyl index (CI), in their article they recommended the use of the specified area under band, SAUB, methodology [39]. The CIs were computed using the formula CI = Area under band 1850 -1650 cm^{-1} / Area under band 1500 -1420 cm^{-1}. The carbonyl index (CI) values, see Figure S6, further underscore the distinction between the control (CI ≈ 1.0) and UV-B treated (CI ≈ 2.0) samples.

Arrow 3 indicates the absorbance peak at 1602 cm^{-1}, which is indicative of conjugated C=C systems. A closer inspection of the signal reveals that the absorbance at 1602 cm^{-1} increases compared to the C-H regions, 3100 – 2840 and 1500-1440 cm^{-1}. Hence, the chemistry of the polystyrene nanoparticles is altered by the UV-B irradiation.

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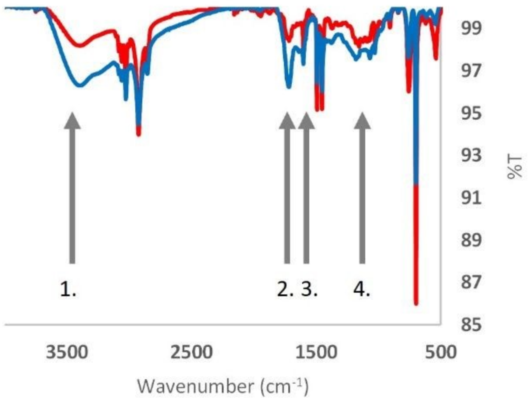

Figure 3. **U**ATR-FTIR spectra for the 53 nm PS-NH_2 particles. Red is the control, placed in a glass petri dish during the experiment, sample. Blue is the UV-B treated, placed in a quartz petri dish during the experiment, sample. The grey arrows indicate some of the changes in the FTIR spectrum.

UV-B exposure of 53 nm amine-modified polystyrene particles – toxicity The untreated 53 nm amine-modified polystyrene particles were, as expected, toxic to *D. magna*, ($\chi^2_{(df=1)}$=24.18, p<0.0001), Figure 4, which has been reported before [32]. The UV-B exposed sample was still toxic to *D. magna*, ($\chi^2_{(df=1)}$=32.81, p<0.0001) compared to the control. However, the toxicity was significantly reduced ($\chi^2_{(df=1)}$=32.81, p<0.0001) compared to the untreated particles. It is likely that the oxidation by UV-B exposure have changed the particles to more resemble polystyrene and oxidized polystyrene than the starting material amine-modified polystyrene. This could explain the decreased toxicity as neither plain nor carboxyl-modified polystyrene nanoparticles are acutely toxic [32].

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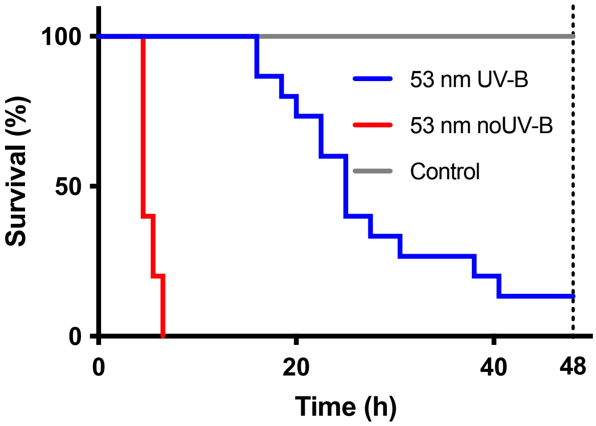

Figure 4. Acute toxicity test with 53 nm amine-modified polystyrene particles. Kaplan-Meier survival curves for *D. magna* exposed to UV-B exposed (blue line) and UV-B shaded (red line) nanoplastics. Grey line shows a control treatment only containing water. Total exposure time is 48 hours as highlighted by the dotted vertical line.

UV-B exposure of 53 nm amine-modified polystyrene particles – dissolved fraction It has been reported that intense UV irradiation of polystyrene results in the release of many different small dissolved organic compounds into the media [25, 26, 40]. We therefore expect that UV-B exposure also can result in the release of small molecules. To further analyse the samples, the small, dissolved molecules were separated from particles by applying the UV-B exposed mixture to a PD10 column. The 53 nm particles elute in fractions 3-5, and the small, dissolved molecules elute in fractions 10-11, Figure 5A. The total amount of carbon and nitrogen was determined using a Shimadzu TOC-V CPH equipped with a nitrogen detector, before and after separation, Table 2. The carbon/nitrogen ratio is higher for the particle fractions but lower for the dissolved molecules after separation, which is consistent with that nitrogen disappear from the surface of the particles.

Table 2. Carbon and nitrogen content in the fractions after separation

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Sample	**Carbon (mg/L)**	**Nitrogen (mg/L)**	**Carbon/Nitrogen**
Unseparated	2614	32.9	79.5
NP fraction	257	2.0	128.5
DM fraction	21.4	1.3	16.5

[1] Diluted 8-10 times compared to unseparated fraction

The separated samples were tested for toxicity with an acute toxicity test, see Figure 5. Interestingly, the fractions containing the dissolved molecules produced from the UV-B exposure, exhibited a significant toxicity compared to the control ($\chi^2_{(df=1)}$=19.41, p<0.0001). In agreement with the first experiment, Figure 4, the UV-B exposed particles are less toxic than untreated particles ($\chi^2_{(df=1)}$=16.35, p<0.0001). The time when immobilization occurs and the resolution between the samples are different, this could be due to several reasons. As we aimed for a higher concentration of dissolved breakdown molecules, the concentration here is 10X higher as compared to the previous test. The experimental design, with a smaller volume compared to the other assay, was limited by the sample volume and aimed at determining if the dissolved compounds are toxic.

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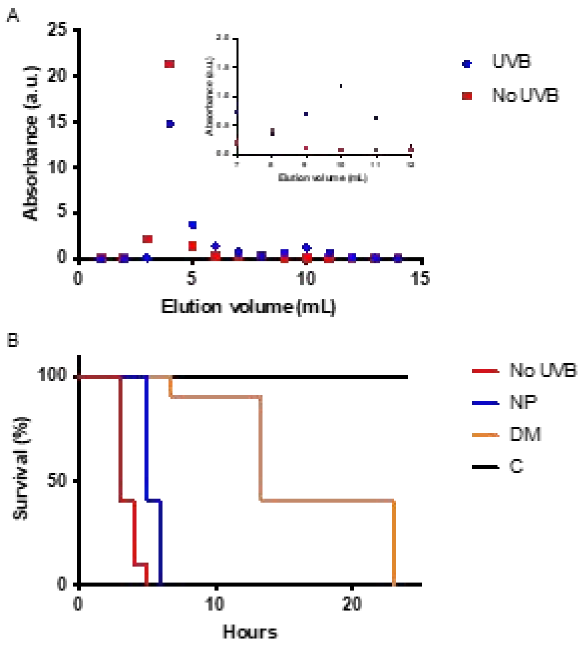

Figure 5. The acute toxicity of the separated samples after passing the PD10 column. The separation of untreated and treated articles by a PD10 column. The acute toxicity of the different fractions (B). In the red is the pooled fractions 3 to 5 of the UV-B shaded sample. In blue is the pooled fractions 3 to 5 of the UV-B exposed sample. In orange is the pooled fractions 10 to 11 of the UV-B exposed sample. In black is the control sample (no particle fraction added).

The dissolved small molecules were analysed by mass spectrometry, Figure S7. Notably in the spectra is a 44-mass unit repeat. This could be an oxidized two carbon unit as C_2OH_4 has a molecular mass of 44. The smallest weight we can detect is 626 which would mean a chain with 28 carbons.

The dissolved fraction was further described with untargeted analysis. The detected compounds are shown in Table 3 and with the structure in Table S1. It is evident that the UV-B exposure results in a very complex mixture of dissolved compounds, including molecules containing nitrogen. There are examples of molecules indicating ring opening resulting in long carbon chains. There are oxidized carbon chains which support the results from the

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mass spectrometry data described above. In four molecules the nitrogen is within closed ring indicating complexed secondary cyclization reactions which have been described before [26]. Sulphone groups are present which is probably from the original polystyrene nanoparticles that are aminated. We have previously shown that aminated polystyrene nanoparticles have a batch dependent FTIR signal, probably due to the level of amination [41]. Three molecules, amine alkyls, 12-Aminododecanoic acid, and 4-Methyl-2-pentylpyridine are labelled with health/environmental hazards. Nine of the identified molecules can be described as amine detergents. The evaluation of the toxicity of 20 amine detergents to *D. magna* showed that alkyl chains with a single nitrogen at the end were more toxic than oxidized amine alkyls [42]. The found amine alkyls may therefore contribute more to the toxicity than the 8 oxidized amine detergents. Prolonged UV irradiation should induce more oxidation and thereby with time decrease the toxicity. Furthermore, the production of amine detergents could affect the physical behaviour and toxicity of the remaining polystyrene nanoparticles [43]. However, many of the other identified molecules may contribute to the toxicity but are to our knowledge not tested.

Table 3. Non-targeted analysis of the compounds in the dissolved fractions 10-11. After UV-B breakdown of the aminated polystyrene nanoparticles the dissolved molecules were separated from the remaining particle and analysed for the contents.

	Name	Molecular Formula	Hazard
1	(2-Neopentylallyl)succinic acid	$C_{12}H_{20}O_4$	
2	(2Z,4S,5S,6S,7R,8Z)-2,9-diphenyldeca-2,8-diene-3,4,5,6,7,8-hexol	$C_{22}H_{26}O_6$	
3	(5-Ethyl-2,2-dimethyl-1,3-dioxan-5-yl)methyl acrylate	$C_{12}H_{20}O_4$	
4	1-Propanamine, 3-(hexadecyloxy)-	$C_{19}H_{41}NO$	

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5	12-(Methylamino)dodecanoic acid	$C_{13}H_{27}NO_2$	
6	12-Aminododecanoic acid	$C_{12}H_{25}NO_2$	Health
7	2-Hydroxypropyl hexanoate	$C_9H_{18}O_3$	
8	3-(Hexadecylamino)propane-1,2-diol	$C_{19}H_{41}NO_2$	
9	3-Ethylaniline	$C_8H_{11}N$	
10	3-[(Acetoxy)methyl]nonan-1-oic acid	$C_{12}H_{22}O_4$	
11	4-Methyl-2-pentylpyridine	$C_{11}H_{17}N$	Environment
12	5-Amino-1,1-dimethylhexyl acetate	$C_{10}H_{21}NO_2$	
13	5-Butyl-2-methylpyridine	$C_{10}H_{15}N$	
14	A-[(2-methylpropoxy)methyl]pyrrolidine-1-ethanol	$C_{11}H_{23}NO_2$	
15	A-hydroxy-p-methoxytoluene-a-sulphonic acid	$C_8H_{10}O_5S$	
16	Amines, C16-22-alkyl	$C_{19}H_{41}N$	Health /Environment
17	C13 Alkyl dimethyl betaine	$C_{17}H_{35}NO_2{}^1$	
18	C15 Alkyl dimethyl betaine	$C_{19}H_{39}NO_2{}^1$	
19	C17 Alkyl dimethyl amine oxide	$C_{19}H_{41}NO^2$	
20	Calcium bis(hydroxybenzenesulphonate)	$C_{12}H_{10}CaO_8S_2$	
21	Cyclohexylmethyl-2,3-Dihydroxy-5-Methyl-Hexylamide	$C_{14}H_{29}NO_2$	
22	Dodec-2-enedioic acid	$C_{12}H_{20}O^4$	
23	Piperidine-1,2-diethanol	$C_9H_{19}NO_2$	
24	TRADECAMIDE	$C_{15}H_{31}NO_2$	

62 nm carboxyl-modified polystyrene nanoparticles

We wanted to further explore the UV-B efficiency in breaking down polystyrene and to see if the process could generate small toxic molecules from a nanoparticle that is not acutely toxic for *D. magna*. The choice fell upon 62 nm carboxyl-modified polystyrene nanoparticles. In contrast to the 53 nm amine-modified polystyrene nanoparticles the 62 nm carboxyl-modified polystyrene nanoparticles exhibit no acute toxicity [32].

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The size of the carboxyl-modified nanoparticles during degradation was followed using DLS, Figure 6A. The effect of the UV-B radiation is slow during the first 20 days; after that, it accelerates, and DLS cannot detect any particles in the treated sample after 79 days of treatment. The degradation of the 62 nm particles is significantly faster than the degradation of the 53 nm particles. A plausible explanation for this is the lower concentration, 50x less, of the particle dispersion in the 62 nm experiment which means that the light will hit each particle in the sample more often. Another difference between the two UV-B experiments is that new UV lamps were used in the 62 nm carboxyl-modified polystyrene particle experiment. Based on our later lamp characterization, see supplementary figure S1, it is clear that the lamp age affects the spectral intensity of the lamp. The combination of lower particle concentration and new UV-B lamps could explain the faster photo-degradation observed in the 62 nm carboxyl-modified polystyrene particle experiment, see Figure 6.

The degradation process of the 62 nm carboxyl-modified polystyrene particles was followed by UATR-FTIR. Figure S8 shows the spectra from five different time points and Figure S6 shows the calculated CI. Compared to the CI for the amine-modified particles, the CI is higher for the carboxyl-modified particles and is decreasing for the first two time points after which it fluctuates. In the later time points (data not shown) the data is difficult to collect.

The samples were too diluted to give a reliable determination of the Zeta potential.

Toxicity test with 62 nm carboxyl-modified polystyrene nanoparticles and its breakdown products

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As shown previously, the degradation products from the amine-modified particles caused acute toxicity. In light of this, we wanted to investigate if the degradation products from a normally benign particle, the 62 nm carboxyl-modified polystyrene particle, would be acutely toxic to *D. magna*. After 79 days of UV-B exposure, there were no detectable particles, indicating complete or near complete particle degradation, hence the sample only contains degradation products. This sample was tested for toxicity to *D. magna*, Figure 6B. As expected, no toxicity was observed for the untreated particles ($p>0.05$) although the concentration of particles was the same as for the amine-modified polystyrene nanoparticles. Likewise, there was no toxicity observed for the UV-B treated sample either ($p>0.05$). Although the concentration of dissolved compounds is unknown the potential concentration is probably higher for the dissolved fraction originating from the carboxyl-modified than from the amine-modified polystyrene particles, since only a small fraction of the amine-modified particles was degraded. This may suggest that the degradation products from the amine-modified polystyrene are much more toxic. However, since the degrading process was faster for the 62 nm carboxyl-modified polystyrene particles, due to lower particle concentration and new lamps, there is a possibility that we missed a point on the degradation road that would be toxic, but the compounds have either evaporated or degraded further before we started the toxicity test. The answer to this question is beyond the scope of this article.

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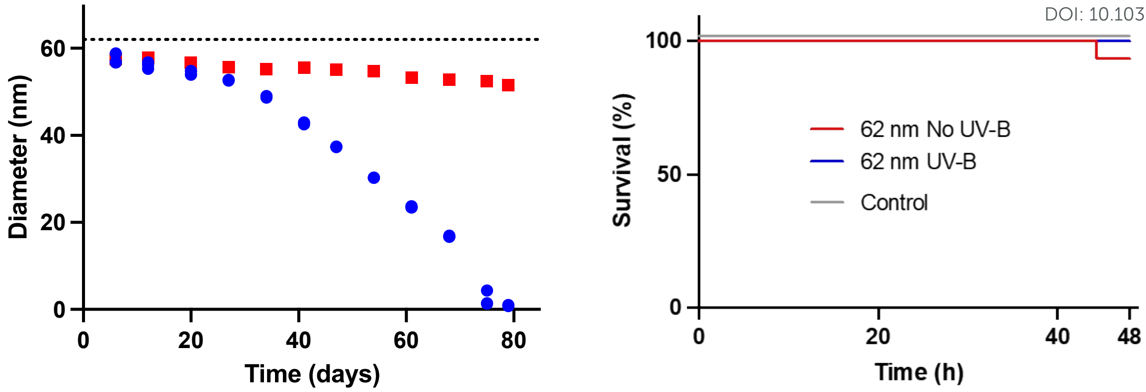

Figure 6. UV-B exposure of 62 nm carboxyl-modified polystyrene particles. Left. Particle size was followed by DLS. The UV-B treated samples are shown in blue, and the UV-B shaded samples are shown in red. The dotted horizontal line represents nominal size of the particles as stated by the manufacturer (i.e. 62 nm). Right. Acute toxicity test with 62 nm carboxyl-modified polystyrene particles. Kaplan-Meier survival curves for *D. magna* exposed to UV-B exposed (blue line) and UV-B shaded (red line) nanoplastics. Grey line shows a control treatment only containing water. Each treatment was replicated 15 times, and the exposure concentration is 5 mg/L. No significant differences were identified, p>0.05.

Conclusions

- UV-B exposure decrease the size of 53 nm amine-modified and 62 nm carboxyl-modified polystyrene particles.
- The toxicity to *D. magna* decreases after UV-B exposure of the 53 nm amine-modified polystyrene particles.
- The UV-B degradation release a complex mixture of small molecules that are toxic when originating from the 53 nm amine-modified nanoparticles.
- The UV-B degradation of the 62 nm carboxyl-modified nanoparticles did not result in any, for *D. magna*, acutely toxic compound.

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Data availability statements

The data supporting this article will be included as part of the Supplementary Information