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New insights into the occurrence of vehicle related compounds in one of Europe's longest rivers

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ABSTRACT

Vehicle Related Compounds (VRCs) are considered emerging contaminants arising from road traffic. These compounds consist of a complex mixture of chemicals related to additives used in various parts of the vehicle such as brakes, tires or different vehicle fluids. The objective of this study was to investigate the occurrence of VRCs in surface waters in one of the longest rivers in Europe (Tagus River, Spain). Samples (n = 89) were collected during 2020–2022 from thirteen locations along the entire river in annual and seasonal campaigns and analyzed by liquid chromatography coupled to high-resolution mass spectrometry. A qualitative suspect workflow including an initial suspect list of 153 environmentally relevant VRCs was developed. Up to 26 compounds presented detection frequencies (DF) above 20 % in the investigated river waters. Vulcanizers, cross-linkers, bittering agents or corrosion inhibitors stand out among the main classes of additives where hexa(methoxymethyl)melamine (HMMM) and derivatives, denatonium benzoate (DB), benzothiazole-2-sulfonic acid (BTSA) and 1-H-benzotriazole (BTR) were the most frequently detected compounds (DF > 75 %). Among the high intensity suspects, the vulcanizers triisopropanolamine (TIPA) and 1,3-diphenylguanidine (DPG) and the corrosion inhibitor 5-methyl-1H-benzotriazole (Me-BTR) were also prominent. The detection of some tire-related markers and the fate of most of the identified VRCs highlighted a common anthropogenic origin, although variable sources for some of them are possible. This study illustrates the capabilities of suspect screening in assessing the presence of VRCs in river water and its potential as a monitoring tool to provide insights into the pollutant load influenced by parameters such as the urbanization degree.

1. Introduction

Road travel is currently one of the most common ways of mobility at a regional level comprising around 70 % of passenger and freight transport in Europe (Eurostat, 2024). Emission of greenhouse gases such as CO_2, CO or NO_x have been historically associated with pollution from road traffic. Nevertheless, the fingerprint from vehicles is much broader and the scientific community and recent regulations such as the future EURO 7 (European Commission, 2022) also have focused on non-gaseous emissions. For instance, tire and road wear, brake abrasion, fuel or leakage of other fluids used in automobiles (i.e. coolants or antifreeze) are well-known sources of vehicle-related compounds (VRCs) (Maurer et al., 2023). Among the latter, vehicle tires are one of the most representative carriers of VRCs, as dozens of additives are added to the rubber to minimize degradation and improve its properties (Johannessen et al., 2022a). Ultimately, these additives are released as pollutants to the environment and distributed into soil (Klöckner et al., 2021; Li et al., 2023; Wang et al., 2024), air (Cao et al., 2022; Johannessen et al., 2022b; Wang et al., 2022), snow (Challis et al., 2021; Maurer et al., 2023; Seiwert et al., 2022) or water (Peter et al., 2022; Rauert et al., 2022a, 2022b; Zhang et al., 2023), where they can have a significant environmental impact (Tian et al., 2021b).

Aquatic ecosystems are precisely one of the main receptors of VRCs acting as ultimate sinks where complex mixtures of several compounds have been reported (Rauert et al., 2022a, 2022b; Tian et al., 2020). Run-off near the source and urban stormwater are processes that play an important role in the arrival of VRCs to the water bodies (Chen et al., 2024; Peter et al., 2020), concretely, up to 20 % of the non-airborne Tire-road Wear Particles (TRWPs) can be transported to surface waters (Baensch-Baltruschat et al., 2021). A fact that exacerbates the problem is

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that not only the additives reach the aquatic ecosystem, but also related impurities or transformation products generated after release do (Chen et al., 2024; Hu et al., 2022; Peter et al., 2020). In some cases, these transformation products can be more harmful than the parent compounds. A representative example of this scenario was the high mortality rate observed in coho salmon associated to the degradation of the tire antioxidant N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) into a quinone-type derivative (6PPD-Q) (Tian et al., 2021b), which has been proven to be also toxic not only to other salmonids (French et al., 2022), but also to other fish species (Brinkmann et al., 2022; French et al., 2022). In recent years, studies on additional species have revealed significant toxic effects related to 6PPD and 6PPD-Q such as neuro-, hepato- or reproductive toxicity in mammals (Hua and Wang, 2023), oxidative damage and physiological disruption in invertebrates (Zhou et al., 2025) or phototoxicity and oxidative stress in algae (Yan et al., 2024).

Furthermore, special attention has been paid to other bicyclic N,N′-*p*-phenylenediamines (PPDs) antioxidants and their degradation products (Cao et al., 2022), as well as, to other kind of VRCs with similar origin (Zhang et al., 2023). Among them, guanidine vulcanizers such as 1, 3-diphenylguanidine (DPG) and 1,3-di-o-tolylguanidine (DTG) (Bautista et al., 2024; Rauert et al., 2022a), compounds related to the cross-linker hexa(methoxymethyl)melamine (HMMM) (Alhelou et al., 2019; Seiwert et al., 2020), benzothiazoles (Müller et al., 2022; Zhang et al., 2023) or benzotriazoles (Herrero et al., 2013) are ubiquitous and predominant VRCs in the environment, where high concentrations have been observed in surface waters (Alhelou et al., 2019; Johannessen et al., 2021; Rauert et al., 2020). The detection of VRCs in the environment is even more relevant because some of the previous contaminants also pose toxic and adverse effects for different organisms (Boisseaux et al., 2024; Obanya et al., 2025; Shi et al., 2019; Thodhal Yoganandham et al., 2025; Xu et al., 2024; Zhou et al., 2025). For example, DPG has shown acute toxic effects that affect development and behavior in aquatic organisms and has demonstrated *in vitro* genotoxicity (Thodhal Yoganandham et al., 2025). Benzotriazoles have also been found to have sublethal effects as endocrine disruptors, as well as, hepato- and neurotoxicity in fishes (Shi et al., 2019; Xu et al., 2024) and have shown to alter the molting frequency in invertebrates (Shi et al., 2019). Similarly, some benzothiazoles have been classified as potential carcinogenic substances and are capable of inducing oxidative stress in aquatic organisms (Obanya et al., 2025).

Regarding the European legislative framework for VRCs, in many cases the initial use of automotive additives is regulated as individual compounds in production processes by REACH regulation. However, later on, there is a significant legislative gap related to their environmental fate (Trudsø et al., 2022). The growing concern about VRCs occurrence and possible associated risks has led to the inclusion in early 2025 of 6PPD and 6PPD-Q in the 5th Watch List of the Water Framework Directive (European Commission, 2025). Nevertheless, many other VRCs still remain out of spotlight, and this is where efforts and advances of the scientific community in the early identification of emerging pollutants can contribute to fulfill this void (Klöckner et al., 2021; Zhao et al., 2023).

Vehicle additives and their related compounds reach the aquatic environments as part of complex cocktails of compounds where, additionally, they co-occur with other contaminants (Tian et al., 2021a). It is therefore necessary to develop specific analytical methods for the monitoring of VRCs in aquatic matrices. Target liquid chromatography MS/MS is often selected as a suitable technique for determining concentration of polar and/or mid-polar VRCs and have provided information on the concentrations of these compounds in aquatic matrices (Zhang et al., 2023) and surface river waters (Rauert et al., 2022a; Zhang et al., 2023) in different regions. However, target analysis leaves out of the picture many other potential contaminants such as contaminants of emerging concern, impurities and/or transformation products. Suspect and non-targeted screening using LC coupled to high-resolution mass spectrometry (HPLC-HRMS) are wide-scope alternatives to identify a greater number of compounds not included in target methods. These procedures are increasingly being used for the analysis of urban micropollutants such as VRCs, as they provide a more open strategy that is highly valuable for obtaining a first overview of environmental samples (Kang et al., 2024). Studies in aquatic compartments have demonstrated the utility of these broad spectrum screenings for conducting exploratory analyses and for assessing and monitoring water quality (Kang et al., 2024; Peter et al., 2022; Tarábek et al., 2024). The detection frequency, number of compounds and HRMS peak areas are considered as qualitative indicators to assess the ubiquity and occurrence of contaminants in several matrices (Dewapriya et al., 2023; Kang et al., 2024; Peter et al., 2022; Tarábek et al., 2024). For example, these parameters allow to establish temporal trends and variations (Tian et al., 2021a) and/or to evaluate the level of contamination and characteristics of a given sample (Tarábek et al., 2024). In particular, suspect screening may mitigate those challenges more commonly encountered in non-targeted analysis, such as the large amount of information, the high number of features or the identification of true unknowns, by focusing on a specific list of hundreds or even thousands of pre-selected or prioritized compounds (Been et al., 2021; Tian et al., 2021a). This makes suspect analysis a powerful tool for the monitoring and screening of a wide range of previously prioritized contaminants. This strategy has been successfully applied in recent years to the analysis of VRCs in aquatic ecosystems (Kang et al., 2024; Seiwert et al., 2020; Tian et al., 2020, 2021a).

In that sense, there is a need to expand knowledge about the presence of VRCs in European aquatic ecosystems where wide-scope analyses to characterize micropollutants not covered by target screening are still scarce. The aim of this study is to contribute to the field, in terms of compound coverage and temporal scope, by carrying out a comprehensive analysis using a suspect screening approach based on HPLC-HRMS to assess the occurrence of VRCs in surface waters from the Tagus River basin in Spain.

2. Material and methods

2.1. Study area and sample collection

The study area comprises the Tagus River basin which is one of the longest fluvial water bodies of the whole Iberian Peninsula (1,007 km) (Instituto Geográfico Nacional (IGN), 2025). The basin has its origin in the center of the peninsula where the Tagus River and its tributaries flow through important municipalities such as Guadalajara (Castilla-La Mancha; 0.27 million inhabitants, 2021), Madrid metropolitan area (3.3 million), Toledo (Castilla-La Mancha; 0.71 million) and Cáceres (Extremadura; 0.39 million) (Instituto Nacional de Estadística (INE), 2025). In general, the Tagus River basin has a relatively constant flow throughout the year, as it is regulated to prevent it from falling below the established ecological flow.

Because of its geographical location, road traffic, associated with both professional and recreational travels, is the main form of transport (Observatorio del Transporte y Logística en España (OTLE), 2025) and a major contributor to the anthropogenic impact in the area. Vehicle travels are mainly concentrated around the main urban centers with average vehicle densities in 2022 of 58,434 vehicles/day (vh/day) in Madrid, 9,546 vh/day in Castilla-La Mancha and 6,786 vh/day in Extremadura (Ministerio de Transportes y Movilidad Sostenible, 2022a).

In the current study, 89 river surface water samples were collected between 2020 and 2022 at thirteen locations (S1 to S13) along the basin (Fig. 1 and Table S1). Three annual sampling campaigns were carried out in autumn (October–November) of each year and, in addition, quarterly campaigns (winter, spring, summer and autumn) were performed in 2022 to assess possible seasonality. Average rainfalls within the basin showed that wet weather periods usually occur during March–April and October–November with average monthly rainfall

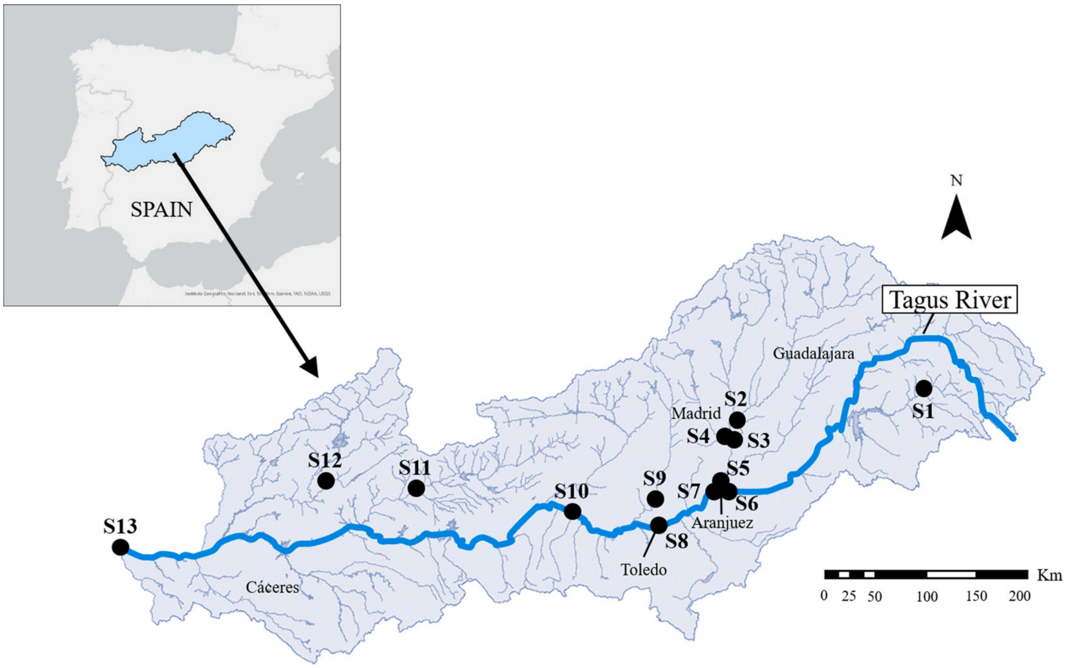

Fig. 1. Location of the selected sampling points S1 to S13 in the Tagus River basin.

exceeding 2–3 mm (Sistema Automático de Información Hidrológica (SAIH Tajo). Ministerio para la Transición Ecológica y el Reto Demográfico. Confederación Hidrográfica del Tajo, 2025).

Samples were classified according to the river flow, number of inhabitants and average daily traffic as sites of low, medium and high anthropogenic impact (Text S1). Table S2 and Fig. S1 provide information about their classification and road networks around each location. Water samples (2L) were collected in polypropylene (PP) bottles previously cleaned with acetonitrile/methanol. After collection samples were immediately transported to the laboratory and frozen at −20 °C until their preparation and analysis within the same week. Further details related to study area and sampling collection and preservation can be found in a previous work (Royano et al., 2023).

2.2. Sample preparation

Samples were processed in a previous study using a generic SPE methodology (Johannessen et al., 2021; Rauert et al., 2020, 2022a; Royano et al., 2023) and the extracts were retrospectively analyzed for the screening of VRCs. Briefly, 1 L of pre-filtered surface water samples were extracted and purified on Oasis HLB cartridges (6 mL, 500 mg; Waters, Milford, MA, USA) adequate for extraction of medium-polarity analytes (Johannessen et al., 2021). Cartridges were previously preconditioned with 10 mL of both methanol and Milli-Q water. Samples were loaded and passed through SPE cartridges and dried under negative pressure for 1 h. Analytes were eluted with 10 mL of methanol and nitrogen-dried to 300 μL in a TurboVap evaporator and, finally, were transferred to glass amber vials. Field blanks were extracted in parallel for each set of samples.

Samples were spiked before extraction with internal standards (ISTDs) available at the time in the laboratory (Tables S3 and S4). These standards were used for normalization of peak areas of detected VRCs in a similar approach as reported in the literature (Kang et al., 2024). In addition, after sample treatment, extracts were spiked with injection standards (ISS) and instrumental analysis was immediately conducted. Good extraction efficiencies (70–120 %) were obtained for different internal standards available at the time of sample preparation in the laboratory (Royano et al., 2023). These values were in accordance with those observed following similar procedures for different VRCs (Rauert et al., 2022a). See quality assurance and quality control details at supplementary material for more information (Text S2).

2.3. Instrumental analysis

Samples were analyzed by ultra-high performance liquid chromatography coupled to HRMS (UHPLC-QTOF HRMS). Chromatographic separation was carried out in a UHPLC SCIEX ExionLC™ AC system (SCIEX, MA, USA) using a C18 Securityguard column (2.1 × 4.6 mm) and a Luna® Omega C18 (1.6 μm, 100 Å, 100 × 2.1 mm i.d) as analytical column from Phenomenex (Torrance, CA, USA). Milli-Q water (2 mM ammonium formate; 0.1 % formic acid) and methanol (2 mM ammonium formate; 0.1 % formic acid) were used as mobile phases at a flow rate of 0.35 mL/min for a total run of 22 min. Gradient details and more information about the chromatographic conditions are included in Table S5.

The chromatographic system was coupled to a X500R QTOF MS/MS mass spectrometer equipped with an electrospray ionization (ESI) source. Positive ionization mode (ESI^+) was preferred for the analysis due to a greater number of features and higher efficiency provided for VRCs detection (Peter et al., 2018). MS/MS high-resolution data were acquired using Data Independent Acquisition method (DIA) that allows obtaining more information and a potential retrospective analysis of the data. Mass spectral runs were performed in the range of 100-1,100 Da for TOF-MS survey and 40-1,100 Da for MS/MS scans in 23 windows. Collision energy (CE) was set at a compromise value of 35 V with a CE spread of ±15 V. Complementary analyses were also performed in Data Dependent Acquisition (DDA) to improve the mass spectral quality in confirmation experiments. Comprehensive details regarding mass spectrometric acquisition are shown in Tables S6 and S7.

2.4. Data processing

All samples were processed using a suspect screening workflow as described in Text S3 and depicted in the general scheme shown in Fig. S2.

A comprehensive review of the extant literature yielded an initial list of 153 suspect compounds, which included relevant environmental VRCs (Table S8). Mass spectral information of suspects was compiled from scientific papers, property mass spectral libraries (NIST, SCIEX All-in-one HRMS) and online mass spectral databases (e.g., MassBank Europe, MassBank of North America, m/z cloud, Human Metabolome Database) including precursor and fragment information to increase confidence in identification.

Features were identified based on the widely accepted 5-confidence level Schymanski scale (Schymanski et al., 2014) (identification levels; IL, 1 to 5), considering a 70 % match with libraries (when available), a maximum accurate mass error of 5 ppm and an isotopic pattern difference among 5–20 % (Zhang et al., 2021). When reference mass spectra were not available for automatic software processing, manual inspection was performed by comparison with compiled mass spectra from literature or online databases (Kang et al., 2024). In-silico fragmentation using ChemSpider tool within SCIEX OS was also used as additional evidence for structural confirmation. Identification workflow followed in the current study was performed according to details provided in Fig. S3.

Reference standards (n = 25) included in Table S3 were used for confirmation of identified compounds in the proposed suspect approach. Unambiguous identification of the compounds detected in the samples was based on the criteria described above (i.e. library match, mass error, isotopic profile) and, in addition, on the coincidence of their retention time (RT±0.3 min) under the same analytical conditions.

Feature-by-feature manual review was conducted to check peak integration, identification and MS interpretation, if needed. Blank subtraction was performed for each feature reported in the peak table and only those features 10 times above the average field blank samples were considered (Krauss et al., 2019). Then, peak areas were normalized by sample-specific ISTD ratio using the average peak area of ISTDs detected in the same sample (Peter et al., 2022) (Table S4). The use of peak areas is considered a valid strategy as qualitative indicator for characterizing the pollutant burden, assessing general trends and/or identifying differences between sampling points (Kang et al., 2024; Peter et al., 2022; Tarábek et al., 2024). This approach can also support the identification of potential sources of emerging contaminants (Peter et al., 2022). The number of detected compounds per sample and detection frequency (DF, %) were also used in the current study as indicators of pollution burden. In this case, DF for each compound is defined as the number of detections of a suspect feature, identified according to Schymanski's scale, divided by the total number of samples.

3. Results and discussion

A total of 46 compounds out of the initial suspect list of 153 VRCs were identified at different confidence levels (Table S9; Fig. S4). A detailed list of the normalized areas for VRCs detected in the analyzed samples is included in Table S10, while mass spectra for all of them are compiled in supplementary material-3.

3.1. Chemical characterization

The majority of the VRCs detected in river waters are related to tires (n = 40), while the rest (n = 6) are used in vehicles for other purposes (e.g. vehicle fluids such as coolants, antifreeze, fuel) (Johannessen et al., 2022a). Among them, we identified parent additives (1 antioxidant, 10 vulcanizers, 4 corrosion inhibitors, 1 cross-linker, 4 antislipering agents, 1 stabilizer, 1 lubricant, 1 bittering agent), but also an important number of miscellaneous compounds such as impurities (2) and transformation products (13).

Fig. 2 shows the normalized areas of the 26 VRCs with DFs above 20 % (Table S11). Box plots for all the 46 identified compounds (Fig. S5), as well as their individual contribution (Figs. S6 and S7) are detailed at supplementary material. In terms of the total number of compounds detected in all samples, the Tagus River surface water was strongly dominated by tire additives used as vulcanizers (sum of all detected compounds, 344, 25 %), corrosion inhibitors (156, 11 %) and transformation products (434 compounds, 31 %) (Figs. S8 and S9).

3.1.1. Predominant compounds

The compounds which generally presented the highest normalized areas were DPG, 5-methyl-1H-benzotriazole (Me-BTR), 1-H-benzotriazole (BTR), triisopropanolamine (TIPA) and denatonium benzoate (DB) (DF > 60 %). Most of them are used as tire additives though other automotive uses cannot be excluded (Li et al., 2023).

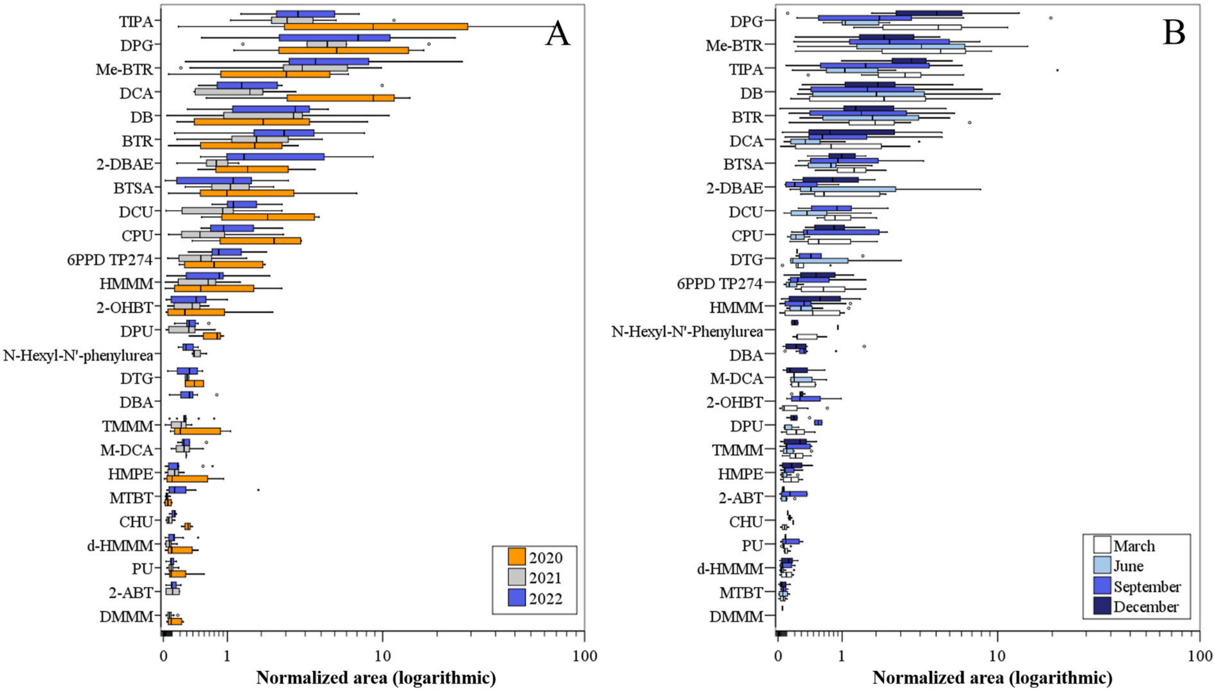

Fig. 2. Box and whisker plots of the normalized area (logarithmic scale) of the 26 VRCs with DF > 20 % in river water in the different sampling campaigns (A: annual and B: seasonal).

BTR, Me-BTR and DPG have been ubiquitously detected in surface waters from different parts of the world (Rauert et al., 2022a; Tarábek et al., 2024), including Spain (Herrero et al., 2013) and in many cases co-ocurring at high concentrations in the same ecosystem (Li et al., 2023; Rauert et al., 2022a; Tian et al., 2020). In contrast, although their environmental levels and distribution do not appear to be negligible (Peter et al., 2020), TIPA and DB have been analyzed less frequently in vehicle-related environmental samples.

DB is primarily used as a food additive due to its exceptional bittering properties, but also added to coolants or antifreeze in the automotive industry as a deterrent to prevent ingestion (Peter et al., 2020). In the current study, DB was detected in more than 80 % of the analyzed samples with significant peak intensities, especially in urban locations (e.g. S3 and S4), being in agreement with recent studies in snowmelt (Maurer et al., 2023) and stormwater (Peter et al., 2020) from roads. The ubiquity and environmental concentrations found in the aquatic system might be supported by its persistency and the difficulties to enhance its degradation in wastewater treatment plants (Lege et al., 2019; Maurer et al., 2023).

BTR and Me-BTR are used as corrosion inhibitors in rubber-based products and their presence in the environment has been linked to road traffic when detected in environmental matrices, such as urban air (Johannessen et al., 2022b), roadside soils (Li et al., 2023) or aqueous matrices including surface waters (Herrero et al., 2013; Rauert et al., 2022a; Tian et al., 2020). As it is shown in Fig. 3F, Me-BTR was consistently detected in greater contribution in river waters. Interestingly, both analytes remain almost undetected in low anthropogenic areas such as S1, S6 and S11, while an important contribution (half of the total normalized area) was observed at S13. It could mainly be due to the high solubility of BTR and Me-BTR compared with other VRCs, which are distributed primarily during storm events (Tian et al., 2020). This could explain why they are found in areas far from the major urban centers (Tian et al., 2021a).

TIPA (DF = 62 %) and DPG (DF = 64 %) are both vulcanizers used as accelerators in the vehicle industry (Chen et al., 2024; McMinn et al., 2024). Related to the latter, DTG (DF = 27 %) was also detected in the Tagus River. The contributions of DTG were relatively lower (Fig. 3A), a fact that could be related to its lower solubility and seems to be consistent with concentrations found in Spanish rivers (Schulze et al., 2019). DPG has been recognized as indicator of tire contamination in waters (Zahn et al., 2019) and also found as the predominant compound in surface waters from Australian urban tributaries (Rauert et al., 2022a), leachates and roadway runoff (Chen et al., 2024; Peter et al., 2018), snowmelt from Canada (Challis et al., 2021) or stormwater runoff in China (Zhang et al., 2023). Interestingly, in the current study, the detection of DPG in S10, S12 and S13 in 2022 resulted in a higher DF and greater contribution to the total normalized area, which could be related to the increased rainfall observed in areas downstream of the basin this year. DPG is well known for its sorption capacity to particulate matter like TRWPs (Kang et al., 2024; Xie et al., 2024), thus its distribution strongly depends on rainfall events. Therefore, concentrations in water bodies may undergo important temporal variations depending on the periods of drought, the precipitation regime or the magnitude of the runoff processes around each sampling (Müller et al., 2022). In the Tagus River, seasonal samplings during 2022 revealed higher DPG areas in the wetter season (March and December, Fig. S10) compared to the dry period (Mann-Whitney U test, $p < 0.05$; Fig. S11). On the other hand, TIPA has been classified as an industrial compound with possible uses in vehicles (Peter et al., 2020), but it is rarely described in the tire wear literature. Its origin from tires could be related to the rubber crumb (U.S. EPA & CDC/ATSDR, 2019) that is sometimes added to asphalt to improve its elasticity and durability, although recently it has been detected in Tire-wear Particles (TWPs) lixiviates (McMinn et al., 2024), which might be a more likely source for aquatic environments. It is therefore plausible to be detected with high frequency in river samples due to runoff during storm events (Peter et al., 2020), especially considering its water solubility. In contrast to DPG, the normalized areas of TIPA were higher in urban areas in 2020, but decreased in subsequent

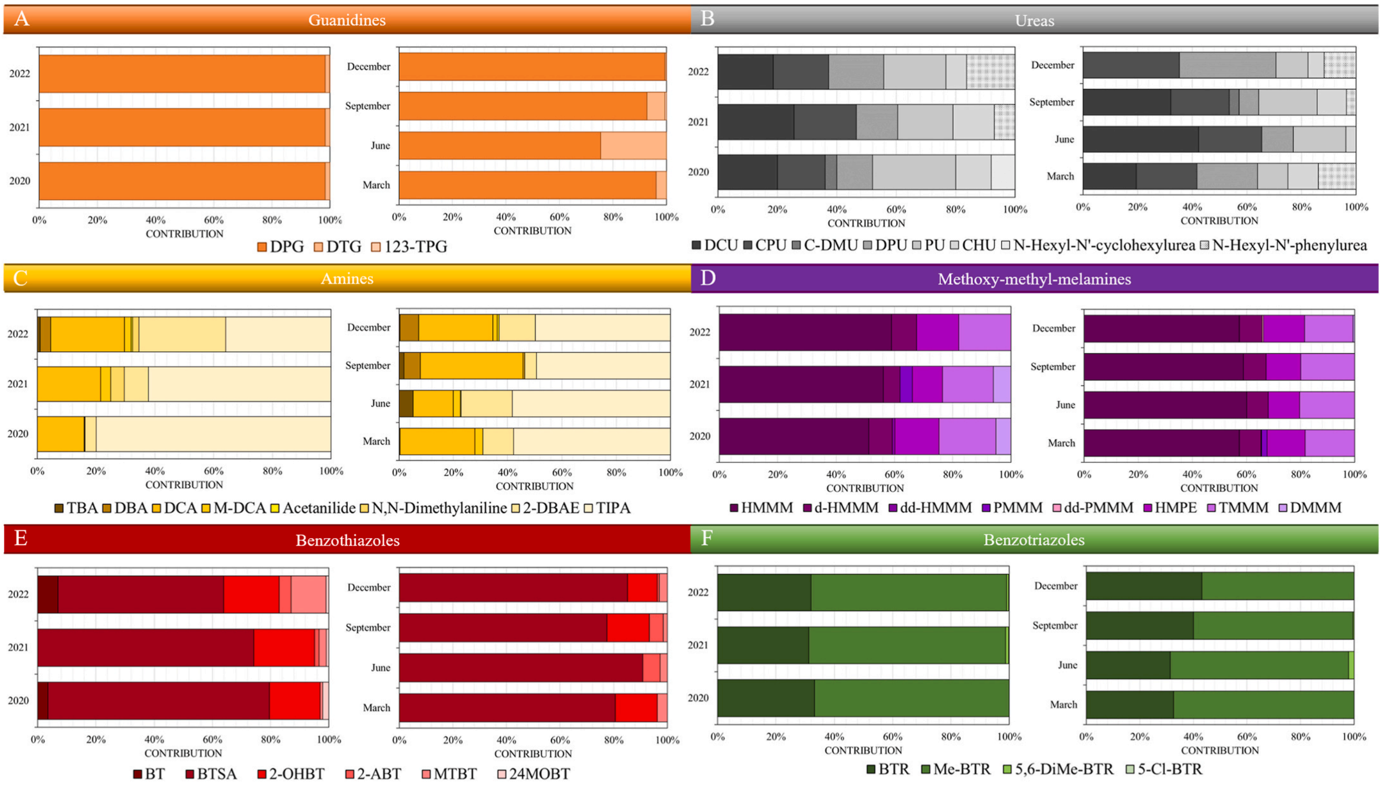

Fig. 3. Compound contributions (%) per chemical class of (A) Guanidines, (B) Ureas, (C) Amines, (D) Methoxy-methyl-melamines, (E) Benzothiazoles and (F) Benzotriazoles in annual (right) and quarterly (left) sampling campaigns.

years (Kruskal-Wallis H test, $p < 0.05$; Fig. S6).

3.1.2. Antioxidants

Antioxidants are added to tires and other products (Cao et al., 2022) to protect the rubber from oxidative degradation caused by factors such as oxygen, ozone, heat, and ultraviolet light. Among them, PPDs such as 6PPD have proven to be particularly effective and it is therefore not surprising that their presence and that of their transformation products has been reported in the environment (Hua and Wang, 2023).

In the present study, 6PPD was not detected in river waters, but traces of 6PPD-Q were found in few samples from 2020 to 2021 (DF = 9 %). 6PPD-Q (Fig. S12) was among the minority compounds showing peak intensities below 5×10^4 cps and low average contributions (<1 % to the total normalized area in each sample). This result would be coherent with the much lower concentration and detection frequency found in river waters for 6PPD in comparison with 6PPD-Q (Johannessen et al., 2021; Zhang et al., 2023), probably due to the stability and short half-life of 6PPD (Lane et al., 2024). The presence of 6PPD-Q is relevant due to it has been recognized as a tire marker in other environmental matrices (Klöckner et al., 2021). It has been previously reported in watersheds of different countries (Challis et al., 2021; Johannessen et al., 2021; Rauert et al., 2022b), but its study in Spain had been limited to recycled tire rubber materials (Duque-Villaverde et al., 2024).

A feature eluting at 12 min was detected in 53 of the samples (6PPD TP274; DF = 60 %) with relatively large peak intensities and a mass spectra that showed characteristic fragmentations of bicyclic amines (Fig. S13). Peak intensity of this feature was 3-fold higher than that of 6PPD-Q. 6PPD TP274 was found in TWPs and stormwaters by Peter et al. (2018) and later confirmed by Zhao et al. as an ozonation product of 6PPD from a ring cleavage of the phenylenediamine ring resulting in an unsaturated dicarbonyl diamide structure (Zhao et al., 2023). The occurrence of 6PPD TP274 is remarkable, as it has been detected in air (Johannessen et al., 2022b), road dust (Klöckner et al., 2021), road run-off and stormwater (Hu et al., 2022; Peter et al., 2018; Zhao et al., 2023) and it has been proposed as a plausible abundant marker for tire contamination (Hu et al., 2022). The results provide additional data on its environmental fate in the aquatic environment and the studied watershed.

Zhao et al. (2023) suggested that the 6PPD TP274 could be formed from N-cyclohexyl-N′-phenyl-1,4-phenylenediamine (6QDI), but the latter chemical was not identified in river samples from this study. Similarly, other PPDs and transformation products remain undetected, although they were included in the original suspect list (Table S8).

3.1.3. Cross-linkers

In tires, cross-linker additives are used to improve rubber properties such as strength, durability and elasticity. HMMM stands out among them, although it is also used in other applications in coatings and plastics products (Alhelou et al., 2019).

In samples from the Tagus River, HMMM has been by far the most frequently detected compound (Fig. S14, DF = 91 %), being within the 15 most intense peak features in each sample, a behavior also observed by other authors (Kang et al., 2024; Krauss et al., 2019; Peter et al., 2018). The normalized areas and contributions of HMMM showed some variability between points along the river which may be influenced by anthropogenic activity and possible industrial discharges (Dsikowitzky and Schwarzbauer, 2015). Its occurrence has been also reported in river waters from Australia (Rauert et al., 2020), North America (Peter et al., 2018), Asia (Peng et al., 2018) or Europe (Alhelou et al., 2019) with concentrations even up to the range of μg/L. However, to date, its presence in Spain has only been associated with wastewater from an automobile industry (Consejo et al., 2005).

Other structurally related compounds belonging to the same family have been identified in the analyzed samples (see Figs. S15–S19). For example, it is remarkable the detection of 3 analytes with large peak areas (DF > 70 %, IL 2a) identified as d-HMMM, hexamethylolmelamine pentamethyl ether (HMPE) and tetra(methoxymethyl)melamine (TMMM) with strong positive correlations among them ($r_s > 0.95$, $p < 0.01$; Table S12), suggesting a common origin thereof. The degradation of HMMM has been addressed previously, pointing out d-HMMM, HMPE and TMMM as possible biotransformation products of the parent compound (Alhelou et al., 2019; Johannessen et al., 2021). Transformation products of lower molecular weight and with an even number of methoxy groups have a higher water solubility and stability, which may lead to an increasing persistence and mobility (Alhelou et al., 2019) and may explain the greater normalized areas observed for HMPE and TMMM in comparison with the formylated analogue d-HMMM.

A fourth less intense peak (DF = 22 %) was identified as di(methoxymethyl)melamine (DMMM, Fig. S19). The eluting region of this feature was consistent with its log D and its mass spectra showed fragments from $[C_5H_7N_6]^+$ (151.0727) and $[C_4H_7N_6]^+$ (139.0735) units (Alhelou et al., 2019). Interestingly, this compound was predominantly detected in samples from 2020 (DF = 61 %) and 2021 (DF = 69 %) in comparison with 2022 (DF = 3 %). Other minor peaks (IL 3; DF < 10 %) were observed in river samples presumed to be double diformylated-HMMM (dd-HMMM), penta(methoxymethyl)melamine (PMMM) and double diformylated-PMMM (dd-PMMM) based on common *m/z* mass fragments of the methoxy-methyl-melamine family and their coherent relative position in the chromatograms (Figs. S20 and S21). These compounds have been also detected in lower concentrations than the major HMMM derivatives in environmental samples (Bobeldijk et al., 2002; Johannessen et al., 2021; Krauss et al., 2019; Peter et al., 2018) and TWP leachates (Müller et al., 2022; Peter et al., 2018).

Transformation products belonging to the whole degradation chain have been reported in surface waters in European (Alhelou et al., 2019; Krauss et al., 2019) and North American rivers (Peter et al., 2018) with a significant (more than half) contribution compared to the HMMM. It is in accordance with the behavior observed in the Tagus River based on normalized peak areas (Fig. 3D), where HMMM contribution was followed by HMPE > TMMM > d-HMMM > DMMM. This result evidences the largest presence of methoxy-methyl-melamine derivatives in the Spanish fluvial ecosystem.

No significant annual and seasonal differences were obtained for this family of compounds based on Kruskal-Wallis H test ($p > 0.05$; Fig. S22).

3.1.4. Vulcanizers and transformation products

Vulcanizers are used in the automobile industry to improve physico-mechanical properties of the materials by accelerating or retarding the vulcanization reaction during polymer synthesis. It constitutes the largest group of compounds (n = 10; 3 guanidines, 4 amines, 2 ureas and 1 benzothiazole) in the analyzed samples having a whole significant contribution (Fig. S9).

The vulcanizer retarder 1,3-diphenylurea (DPU; DF > 40 %) was found simultaneously with other urea compounds with similar structural backbone, but a more diffuse use in tires like 1-cyclohexyl-3-phenylurea (CPU) and 1,3-dicyclohexylurea (DCU) (DF > 50 %) (Peter et al., 2018). These contaminants have been described as common pollutants in water samples affected by traffic related pollution and often co-occur with other VRCs such as HMMM (Peter et al., 2018; Rauert et al., 2020). Interestingly, a strong correlation between the aforementioned urea additives with cross-linkers related to HMMM was observed (Table S12), indicating a possible common origin in the Tagus River.

Benzothiazole (BT) has been related with tire contamination and classified as a vulcanization accelerator (Zhang et al., 2023) and/or a possible transformation product (Müller et al., 2022). This compound was rarely detected in the Tagus River (only 4 samples: 2020 -S2, S6, S9- and 2022 -S10-). In general, low intensities and poor peak shapes were observed, possibly due to the fact that its analysis is also susceptible by gas chromatography. Its presence in vehicle-impacted matrices does not seem to be as dominant and widespread as that of other VRCs, but it has been found in high concentrations (0.2–15 μg/L) in runoff water samples

(Zhang et al., 2023).

Four other compounds structurally related to BT were detected in samples from the Tagus River classified as vulcanizers, transformation or reaction products (Fig. 3E). 2-benzothiazole sulfonic acid (BTSA) presented the highest frequency of detection (78 %) and a high normalized peak area (among the top 10 most abundant compounds), which is coherent with previous investigations and may be considered one of the most recalcitrant compounds of the tire-related benzothiazole derivatives (Maurer et al., 2023; Seiwert et al., 2020). As for other VRCs, peak intensities were typically higher near urban centers, but a higher dispersion was observed with occasional detections also at points of lower anthropogenic influence (S1, S6, S11 and S13).

2-hydroxybenzothiazole (2-OH-BT), 2-aminobenzothiazole (2-ABT), 2-(methylthio)benzothiazole (MTBT), and 2-morpholin-4-yl-benzothiazole (24-MoBT) presented a lower DF and co-exist in aquatic matrices in relevant concentrations (Kloepfer et al., 2005; Zhang et al., 2023). 2-OH-BT, a transformation product of 2-mercaptobenzothiazole, was also identified as the compound with the highest contribution after BTSA, a fact coherent as reported elsewhere (Zhang et al., 2023).

3.2. Spatio-temporal distribution and sources

Samples collected at different sampling points along the basin were compared to identify possible trends or factors contributing to VRC occurrence in the Tagus River basin such as specific characteristics and/or possible local sources of contamination.

Higher median values were observed for those locations with a high anthropogenic impact ($p < 0.05$; Fig. 4), particularly, where the main urban settlements are located (city of Madrid; S2, S3, S4 and S5). Among them, those points located in the metropolitan area (S3 and S4) stand out in terms of normalized area and number of detected compounds when compared with S2 (upstream) and S5 (downstream). As would be expected, these results clearly evidence that population density and road traffic are major sources of these pollutants in the aquatic ecosystem. Some of the VRCs detected in this study such as DPG, methoxy-methyl-melamines, 3-cyclohexyl-1,1-dimethylurea (C-DMU) or dibenzylamine (DBA) have indeed been proposed as chemical indicators of urban water quality as they have shown differences in runoff samples according to the urbanization level (Peter et al., 2022).

Notably, S7, S8 and S9, located downstream of the city of Madrid, showed a lower pollutant load in terms of normalized area, but a high number of detected VRCs (up to 27 per sample). S7, located in the Jarama River (Tagus River tributary), collects the flow from the city of Madrid. However, the normalized areas observed at this point decrease compared to S5 due to the dilution associated with the fresh input of the Tagus River from less polluted areas (S6). Site S8 is the same river as S7 with no additional inputs among these two locations, then rather similar normalized areas and chemical profiles were observed between these points (Fig. S23). S9 has significantly lower traffic and population density than the previous points, but the low flow of the river at this point contributes to median areas similar to that observed upstream. Downstream (S10 to S13), the distance from the main sources (roads and large urban centers), dilution by new inputs from areas with less traffic, and possible degradation processes result in lower normalized areas. VRCs detected in these less urbanized areas could also have some contribution from agricultural activities (Tian et al., 2021a).

No general temporal or seasonal differences were obtained considering both the total number of compounds and normalized areas ($p > 0.05$; Figs. S24 and S25), although higher areas have been observed for few individual compounds in wet weather samples (6PPD TP274, DPG, CPU and TIPA; $p < 0.05$). This general behavior could be influenced by several factors. On one hand, the Tagus River is a heavily dammed river and, as other Mediterranean basins, its flow is highly regulated to ensure the ecological biodiversity and water quality (Royano et al., 2023). On the other hand, traffic conditions around the watershed tend to decrease during summer (Ministerio de Transportes y Movilidad Sostenible, 2022b) when precipitations are minimal, while the opposite occurs during the wet period, thereby minimizing seasonal differences. Limitations in establishing spatio-temporal trends for some VRCs due to different possible sources for these compounds have been reported in literature (Rauert et al., 2020).

Further data analysis to characterize the chemical profile of the sampling points was performed using Principal Component Analysis (PCA) (Tables S13 and S14). The first component (PC1, 55 % of the variance; Fig. S26) may be related to tire derived compounds and the anthropogenic impact at each sampling location since less impacted locations clustered with negative contributions of PC1. The results in this component revealed similar weights for several VRCs (Table S13) which suggest similar sources, transformation processes and/or environmental fate (Wang et al., 2025). In PC2 (11 % of the variance), DTG shows a significant contribution to this component pointing a potential anthropogenic origin related to vehicular traffic, but less associated to tires in this study. Indeed, DTG separated from other guanidines such as DPG, a compound with which it has shown a weak correlation in other studies in traffic-related samples (Li et al., 2023). The corrosion inhibitors BTR and Me-BTR also stand out in this component and remain separated from other VRCs, which could be related to its distribution by other mechanisms beyond storm water runoff (Tian et al., 2020) or their widespread use in various products (Xie et al., 2024). Other non-tire compounds as the bittering agent DB (Peter et al., 2020) or 2-N-dibutylaminoethanol (2-DBAE), used as a possible stabilizer in fuels (ECHA, 2025), also have a strong weight in PC2. Interestingly, in PC3 (8 %), the

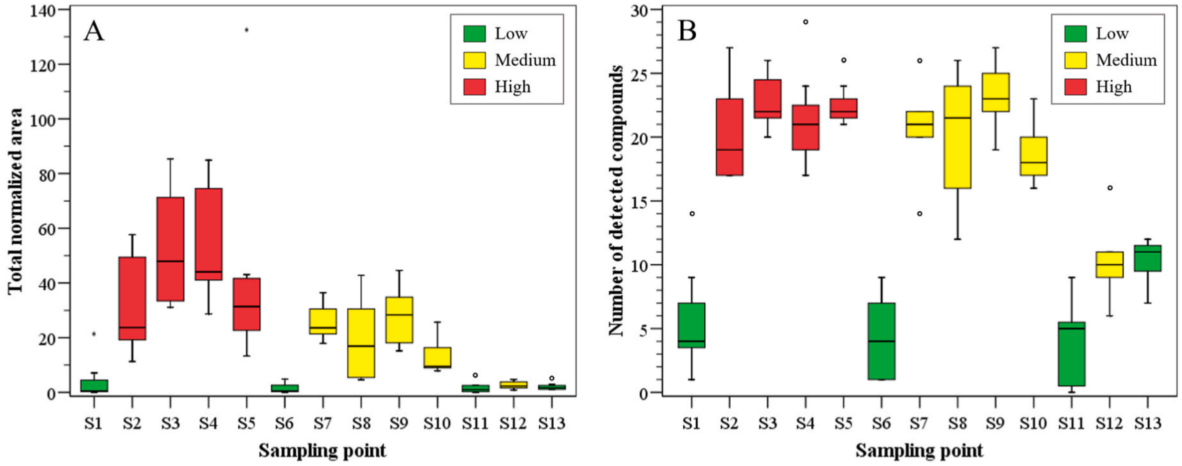

Fig. 4. Box and whisker plots of the (A) $\sum$VRCs (as normalized area) and (B) number of detected compounds at each sampling location.

samples from the urban location S4, are grouped mainly in the negative part of the loading plot (Figs. S27 and S28) which would point local pollution sources. The origin from vehicles appears less in this component, probably due to multiple sources of some VRCs (Li et al., 2023; Tian et al., 2021a; Xie et al., 2024). For example, positive contribution to this component was observed in the score plot for N-hexyl-N'phenylurea and DBA. The former shows one of the weakest correlations with other VRCs (Table S12), suggesting a more diffuse use in vehicles. DBA is used as a vulcanizing agent in tires, although other possible direct and indirect sources have been proposed (Kuntz et al., 2024).

4. Conclusions

A wide variety of VRCs with different uses in the automobile industry have been reported using a suspect analysis workflow applied to surface river waters collected from one of the largest fluvial ecosystems in the Iberian Peninsula. 26 of the detected VRCs showed DF > 20 %, among which, vulcanizers, corrosion inhibitors or transformation products stand out, with some of these being reported for the first time in Spanish surface waters.

This analysis revealed the widespread distribution (DF > 60 %) and prevalence of compounds such as DPG, Me-BTR, BTR, TIPA and DB. Additionally, the presence of certain markers of contamination by tire-derived compounds was identified, including the transformation product of the antioxidant 6PPD, 6PPD TP274, and the cross-linker HMMM. The latter was the most frequently detected compound in the analyzed samples (DF > 90 %), and, alongside it, several transformation products belonging to the same family, such as d-HMMM, HMPE, TMMM and DMMM (DF > 20 %) have also been identified.

The qualitative approach used in this work allowed to obtain a general overview of the distribution and contaminant load in the analyzed river waters. Normalized areas were consistently higher in urbanized locations in comparison with agricultural and remote ones, indicating that higher traffic density around major urban centers is a key factor to VRC occurrence. Nevertheless, further research is required to better characterize the environmental fate and concentration levels of these compounds within the European aquatic environment.

CRediT authorship contribution statement

Juan Escobar-Arnanz: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Silvia Royano:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Irene Navarro:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Adrián de la Torre:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **María Ángeles Martínez:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123158.

Data availability

Data will be made available on request.

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