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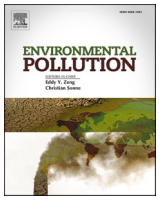

Environmental risks of breakdown nanoplastics from synthetic football fields☆

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ABSTRACT

The widespread use of synthetic turf in sports has raised health concerns due to potential risks from nanoplastic inhalation or ingestion. Our research focused on detecting nanoplastics in drainage water from a synthetic football field and evaluating the toxicity of these materials after mechanical fragmentation. We collected and analysed drainage water samples for polymer content and subjected high-density polyethylene (HDPE) straws and ethylene propylene diene monomer (EPDM) granules used on synthetic football fields, to mechanical breakdown to create nanoplastics. The results indicated the presence of trace amounts of EPDM in the water samples. Furthermore, the toxicological assessment revealed that the broken-down nanoplastics and leachate from the surface of EPDM rubber granules exhibited high toxicity to *Daphnia magna*, while nanoplastics from the inner material exhibited no significant toxicity. The findings highlight the urgent need for future research to identify these specific toxic agents from the surface of EPDM granules.

1. Introduction

Synthetic turf is becoming more prevalent, gradually replacing natural grass on sports grounds, especially football fields (Kemikalieinspektionen, 2006). These synthetic turfs are engineered to emulate the appearance and characteristics of natural grass, including resistance to sliding and slipping, energy recovery, shock absorption, vertical deformation, and wear resistance (Cheng et al., 2014). The Fédération Internationale de Football Association (FIFA) has established standards for synthetic field quality, primarily focusing on enhancing the game and safeguarding players, clubs, and associations by endorsing products that adhere to high-quality standards. Synthetic fields are marketed as a more affordable and ecologically benign alternative, attributed to water savings, the absence of a need for fertiliser or pesticides, and the reuse of rubber from scrap tires (Cheng et al., 2014; Russo et al., 2022). However, synthetic fields can potentially have a negative impact on human health and the environment due to the contaminants released by the tire rubber infill (Gomes et al., 2021; Krüger et al., 2012; Li et al., 2010; Menichini et al., 2011; Murphy and Warner, 2022; Van Ulirsch et al., 2010).

Synthetic fields are constructed with a carpet backing that has perforations to retain the fibre and includes an integrated drainage system (Cheng et al., 2014; Harris, 2008). Plastic grass is typically made from high-density polyethene, nylon, or a combination of both. The infill, added to the plastic grass on the fields, is estimated to consist of recycled rubber in 95 % of synthetic fields, according to the United States Environmental Protection Agency's Office of Research and Development (EPA/ORD) and the Centers for Disease Control and Prevention's Agency for Toxic Substances and Disease Registry (CDC/ATSDR) (EPA/ORD and CDC/ATSDR, 2019). Approximately 80,000–130,000 tonnes of recycled styrene-butadiene rubber (SBR) infill (Table S1) are used annually on European sports fields, as reported by the European Chemicals Agency (ECHA, 2017). Alternative infill materials may include ethylene propylene diene monomer rubber (EPDM) and thermoplastic elastomer (TPE) (ECHA, 2017). As of 2015, EPDM and TPE constituted 0.3% and 1% of synthetic turf infill globally, respectively, with their use varying significantly by region. In countries like Germany and Scandinavia, the usage of EPDM or TPE is significant (ECHA, 2017). As reported by the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), EPDM is used in 10% of all synthetic turf fields in the Netherlands (RIVM, 2017). It is known that EPDM granules contain a

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variety of chemicals, including paraffinic oils virtually free of Polycyclic Aromatic Hydrocarbons (PAHs), ZnO at a concentration of 1–2 wt%, and ultra-accelerators (ECHA, 2017). However, the exact composition of EPDM granules remains undisclosed due to industrial confidentiality practices, further complicating the detailed analysis and understanding of its environmental concentrations.

Both the plastic grass and the rubber infill are susceptible to degradation from various environmental factors, including oxygen, water, heat, ultraviolet (UV) radiation, and abrasion (Cheng et al., 2014), and can break down into micro-sized and even nano-sized particles. These tiny particles pose a risk as they can be inhaled, ingested, or dermally absorbed by users and wildlife (Cheng et al., 2014). Additionally, maintenance-related watering and rainfall can lead to leachate containing microplastics (Cheng et al., 2014), and potentially nanoplastics. Nanoplastics, in particular, are a concern as they can penetrate biological barriers and cell membranes, potentially posing a greater hazard to aquatic life than microplastics (Shi et al., 2024; Besseling et al., 2017). In previous studies, we have established a mechanical breakdown process to produce and isolate nanoplastics from various plastics (Ekvall et al., 2022; Ekvall et al., 2019). Here we apply a similar methodology to breakdown EPDM granules and polyethylene straws collected from an actively used football field to address two principal questions: (1) Are nanoplastics or any other material produced or leached from the breakdown process toxic to the water flea, *Daphnia magna*? And (2) Can nano-sized EPDM or HDPE be detected in the drainage water from the synthetic football field? The significance of this research lies in its potential to inform risk assessments and environmental management regulations regarding the use of synthetic turf, by elucidating the ecological risks posed by the release of nanoplastics into aquatic ecosystems.

2. Materials and methods

2.1. Materials acquisition and preparation of breakdown nanoplastics

Straws (HDPE) and granules (EPDM) were collected from Veberöds AIF football field (55°38′01.4″N 13°30′00.4″E) in Veberöd, Lund municipality, Sweden. All materials were thoroughly cleaned and debris was removed. The straws and granules were mechanically processed to simulate natural breakdown at high concentrations, following procedures from our previous studies (Ekvall et al., 2022; Ekvall et al., 2019). Two grams of either straw or granules were combined with 100 mL of tap water and blended for 2 min at maximum speed in a mini chopper (Bosch ErgoMixx 600 W food blender, Gerlingen, Germany). Subsequently, 50 mL of this mixture was filtered through a 0.8 μm syringe filter (Whatman, UK) into a separate glass container. This step eliminates macro- and microplastics. The residual mixture was replenished with an additional 50 mL of tap water and pulverised again. This process was repeated until the requisite volume of nanoplastics was obtained, such as 100 mL for the toxicity test of breakdown nanoplastics from straws and granules and 600 mL for the test of different size fractions of nanoplastics. From Fig. S4, the “breakdown" mechanism appears to be only surface-level, independent of the starting material. These nanoplastic samples were then characterised and used in the toxicity test on *Daphnia magna*.

2.2. Characterisation of nanoplastics formed after the breakdown

Transmission electronic microscopy (TEM) was used to evaluate the primary size of the nanoparticles in the various granule fractions. The images were generated on a JEOL JEM-1400 PLUS microscope at 100 kV (JEOL Ltd., Japan) using TEM Centre for JEM-1400 Plus software. Place 10 μL sample onto a single slot grid coated with pioloform (Ted Pella, Cu, Pelco Slot Grids, USA). Before analysis, the samples were placed aside to allow the water to evaporate for several days.

Nanoparticle tracking analyses (NTA) were used to evaluate the size and concentration of the nanoplastics in the drain water and breakdown water using NanoSight LM10 (Amesbury, UK). The acquired recordings were analysed using the programme NanoSight NTA 3.1 in a standard analysis setting for 30 s at camera level 13. All data supplied is an average of five different recordings.

Zeta potential measurements were performed in tap water at 25 °C using a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK). Measurements were repeated and averaged for three consecutive analyses of the same sample. In addition, the pH of all samples was measured on a Seven Compact (Mettler Toledo, Columbus, USA) pH meter.

Attenuated total reflectance Fourier-transform Infrared spectroscopy (ATR-FTIR) was conducted on a Spectrum Two FTIR spectrometer (PerkinElmer, Waltham, USA) with a UATR HR unit. The samples (10 μL) were added to the crystal and evaporated for 30 min, and then another 10 μL were added and evaporated until enough materials were suitable for the spectrum. Each sample was scanned and captured using 64 scans with a Spectral resolution of 4 cm^{-1} and a range of 450–4000 nm by Spectrum version 6.2.0. Each spectrum has had the water background signal removed. The instrument crystal was carefully cleaned between measurements.

2.3. Culturing of D. magna

The culture of the water flea, *Daphnia magna*, utilised originated from Lake Bysjön in southern Sweden (55°40′31.3″N 13°32′41.9″E) and has been preserved in the lab for hundreds of generations. Their diet consisted primarily of the green algae Scenedesmus sp., which was supplied to the culture thrice weekly. All cultures were maintained at a constant temperature of 18 °C with a 16:8 h light/dark cycle.

2.4. Toxicity of breakdown nanoplastics from straws and granules

Three groups of *Daphnia magna*, with 10 replicates each, were exposed to 5 mL of breakdown nanoplastics from straws, granules, or a control (tap water), in uncovered 15 mL Falcon tubes. This relatively small volume was chosen to minimise nanoplastic dilution during the breakdown process, thereby enhancing replication and allowing for more trials. The test animals, synchronised within 24 h, were randomly assigned to the different treatment and control groups. The tubes were labeled and randomly dispersed at 18 °C with a 16:8 h light/dark cycle. Consistent with The Organisation for Economic Co-operation and Development (OECD) guideline 202, the animals were not fed, and the medium was not changed during the exposure time. Immobilisation of the *Daphnia magna* was visually monitored daily. We decided to prolong the observation duration in light of the results of Baumann et al. (2014) and the potential risk of underestimating toxicity, particularly for specific substances like nanoparticles, within the parameters of a standard 48-h test. Taking suggestions from Baumann et al. (2014), who used 96 h, we continued our investigation until immobilisation was identified in each sample. This longer time range made it possible to analyse toxicity more comprehensively and conclusively.

2.5. Granules nanoplastics from different consecutive breakdowns

In the toxicity test of nanoplastics from granules, we observed a discrepancy in toxicity based on the volume of nanoplastics produced. Smaller volumes of breakdown nanoplastics were toxic, whereas larger volumes were non-toxic. To investigate this, we conducted a test on the toxicity of granules nanoplastics from different consecutive breakdowns. The breakdown procedure mirrored our previous method. After each round of pulverising and filtering with a 0.8 μm syringe filter, we collected 50 mL of the mixture, referring to each as “1st", “2nd", and so forth, up to the “10th". These sequential samples of nanoplastics, along with a Control sample (tap water), were then used in the *Daphnia magna* toxicity test as described in Section 2.4. Nanoplastic concentration and size were characterised using NTA.

2.6. Toxicity of size-fractionated nanoplastics from granules

To gain further insight into the toxicity of nanoplastics from EPDM granules, nanoplastics obtained from the initial three breakdowns (outer layer of granules, which were found to be toxic) were separated into different size fractions. For reference, nanoplastics derived from the fourth and subsequent breakdowns (inner layer of granules), which were deemed non-toxic, were also fractionated. 600 mL of each sample was processed using a VivaFlow device equipped with a 10 kDa Hydrosart Ultrafiltration Membrane filter (Vivaflow® 50R, HY, Sartorius, Göttingen, Germany) operating at a flow rate of approximately 250 mL/min. After filtration, the breakdown nanoplastics were split into two size fractions: (1) Vivaflow fraction (VF): the larger size fraction, containing particles larger than 10 kDa. This portion (60 mL) was concentrated to 10% of its original volume and then diluted back to 600 mL. This concentration-dilution cycle was repeated three times; (2) Filtrate fraction (FT): the smaller size fraction, composed of particles smaller than 10 kDa and dissolved substances. This fraction (540 mL) was collected directly after the filtration. For the toxicity test, we used the third cycle VF (60 mL, dense concentration of pure nanoparticles, with 0.1% of substances smaller than 10 kDa) and the first cycle FT (540 mL, containing substances smaller than 10 kDa at original concentration). These samples, along with a Control sample (tap water), were characterised and used in the *Daphnia magna* toxicity test.

2.7. Granules and their sonication-derived leachates

To further determine the sources of toxicity in granules, we implemented a sonication process designed to extract embedded additives and absorbed chemicals. Two grams of granules were placed in a beaker with 100 mL of tap water, sealed with parafilm, and subjected to sonication for 6 h each day over five consecutive days. In parallel, a control was established where 100 mL of tap water without granules was also subjected to the same sonication process. The sonication process yielded two primary results: (1) the leachate, which contained the extracted additives and chemicals; (2) the sonicated granules, largely free of these substances, were further broken down into nanoplastics as mentioned above. In addition to these, nanoplastics derived from the outer layer of granules were included as another comparison point. We characterised the leachate and the nanoplastics derived from the sonicated granules and employed them in a toxicity test.

2.8. Separation and characterisation of nanomaterial from drainage water

Three distinct drainage water samples were collected, each of 5000 mL, labeled as “Before rain”, “During rain”, and “After rain”, representing the conditions under which the samples were taken at the drainage system from Veberöds AIF football field (55°38′01.4″N 13°30′00.4″E) in Veberöd, Lund municipality, Sweden. The drain water was first filtered through a 1 μm filter sheet to eliminate larger debris, microparticles, and other materials from synthetic turf. Then the samples were separated and concentrated using a VivaFlow device with a 10 kDa filter as mentioned above, but concentrated to one-hundredth of their original volume. The concentrated samples (VF, 50 mL) and the filtrate (FT, 4950 mL) were then lyophilised to remove water, and the dry weights of the resulting samples were determined. Ultrapure water and tap water were also concentrated similarly to serve as controls. The chemical structure of drain water and Controls was analysed using FTIR after lyophilisation. Then, 1 g the lyophilised materials were resuspended in 1 mL ultrapure water and dialysed against ultrapure water using a 3.5 kDa MWCO dialysis membrane (Spectra/Por) to remove the salts and other small unwanted molecules. The six lyophilised samples and their six resuspended and dialysed counterparts were then characterised.

2.9. Statistical analysis

Data were analysed using the statistical computing software GraphPad Prism version 10. The survival analysis was performed with the Log-rank (Mantel-Cox) test and the Gehan-Breslow-Wilcoxon test. A p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Characterisation of raw materials

Although EPDM rubbers might range in composition, they all contain the same EPDM polymer. The precise composition of the EPDM granules is proprietary to the manufacturer. Therefore, our initial step was to establish a reference ATR-FTIR spectrum of the EPDM granules, Fig. 1a. From the spectra, we conclude that their surface is mainly composed of EPDM, silica, and $CaCO_3$ and that the polymer at the surface is oxidised, see Fig. 1 and Fig. S1. The chemical modification and leaching of fillers were estimated by cutting the granules in half and obtaining ATR-FTIR spectra of the newly exposed surface (Fig. S2). The spectra indicate that the pristine surface is less oxidised and contains more $CaCO_3$, Figs. S2a and d. In Fig. S3a, the ATR-FTIR spectrum of the “straws" is compared to that of a non-colored HDPE bag, revealing that the “straws" are primarily composed of HDPE.

3.2. Characterisation of nanoplastics formed after the breakdown

The two investigated materials underwent mechanical breakdown. Due to the inherent properties of materials, the chopper predominantly abraded the outer layer while the overall structure of the materials

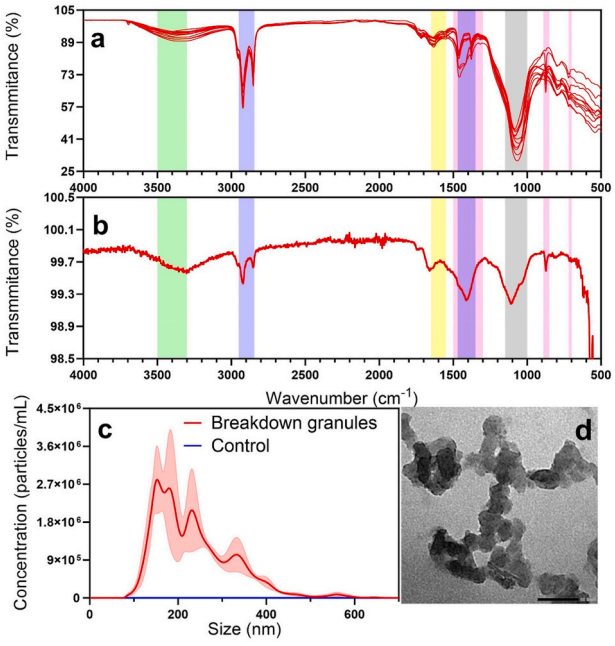

Fig. 1. Characterisation of intact granules and produced nanoplastics. **a.** the FTIR spectrum of eleven individual intact EPDM granules. The colored regions represent signals mainly from EPDM (blue), oxidised EPDM (green), $CaCO_3$ (pink), silica (grey). **b.** the FTIR spectrum of nanoplastics formed after a breakdown of EPDM granules. **c.** size distribution of nanoplastics from breakdown granules (red) or Control (tap water, blue). The dark line is the mean value of five recordings, and the light colour border surrounding it shows the standard deviation. No particles were detected in the Control. **d.** TEM images of breakdown granules. The scale bar is 50 nm with a magnification of 150k times. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

largely remained intact, indicative of a surface abrasion or surface breakdown process. The resulting breakdown solutions are depicted in Fig. S4. The mean size and estimated particle concentration for the HDPE straws, determined by NTA, were 221 ± 6 nm and $6.3 \times 10^8 \pm 2.4 \times 10^7$ particles/mL (Fig. S3b). The estimated particle concentration is close to previously published results for an HDPE bag, although the mean size is approximately twice that of the HDPE bag (Ekvall et al., 2022).

The size distribution of nanoplastics from EPDM granules is shown in Fig. 1c. The mean size is 233 ± 6 nm, with a concentration of $4.3 \times 10^8 \pm 5.8 \times 10^7$ particles/mL. When well-defined individual nanoplastics were identifiable in TEM, small primary particles in the breakdown solution appeared irregularly spherical or oval, with most less than 50 nm in size (Fig. 1d).

The chemical composition of nanoplastics formed after the breakdown of granules was determined by ATR-FTIR (Fig. 1b). The stretching vibration of the saturated C–H bond in methyl and methylene at 2850 cm^{-1} and 2925 cm^{-1}; and the deformation vibration of methylene at 1377 cm^{-1} and 1462 cm^{-1}, indicative of EPDM (He et al., 2016), are visible in Fig. 1b. The signals between 3200 cm^{-1} and 3500 cm^{-1} (OH) and around 1700 cm^{-1} (C═O) are, compared to the peaks at 2850 cm^{-1} and 2925 cm^{-1} in the breakdown nanoplastics than in the granules, indicating more oxidation. Like the EPDM granules in Fig. 1a, the breakdown nanoplastics also contain $CaCO_3$ and silica, represented by pink and grey areas, respectively, in Fig. 1b.

Furthermore, the stretching vibration C–H peak ratio is calculated by dividing the strength of the symmetric stretching vibration of the C–H peak (~2850 cm^{-1}) by the strength of the asymmetric stretching vibration of the C–H peak (~2920 cm^{-1}). According to the analysis of the FTIR graph presented by He et al. (2016) the calculated stretching vibration C–H peak ratio of pure EPDM is approximately 0.71. The stretching vibration C–H peak ratio of intact granules is 0.73 ± 0.01. The ratio of nanoplastics formed after breakdown is 0.57, Table S2. Hence, the generated particles have a lower ratio compared to bulk-formed granules, suggesting that more of the symmetric stretching vibration of the C–H is broken down during the grinding process compared to the asymmetric stretching vibration.

3.3. Toxicity of breakdown nanoplastics from straws and granules

The toxicity of the breakdown nanoplastics was tested by exposing *Daphnia magna* to dispersions of nanoplastics derived from both straws and granules. These experiments were conducted in the absence of food until complete immobilisation of the animals occurred. The results showed that nanoplastics from the granules, at a concentration of $4.3 \times 10^8 \pm 5.8 \times 10^7$ particles/mL, were significantly toxic to *Daphnia magna* (Log-rank: $\chi^2_{(1)} = 5.018$, $p = 0.0251$; Gehan-Breslow-Wilcoxon: $\chi^2_{(1)} = 4.000$, $p = 0.0455$). There was no significant difference in survival between the zooplankton exposed to nanoplastics from the straws and the Control (Log-rank: $\chi^2_{(1)} = 0.2959$, $p = 0.5864$; Gehan-Breslow-Wilcoxon: $\chi^2_{(1)} = 0.1342$, $p = 0.7141$), Fig. 2.

The lack of shown toxicity from the nanoplastics from straws aligns with previously published results as breakdown HDPE is not acutely toxic (Ekvall et al., 2022). The straws have a more complex surface chemistry and composition than the bag according to the ATR-FTIR spectra, see Fig. S3a. Since the fraction containing nanomaterials was not acutely toxic to the *Daphnia magna,* and the long-term toxicity of breakdown products from HDPE has been explored in previous studies (Ekvall et al., 2022), we decided to focus on the granules in this study.

3.4. Toxicity of granules nanoplastics from different consecutive breakdowns

Toxicity tests were conducted on nanoplastic dispersions derived from ten consecutive surface breakdowns of the same bulk material. The results revealed that the dispersions from the initial three breakdowns

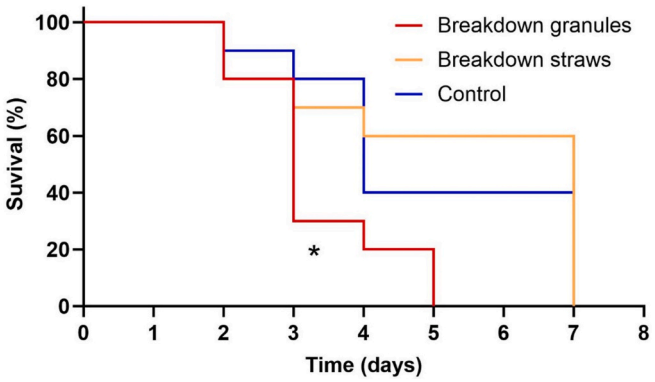

Fig. 2. Survival curves for *Daphnia magna* exposed to granules breakdown nanoplastics (red line), straws breakdown nanoplastics (orange line), and Control with clean water (blue line). Asterisks denote statistically significant differences compared to the control group throughout the entire exposure period, $*p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were toxic to *Daphnia magna* when compared to the control group (Fig. 3). However, nanoplastics from the fourth to the tenth breakdowns did not exhibit any significant impact on survival rates compared to the control group (Fig. 3; Fig. S5; Table S3). These toxicity results for the breakdown products from EPDM nanoplastics suggest that the toxic components are predominantly located on the surface of the granules. Consequently, a comparative analysis was performed to study the properties of granules from the early breakdowns versus those from the later breakdowns.

During the surface breakdown of the granules, we observed that consecutive breakdowns of the same granules resulted in less turbid breakdown solutions, Figs. S4a and S4b. Following three breakdowns, the number and size of the EPDM nanoplastics decreased, Fig. S6 and Table S4. The TEM images did not reveal any notable differences among the nanoplastics from the 1st breakdown (Fig. 1d) and those from the 10th breakdown (Fig. S7). Furthermore, z-potential measurements of nanoplastics from these two stages showed that the nanoplastics from the 10th breakdown have a more distinct z-potential compared to those from the 1st breakdown (Fig. S8). The particles from the 1st breakdowns exhibited a broad distribution in zeta potential, with multiple peaks and an average value of −35 mV. This wide distribution likely indicates a complex chemical composition and a heterogeneous distribution of surface charges on these particles. Contrarily, the particles from the 10th breakdowns displayed a narrower peak in their zeta potential distribution, with a mean value of −19 mV. This relatively tight distribution

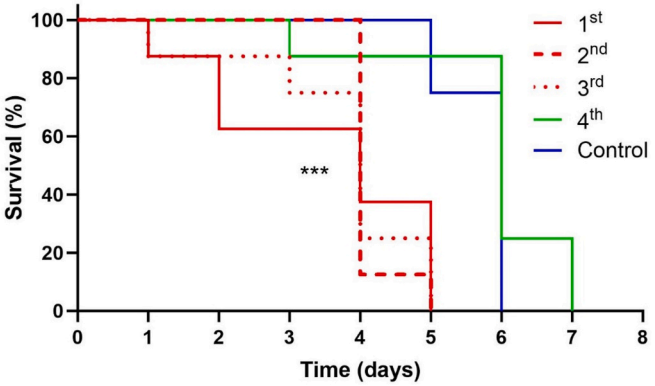

Fig. 3. Survival curves for *Daphnia magna* exposed to particles separately collected from the initial four consecutive breakdowns of granules. Asterisks denote statistically significant differences compared to the control group throughout the entire exposure period, $***p < 0.001$.

could imply a more consistent chemical composition and a more homogeneous distribution of surface charges. Fig. S2e shows the ATR-FTIR spectra for EPDM nanoplastics of the 1st and 10th breakdown of the same granules. The nanoplastics from the 1st breakdown contain more $CaCO_3$ and silica and more absorption for O–H and C═O, which imply more oxidation. However, it is hard to find any substantial difference in the FTIR spectra to explain the difference in toxicity.

Following the third mechanical breakdown, Table S4 shows a decreasing concentration of nanoparticles, indicating that concentration was a toxicity factor, but not the sole determinant. Although concentrations were elevated in the 2nd and 3rd breakdowns compared to the 1st, Fig. 3 shows a higher mortality rate during the 1st breakdown. The observed toxicity may arise from various factors, such as the chemical treatment of the granule surface, surface oxidation, the size and shape of the resulting nanomaterial, or a combination of these factors. Another plausible explanation for the higher lethality of nanoplastics from the granule surface is that the surface may absorb environmental toxins like lead, cadmium, and arsenic. Given that the inner core of rubber granules is shielded from these external factors, the risk of toxicity is presumably reduced. Previous studies on EPDM and other crumb rubber infills have mainly focused on the chemicals leached from these materials. For instance, EPDM and other infills from pitches in six countries revealed a wide range of harmful toxins, such as benzo[*a*]pyrene and zinc frequently exceeding the set limits (Gomes et al., 2021). Other studies involving 46 rubber granule-based materials and EPDM used to fill synthetic turf fields showed that benzothiazole and methyl isobutyl ketone were the most prevalent components (Sakai et al., 2022). Notably, a study found that EPDM leachate had a significant impact on *Daphnia magna* (EC_{50} < 0.4% leachate) (Krüger et al., 2013). The EPDM leachate in that study exhibited high turbidity (Krüger et al., 2013), akin to our observations of the water fraction after the first mixing of the granules (Fig. S4a). However, the turbidity disappeared after filtering through a 0.8 μm filter.

3.5. Toxicity of size-fractionated nanoplastics from granules

To distinguish between toxicity from nanoplastics derived from the outer layer of the granules and leached additives we attempted to separate these two fractions by size using a Vivaflow system. This resulted in two fractions: Filtrate fraction (FT, all the liquid that passed the 10 kDa filter) and nanoplastics (VF, particles larger than 10 kDa, and theoretically less than 1% FT). Fig. S9 displays the mean size and estimated particle concentration for the granules breakdown and their VF fractions, as measured by NTA. Particles were not detected in the FT fractions due to the limitations of the NTA method. Both fractions exhibited similar toxicity to *Daphnia magna* (Fig. S10, Table S5), indicating that both the nanoplastics and leachate are toxic. In contrast, the size fractionation of nanoplastics from the inner layer of granules appeared, as expected, to be non-toxic to zooplankton. This shows that the toxicity of the FT fraction from the outer granules is not derived from the Vivaflow system.

3.6. Granules and their sonication-derived leachates

The mean size and estimated particle concentration for granules and their leachate, as determined by NTA, are shown in Fig. S11. The toxicity results indicated that both the leachate and the nanoparticle fraction of the leachate, obtained after filtering through a 0.8 μm filter, exhibited toxicity to *Daphnia magna* (Fig. 4, Table S6). Notably, there was no discernible difference in toxicity between these two leachate samples. Consistent with the results presented in Fig. 2, the nanoplastics derived from the outer layer of granules also displayed toxicity to *Daphnia magna*.

In summary, these findings suggest that the nanoplastics derived from the relatively pure inner EPDM layer appear to be non-toxic. Furthermore, nanoplastics from the outer layer, exposed to the natural environment and subjected to weathering, oxidation, and other complex transformations, could also absorb toxic substances and therefore

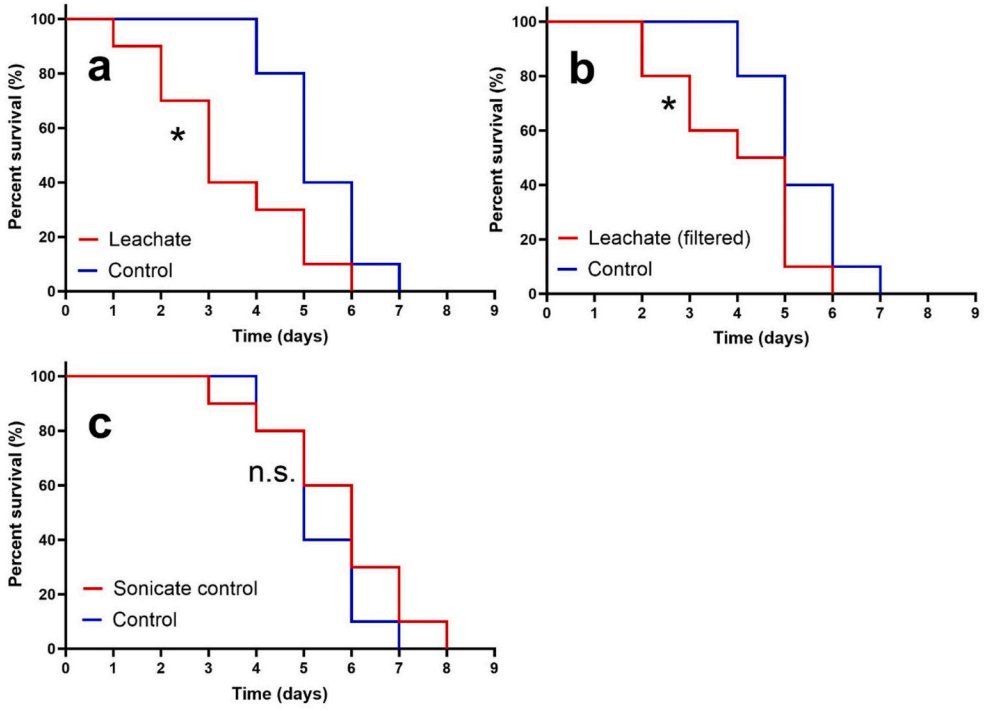

Fig. 4. Survival curves for *Daphnia magna* exposed to granules and their sonication-derived leachates. Five treatments (red line) compared to a control group (blue line). **a.** Leachate: the liquid obtained after subjecting the granules to the sonication process. **b.** Leachate (filtered): the leachate fraction obtained after filtering through a 0.8 μm filter. **c.** Sonicate control: the control sample where only tap water was subjected to the same sonication process as the granules. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

potentially increase the toxicity of the changed EPDM. Our results underscore the need for a deeper understanding of the different constituents of EPDM granules and their potential roles in toxicity. They also highlight the necessity for more sophisticated testing methodologies to discern these effects.

3.7. Separation and characterisation of nanomaterial from drain water

To address the second main question, can nanoplastics be detected in synthetic football field leachate, samples were collected at three distinct times: “Before rain", “During rain", and “After rain" representing different drainage water conditions. The collected samples underwent a filtration process using a 1 μm mesh filter (Filtered). Subsequently, the samples were concentrated, resulting in two fractions: a VF fraction (molecules larger than 10 kDa) and a FT fraction (molecules smaller than 10 kDa). Both fractions underwent lyophilisation, resuspension, and dialyses against ultrapure water. The resulting five samples (1. Filtered, 2. VF, 3. FT, 4. Dialysed VF, and 5. Dialysed FT) were characterised with NTA, see Fig. S12 and Table S7.

Particles were only detectable in the 100x concentrated VF fractions. A comparison between the VF samples before and after lyophilisation, Fig. S12 panels 2 and 3, revealed the formation of aggregates during the lyophilisation process.

The lyophilisation of the VF and FT fractions produced a white powder, see Fig. S13. Control experiments demonstrated that ultrapure water did not generate any detectable powder, while tap water did, see Fig. S13. However, the “After rain” sample contained approximately four times more material in the VF fraction, and the FT fraction had between three and four times more material than the corresponding tap water fractions, see Table S8. This significant increase in material in the drainage water sample compared to the tap water control confirms the presence of material leaked from the synthetic football field.

Fig. 5a and b presents the ATR-FTIR spectra for the lyophilised VF and FT samples. The spectra for both fractions are primarily dominated by absorption peaks associated with $CaCO_3$ and silica, chemicals leaked from the EPDM granules. This composition is consistent with the white

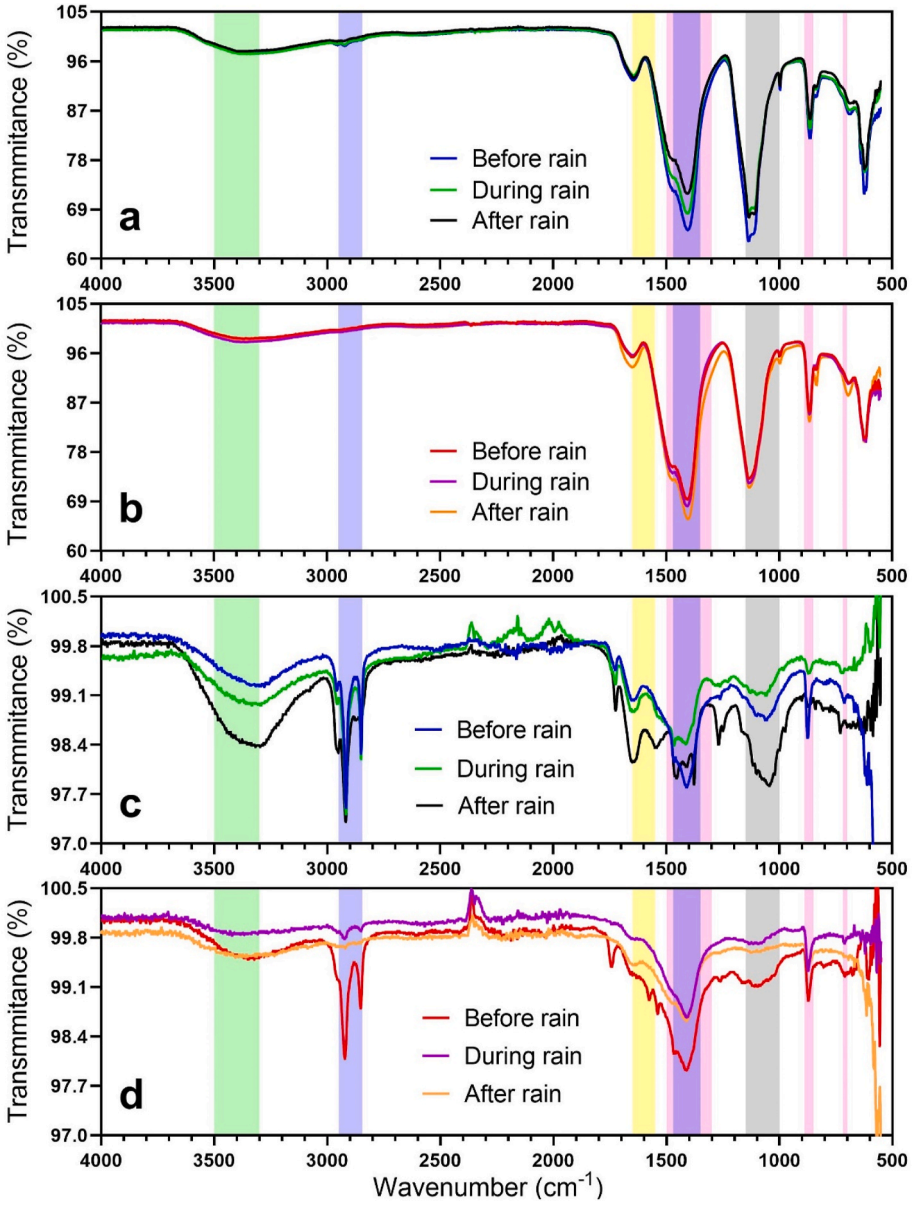

Fig. 5. ATR-FTIR spectra of drainage water. **a.** the lyophilised Vivaflow fraction (VF) of drain water comprised molecules larger than 10 kDa. **b.** the lyophilised Filtrate fraction (FT). **c.** the dialysed VF (resuspended to 1 mg/mL), the concentration is about 500 times higher than the original concentration. **d.** the dialysed FT (resuspended to 1 mg/mL), the concentration is about 3 times higher than the original concentration.

powder observed in Fig. S13. In the spectrum for the VF sample, four signals corresponding to wavenumbers expected for EPDM can be observed (Fig. 5c). The ratio between the peaks at wavenumber 2925 cm^{-1} and 2850 cm^{-1} ranges from 0.68 to 0.53 (Table S2), which is close to the value for EPDM nanoplastics. Furthermore, the fingerprint region for the "After rain" sample is more complex (Fig. 5c).

The dialysed FT samples "Before rain" and "During rain" display the four absorption peaks characteristic of EPDM, with ratios between peaks at 2925 cm^{-1} and 2850 cm^{-1} (Fig. 5d; Table S2) of 0.64 and 0.62, respectively. However, there was no detectable absorbance for the "After rain" dialysed FT sample.

The findings strongly suggest that EPDM nanoplastics and smaller polymers are being released from the synthetic football field. These nanoplastics and smaller polymers likely originate from the granules' outer layer, which is exposed to the natural environment and subject to complex transformations from various factors, potentially becoming toxic over time.

Toxicological evaluations of leachates from EPDM granules are complex, as it consists of both naturally derived substances and anthropogenic pollutants. This complicates the identification of specific compounds using techniques like FTIR spectroscopy. The dialysis of our sample preparation, while isolating particulates, likely excluded soluble plastic additives, restricting our examination largely to the insoluble components. Existing literature on EPDM leachate is notably limited. Our literature search yielded only the study by Krüger et al. (2013). In their investigation, EPDM leachate exhibited a significant toxic effect on *Daphnia magna*, with an EC_{50} value of less than 0.4% leachate concentration. However, they were unable to establish correlations between the ecotoxicity potential of the leachates and zinc and PAH concentrations, implying that unmeasured components in the leachate may contribute to the observed effects (Krüger et al., 2013).

Consequently, it's important to understand that rainwater and the act of spraying synthetic turf fields with water can result in the release of leachate. This leachate is a complex mixture not just limited to infills, additives, and microplastics. It also contains transformed EPDM nanoplastics and smaller oligomers, thus broadening the scope of pollution and potential environmental impact. These findings highlight the need for further research and regulatory measures to address nanoplastic pollution from synthetic turf fields.

4. Conclusions

Our investigation into the environmental impact of synthetic football fields has revealed the presence of EPDM nanoplastics in the drainage water, highlighting an often overlooked pathway for environmental contamination. The toxicity is localized to the surface layer of the EPDM granules, which, upon mechanical breakdown, demonstrated high toxicity to the freshwater organism. In contrast, nanoplastics originating from the inner core of the EPDM granules were found to be non-toxic. These results suggest that the surface of EPDM granules, which is more likely to interact with external environmental factors and undergo weathering and oxidative processes, may become more chemically complex and absorb additional toxic substances. Consequently, this surface-associated toxicity could pose a greater risk to aquatic life. Our study signals the need for closer scrutiny of synthetic turf's environmental effects. While the specific substances responsible for this increased toxicity were not identified within this study, the observed toxicity strengthens the need for future research to pinpoint the exact toxic origin.

CRediT authorship contribution statement

Jing Hua: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Martin Lundqvist:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Shanti Naidu:** Data curation. **Mikael T. Ekvall:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Tommy Cedervall:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGTP in order to improve language in the otherwise completed manuscript. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tommy Cedervall reports financial support was provided by Mistra, Swedish Environmental protection Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.123652.

References

Baumann, J., Sakka, Y., Bertrand, C., Köser, J., Filser, J., 2014. Adaptation of the Daphnia sp. acute toxicity test: miniaturization and prolongation for the testing of nanomaterials. Environ. Sci. Pollut. Res. Int. 21 (3), 2201–2213. https://doi.org/10.1007/s11356-013-2094-y.

Besseling, E., Quik, J.T.K., Sun, M., Koelmans, A.A., 2017. Fate of nano- and microplastic in freshwater systems: a modeling study. Environ. Pollut. 220, 540–548. https://doi.org/10.1016/j.envpol.2016.10.001.

Cheng, H., Hu, Y., Reinhard, M., 2014. Environmental and health impacts of artificial turf: a review. Environ. Sci. Technol. 48 (4), 2114–2129. https://doi.org/10.1021/es4044193.

ECHA, 2017. Annex XV Report: an Evaluation of the Possible Health Risks of Recycled Rubber Granules Used as Infill in Synthetic Turf Sports Fields. Final (europa.eu).

Ekvall, M.T., Gimskog, I., Hua, J., Kelpsiene, E., Lundqvist, M., Cedervall, T., 2022. Size fractionation of high-density polyethylene breakdown nanoplastics reveals different toxic response in Daphnia magna. Sci. Rep. 12 (1), 3109. https://doi.org/10.1038/s41598-022-06991-1.

Ekvall, M.T., Lundqvist, M., Kelpsiene, E., Šileikis, E., Gunnarsson, S.B., Cedervall, T., 2019. Nanoplastics formed during the mechanical breakdown of daily-use polystyrene products. Nanoscale Adv. 1 (3), 1055–1061. https://doi.org/10.1039/C8NA00210J.

EPA/ORD and CDC/ATSDR, 2019. Synthetic turf field recycled tire crumb rubber research under the federal research action plan. Synthetic Turf Field Recycled Tire Crumb Rubber Research Under the Federal Research Action Plan Final Report: Part 1 - Tire Crumb Characterization Volume 1 (epa.gov).

Gomes, F.O., Rocha, M.R., Alves, A., Ratola, N., 2021. A review of potentially harmful chemicals in crumb rubber used in synthetic football pitches. J. Hazard Mater. 409, 124998 https://doi.org/10.1016/j.jhazmat.2020.124998.

Harris, M.E., 2008. Polymers in the field and track. J. Chem. Educ. 85 (10), 1323. https://doi.org/10.1021/ed085p1323.

He, Y., Zhang, X., Guan, J., 2016. Preparation and properties of EPDM-based composite coatings with low infrared emissivity. J. Energy Eng. 142 (4), 04016011 https://doi.org/10.1061/(ASCE)EY.1943-7897.0000349.

Kemikalieinspektionen, 2006. Synthetic turf from a chemical perspective : a status report, Kemikalieinspektionen, Sundbyberg. PM 3/06: Synthetic turf from a chemical perspective - a status report - Kemikalieinspektionen.

Krüger, O., Kalbe, U., Berger, W., Nordhauβ, K., Christoph, G., Walzel, H.P., 2012. Comparison of batch and column tests for the elution of artificial turf system components. Environ. Sci. Technol. 46 (24), 13085–13092. https://doi.org/10.1021/es301227y.

Krüger, O., Kalbe, U., Richter, E., Egeler, P., Römbke, J., Berger, W., 2013. New approach to the ecotoxicological risk assessment of artificial outdoor sporting grounds. Environ. Pollut. 175, 69–74. https://doi.org/10.1016/j.envpol.2012.12.024.

Li, X., Berger, W., Musante, C., Mattina, M.I., 2010. Characterization of substances released from crumb rubber material used on artificial turf fields. Chemosphere 80 (3), 279–285. https://doi.org/10.1016/j.chemosphere.2010.04.021.

Menichini, E., Abate, V., Attias, L., De Luca, S., di Domenico, A., Fochi, I., Forte, G., Iacovella, N., Iamiceli, A.L., Izzo, P., Merli, F., Bocca, B., 2011. Artificial-turf playing fields: contents of metals, PAHs, PCBs, PCDDs and PCDFs, inhalation exposure to PAHs and related preliminary risk assessment. Sci. Total Environ. 409 (23), 4950–4957. https://doi.org/10.1016/j.scitotenv.2011.07.042.

Murphy, M., Warner, G.R., 2022. Health impacts of artificial turf: toxicity studies, challenges, and future directions. Environ. Pollut. 310, 119841 https://doi.org/10.1016/j.envpol.2022.119841.

RIVM, 2017. Evaluation of Health Risks of Playing Sports on Synthetic Turf Pitches with Rubber Granulate. National Institute of Public Health and the Environment (RIVM), Netherlands. Evaluation of health risks of playing sports on synthetic turf pitches with rubber granulate 2017-0016 (rivm.nl).

Russo, C., Cappelletti, G.M., Nicoletti, G.M., 2022. The product environmental footprint approach to compare the environmental performances of artificial and natural turf. Environ. Impact Assess. Rev. 95, 106800 https://doi.org/10.1016/j.eiar.2022.106800.

Sakai, S., Tahara, M., Kubota, R., Kawakami, T., Inoue, K., Ikarashi, Y., 2022. Characterization of synthetic turf rubber granule infill in Japan: volatile organic compounds. Sci. Total Environ. 838, 156400 https://doi.org/10.1016/j.scitotenv.2022.156400.

Shi, C., Liu, Z., Yu, B., Zhang, Y., Yang, H., Han, Y., Wang, B., Liu, Z., Zhang, H., 2024. Emergence of nanoplastics in the aquatic environment and possible impacts on aquatic organisms. Sci. Total Environ. 906, 167404 https://doi.org/10.1016/j.scitotenv.2023.167404.

Van Ulirsch, G., Gleason, K., Gerstenberger, S., Moffett, D.B., Pulliam, G., Ahmed, T., Fagliano, J., 2010. Evaluating and regulating lead in synthetic turf. Environ. Health Perspect. 118 (10), 1345–1349. https://doi.org/10.1289/ehp.1002239.