



**HAL**  
open science

## Leaching of chemicals and DOC from tire particles under simulated marine conditions

Aurelio Foscari, Natascha Schmidt, Bettina Seiwert, Dorte Herzke, Richard Sempéré, Thorsten Reemtsma

► **To cite this version:**

Aurelio Foscari, Natascha Schmidt, Bettina Seiwert, Dorte Herzke, Richard Sempéré, et al.. Leaching of chemicals and DOC from tire particles under simulated marine conditions. *Frontiers in Environmental Science*, 2023, 11, 10.3389/fenvs.2023.1206449 . hal-04570245

**HAL Id: hal-04570245**

**<https://amu.hal.science/hal-04570245>**

Submitted on 7 May 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.




Distributed under a Creative Commons Attribution 4.0 International License

## Author's proof

Before checking your proof, **please read the instructions below**

- Carefully read the entire proof and mark all corrections in the appropriate place, using the Adobe Reader commenting tools (**Adobe Help**). Do not use the Edit tool, as direct edits to the text will be ignored; annotate your corrections instead.
- Provide your corrections in a single PDF file or post your comments in the Production Forum, making sure to reference the relevant query/line number. Upload or post all your corrections directly in the Production Forum to avoid any comments being missed.
- We do not accept corrections via email or in the form of edited manuscripts.
- Do not provide scanned or handwritten corrections.
- Before you submit your corrections, make sure that you have checked your proof carefully, as once you approve it you won't be able to make any further corrections.
- To ensure timely publication of your article, please submit your corrections within 48 hours. We will inform you if we need anything else; do not contact us to confirm receipt.





Do you need help? Visit our **Production Help Center** for more information. If you can't find an answer to your question, contact your Production team directly by posting in the Production Forum.

**NOTE FOR CHINESE-SPEAKING AUTHORS:** If you'd like to see a Chinese translation, click on the  symbol next to each query. **Only respond in English** as non-English responses will not be considered. Translated instructions for providing corrections can be found **here**.

### Quick checklist

- Author names** - Complete, accurate and consistent with your previous publications.
- Affiliations** - Complete and accurate. Follow this style when applicable: Department, Institute, University, City, Country.
- Tables** - Make sure the meaning/alignment of your Tables is correct with the applied formatting style.
- Figures** - Make sure we are using the latest versions.
- Funding and Acknowledgments** - List all relevant funders and acknowledgments.
- Conflict of interest** - Ensure any relevant conflicts are declared.
- Supplementary files** - Ensure the latest files are published and that no line numbers and tracked changes are visible. Also, the supplementary files should be cited in the article body text.
- Queries** - You must reply to all **typesetter's queries below** in order for production to proceed.
- Content** - Read all content carefully and ensure any necessary corrections are made, then **upload them** to the Production Forum.

## Author queries form

Query no.	Details required	Author's response
<b>Q1</b>	Confirm whether the article title is correct. 	
<b>Q2</b>	If the following authors would like their LOOP profile to be linked to the final published version, ensure that they <b>register</b> with Frontiers at the provided link, and provide us with the URLs to their profile(s). If just an email address is provided, the profile link will not be added to the article. Non-registered authors and authors with profiles set to "Private" will have the default profile image displayed. Note that we will not be able to add profile links after publication. Aurelio Foscarì 	
<b>Q3</b>	The citation and surnames of all of the authors have been highlighted. Check that they are correct and consistent with your previous publications, and correct them if needed. Please note that this may affect the indexing of your article in repositories such as PubMed. If adding/removing authors, or changing the order of this list, please provide us with a signed <b>Authorship Change form</b> , which should be uploaded as a "Related Article" file type with your Author's Proof Corrections. 	
<b>Q4</b>	There is a discrepancy between the styling of the author names in the submission system and the manuscript. We have used [Bettina Seiwert] instead of [BETTINA SEIWERT]. <b>Please</b> confirm that it is correct. 	

Query no.	Details required	Author's response
Q5	Confirm that all author affiliations are correctly listed. Per our style guidelines, affiliations are listed sequentially and follow author order. Requests for non-sequential affiliation listing will not be fulfilled. Note that affiliations should reflect those at the time during which the work was undertaken. If adding new affiliations, specify if these should be listed as a present address instead of a regular affiliation. 🌐	
Q6	Confirm that the email address in your correspondence section is accurate. Any changes to corresponding authors requires individual confirmation from all original and added/removed corresponding authors. 🌐	
Q7	The abstract should ideally be structured according to the IMRaD format (Introduction, Methods, Results and Discussion). Provide a structured abstract if possible. 🌐	
Q8	Confirm that the keywords are correct, and keep them to a maximum of eight and a minimum of five. (Note: a keyword can be made up of one or more words.) 🌐	
Q9	Check if the section headers (i.e., section leveling) have been correctly captured. 🌐	
Q10	Check that all equations and special characters are displayed correctly. 🌐	
Q11	Confirm that the Data Availability statement is accurate. Note that this statement may have been amended to adhere to our Publication Ethics guidelines. 🌐	
Q12	Confirm that the details in the "Author Contributions" section are correct. We have added the sentence "All authors contributed to the article and approved the submitted version." 🌐	
Q13	Please confirm if this should be moved to the Funding/Acknowledgments section: "XXXX." 🌐	
Q14	Confirm if the text included in the Conflict of Interest statement is correct. Please do not suggest edits to the wording of the final sentence, as this is standard for Frontiers' journal style, per our <a href="#">guidelines</a> . 🌐	
Q15	Ensure that any supplementary material is correctly published at this link: <a href="https://www.frontiersin.org/articles/10.3389/fenvs.2023.1206449/full#supplementary-material">https://www.frontiersin.org/articles/10.3389/fenvs.2023.1206449/full#supplementary-material</a> . If the link does not work, you can check the file(s) directly in the production forum; the published supplementary files appear in green. Please make sure all Supplementary files are cited. Please also provide captions for these files, if relevant. If you have any corrections, please provide new files and republish them in the forum. Frontiers will deposit ALL supplementary files to FigShare and they will receive a DOI. Notify us of any previously deposited material. If the Supplementary Material files contain identifiable or copyright images, please keep in mind that it is your responsibility, as the author, to ensure you have permission to use the images in the article. Please check this <a href="#">link</a> for information on author responsibilities and the publication images. 🌐	
Q16	Ensure that all the figures, tables, and captions are correct, and that all figures are of the highest quality/resolution. If necessary, you may upload improved figures to the Production Forum. Please note that figures and tables must be cited sequentially, per the <a href="#">author guidelines</a> . 🌐	
Q17	If you decide to use previously published and/or <a href="#">copyrighted figures</a> in your article, please keep in mind that it is your responsibility as the author to obtain the appropriate permissions and licenses to reproduce them, and to follow any citation instructions requested by third-party rights holders. If obtaining the reproduction rights involves the payment of a fee, these charges are to be paid by the authors. 🌐	



## OPEN ACCESS

## EDITED BY

Tania Martellini,  
University of Florence, Italy

## REVIEWED BY

Cassandra Rauert,  
The University of Queensland, Australia  
Shangwei Zhang,  
Beijing Normal University, China

## \*CORRESPONDENCE

Thorsten Reemtsma,  
✉ thorsten.reemtsma@ufz.de

†These authors have contributed equally  
to this work and share first authorship

RECEIVED 15 April 2023

ACCEPTED 12 June 2023

PUBLISHED XX XX 2023

## CITATION

Foscari A, Schmidt N, Seiwert B, Herzke D,  
Sempéré R and Reemtsma T (2023),  
Leaching of chemicals and DOC from tire  
particles under simulated  
marine conditions.  
*Front. Environ. Sci.* 11:1206449.  
doi: 10.3389/fenvs.2023.1206449

## COPYRIGHT

© 2023 Foscari, Schmidt, Seiwert, Herzke,  
Sempéré and Reemtsma. This is an open-  
access article distributed under the terms  
of the [Creative Commons Attribution  
License \(CC BY\)](#). The use, distribution or  
reproduction in other forums is  
permitted, provided the original author(s)  
and the copyright owner(s) are credited  
and that the original publication in this  
journal is cited, in accordance with  
accepted academic practice. No use,  
distribution or reproduction is permitted  
which does not comply with these terms.

# Leaching of chemicals and DOC from tire particles under simulated marine conditions

Aurelio Foscari<sup>1†</sup>, Natascha Schmidt<sup>2,3†</sup>, Bettina Seiwert<sup>1</sup>,  
Dorte Herzke<sup>2</sup>, Richard Sempéré<sup>3</sup> and Thorsten Reemtsma<sup>1,4\*</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research—UFZ, Department of Analytical Chemistry, Leipzig, Germany, <sup>2</sup>Norwegian Institute for Air Research (NILU), Tromsø, Norway, <sup>3</sup>CNRS, IRD, Mediterranean Institute of Oceanography (MIO), Aix-Marseille University, Université de Toulon, Marseille, France, <sup>4</sup>Institute for Analytical Chemistry, University of Leipzig, Leipzig, Germany

Tire wear particles (TWPs) represent one of the major anthropogenic pools of particles ending up in the environment. They contain a large variety of chemicals, a part of which may be released into the environment through leaching, although the influence of sunlight and other environmental factors during this process is still unclear. This laboratory study compares the leaching of organic compounds from TWP in seawater in the dark and under artificial sunlight for 1) cryo-milled tire tread (CMTT), 2) 'virgin' crumb rubber (VCR) and 3) crumb rubber immersed in the sea for  $\geq 12$  months prior to the experiments (WCR). Leachates were analyzed for dissolved organic carbon (DOC) and 19 tire-derived chemicals, benzothiazoles and phenylguanidines as well as phenyldiamines by liquid chromatography-high resolution-mass spectrometry. For DOC and most chemicals, the amounts released decreased in the order CMTT > VCR > WCR and increased when leaching occurred under artificial sunlight. Sunlight also led to the formation of 23 transformation processes related to 1,3-diphenylguanidine (DPG). In contrast, 4-hydroxydiphenylamine (4-HDPA) and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6-PPDQ) were found in lower amounts upon sunlight exposure. The 19 quantified chemicals, however, did only account for 6%–55% of the DOC in the leachates; most of the DOC, thus, remained unexplained. This study highlights that the amount of chemicals leached from tire particles depends upon their aging history and may be modulated by environmental conditions.

## KEYWORDS

photodegradation, transformation products (TPs), ocean, automobile, additives, antioxidants, vulcanization accelerators

## 1 Introduction

Tire wear particles (TWPs) originate from the abrasive friction between roads and the treads of tires from car, truck, bicycle or e-scooter tires. The particles originating from this process are usually further referred to as tire and road wear particles (TRWP), since they include road materials as well as the original tire material.

Tires are commonly made of styrene-butadiene-rubber (SBR) and butadiene rubber (BR) and can contain high amounts of filling agents, vulcanization agents (e.g., benzothiazoles and benzothiazoles) and other additives, such as zinc (Wagner et al., 2018). Zn has therefore previously been proposed as an elemental marker for the detection of TWP in particulate samples (Klößner et al., 2019). Currently, a widely used

antioxidant, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD) and its derivative 6-PPD quinone (6-PPDQ) receive growing attention, since a study reported 6-PPDQ to induce acute mortality in juvenile coho salmon (*Oncorhynchus kisutch*) (Tian et al., 2021), with a revised LC<sub>50</sub> level of 95 ng/L (Tian et al., 2022). Interestingly, a follow-up study confirmed that short-term (<24 h) exposure to TWP leachate was acutely lethal in adult coho salmon, but not in adult chum salmon (*Oncorhynchus keta*), a closely related species, raising questions concerning the mechanisms behind these inter-species differences (McIntyre et al., 2021). Environmental data on TWP additives are still scarce and laboratory scale experiments have been used to describe the chemical composition of tire leachables (Müller et al., 2022). A study investigating TWP additives in surface waters of an urban tributary in Australia reported a maximum total concentration of 2,760 ng/L for 15 chemicals after a storm event, with 1,3-diphenylguanidine (DPG) being the dominant compound (1,079 ng/L). While a correlation between the presence of SBR, BR and additives was observed, the authors noted that for most of the detected chemicals other sources than tires exist and might have contributed to the observed concentrations (Rauert et al., 2022).

Estimations on the amount of TRWP generated by automotive traffic are available (Baensch-Baltrusch et al., 2020; Wagner et al., 2022), but the percentage to which it reaches the marine environment is not clear, yet. It, certainly, depends on regional aspects such as the distance between urban source regions and the receiving ocean and on their connectivity. The leaching of chemicals from tire particles can be influenced by biotic (biodegradation) and abiotic (e.g., oxidation, mechanical degradation) factors (Wagner et al., 2022). Photodegradation studies on TWP have been performed to investigate the influence of photoaging on both particles and related chemicals (Unice et al., 2015; Fohet et al., 2023). These studies were, however, performed in dry conditions and dedicated studies simulating marine environmental conditions have not been published, yet.

Therefore, our objective was to investigate the leaching behavior of three different TWP materials in natural seawater when exposed to artificial sunlight to simulate processes expected to occur when tire particles reach the marine environment. This study was performed with cryo-milled tire tread (CMTT) and end-of-life tires ground into crumb rubber granulate for use in artificial sports fields.

## 2 Material and methods

### 2.1 Chemicals

All chemicals were of analytical grade and used as received without further purification. Methanol, acetonitrile, and formic acid for UPLC-MS analysis were provided by Biosolve (Valkenswaard, Netherlands) whereas ultrapure water was obtained from a Merck MilliQ Integral 5 system (Merck, Darmstadt, Germany). All standards used are listed in Supplementary Table S1, with purity grade and supplier information.

### 2.2 Artificial sunlight exposure

Surface seawater was collected in the Bay of Marseille (France) in July 2021 using pre-combusted glass bottles, then filtered in the laboratory through a 0.22 μm polyethersulfone (PES) filter pre-rinsed with Milli-Q water and filled into 50 mL quartz tubes containing 1 g/L of the test material; i.e., 50 ± 2 mg per tube. The samples were exposed in laboratory conditions to artificial sunlight using a Suntest CPS + system (ATLAS) equipped with a daylight filter according to (Para et al., 2010). Irradiance intensity was set at 300 W/m<sup>2</sup> (300–800 nm) and temperature at 22°C ± 2°C, both representing environmental conditions as encountered in Marseille (France) during summer (reference data from July 2021: monthly mean solar radiation = 298 W/m<sup>2</sup>, monthly mean surface seawater temperature = 22°C). The quartz tubes were hereby partially submerged in a basin connected to a Minichiller® (Huber Kältemaschinenbau AG) inducing a constant water flow to ensure the maintenance of the desired temperature. In parallel, samples were kept under dark conditions by using the same experimental set-up and temperature, but in complete darkness. At each sampling timepoint, three replicates of UV exposed and dark condition samples were sacrificed, each. Hereby, 10 mL were collected for dissolved organic carbon (DOC) analysis, acidified (20 μL of 85% H<sub>3</sub>PO<sub>4</sub> acid) and stored in glass vials in the dark at 4°C until analysis within a month by using a TOC-5000 total carbon analyzer (Sohrin and Sempéré, 2005). The natural seawater collected at the surface in the Bay of Marseille exhibited a DOC value of 76.8 (±16.4) μM, which is consistent with DOC concentrations previously measured in the same area (72.5–90 μM in summer-fall; Paluselli et al., 2018). This background DOC concentration from the seawater was subtracted from the DOC values observed in the leachates, to further investigate the DOC which derived from the test materials.

The remaining volume was stored in amber glass vials in the dark at 4°C until analysis of organic compounds using UPLC-TOF-MS (see section 2.5). Samples were collected at three sampling timepoints: after (t<sub>1</sub>) 66 h, (t<sub>2</sub>) 158 h and (t<sub>3</sub>) 336 h, representing 6, 14.4 and 30.6 simulated days, respectively. Simulated days were calculated using the following equation from Gewert et al. (2018): **Q10**

$$\text{Simulated days} = \frac{\text{Total irradiance exposed}}{\text{Mean European irradiance}} \times 365$$

where the total irradiance exposed is the product of the intensity (in W/m<sup>2</sup>) and the number of hours of exposure (in h) and the European mean irradiance equals 1,200 kWh/m<sup>2</sup> per year

### 2.3 Test material

Three types of test material were used for this study. The “CMTT” comprises a mix of tire tread from 20 different, new tires (see Supplementary Table S2), which were homogenized through a ball milling in cryogenic conditions (Mixer Mill MM 400, Retsch, Germany) and represents small particles as they can be found in road runoff. The size distribution and particle shape images are provided in Supplementary Figure S1. The two other test materials are made of crumb rubber granulate as used in artificial sports fields or playgrounds. The original material (hereafter called “virgin crumb rubber”, VCR) was sourced from a sports field in

Tromsø (Norway) prior to application on the field and has previously been used for leaching and toxicity testing (Halsband et al., 2020).

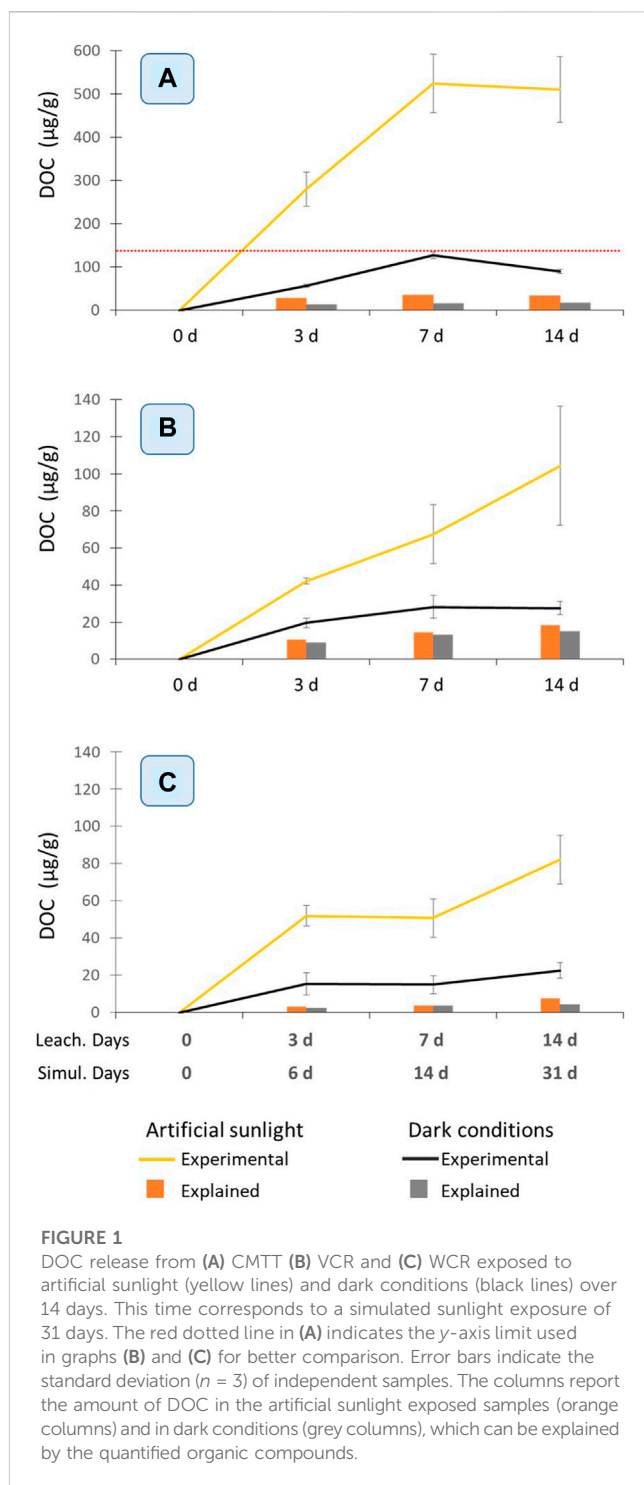
Finally, part of the VCR was exposed *in situ* to natural seawater in Tromsø for 12–18 months (hereafter called “weathered crumb rubber”, WCR) to create a test material of high environmental relevance. For this, the particles were placed in a cylindrical stainless-steel container covered with a mesh and submerged in surface waters (3–4 m depth). The outside of the container was mechanically cleaned at regular intervals to remove algal and bivalve growth and permit the sunlight to transpire. While the choice of stainless steel limited the exposure of the contained particles to natural sunlight, it was considered as preferable to plastic materials to avoid a chemical contamination of the test material. The particles exposed for 12 and for 18 months were mixed together to have sufficient material to perform the experiment. After recovery from the sea, the WCR was not modified (i.e., no removal of the biofilm or other cleaning), but stored in dry conditions prior to use.

## 2.4 QA/QC

All glassware was heated (450°C, 6 h) prior to use and covered with burnt aluminum foil. Plastic and rubber materials were avoided at all times. All sample processing steps were conducted in an ISO class 6 clean room equipped for organic trace analysis. Cotton lab coats and nitrile gloves were worn during sample handling. Instrument and water media blank controls performed during the sample analysis were below LOD/LOQ levels or otherwise subtracted from the sample values.

## 2.5 Analysis with UPLC-TOF-MS

Aqueous samples were analyzed for 19 tire-related chemicals (Supplementary Table S1) by ultra-performance liquid chromatography time-of-flight mass-spectrometry (LC-TOF-MS). The instrument consisted of an ACQUITY UPLC-System (HSS T3 column; 100 × 2.1 mm, 1.7 μm) coupled to a XEVO XS Q-TOF-MS (Waters GmbH, Eschborn, Deutschland) as described elsewhere (Klößner et al., 2021; Klößner et al., 2020). A flow rate of 0.45 μL/min, a column temperature of 45°C and a capillary voltage of 0.7 kV (positive ionization mode) were applied. The source temperature was set at 140°C, while the desolvation temperature was set at 550°C. The sampling cone voltage was 20 V and the source offset was 50 V. Nitrogen was used as a cone gas and argon as a collision gas. The desolvation gas flow was set at 950 L/h and the scan time at 0.15 s. A collision energy of 4 eV (molecular ions) and 15–35 eV (fragment analysis) was applied. The mobile phase consisted of A) water with 0.1% formic acid and B) methanol with 0.1% formic acid. The solvent gradient was as follows: 0 min 2% B, 12.25 min 99% B, 15.00 min 99% B, 15.10 min 2% B, 17.00 min 2% B. A volume of 10 μL of the leachates was directly injected into the LC-HRMS system and the first minute was forwarded to waste, not to the MS. The target substances (Supplementary Table S1) were quantified using an external calibration, which was comparable to seawater (checked separately). Therefore, TargetLynx was used with 0.01 Da mass



accuracy and 0.1 min accurate retention time and a calibration range from 0.03 to 30 μg/L.

## 2.6 Identification of transformation products

Identification of transformation products (TPs) was done by evaluating the RPLC-MS data in a retention time window of 1–10 min and a mass range of  $m/z$  50–1,200. MarkerLynx was

used to perform the peak picking for the molecular ion trace (function 1) with 0.1 min deviation in retention time and 0.01 Da deviation in exact mass. The marker table with relative intensities (to the total marker intensity) was exported to Excel and all further evaluations were performed there. The peaks with a relative intensity above 1 and present in the leachates compared to controls were selected. Sum formulas were assigned by using a mass tolerance of 5 ppm and an elemental composition of C (0–100), H (0–100), N (0–20), O (0–20), S (0–3) and Na (0–2), and by taking into account the fragment ions. The fragment ions were used, if available, to assign the elemental composition. For DPG related TPs the fragments were screened for those containing m/z 212.118 (C<sub>13</sub>H<sub>14</sub>N<sub>3</sub>), 195.0922 (C<sub>13</sub>H<sub>11</sub>N<sub>2</sub>), 131.0599 (C<sub>8</sub>H<sub>7</sub>N<sub>2</sub>) and/or 119.06 (C<sub>7</sub>H<sub>7</sub>N<sub>2</sub>), 106.0648 (C<sub>7</sub>H<sub>8</sub>N). DCH related TPs were identified by having the fragment m/z 180.1755 (C<sub>12</sub>H<sub>22</sub>N). The integration of the areas was done in TargetLynx.

## 3 Results and discussion

### 3.1 Release of dissolved organic carbon

All three test materials released DOC into the seawater in the range of 20–500 µg/g within 14 days (Figure 1). Under dark conditions CMTT released the highest amount of organic carbon (140 µg/g; Figure 1A), followed by WCR and VCR (23–28 µg/g). The stronger DOC release from CMTT may in part be due to a higher content of low molecular weight additives in that material (see section 3.2) and its significantly lower mean particle size (180 µm) compared to VCR and WCR (3 mm). A higher DOC concentration (316 µg/g) was previously reported for a freshly pulverized crumb rubber after 3 days of leaching, after which the concentration decreased (Selbes et al., 2015). The authors suggested that freshly generated particles would release more DOC; this may explain their higher values compared to the crumb rubber in this study.

It is noteworthy that the VCR released only slightly higher amounts of organic carbon (20%–25%) than the same material after being exposed to marine water for about 1 year. This suggests that the pool of organic compounds that can be released from crumb rubber is large and is not much depleted within 1 year. The release rate of organic compounds from the rubber into the surrounding water is determined by the (low) diffusion speed of these compounds from the interior of the particles towards the surface (Huntink, 2003). A part of the organic matter released from WCR may, however, also be natural organic matter adsorbed to it during the exposure period.

For CMTT there is a slight decrease in average DOC concentrations from day 7 to day 14 (Figure 1A), which may reflect the onset of biodegradation of some of the organic chemicals released from the material, as the experiments were not run under sterile conditions.

For all three materials parallel exposure to artificial sunlight drastically increased the release of organic matter over 14 days by a factor of 3–5 compared to the dark conditions (Figure 1.). Several hypotheses might be made to explain this observation: 1) The UV exposure could induce an aging and loosening of the rubber matrix, which would increase its accessibility for water and speed up leaching of organic compounds. 2) A sunlight induced

transformation of tire constituents with a relatively lower tendency for leaching into TPs of lower molecular weight and higher polarity, with a stronger leaching tendency might occur. 3) While the temperature within the irradiation chamber was controlled (22°C ± 2°C), the black color of the particles might have led to a higher temperature of the particles themselves exposed to artificial sunlight, supporting the leaching compared to the dark conditions. A combination of these aspects as well as other factors may be responsible for the enhancing effect of sunlight exposure.

While the leaching of chemicals from tire material has been studied (Halsband et al., 2020; Tian et al., 2021; Jeong et al., 2022; Müller et al., 2022), the total amount of organic matter measured as DOC has been reported so far only for crumb rubber (Selbes et al., 2015) but not for a smaller dimensional size range of tire particles.

### 3.2 Leachate composition

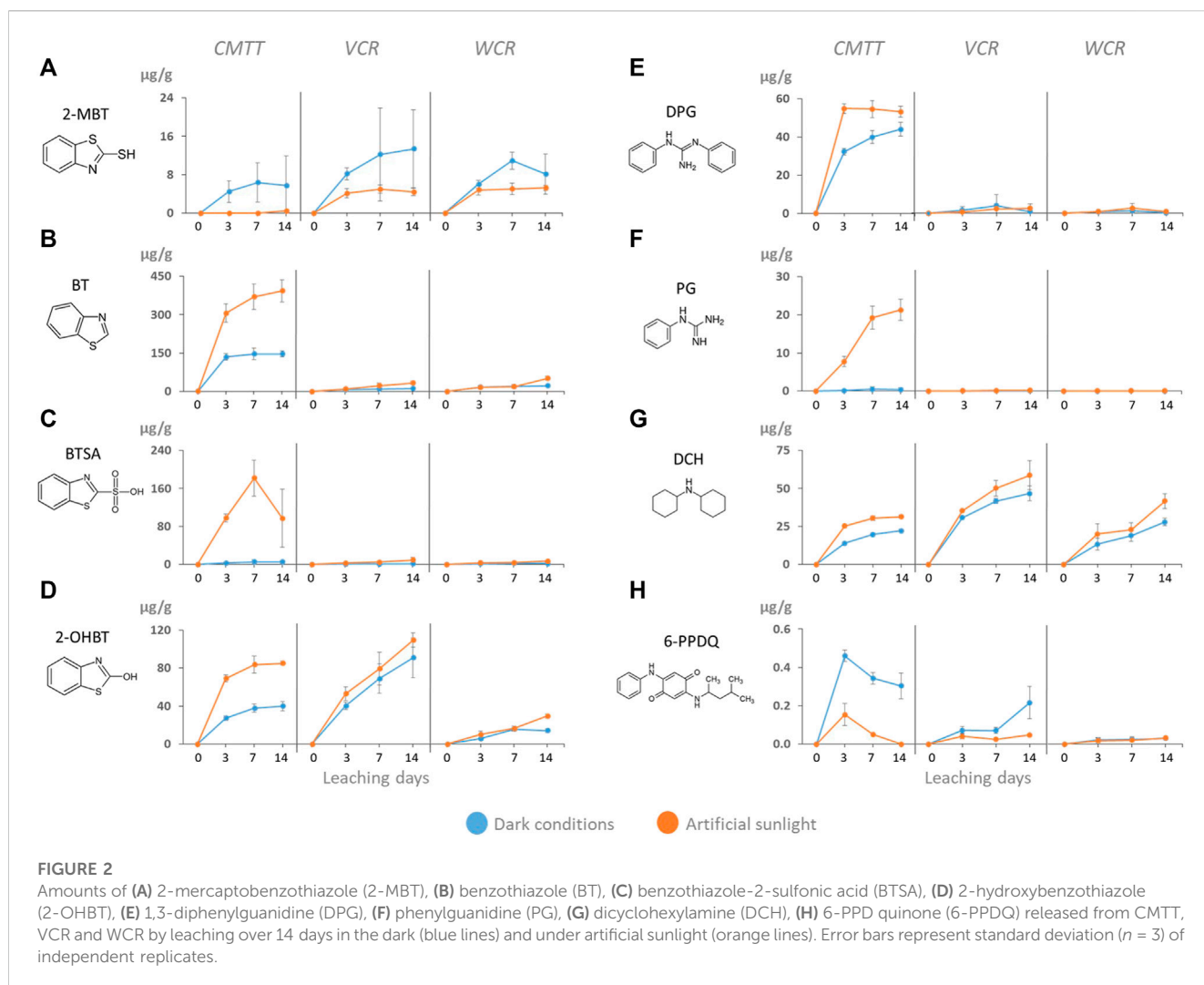
A total of 19 tire-related chemicals were quantified in the leachates based on LC-HRMS analysis (Supplementary Table S1). Among them were the vulcanization accelerators 2-mercaptobenzothiazole (2-MBT), benzothiazole (BT), benzothiazole-2-sulfonic acid (BTSA), 2-hydroxybenzothiazole (2-OHBT), 1,3-diphenylguanidine (DPG) and its degradation product phenylguanidine (PG), and dicyclohexylamine (DCH), as well as the antioxidant N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD) and its hydrolysis and oxidation products 4-hydroxydiphenylamine (4-HDPA) and 6-PPD quinone (6-PPDQ), respectively.

#### 3.2.1 Vulcanization accelerators

Benzothiazoles were the most abundant substances found in the leachates (from 58 to 342 µg/g and from 111 to 589 µg/g in dark and sunlight exposed conditions, respectively). 2-MBT is a prominent transformation product of the vulcanization accelerators found in tires. Similar amounts were released from the three materials, even after 1 year of exposure to marine waters (WCR). This suggests that the reservoir of 2-MBT in the materials is large enough not to be exhausted in this period of time. For all three materials 2-MBT leaching was lower during sunlight exposure, likely due to direct or indirect photodegradation of 2-MBT (Zajčková and Párkányi, 2008). This photodegradation may have occurred either on the particle surface before 2-MBT was released or in solution after its release.

BT, BTSA and 2-OHBT all are putative transformation products of MBT. For BT and BTSA, the leached amounts were significantly higher from CMTT, compared to VCR and WCR, especially under sunlight exposure (CMTT 160–350 µg/g, VCR and WCR 6–50 µg/g). CMTT with its much smaller particle size offers more surface per mass. This enhances both, release of chemicals from the surface as well as transformation reactions on the surface. And it might explain why much higher amounts of BT and BTSA were detected in CMTT samples exposed to artificial sunlight (Figures 2B,C), even though the amount of the parent compound, 2-MBT, in solution under dark conditions was even lower than for VCR and WCR samples (Figure 2A).

BT was also detected in 14-day leachates of crumb rubber in seawater, at concentrations of 80 ± 46 µg/g (Halsband et al., 2020)



when considering the same crumb rubber concentration as used in our study (1 g/L). This is comparable to our results for VCR and WCR after 14 days of leaching (11–50 µg/g, depending on the conditions).

Furthermore, 2-aminobenzothiazole (NH<sub>2</sub>-BT) was determined in the leachates (0.2–2 µg/g). As for BTSA and BT, sunlight exposure increased the release of NH<sub>2</sub>-BT from CMTT more strongly than from VCR and WCR (Supplementary Figure S2).

For 2-OHBT the difference between CMTT and the other materials is less strong (WCR) or even absent (VCR); also, the difference between dark and light leaching conditions is less pronounced (Figure 2D). This, likely, indicates that the formation of 2-OHBT occurred inside the tire particles and is only partially due to sunlight exposure during the leaching. The amount of 2-OHBT released from WCR was approximately 20% of that released from the VCR material (Figure 2D). It appears that a significant proportion of 2-OHBT leached out of the WCR during the 1 year of weathering in marine waters. Such a strong difference between WCR and VCR was not visible for BT and BTSA, supporting the assumption that they are formed by different processes than 2-OHBT. On a molar basis, the enhancing effect of sunlight exposure, however, was equally

strong for VCR (+118 nM) and WCR (+100 nM) probably due to their similar particle size range, while it was three times higher for CMTT (+300 nM). 1,3-diphenylguanidine (DPG) is another curing agent and a prominent chemical in tire rubber and tire leachates (Schulze et al., 2019; Zahn et al., 2019; Johannessen et al., 2021; Müller et al., 2022). This was visible for CMTT with 40–50 µg/g being released under dark and sunlight conditions (Figure 2E). Release of DPG from VCR and WCR was one order of magnitude lower, with 3–4 µg/g. The transformation of DPG to PG is a hydrolytic process. Nevertheless, sunlight exposure appears to support this transformation, as the PG concentration in the CMTT leachate continuously increased from 0.3 to 21 µg/g in artificial sunlight conditions (Figure 2F). Besides PG, a large number of transformation products have previously been reported to occur in tire leachates (Müller et al., 2022). Fohet et al. (2023) reported two DPG TPs, one being more stable (C<sub>19</sub>H<sub>23</sub>N<sub>3</sub>, 13 days) and the other less stable (C<sub>19</sub>H<sub>23</sub>N<sub>3</sub>O, 4 days) than DPG (8 days) under photoaging conditions.

To search for additional DPG related TPs a screening was performed in the LC-HRMS data of the CMTT leachates based on characteristic fragment ions (see section 2.6). In total, 23 putative



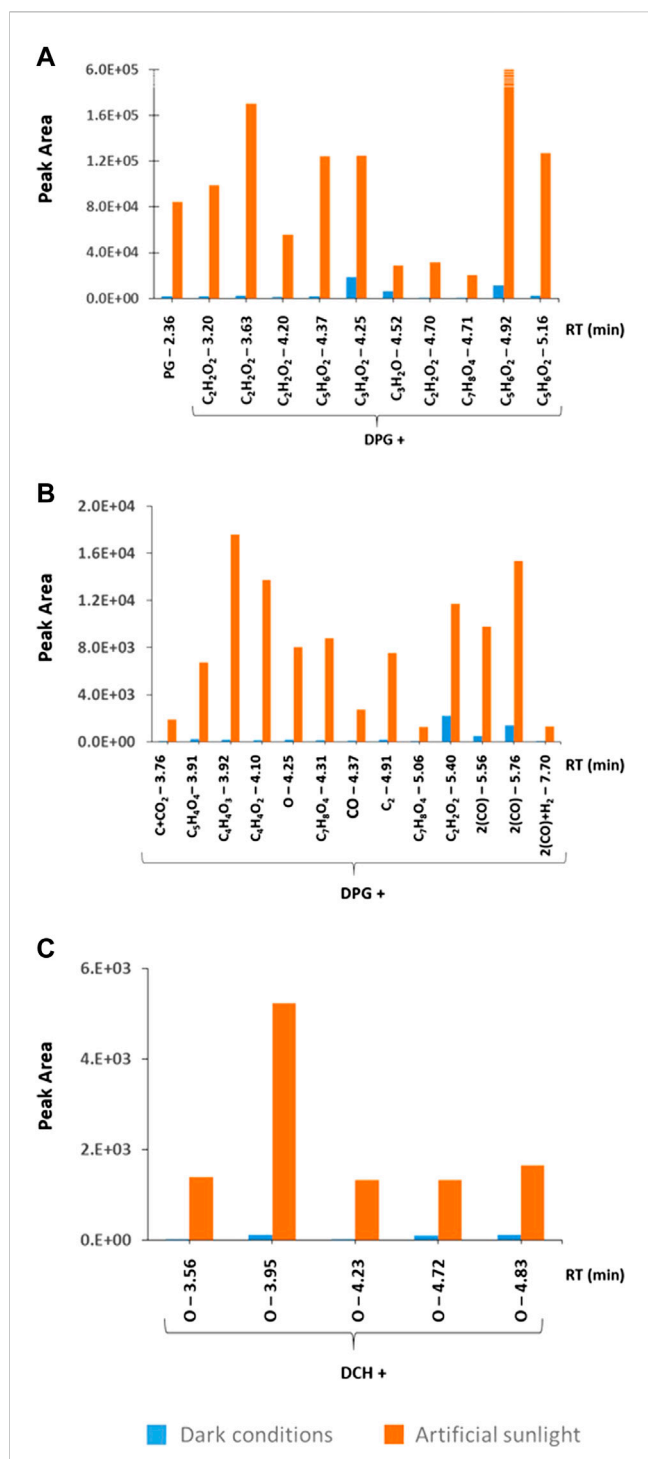


FIGURE 3

Peak areas of DPG transformation products (TPs) detected in the leachates of CMTT after 14 days (A) at high intensity and (B) low intensity, and (C) TPs of DCH. The peak intensities in the artificial sunlight exposed samples (orange color) are compared with the dark condition samples (blue color), highlighting the impact of UV irradiance on the formation of TPs from the considered parent compounds.

TPs were detected in the leachate after 14 days. Just one of these TPs (DPG + C<sub>3</sub>H<sub>3</sub>O<sub>2</sub>) corresponds to those reported by (Müller et al., 2022); all the others have not been reported before. Among others,

five isomers with the formula DPG + C<sub>2</sub>H<sub>2</sub>O<sub>2</sub> and three isomers corresponding to DPG + C<sub>5</sub>H<sub>6</sub>O<sub>2</sub> were detected (Figure 3; details provided in the Supplementary Table S4). Such sets of isomers point at their formation by radical processes, as those are often of limited regio-selectivity. It is not clear, if these 23 TPs were formed directly from DPG, or if they were formed from DPG related transformation products already present in the tire material. It has been shown that only 50% of the initial amount of DPG in a starting material may remain unaltered after rubber curing (Unice et al., 2015). Artificial sunlight strongly supported the formation of these TPs, as they were hardly detectable under dark conditions (Figures 3A, B).

DCH was the amine with the highest intensity found in all the leachates and is suspected to be formed by hydrolysis from different vulcanization accelerators, such as N,N-dicyclohexylbenzothiazole-2-sulphenamide (Seiwert et al., 2020). An amount of 20–50 µg/g was released from the three tested materials (Figure 2G). The lack of a difference between the three materials is unexpected. However, the concentration of a chemical detected in the leachate is not only a consequence of its concentration in the test material, but governed by several parallel processes such as the migration from the particles interior to its surface, the release from the particles into solution, the formation of precursor compounds and its transformation to TPs, either in the particles or in solution. For all three materials, artificial sunlight led to slightly increasing concentrations of DCH of approx. 25% (Figure 2G). Furthermore, a screening for DCH TPs was performed in the sunlit leachate, which all carried an additional hydroxy group (Figure 3C). These TPs were hardly detected in the dark condition samples.

Another chemical often reported is hexamethoxymethylmelamine (HMMM) (Alhelou et al., 2019). It was detected in all leachates (0.5–1.8 µg/g), exhibiting a relatively stable behavior in all samples (CMTT, VCR and WCR), with comparable concentrations both in dark and artificial sunlight conditions (Supplementary Table S3).

### 3.2.3 Antioxidants

Among the antioxidant compounds detected, 4-hydroxydiphenylamine (4-HDPA), a hydrolysis product of 6-PPD (Unice et al., 2015), was most abundant, with higher amounts released from CMTT than from VCR and WCR (Supplementary Figure S1). Since 6-PPD and diphenylamine react rapidly with reactive oxygen species and as 4-HDPA is also reactive (Seiwert et al., 2022), the 4-HDPA concentrations in the CMTT leachates under sunlight exposure were extremely low, with near-zero concentration values during the whole experiment.

The 6-PPD oxidation product 6-PPDQ (Tian et al., 2021) was detected in all leachates, with the amounts following the order CMTT (0.4 µg/g) > VCR (0.2 µg/g) > WCR (0.03 µg/g) (Figure 2H). The concentrations were always lower in the samples exposed to artificial sunlight, according to the reactivity of 6-PPDQ towards oxidative species. However, 6-PPDQ quantification was compromised by its adsorption to the PES membrane filters (>50%) used during the experimental procedure (Supplementary Figure S3). This data therefore needs to be considered with caution.

**TABLE 1** Percentages of the DOC contributions which can be explained by the quantified compounds under artificial sunlight (light orange) and dark conditions (grey) by CMTT a), VCR b) and WCR c).

	t1 (3 days)		t2 (7 days)		t3 (14 days)	
a)	Light	Dark	Light	Dark	Light	Dark
DOC Explained (%)	10	22	7	12	7	18
Vulcanization Accelerators (%)	9	20	6	11	6	17
Antioxidants (%)	1	2	1	1	1	1
	t1 (3 days)		t2 (7 days)		t3 (14 days)	
b)	Light	Dark	Light	Dark	Light	Dark
DOC Explained (%)	25	46	22	47	18	55
Vulcanization Accelerators (%)	24	25	21	46	17	54
Antioxidants (%)	1	1	1	1	1	1
	t1 (3 days)		t2 (7 days)		t3 (14 days)	
c)	Light	Dark	Light	Dark	Light	Dark
DOC Explained (%)	6	16	8	25	9	19
Vulcanization Accelerators (%)	5	15	7	24	8	18
Antioxidants (%)	1	1	1	1	1	1

### 3.3 Contributions of target compounds to DOC

For the 19 tire related chemicals quantified in the leachates (Supplementary Table S1), their contribution to the DOC amount was calculated, grouped into vulcanization accelerators (15 compounds, including thiazoles and guanidines) and antioxidants (4 phenylenediamine compounds) (Table 1). For all three tire materials, the explainable DOC is always significantly higher for the leachate generated in the dark than under sunlight exposure. This corresponds to the much higher DOC in the leachate with artificial sunlight. Obviously, sunlight exposure leads to the release of larger amounts of yet unknown compounds. Sunlight exposure transforms parent chemicals into TPs, many of which are either unknown or not available as reference compounds and are, therefore, not considered in this balance. A typical example for this are the 23 TPs of DPG, which were detected by LC-HRMS screening in the sunlit CMTT leachate, but could not be quantified (Figure 3, Supplementary Table S4). The oxidative transformation of 6-PPD has been shown to lead to an even larger number of TPs (Seiwert et al., 2022), three of which are available as standards and have been included in this study.

In all three tire materials, vulcanization accelerators played a predominant role in the explainable DOC. On the contrary, antioxidants explain only 1% of DOC in most cases. This is not only because of the lower number of quantified antioxidant compounds, but also due to the fast degradability of antioxidants through hydrolysis and oxidation, which may lead to an extremely high number of possible TPs (Seiwert et al., 2022).

For the CMTT leachates (Table 1), the explainable DOC during the UV exposure was 10% in the first timepoint but then decreased

to 7% as the amount of DOC significantly increased. In dark conditions, the explainable DOC corresponds to almost the double compared to the UV exposed samples. While the smaller particle size of the CMTT favors the release of all chemicals compared to the coarser VCR and WCR material, this effect can be expected to be stronger for chemicals with a lower diffusion coefficient, i.e., compounds of higher molecular weight, e.g., oligomers of HMMM and poly (1,2-dihydro-2,2,4-trimethylquinoline) (TMQ). These compounds were not included in the target list, which may explain the lower percentage of explained DOC for CMTT.

The WCR material, which has been weathered in marine water for about 1 year, may additionally release natural organic matter into the leachate, which was taken up during this long-time exposure to seawater. As a consequence of these different trends, the highest percentage of “explainable” DOC was obtained for VCR in the dark (55%) and the lowest percentages for CMTT and WCR under sunlight exposure (7%–8%).

## 4 Conclusion

This study investigated the leaching of tire materials (CMTT, VCR and WCR) while simulating marine environmental conditions as encountered in Europe during summer. DOC concentrations found in leachates increased from WCR over VCR to CMTT. For all three materials DOC increased significantly upon sunlight exposure, highlighting that sunlight favors the release of organic compounds from tire materials. Likely, particle aging and transformation of chemicals into more soluble products contribute to this enhanced leaching.

Of the 19 tire-derived chemicals that were quantified, BT, BTSA, 2-OHBT, DPG and DCH were released in largest amounts. Exposure of the tire materials to artificial sunlight during leaching increased the release of TPs such as BT, BTSA, and PG and led to the occurrence of 23 TPs of DPG and 5 TPs of DCH in the CMTT leachate, most of which were absent in the dark condition leachate. Accordingly, highly reactive chemicals, such as 2-MBT, 4-HDPA and 6-PPDQ, decreased in concentration. Although a total of 19 tire related chemicals were quantified, the largest proportion of the organic carbon released from the three tire materials remained of unknown origin.

This study outlines that the nature of chemicals released from tire material depends on the history of the tire particles and the leaching conditions. It indicates that the release of chemicals from larger tire particles, such as crumb rubber (3 mm mean diameter), can be a slow process taking several years.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed **Q11** to the corresponding author.

## Author contributions

AF, NS, DH, RS, and TR contributed to the conception and design of the study. AF and BS performed the chemical analysis. AF and NS wrote the first draft of the manuscript. All authors **Q12** contributed to the article and approved the submitted version.

## References

- Alhelou, R., Seiwert, B., and Reemtsma, T. (2019). Hexamethoxymethylmelamine – a precursor of persistent and mobile contaminants in municipal wastewater and the water cycle. *Water Res.* 165, 114973. doi:10.1016/j.watres.2019.114973
- Baensch-Baltruschat, B., Kocher, B., Stock, F., and Reifferscheid, G. (2020). Tyre and road wear particles (TRWP) - a review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. *Sci. Total Environ.* 733, 137823. doi:10.1016/j.scitotenv.2020.137823
- Fohet, L., Andanson, J. M., Charbouillot, T., Malosse, L., Lereboure, M., Delor-Jestin, F., et al. (2023). Time-concentration profiles of tire particle additives and transformation products under natural and artificial aging. *Sci. Total Environ.* 859, 160150. doi:10.1016/j.scitotenv.2022.160150
- Gewert, B., Plassmann, M., Sandblom, O., and Macleod, M. (2018). Identification of chain scission products released to water by plastic exposed to ultraviolet light. *Environ. Sci. Technol. Lett.* 5, 272–276. doi:10.1021/acs.estlett.8b00119
- Halsband, C., Sørensen, L., Booth, A. M., and Herzke, D. (2020). Car tire crumb rubber: Does leaching produce a toxic chemical cocktail in coastal marine systems? *Front. Environ. Sci.* 8, 1–15. doi:10.3389/fenvs.2020.00125
- Huntink, N. M. (2003). *Durability of rubber products*, PhD thesis. Enschede, Netherlands: University of Twente.
- Jeong, Y., Lee, S., and Woo, S. H. (2022). Chemical leaching from tire wear particles with various treadwear ratings. *Int. J. Environ. Res. Public Health* 19, 6006. doi:10.3390/ijerph19106006
- Johannessen, C., Helm, P., and Metcalfe, C. D. (2021). Detection of selected tire wear compounds in urban receiving waters. *Environ. Pollut.* 287, 117659. doi:10.1016/j.envpol.2021.117659
- Klöckner, P., Reemtsma, T., Eisentraut, P., Braun, U., Ruhl, A. S., and Wagner, S. (2019). Tire and road wear particles in road environment – quantification and assessment of particle dynamics by Zn determination after density separation. *Chemosphere* 222, 714–721. doi:10.1016/j.chemosphere.2019.01.176

## Acknowledgments

We thank Catherine Guigue and the PACEM-MIO platform for DOC analysis and Laure Papillon for seawater collection. The research was conducted as part of the JPI Oceans ANDROMEDA project. We thank the French Agence Nationale de Recherche (ANR-19-383 JOCE-0002-01), the German Ministry for Education and Research (BMBF; FKz 03F0850A) 384 and the Norwegian Research Council (311313/E40) for funding. **Q13**

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. **Q14**

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2023.1206449/full#supplementary-material> **Q15**

- Klöckner, P., Seiwert, B., Eisentraut, P., Braun, U., Reemtsma, T., and Wagner, S. (2020). Characterization of tire and road wear particles from road runoff indicates highly dynamic particle properties. *Water Res.* 185, 116262. doi:10.1016/j.watres.2020.116262
- Klöckner, P., Seiwert, B., Weyrauch, S., Escher, B. I., Reemtsma, T., and Wagner, S. (2021). Comprehensive characterization of tire and road wear particles in highway tunnel road dust by use of size and density fractionation. *Chemosphere* 279, 130530. doi:10.1016/j.chemosphere.2021.130530
- McIntyre, J. K., Prat, J., Cameron, J., Wetzel, J., Mudrock, E., Peter, K. T., et al. (2021). Treading water: Tire wear particle leachate recreates an urban runoff mortality syndrome in coho but not chum salmon. *Environ. Sci. Technol.* 55, 11767–11774. doi:10.1021/acs.est.1c03569
- Müller, K., Hübner, D., Huppertsberg, S., Knepper, T. P., and Zahn, D. (2022). Probing the chemical complexity of tires: Identification of potential tire-borne water contaminants with high-resolution mass spectrometry. *Sci. Total Environ.* 802, 149799. doi:10.1016/j.scitotenv.2021.149799
- Paluselli, A., Fauvelle, V., Schmidt, N., Galgani, F., Net, S., and Sempéré, R. (2018). Distribution of phthalates in Marseille Bay (NW Mediterranean sea). *Sci. Total Environ.* 621, 578–587. doi:10.1016/j.scitotenv.2017.11.306
- Para, J., Coble, P. G., Charrière, B., Tedetti, M., Fontana, C., and Sempéré, R. (2010). Fluorescence and absorption properties of chromophoric dissolved organic matter (CDOM) in coastal surface waters of the northwestern Mediterranean Sea, influence of the Rhône River. *Biogeosciences* 7, 4083–4103. doi:10.5194/bg-7-4083-2010
- Rauert, C., Charlton, N., Okoffo, E. D., Stanton, R. S., Agua, A. R., Pirrung, M. C., et al. (2022). Concentrations of tire additive chemicals and tire road wear particles in an Australian urban tributary. *Environ. Sci. Technol.* 56, 2421–2431. doi:10.1021/acs.est.1c07451
- Schulze, S., Zahn, D., Montes, R., Rodil, R., Quintana, J. B., Knepper, T. P., et al. (2019). Occurrence of emerging persistent and mobile organic contaminants in European water samples. *Water Res.* 153, 80–90. doi:10.1016/j.watres.2019.01.008

Seiwert, B., Klöckner, P., Wagner, S., and Reemtsma, T. (2020). Source-related smart suspect screening in the aqueous environment: Search for tire-derived persistent and mobile trace organic contaminants in surface waters. *Anal. Bioanal. Chem.* 412, 4909–4919. doi:10.1007/s00216-020-02653-1

Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., and Reemtsma, T. (2022). Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. *Water Res.* 212, 118122. doi:10.1016/j.watres.2022.118122

Selbes, M., Yilmaz, O., Khan, A. A., and Karanfil, T. (2015). Leaching of DOC, DN, and inorganic constituents from scrap tires. *Chemosphere* 139, 617–623. doi:10.1016/j.chemosphere.2015.01.042

Sohrin, R., and Sempéré, R. (2005). Seasonal variation in total organic carbon in the northeast Atlantic in 2000–2001. *J. Geophys. Res. Oceans* 110, C10S90–11. doi:10.1029/2004JC002731

Tian, Z., Gonzalez, M., Rideout, C. A., Zhao, H. N., Hu, X., Wetzel, J., et al. (2022). 6PPD-Quinone: Revised toxicity assessment and quantification with a commercial standard. *Environ. Sci. Technol. Lett.* 9, 140–146. doi:10.1021/acs.estlett.1c00910

Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., et al. (2021). A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* 371, 185–189. doi:10.1126/science.abd6951

Unice, K. M., Bare, J. L., Kreider, M. L., and Panko, J. M. (2015). Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. *Sci. Total Environ.* 533, 476–487. doi:10.1016/j.scitotenv.2015.06.053

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., and Reemtsma, T. (2018). Tire wear particles in the aquatic environment - a review on generation, analysis, occurrence, fate and effects. *Water Res.* 139, 83–100. doi:10.1016/j.watres.2018.03.051

Wagner, S., Klöckner, P., and Reemtsma, T. (2022). Aging of tire and road wear particles in terrestrial and freshwater environments – a review on processes, testing, analysis and impact. *Chemosphere* 288, 132467. doi:10.1016/j.chemosphere.2021.132467

Zahn, D., Mucha, P., Zilles, V., Touffet, A., Gallard, H., Knepper, T. P., et al. (2019). Identification of potentially mobile and persistent transformation products of REACH-registered chemicals and their occurrence in surface waters. *Water Res.* 150, 86–96. doi:10.1016/j.watres.2018.11.042

Zajíčková, Z., and Párkányi, C. (2008). Photodegradation of 2-mercaptobenzothiazole disulfide and related benzothiazoles. *J. Heterocycl. Chem.* 45, 303–306. doi:10.1002/jhet.5570450201