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Past, present and future considerations

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Published in:
Science of the Total Environment

DOI:
[10.1016/j.scitotenv.2019.135694](https://doi.org/10.1016/j.scitotenv.2019.135694)

Publication date:
2020

Document Version
Peer reviewed version

Citation for published version (APA):

Halle, L. L., Palmqvist, A., Kampmann, K., & Khan, F. (2020). Ecotoxicology of micronized tire rubber: Past, present and future considerations. *Science of the Total Environment*, 706, Article 135694.
<https://doi.org/10.1016/j.scitotenv.2019.135694>

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PII: S0048-9697(19)35689-X

DOI: <https://doi.org/10.1016/j.scitotenv.2019.135694>

Reference: STOTEN 135694

To appear in: *Science of the Total Environment*

Received date: 16 September 2019

Revised date: 12 November 2019

Accepted date: 21 November 2019

Please cite this article as: L.L. Halle, A. Palmqvist, K. Kampmann, et al., Ecotoxicology of micronized tire rubber: Past, present and future considerations, *Science of the Total Environment* (2019), <https://doi.org/10.1016/j.scitotenv.2019.135694>

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Ecotoxicology of micronized tire rubber: past, present and future considerations

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Keywords: microrubber; elastomer; tire/tyre wear; TWP; polymer; microplastic.

Abstract

Micronized tire rubber has recently come into focus as black particles that are found in microplastic (MP) samples worldwide. These particles have been found in all environmental compartments with the most likely source being the abrasion of car tires on road surfaces. Thus, it is well founded that tires are a source of MPs and that tire abrasion is a primary source of anthropogenic particulates. Currently, the impact of tires has been viewed through the lens of particulate pollution together with MPs, but this is a relatively new direction for this topic. Previously ecotoxicological research into the environmental consequences of tires has primarily been related to the leached chemicals from tire particulates. This paper aims to (i) highlight similarities and differences of micronized rubber particles with the existing suite of polymer contaminants termed as ‘microplastics’ or ‘plastic debris’, (ii) survey the existing literature on environmental presence, fate, and interaction of micronized rubber particles with biota, and lastly (iii) present future research needs that require consideration in order to move this research area forward. Existing knowledge gaps that require attention include; determining the environmental presence and fate of micronized rubber within different environmental compartments, understanding the interaction of rubber particles with biota, particularly as potential impacts have so far been attributed solely to the leachate, and evaluating whether standard ecotoxicological protocols need to be adapted for particulate contaminants in general and specifically to suit rubber particulates and leachate.

1. Introduction

Micronized tire (alternative spelling 'tyre') rubber particles have been found in all environmental compartments, with the most likely source being the abrasion of car tires on road surfaces (Wik and Dave, 2009; Kole et al., 2017). Thus, it is well founded that tires are a source of microplastics (MPs) and that tire abrasion is a primary source of anthropogenic particulates. Owing to the apparent ubiquity of tire debris, tire-related ecotoxicology is not a new topic per se, but rather a topic that seems to resurface with shifting foci. Currently, the impact of tires are being viewed through the lens of particulate pollution together with MPs, but this is a relatively new direction for the topic as previous ecotoxicological research into the environmental consequences of tires has primarily been related to the leached chemicals from tire particulates (Hartwell et al., 1998; Wik and Dave, 2006a; Turner and Rice, 2010).

In order to summarize the current status and outline the future direction of rubber-related research, it is first necessary to appreciate the long history of research in this field (Table 1). Starting in 1895, the first pneumatic car tire was produced by the Michelin Brothers in France and by the 1950s the modern tire used today, the radial tire, was produced. The first research paper on emission and identification of rubber particles in road dust emerged in 1966 (Thompson, 1966), and few years later considerations on the sizes of particles, abrasion patterns and the potential toxicity of tire particles followed (Cardina, 1974; Dannis, 1974; Pierson and Brachczek, 1974). Attention was then redirected to an increased recognition on the process of tire degradation and its derived products during the 1980s, for instance the release of heavy metals along highways in connection to rubber particles (Williams and Cadle, 1980; US EPA, 1981; Rowley et al., 1984; Warren and Birch, 1987). The focus then

shifted in the 1990s to concentrate predominantly on the impact of scrap tires in aquatic environments by examining the suite of chemicals in the water phase originating from scrap tires, referred to as leachate (Day et al., 1993; Maltby et al., 1995b, 1995a; Hartwell et al., 1998; Davis et al., 2001). In the first decade of the new century, research was directed towards repurposing of tire debris in turf fields and playgrounds (Källquist, 2005), and finally, micronized tire rubber has become grouped with other polymers described as ‘microplastics’ in the 2010s. More specifically, in 2015 and 2017 it was estimated, based on calculations of mileage data, emission factors for passenger car tires and commercial vehicles, and average tire lifespan and wear data, that micronized tire rubber particles represents the majority of MPs in the environment (Lassen et al., 2015; Boucher and Friot, 2017; Kole et al., 2017).

The global production of rubber used in the tire industry has risen from 25.1 million tonnes in 2011 to 29.1 million tonnes in 2018 (MREPC, 2019), a 15.9 % rise over the course of 8 years. The annual production of 2018 can be almost equally divided into two classes of rubber, the natural rubber (latex) from the rubber tree, *Hevea brasiliensis* (14.0 million tonnes), and the synthetic rubber, primarily styrene-butadiene rubber (SBR, 15.1 million tonnes) (MREPC, 2019). Synthetic rubber is the primary component of a passenger car tire and the particulate emissions that are released from tires are split into airborne or non-airborne emissions. It has been estimated that 90.0-99.9% of the total emissions released from car tire abrasion are non-airborne (Unice et al., 2013), whilst research on airborne particulates heavily outweighs research on tire particulates in other environmental compartments (Pierson and Brachczek, 1974; Kim et al., 1990; Adachi and Tainosho, 2004; Kreider et al., 2010; Abbasi et al., 2017). Recently, it has been estimated that 18 % of micronized tire rubber is

discharged from land to freshwater (Unice et al., 2019a, 2019b), underlining the importance of research within this area.

Recent reviews on micronized tire rubber particles have focused primarily on the generation, the physicochemical properties of released rubber particles, and the occurrence, detection and analysis of the particles in the environment (Kole et al., 2017; Wagner et al., 2018). Although recognizing the need for biological effects data, this has not been the main aim of these papers. The present paper aims to place ecotoxicology of micronized rubber particles within the broader landscape of MP research. In doing so we (i) highlight similarities and differences of micronized rubber particles with the existing suite of polymer contaminants termed as ‘microplastics’ or ‘plastic debris’, (ii) survey the existing literature on environmental presence, fate, and interaction of micronized rubber particles with biota, and lastly, (iii) present future research needs that require consideration.

2. Placing micronized tire rubber in the polymer landscape - relationship to other polymer particulates

The ecotoxicology of micronized tire rubber, in its latest incarnation as part of the MP dialogue, whilst still in its infancy, has a great deal of terminological baggage; and perhaps the first research gap that needs addressing is the clarification of a common nomenclature. Primarily, the placement of micronized tire rubber in the polymer landscape needs to be established to further elucidate the relationship between different polymer types (Figure 1).

Particulate debris can be comprised of natural molecules (e.g. sand or dust particles) or be anthropogenic in origin. The umbrella term ‘anthropogenic polymer particulates’ is used to

describe all man-made polymer particulates. These can be organized in different ways, the most common being either thermoplastic or thermoset polymers. Thermoplastic elastomers and thermoset plastics also exist, but for the purposes of this paper the thermoplastic or thermoset division is considered appropriate. The term ‘thermoplastic’ applies to polymers with the ability to soften when heated and harden when cooled. This includes most plastic polymers, defined as high molecular weight polymers that can be shaped by flow (ISO, 2013). These are the plastic polymers that eventually become environmental plastic debris or MPs covering a range of sizes (e.g. nano, micro, meso, macro). The more recently established traits that are relevant for describing plastic debris are also highly relevant for micronized tire rubber, namely, chemical composition, origin (primary or secondary), solubility, size, shape (morphology), color and additives (Frias and Nash, 2019; Hartmann et al., 2019; Rochman et al., 2019). The thermoset polymers, on the other hand, are irreversibly hardened by curing (cross-linking). Most elastomers fall under thermoset polymers and are described as macromolecules with the ability to rapidly return to their initial dimension after deformation (ISO, 2012). They can be divided into either natural or synthetic elastomers, with a slight overlap between the two since a synthetic version of natural rubber can be produced. Both natural and synthetic elastomers are present in tires. Natural elastomers are commonly known as latex rubber, whereas the synthetic elastomers are all other forms of elastomers, such as styrene, butadiene and many others used for different purposes.

The nomenclature for tire rubber particles has often been defined within individual publications, with terms and abbreviations being applied inconsistently across the literature; Fauser et al. (2002) used two terms to describe particulate matter from tire abrasion e.g. ‘tire tread abrasion’ and ‘tire debris’. Wik (2007) primarily used the phrase ‘tire rubber’ to

describe the complex mixture of the variety of chemicals present in tires, but terms such as ‘worn tread rubber’, ‘tire wear particles’ and ‘tire components’ were also presented in later work (Wik and Dave, 2009). Kreider et al. (2010) used three terms to describe particulate matter from different compartments, ‘roadway particles’ (RP), ‘tread particles’ (TP) and ‘tire wear particles’ (TWP). RP was used to describe road surface particles found in the environment, TP was used to describe particles from tire treads and TWP described the combination of road, environment and tire tread. Marwood et al. (2011) used a combination of all of the above with the term ‘tire and road wear particle’ (TRWP) referring to a mixture of tire tread particles and ‘road dust’ e.g. brake, clutch and road wear, much like what Kreider et al. (2010) termed TWP. More recently, Wagner et al. (2018) used the term ‘tire wear particles’ (TWP) to describe a specific part of traffic related non-exhaust particulate emissions, namely particulates formed from tires undergoing friction on the road. Lastly, in life cycle assessments and regulatory or political contexts the term ‘end-of-life tires’ (ELT) have been used to describe worn tires that are repurposed in different scenarios (WBCSD, 2008). Most recently, Hüffer et al. (2019) used the term ‘tire crumb rubber’ (TCR) to describe the fraction of recycled and shredded end-of-life tires, a term previously referenced in Alamo-Nole et al. (2012).

Thus, there is a clear need to clarify the terminology to describe different particulate forms of micronized tire rubber and how they should be applied. Within Figure 1, we propose the umbrella term microrubber (MR) to cover all rubber particles in the micrometer size-range (up to 1 mm). This encompasses micronized elastomer particles from e.g. shoe soles to tires and are particles either abraded or weathered from larger products. Below MR, we propose to place three tire-related terms, all of which are found in the literature, but are often used

synonymously and therefore must be defined in each publication: (1) tire-road-wear particles (TRWP), (2) tire-wear particles (TWP) and (3) end-of-life-tires (ELT). We propose that TRWPs include all particles abraded from tires when used on road. This is the most likely form to be sampled from the environment. TWPs does not include road wear and is therefore the most likely form to be generated for laboratory experiments, by manual abrasion or by simulation, and is thus less likely to be sampled from the environment. This definition of TWP also includes the term ‘tread particle’ (TP) as is described in Fauser et al. (2002), Kreider et al. (2010) and Klöckner et al. (2019). ELTs represent worn tires that are repurposed for alternative applications such as artificial turf. When repurposing, ELTs are ground into ‘crumb rubber’, which is separated by size (defined by ISO) into chip rubber (10-50 mm), granulated rubber (1-10 mm), ground vulcanized rubber (0.1-1 mm) and fine powdered rubber ≤ 0.1 mm (ISO, 2012).

In order to adopt a consistent nomenclature, in this paper, we will use these terms as follows: MR to compare to MPs, TWP when discussing results from laboratory-generated particles and TRWP when considering the presence and fate of tire particles found in the environment.

2.1. Physicochemical characteristics of particles - microrubber compared to microplastic

Different physical and chemical characteristics of polymer particulates impact their fate and effects in the environment, making it important to distinguish between polymer particle types and their differences in composition, origin, size, morphology and density. MR shares some physical characteristics with MPs, but in some important regards MR differs. All polymers

are built by linking repeating monomer units to form the polymer backbone, varying monomer units are the fundamental difference between polymer types. Natural rubber is commonly synthesized by linking isoprene units resulting in 2-methyl-1,3-butadiene, where the natural form is always in cis-configuration, that is cis-1,4-polyisoprene (Fried, 2003). The chemical composition of MR from tires is a mixture of various polymers, with the primary elastomer in a passenger car tire being a 1:3 mixture of styrene:butadiene (synthetic) rubber, whereas the natural form of rubber is more prevalent in truck and other heavy-duty tires (Hirata et al., 2014).

MPs arise from either primary or secondary sources as plastic pellets or particles abraded from larger items, but MRs will mainly be of secondary origin. This can be either as particulates from tire abrasion or repurposed rubber granulate, ground from whole worn tires (ELTs). The size and morphology of polymer particles may play an important role in the hazard potential of the particle (Wright et al., 2013). Larger fragments could cause physical harm to organisms and smaller particles might penetrate the cellular barriers as seen with nanoparticles (Geiser et al., 2005; García-Alonso et al., 2011; Gaspar et al., 2018).

Depending on the ruggedness of fragmentation or abrasion from the primary polymer product, and the presence of longer fibrous material compared to finer spherical particles, implications on ingestion and gut retention time might also be affected differently between MPs and MRs (Wright et al., 2013) (see section 3.2.). Densities of MPs range from 0.85 to 1.45 g cm⁻³ (Quinn et al., 2017), whereas densities of MR particles has been determined in a closer range between 1.15-1.20 g cm⁻³ (Degaffe and Turner, 2011; Dumne, 2013; Banerjee et al., 2016; US FHA, 2016). Albeit MP particles spans a wider range of densities, allowing some particles to float, sediment has been acting as a sink, retaining a great deal of MP debris

(Browne et al., 2011). This scenario is also most likely for MRs, but despite their density, that would theoretically make all MR particles sink, they have been located in all environmental compartments (Wik and Dave, 2009). One modeling scenario that might explain this show that size, shape and aggregation have been estimated to have greater influence than density and biofilm production on the fate of micro-sized particles in freshwater (Besseling et al., 2017).

The ratio of crystalline to amorphous regions in polymers could influence the sorption of organic contaminants to different polymers, as amorphous regions are prone to interact with foreign substances, contrary to crystalline areas (Fried, 2003). All polymers consist of both crystalline and amorphous regions, and therefore organic contaminants have more or less affinity towards micro-sized polymer particles depending on polymer type (Velez et al., 2018; Hüffer et al., 2019). Elastomers tend to comprise of more amorphous than crystalline regions, hence MR particles have previously been examined for their potential to retain organic compounds due to this amorphous nature. ELTs from used tires have thus been shown to reduce the amount of volatile organic compounds present in field construction and as ‘leachate collection material’ (e.g. using ELTs to collect the chemicals leached from other sources) in municipal solid waste landfills and water (Gunasekara et al., 2000; Edil et al., 2004; Lisi et al., 2004; Aydilek et al., 2006; Alamo-Nole et al., 2011, 2012). This hypothesis has also been tested on metal solutions where different mechanisms in tires are prominent in absorbing metals, most noticeably an ion exchange mechanism that displace zinc(II) with cadmium or mercury, and lead seems to use a separate mechanism (Rowley et al., 1984). A plausible site for metal and MR particle interactions is the locations of carbon black molecules in tires (Snoeyink and Weber, 1967). MR thus has a great affinity for both organic

contaminants and metals, and therefore an increased chemical load could be expected in MR particulates. The changing nature of different polymers potential to adsorb and absorb contaminants renders it difficult to evaluate possible ecological and toxicological implications of different polymers under the same scope. Especially since it is not determined if this affinity of surrounding contaminants to MR will serve to make them more or less bioavailable.

2.2. Chemical characteristics of released chemicals

In addition to distinguishing between the physical characteristics of polymer types, there are differences in their chemical characteristics. The suite of leached chemicals will invariably differ between polymer material and with ambient abiotic factors (temperature, pH, salinity, and UV exposure) which will influence the physicochemical dynamics of the process (Hüffer et al., 2019). MPs contain a variety of additives including plasticizers, flame-retardants, pigments, biocides, antioxidants, stabilizers and fillers depending on form, color or function (Fried, 2003). Like MPs, MRs contains a variety of additives that are present in tires depending on brand and type, some of which differ from the chemicals in MPs. These include carbon black, curing agents, fillers (e.g. silica, CaCO_3), extender oils and softeners (PAHs), pigments, metals (especially zinc) and several different antioxidants, biocides and oxonates. The latter three include compounds such as aniline, benzothiazole and methylbenzothiazole. This variety of MR additives result in >100 organic compounds present on a chromatogram of a MR sample scan on GC-MS (Abernethy et al., 1996) making it extremely complex to analyze which groups of compounds are of more interest when assessing environmental risk and potential biological impacts of MR.

Different studies have employed different extraction protocols to produce leachates, though as yet there is no standard definition of what MR leachate contains or the concentrations of the various chemical components. Usually, higher temperature and longer equilibrium time helps maximize the portion of leached chemicals, and continuous suspension and shaking can hasten this process. To mimic the temperature of a road-surface on a warm summer's day Wik and Dave (2006) prepared leachate at 44°C, this same temperature was used by Marwood et al. (2011). Differing salinities for the leaching of shredded ELTs in three sequential 7-d leaching periods has also been used by Hartwell et al. (1998). They found that toxicity decreased with increasing salinity demonstrating the importance of the extraction conditions. Under environmentally relevant conditions, the suite of chemicals leached out from polymer particulates include i) the chemicals used for manufacturing of different polymer materials, ii) the chemicals added to polymer materials, such as additives and fillers and iii) the pollutants possibly adhered to the particulates during their lifecycle and later weathering. How these interact with each other and the ambient environment is key to understanding potential toxicity resulted from chemicals leached from MR.

2.3 Trojan horse and vector mechanisms

The role of particulate contaminants in chemical transfer has been widely studied and two main mechanisms have been described namely, 'Trojan horse' and vectors. The 'Trojan horse' terminology was introduced into particulate ecotoxicology primarily as a description of how engineered nanoparticles, usually with a metal or metalloid constituent, may cross a biological membrane and release their metal intracellularly via dissolution (Lubick, 2008;

Luoma et al., 2014). The localized increase in labile metal would then be available to cause cytotoxicity. Whilst MPs also contain additives, the main topic of research in relation to chemical transfer for plastics has been its role in transfer of sorbed exogenous chemicals from the environment to biological tissue, the so-called ‘vector effect’ (Syberg et al., 2015). Here, MPs depending on their degree of crystalline structure can sorb hydrophobic organic contaminants or other contaminants such as trace metals or pharmaceuticals from the environment and deliver them into an organism after the MP is ingested (Syberg et al., 2015; Ziccardi et al., 2016). The release of the sorbed chemical within the gastrointestinal environment may result in localization and/or damage that would not have been possible if the organism had encountered the chemical in the water column (Besseling et al., 2013; Khan et al., 2015, 2017a). In this way the uptake route of a chemical may be altered from waterborne uptake to dietary uptake. Thus the main difference between ‘Trojan horse’ and vector effects are whether the chemical is part of the particle or whether it is picked up from the environment. If MR particles act more like a Trojan horse or a vector is not known at this stage, but both mechanisms are equally relevant to investigate since chemicals are added from production and have recently been shown to be sorbed from the environment (Hüffer et al., 2019). Understanding how MR is capable of delivering chemical loads to biota via either or both ‘Trojan horse’ and vector mechanisms is an important step in furthering our knowledge of how MR behaves in the environment and interacts with biota.

3. Environmental presence and fate of microrubber, and interaction of microrubber with biota – current research status

3.1 Presence and detection of microrubber particles and leachate

Tires are produced using different formulations and ratios of natural and synthetic elastomers under different national legislations in order to change properties of wear, grip and rolling resistance. This contributes to the diversity of particle pollution in the environment, as has been already suggested for MPs (Rochman et al., 2019). Collecting MP samples in the environment is primarily conducted in water (manta trawl), sediment (density separation) or biota (tissue digestion) via a variety of sampling techniques each suitable for the particular environmental compartment (Lusher et al., 2017; Hurley et al., 2018; Simon et al., 2018). As yet, MP sampling lacks a standardized set of protocols and expression of concentration, for instance MPs are normalized to both volume and area from the water compartment, which often renders direct comparisons between studies difficult. Similar sampling methods and lack of standardization is met when working with MR, but later analysis is made more complicated by the presence of carbon black, which absorbs almost all light, decreasing the reflection and transmission used to measure and determine polymer type (FTIR, Raman). Thus to date, the presence of MR in the environment has rarely been verified by sampling, but instead has been estimated from production loads and calculated emissions, or detected in environmental compartments by the use of specific marker chemicals that are already present in tires.

Among the few sampling papers on MR, the majority focus on the airborne fraction, even though estimates show that 90.0-99.9% of MR produced from driving will not be in the airborne fraction (Unice et al., 2013). Over the years, several emission estimates have been

calculated for different countries based on expected life time of tires, average driving patterns and data on tires sent for repurposing, an extensive and updated compilation of these data are found in Kole et al. (2017). Detection of MR in environmental compartments based on marker chemicals have been investigated on/off since the 1980s, although, finding a compound or a group of compounds that is derived solely from MR and not from other anthropogenic sources is challenging. The most commonly used marker compounds are benzothiazoles (Spies et al., 1987; Kim, 1990; Kumata et al., 1997; Kumata et al., 2002; Ni et al., 2008) and zinc (Fauser et al., 1999; Councell et al., 2004; Klöckner et al., 2019). Few papers on wastewater and outlet gradients from factories list some of the compounds found in water downstream from tire-production plants (Dzikowitzky and Schwarzbauer, 2014). A suite of chemicals associated to the tire industry, especially benzothiazole, were measured 100 km downstream of a tire production plant (Puig et al., 1996), indicating that some of these compounds have an ability to persist in the aquatic environment. Therefore, evaluating and especially quantifying the presence of MR based on single-substances as sole proxies should be done cautiously to avoid inaccurate estimations. One of the reasons for the lack of data on the natural concentrations of TRWP is the need for protocols and unity in chemical analysis of TRWP in different environmental matrices. The presence of tire debris has been measured with a variety of methods, especially in air, but methods for determining presence in other compartments are lacking along with reliable quantification (discussed further in section 4.1.).

Few papers have tried comparing the laboratory made TWP to MR particulates sampled in the environment (Kreider et al., 2010; Wagner et al., 2018) in an effort to establish relevance when extrapolating from laboratory conditions to realistic environmental scenarios. Wagner

et al. (2018) clearly illustrates the size and morphology differences of MR particles sampled by mechanical shredding, road simulation and road runoff. This differing morphology is controlled by many factors such as velocity, force and from which surface abrasion occurs on. The morphology of MR has generally been described as elongated, jagged, warped, porous, near spherical and irregular (Camatini et al., 2001; Kreider et al., 2010). These descriptions align with MR particles we sampled off the roadside, as a pilot experiment, along a freeway in Denmark (Elsinore freeway, 55°49'50.7"N 12°31'25.7"E) (Figure 2). The road-side sample (A) was collected by using a gel-tape to collect abraded particles present on the road surface and the water sample (B) was collected by placing a tube under the grills leading water off the freeway, to sample all floating particles (it was raining during sampling). These were compared to laboratory produced particles made with a cryomill (Retsch) (C) or by abrasion on a coarse grinding stone (D). The latter used for toxicology experiments of both particles and leachate in Khan et al. (2019). The morphology of particles, sampled in the environment or laboratory generated, were diverse, generally exhibiting more elongated and rugged particles than spherical ones. As Wagner et al. (2018) explains, TRWP are most likely not just pristine material, rather a multicomponent blend of particles differing in physical and chemical properties from the original tire material. This could result in a distinctive chemical load for each particle that would possibly influence the behavior and subsequent effects of that particle in the environment.

It is important to realize that particulate exposure is a mixture of the particle as well as the chemicals leached within a given timeframe. Thus, particle exposure will never be just particle exposure, rather a combination of particle and leachate exposures. The bioavailability of the particle and of the released chemicals likely differ within environmental compartments.

All of the individual compounds in the leachate can be altered by abiotic factors and influence each other (i.e. competition and complexation). Furthermore, leachate is not one single component, but rather a complex suite of chemicals with diverse affinities for various environmental compartments. Thus, there is no one fate for MR, rather there are several fates; one of the particle itself, and numerous for each single chemical leached out, governed by the ambient environment of a given compartment.

Such complexities in the chemical analysis and fate determination of MR and its component chemicals highlight the complicated nature of MR as a contaminant, whilst its apparent ubiquity in nature suggest that solutions to determining environmental presence and fate need to be quickly realized. Moreover, there is mounting evidence that a likely fate for MR is ingestion by aquatic biota and the impacts of this need to be addressed as discussed in the following sections.

3.2. Ingestion and gut retention of microrubber particles

Proof of TWP ingestion by benthic invertebrates has been recently published (Redondo-Hasselerharm et al., 2018a; Khan et al., 2019), although it was mentioned as an observation in earlier work by Wik and Dave (2009). Redondo-Hasselerharm et al. (2018a) used a method of tissue digestion (Löder et al., 2017), modified with a chitinase step to remove the chitine rich exoskeleton, to separate TWP from the bodies and faeces of *Gammarus pulex*. An average of 2.5 and 4 TWP per organism were found in bodies and faeces, respectively, after exposure to 10% TWP in sediment. Comparing this to earlier data on a similar experiment with MPs (Redondo-Hasselerharm et al., 2018b) showed, that although the total number of

ingested particles were similar for MPs and TWPs, the number of TWPs retained in the gut was higher (Redondo-Hasselerharm et al., 2018a), indicating a longer gut-retention time for TWPs than MPs. Similarly, providing evidence for ingestion of TWPs by invertebrates, Khan et al. (2019) used a qualitative method by taking pictures of *Hyalella azteca* ingesting and depurating TWP after exposure in water-only treatments. A gut retention time of TWPs for *H. Azteca* was established at 24-48 hours. Earlier studies on *H. Azteca* exposed to MP particles and fibers showed that it took a significantly greater amount of time to excrete MP fibers than MP particles or natural food items. Also, the gut clearance time for fibers increased in a dose-dependent manner (Au et al., 2015). These observations suggest MRs may possibly have a longer gut retention time than MPs, but such inferences need to be verified. It is necessary to understand the underlying mechanisms behind gut retention time, in order to properly assess the possible risks associated with aquatic biota ingesting different types, sizes and shapes of polymer particulates. The likely release of toxicants from MR particles to organisms may also change when particles are ingested and enter the gastrointestinal tract.

Figure 3 depicts the passage of MR through the gastrointestinal tract of the crustacean opossum shrimp commonly called mysids (Family: *Mysidae* sampled in Herslev Harbour in Roskilde Fjord, Denmark 55°40'40'' N 11°59'09''). Individuals were exposed to TWP (generated as described in Khan et al., 2019) dispersed in water for a period of 10 hours and then moved to clean water in order to depurate for the following 48 hours.

Ingestion, depuration and fecal pellets produced were observed by light microscopy. The stomach fills within the hour and the entire gut within 5 hours. The gut appeared empty after

6 hours of depuration, but the fecal pellets still contained TWP fragments at 48 h (Figure 3). In keeping with other studies (Redondo-Hasselerharm et al., 2018a; Khan et al., 2019), MR was shown to be ingested by mysids. These observations also indicate that the gut transit alone may not be sufficient for determining gut retention time since fecal pellets still contained TWP long after the GI tract had appeared empty. These observations echo the need to further investigate gut passage and retention times in a range of aquatic organisms and furthermore determine whether chemicals are leached following MR ingestion or during the timeframe that MR is retained in the gastrointestinal tract.

3.3. Toxicity and adverse effects of microrubber particles and leachate

Over the past 30 years toxicity and effects of MR have been examined in the aquatic compartment, primarily focusing on the leachate fraction (Hartwell et al., 1998; Gualtieri et al., 2005; Wik and Dave, 2005b, 2006a; Wik, 2007; Marwood et al., 2011). Few authors have also studied the effect of whole tires (Kellough, 1991; Day et al., 1993; Abernethy, 1994) and more recently research on effects of TWP have emerged (Redondo-Hasselerharm et al., 2018a; Khan et al., 2019). In addition, few articles on terrestrial ecotoxicology on the use of crumb rubber from artificial turf have been published (Pochron et al., 2017, 2018).

Recently, it has been suggested that leachate is not the only part of the rubber to cause an adverse effect, but that the particle itself may cause acute mortality and during longer-term exposures impact total reproductive output and growth (Khan et al., 2019). To better understand the different modes-of-action a compilation of ecotoxicological literature comparing MR particulates and MR leachates is shown in Table 2. Three main endpoints are

used to group the different information: short-term effects, ingestion and gut retention time, and long-term effects. Apart from listing current data this table also i) highlights the differences in units when reporting data, ii) localizes similarities and dissimilarities in experimental set-up and exposure route, and iii) underlines the positions at which there is a current lack of data. To date, the only study examining acute toxicity of MR particulates in the aquatic environment is Khan et al. (2019), whereas there are several studies using different experimental organisms on the acute toxicity of MR leachates. It is still unclear if the particle itself or the leached contaminants are the primary cause of toxicity, which supports the need for further examination of the interpretation of particulate toxicity. TWP leachate studies have been conducted in different aquatic environments with a variety of species, resulting in large variations in effects that are attributed to differences in tire composition, TWP and leachate generation, and species sensitivity; these are summarized by Wik and Dave (2009) and Wagner et al. (2018). Studies on ingestion and gut retention time are obviously only available for MR particulates, and thus there are a very limited number of studies, all of which have been published recently, with the exception of the observation of *Daphnia magna* ingesting MR particles made back in 2009 by Wik and Dave. Long-term studies are scarce and to date lack comparability. Shortly, TWP mixed into sediment does not seem to have an effect on *Gammarus pulex*, *Asellus aquaticus*, *Tubifex spp.* or *Lumbriculus variegatus* (Redondo-Hasselerharm et al., 2018a), whereas TWP in the aqueous phase significantly reduced total reproductive output and growth in *Hyaella Azteca* (Khan et al., 2019). Long-term effects on *Ceriodaphnia dubia* exposed to a series of diluted TWP leachate resulted in an estimated EC₅₀ in the range of 0.01-1.8 g rubber/L (Wik et al., 2009), which conceivably describes the range of difficulties in working with leachates and unevenness of exposure concentrations rather than realize an environmental threshold for leachate exposure.

Terrestrial ecotoxicology studies have been conducted on soil microbes and earthworms, *Eisenia fetida*, using virgin and weathered crumb rubber. No effects were found on soil microbes exposed to virgin crumb rubber and the health of the microbial population was not affected. Earthworms were similarly not affected in regard to survival or ability to cope with light or heat stress, but a 14% reduction in growth was observed after 33 days (Pochron et al., 2017). Exposing *E. fetida* to aged crumb rubber however, showed a decrease in survival time (16.2 %) under acute stress tests, but showed no effects on growth (Pochron et al., 2018). In some instances it seems that worn tires and their particulates exhibit greater toxicity compared to their pristine counterparts (Day et al., 1993; Pochron et al., 2018).

The summation of the biological impacts resulting from MR particle and/or leachate exposures indicates current difficulties in forming generalizations. Table 2 highlights the knowledge gaps that persist and can be used to ascertain the direction to which future research should be focused, in order to further understand the toxicities and adverse effect of MR exposure and delineate particle and leachate effects.

4. Future considerations of microrubber

In the previous sections, we have addressed some of the inconsistencies, concerns and possible pitfalls that may arise if the environmental risk assessment of MR is based mainly on direct read-across from observations for MPs. In order to confront the ambiguities from the areas where MR deviate slightly from other polymers, different research priorities are needed for MR. These are described below based on environmental presence and fate, interaction of rubber particles with biota, and standardization of test methods. As stated previously, the first

gap that needed closure is uniformity in the terminology, which is why we have proposed the umbrella term MR to cover all rubber particles up to 1 mm, along with the terms TWP, TRWP and ELT to more precisely define specific MR areas of interest (Figure 1).

4.1 Environmental presence and fate of microrubber particles and leachate

The presence of tire debris has already been measured with a variety of methods, especially in the air compartment (Pierson and Brachczek, 1974; Kim et al., 1990; Adachi and Tainosho, 2004; Kreider et al., 2010; Abbasi et al., 2017), but chemical analysis methods for determining presence in all matrices (sediment, water, biota, soil and air) are lacking along with reliable quantification procedures. Furthermore, MR composition will change over time when exposed to the environment as new equilibria forms. This ageing and change of MR composition should be taken into consideration when quantifying MR. Marker compounds have been used to estimate MR concentrations in soil, sediment, air and riverine runoff (Spies et al., 1987; Maltby et al., 1995b, 1995a; Councell et al., 2004; Ni et al., 2008), but uncovering tire-related chemicals that are not already distributed through anthropogenic sources is challenging. Incorporating already established protocols and techniques used for MPs could be a crucial step towards rearranging current protocols and fitting them to also suit MR analysis. Overall, sampling and detection techniques used for MPs should be largely applicable for MR after taking a few considerations into account.

Collecting MP samples in the environment is done via a variety of sampling techniques each suitable for the particular environmental compartment, with subsequent digestion procedures when needed, for example different general protocols for tissue digestion, oxidation of

organic matter, and density separation. Density separation is commonly used for separating MPs from sediment samples (Claessens et al., 2013) and has recently also been optimized for identifying MR in combination with Zn determination (Klöckner et al., 2019).

Visual identification is commonly used as a first-step in the identification of MPs and is also possible for MR, but can be complex in field sample matrices containing other dark and black fragments such as sediment and soil. Pyrolysis GC-MS is a method commonly used for all polymers, and it also works well for elastomers. Coupled with an extra MS it is possible to first get a signal from the polymer backbone, and then get a signal from the organic constituents of the particles (e.g. PAHs, PCBs or antioxidants). The only negative to the method is that the sample is destroyed by pyrolysis so it cannot be used for further analysis. GC-MS without the pyrolysis is also useful in identifying organic contaminants (not the polymer backbone) from MR particles, but the number of peaks on the chromatogram takes an experienced eye to understand. Sample purification and preparation may ease the chromatogram analysis, but the use of purification columns and pre-clean-up methods depend on what few MR related chemicals are targeted, leaving out a cascade of chemicals that are not targeted. This underpins the importance of choosing the right chemical or group of chemicals when indirectly investigating presence and quantity, as the type and brand of tires will also differ in chemical content.

Confirmation of MP particles by Raman spectroscopy and FTIR identification is now routinely expected in scientific studies. However, the identification of MR particles with these methods is challenging due to the presence of carbon black. Since the carbon black

content in MR is a fraction of the total it is technically possible to measure, but it is extremely time consuming compared to MP particles, these two methods are thus suboptimal for identification of MR (Murakami, 2009). Most of the methods and procedures used on MPs primarily consider the particle itself, mainly by investigating and analyzing the polymer backbone, and does not by default incorporate the chemical load of the particle, or the chemical fraction leached out to the particle's surroundings. It is apparent that MP research is skewed towards investigating the particle but not so much the leached chemicals, contrary to MR research, where the leached fraction is usually investigated rather than the particle. To obtain a more complete understanding, the listed methods should be incorporated with each other to understand both the particulate and leached portions, and their interactions.

There are many different courses of action to solve a problem and answer a research question, one way would be developing analytical methods to amplify the details of individual components, another, would be generalizing to understand the whole. GC-MS and ICP-MS can quantify single MR related compounds in water or air matrices (Zheng et al., 2002; Marwood et al., 2011; Turner and Hallett, 2012), but additional preparatory steps would be needed to look at sediment, soil or biota. The downside to looking for single substances in complex biological matrices is their trace amounts, the prerequisite of metabolic stability, and actually detecting differences significant to the surrounding compartment based on one most likely anthropogenic compound. The use of better separation techniques (e.g. quadrupoles or tandem MS (3D chromatograms)) could help establish MR particle and leachate 'fingerprints' in abiotic samples, that is unique patterns of present chemicals that can be attributed to one source. This measure would not be based on the presence of one anthropogenic compound or a ratio of one compound to another, but by comparing 3D

chromatograms, each with several layers of separation. This technique is already used in oil-determination processes where extremely complex samples are analyzed and differentiated, despite their similarity, with utmost precision (Christensen and Tomasi, 2007; Liu et al., 2009). Something similar could be configured to analyze MR samples with all the complexity they hold.

Determination of prevalence and fate of MR particles in the environment require the development of distinctive protocols for each environmental compartment, paired with a unity in result presentation. This uniformity in protocols would further support the later comparison between studies, without neglecting that one compound may be of more importance in one compartment than another. Presenting limits of detection and quantification for each method used should be mandatory to streamline the importance of each finding and ensure a proper analytical approach. Likewise, the use of strict quality assurance and control protocols to prevent contamination by the use of field- and procedural blanks, along with validation tests on recovery protocols should always be presented as an important part of the research. It is therefore of paramount importance to understand and, if needed, distinguish the different polymers influence on environmental presence, fate and toxicity in different biological compartments on all levels of biological organization.

4.2 Interaction of microrubber particles and leachate with biota

Studies on determination of ingestion- and egestion rates in different organisms along with gut retention time are required to fully understand the potential for chemical transfer from gut to tissue. This is needed for MR particulates and other polymer types, as comparison between

the different types, sizes and shapes would be most beneficial for future analysis and assessment of the internal fate of polymer particulates. As described in section 3.2 there seem to be a tendency for fibrous materials to possibly have a longer gut retention time than spheres or pellets (Wright et al., 2013; Au et al., 2015; Redondo-Hasselerharm et al., 2018a), and the consequence of this needs to be evaluated and compared across polymer types. Additional studies focusing on ingestion, gut retention time and egestion of MR particles in different organisms are needed to explore how the mechanisms of different feeding strategies might influence later toxicological responses.

Furthermore, studies on short term effects of organisms exposed to MR particles through sediment, water and soil are very much needed. Currently mortality (and survival) are the only investigated acute endpoints, so investigations on growth, behavior or sub-organismal parameters like gene expression or biomarker indicators would be very valuable for future studies. Considering the inconsistencies among long-term studies on MR particles and leachate, there is a dire need for more experimental research on effects in different organisms exposed in different matrices. The few studies that have been conducted vary in how they are performed, which exposure routes are investigated and which organisms are used and are thus not readily comparable (Table 2). Lastly, mathematical modelling and extrapolation from individual to population should be done to estimate possible effects on higher levels of biological organization. There is already a comprehensive amount of studies on MR leachate, mainly acute interaction with biota, therefore MR particle ecotoxicology, especially acute effects and ingestion, should be prioritized until more streamlined protocols for leachate exposure are established.

4.3 Laboratory tests and protocols for particulates and leachates

Standard methods for measuring the hazard potential of polymer particulates in general are needed in order to compare across studies and their findings. The standard toxicity methods should include both a protocol on particulates and leachates for water and sediment, and standard chemical analysis methods should be developed for all compartments. Adjustment of already existing guidelines on soluble contaminants could be fine-tuned to suit a leachate guideline, whereas a guideline on particulates would most likely need to be newly developed (Khan et al., 2017b). There are however, additional challenges for analyzing MR using a standardized protocol, as all particles are generated differently and there is a lack of knowledge on particle size and distribution. Therefore, there are currently no standardized environmentally relevant particles that laboratories can buy from manufactures. For MP experiments a number of spherical beads are available in different colors, densities and sizes that can be tracked by chemical labels (such as specific metals or C₁₄) or fluorescence. Fluorescence makes it possible to visually follow ingestion, gut retention and transfer of some particles, with some limitations as the method has recently proven to be flawed by dye disassociating from the particle and leaching to surrounding tissue, so this method should be used with caution (Schür et al., 2019). This, however is not possible for MR particles without changing their elemental parameters/physicochemical composition and other methods will have to be sought to commercialize standardized MR particles. A measure of effect or presence of MR could be obtained indirectly by using biomarkers of stress response or metal detoxification systems in biota, or using chemical markers that originate from elastomer polymers found specifically in the tire industry, distinguishing them from other polymers.

Another issue that arises when estimating amounts of polymer particles in environmental samples is unity in data presentation, which has been addressed before (Lusher et al., 2017; Rochman et al., 2017, 2019; Hartmann et al., 2019). Should particulate data be presented as number and size of particles, weight, sum of chemical load, or all of the above? This issue is relevant for all kinds of polymer particles in all size ranges, from nano (<100 nm) to meso (5 mm > 2.5 cm), and structures, from fibers to particles. Therefore it will evidently be a reoccurring issue in all scientific communities regarding risk assessment and toxicology interpretation of particulates. Until the major driving forces determining toxicity are established, and if weight or particle number are of most importance in this regard, both units should be featured, to avoid extrapolation on a wrong foundation. Several papers have acknowledged the need for research within the MR area and listed several important research gaps including; using tire wear particles in eco-toxicity studies, considering the effects of particulates in general and finally assessing the risk related to MR in the aquatic environment (Wik, 2007; Wik and Dave, 2009; Wagner et al., 2018). Until the toxicological mechanisms have been unraveled, we suggest that results are presented as both weight and numbers of particles.

Conclusion

It is certain that tire abrasion, as a source of MR release to the environment, is one of the more difficult sources to control and limit, and therefore knowledge on the extent and possible impacts of MR pollution is of great importance. Considering the increasing amount of tire debris, the knowledge gaps and the many questions that needs answering the described ambiguities emphasize the challenges ahead. There is a need for uniformity in the polymer debris discourse, and in the research required to estimate toxicological impact of MR in the

environment. There is a pressing need to improve methods for chemical analysis of MR and its components in the environment in order to (1) fill knowledge gaps on environmental presence and fate (including degradation), (2) uncover the toxicity of both particles and leachate, and (3) create or refine protocols for toxicity testing for particles in general, and specifically for MR.

Declaration of competing interest

None.

Acknowledgement

We would like to thank laboratory personnel Torben Brandt Knudsen and Mette Flodgaard (Roskilde University, Department of Science and Environment) for the picture of tire wear particles generated by cryomill.

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Figures Legends

Figure 1. Polymer landscape.

Nomenclature on anthropogenic polymer particulates is divided into two main polymer groups; thermoplastic and thermoset that are precursors to plastics and elastomers,

respectively. Thermoplastic elastomers and thermoset plastics also exist, but for the purposes of this paper thermoplastic/thermoset division is considered appropriate. Terminology of elastomer debris have been outlined generally as microrubber (MR) which serve as an umbrella term covering all micronized rubber particles. MR is then divided into three sub-groups: tire wear particles, tire-road-wear particles and end-of-life tires. End-of-life tires are further extended to different sizes of crumb rubber used in the re-cycling industry.

Figure 2. Diversity of rubber from laboratory production to sampling along a road.

Images used to visualize the ruggedness and size and shape differences of abraded microrubber particles, either sampled in nature (tire-road-wear particles, TRWP) or produced in a laboratory (tire wear particles, TWP). **A:** TRWP (circled in blue) sampled on gel-tape from road surface. **B:** Water sample of TRWP (circled in blue) collected from road surface drain. **C:** Laboratory generated TWP by cryogrinding in liquid nitrogen. **D:** Laboratory generated TWP produced by pressing a tire on a coarse grinding stone as used in Khan, et al. (2019).

Figure 3. Mysid ingestion and depuration of tire wear particles.

Ingestion and depuration of tire rubber particles by the crustacean opossum shrimp (from the *Mysidae* family). Ingestion of laboratory generated tire wear particles (TWP) is demonstrated over 5 h ($t = 0, 1, 2, 3, 4, 5$) during which time shrimps indiscriminately consume tire rubber. The stomach fills up within the hour (**B**) and the length of gastrointestinal tract is completely filled by 5 hours (**F**). G-L represents depuration at $t = 0, 1, 2, 6, 12$ and 48 hours. Picture M represents a 'control fecal pellet' ($t = 0$) after spirulina ingestion only, and N-R shows fecal

pellets corresponding to the depuration pictures at $t = 1, 2, 6, 12$ and 48 hours of depuration. The depuration pictures show a TWP filled stomach after ingestion and depuration seem to occur after 6 hours (**J**) but the corresponding fecal pellets still show fragments of TWP, which is present up to 48 hours after depuration (**R**), indicating that the particles are retained in the gut and stomach longer than visible by spectroscopy of the organism. Scale bars are 1 cm for all mysid pictures and 1000 μm on fecal pellet pictures.

Table 1. A brief history of tire related research. Timeline of major events in tire-related ecotoxicology research per decade since the 1960s, underlining the shifting foci within this research area. Future considerations and recommendations are outlined for the next decade (2020s).

Decade	Findings	References
1960s	First identification of tire rubber in road dust published ¹ .	¹ Thompson, 1966
1970s	Rubber dust studied as an airborne particulate ² . Particle size of tire tread in atmospheric dust was determined ³ . Rubber dust emissions from the normal wear of tires were estimated ⁴ . Products of tire wear emissions were investigated ⁵ and characterized in both laboratory and field studies ⁶ . First toxicity experiment of whole tires on reef fish was conducted ⁸ .	² Pierson and Brachczek, 1974; ³ Cardina, 1974; ⁴ Dannis, 1974; ⁵ Raybold and Byerly, 1972; ⁶ Williams and Cadle, 1978; ⁷ Cadle and Williams, 1978; ⁸ Stone et al., 1975
1980s	Environmental degradation of tire wear particulates was further characterized ^{9, 10} . In-depth mechanisms of antioxidant leaching from truck tires ¹¹ and metal adsorption from aqueous solutions by waste tire rubber ¹² along with toxic hazards of rubber chemicals ¹³ was investigated. First publication using benzothiazoles as an indicator (biomarker) of street run-off on estuarine sediments was published ¹⁴ and heavy metals in roadside dust along a highway was investigated ¹⁵ .	⁹ Williams and Cadle, 1980; ¹⁰ US EPA, 1981; ¹¹ John et al., 1984; ¹² Rowley et al., 1984; ¹³ Nutt, 1984; ¹⁴ Spies et al., 1987; ¹⁵ Warren and Birch, 1987
1990s	Road dust and mechanisms and simulations of rubber abrasion was still under investigation ¹⁶⁻¹⁸ but runoff and leachate research became a more prevalent topic of interest. Effects of whole scrap tires from automobiles in water continued ¹⁹⁻²¹ and leachate toxicity to aquatic biota ^{22, 23} was investigated. Tire leachate toxicants were identified and risk assessed ²⁴ whilst the effects of motorway runoff on freshwater ecosystems were investigated ^{25, 26} . Research on the environmental chemistry of benzothiazoles derived from rubber continued ²⁷ and the use of benzothiazoles as a biomarker expanded ²⁸ , organic zinc was now used as a tracer to quantify tire tread particles ²⁹ . Sampling and analysis of emissions in the rubber industry was investigated ³⁰ and a technical guideline on proper disposal and reuse of worn tires was published ³¹ .	¹⁶ Rogge et al., 1993; ¹⁷ Fukahori and Yamazaki, 1994; ¹⁸ Stalnaker et al., 1996; ¹⁹ Kellough, 1991; ²⁰ Abernethy, 1994; ²¹ Hartwell et al., 1998; ²² Day et al., 1993; ²³ Evans, 1998; ²⁴ Nelson et al., 1994; ²⁵ Maltby et al., 1995b; ²⁶ Maltby et al., 1995a; ²⁷ Reddy and Quinn, 1997; ²⁸ Kumata et al., 1997; ²⁹ Fauser et al., 1999; ³⁰ Giese, 1999; ³¹ Secretariat of the Basel Convention, 1999
2000s	Single groups of chemicals, derived from tire	³² Rauterberg-Wulff, 2003;

	<p>leachates, were investigated. These included studies on PAHs³²⁻³⁷, metals, especially zinc³⁸⁻⁴⁴, and bezothiazoles⁴⁵⁻⁴⁷. Toxicity studies on 'complete' tire leachate was also heavily investigated this decade⁴⁸⁻⁵². First observation of rubber ingestion by <i>Daphnia magna</i> was published⁵⁰. Toxicity studies were conducted on crustaceans, fish and human cells^{34, 53-57}. Life cycle assessment of tires was introduced⁵⁸. Micronized rubber from tire abrasion was introduced as part of the microplastic concerns in the form of artificial turf^{36, 59-60}.</p>	<p>³³European Commission, 2003; ³⁴Stephensen et al., 2005; ³⁵Gadd and Kennedy, 2003; ³⁶Nilsson et al., 2005; ³⁷Van Dolah et al., 2005; ³⁸Davis et al., 2001; ³⁹Smolders and Degryse, 2002; ⁴⁰Adachi and Tainosho, 2004; ⁴¹Council et al., 2004; ⁴²Blok, 2005; ⁴³Hjortenkrans et al., 2007; ⁴⁴Johansson et al., 2009; ⁴⁵Kumata et al., 2002; ⁴⁶Kloepfer et al., 2005; ⁴⁷Ni et al., 2008; ⁴⁸Wik and Dave, 2005; ⁴⁹Wik and Dave, 2006; ⁵⁰Wik and Dave, 2009; ⁵¹Wik et al., 2009; ⁵²Wik, 2007; ⁵³Gualtieri et al., 2005c; ⁵⁴Gualtieri et al., 2005a; ⁵⁵Stephensen et al., 2003; ⁵⁶Beretta et al., 2007; ⁵⁷Mantecca et al., 2007; ⁵⁸BLIC, 2001; ⁵⁹Källquist, 2005; ⁶⁰Nilsson and Malmgren-Hansen, 2008</p>
2010s	<p>Chemical and physical characterization of particles continued⁶¹ and articles and reviews on traffic related emissions and heavy metal pollution was released^{44, 62-70}. Toxicity studies on more trophic levels was conducted to further risk assess tire wear leachate^{71, 72}. Research on biomarkers seemed to fade but most importantly for this decade, tire wear was now regarded as a stealthy source of microplastics in the environment and new emission estimations were calculated for a number of countries⁷³⁻⁷⁵. Ingestion of tire wear particles was determined in freshwater and estuarine invertebrate species⁷⁶⁻⁷⁸.</p>	<p>⁶¹Kreider et al., 2010; ⁶²Huber et al., 2016; ⁶³Hwang et al., 2016; ⁶⁴Grigoratos and Martini, 2014; ⁶⁵Grigoratos and Martini, 2015; ⁶⁶Kwak et al., 2013; ⁶⁷Hilliges et al., 2017; ⁶⁸Abbasi et al., 2017; ⁶⁹Loganathan et al., 2013; ⁷⁰Pant and Harrison, 2013; ⁷¹Turner and Rice, 2010; ⁷²Marwood et al., 2011; ⁷³Lassen et al., 2015; ⁷⁴Kole et al., 2017; ⁷⁵Wagner et al., 2018; ⁷⁶Redondo-Hasselerharm et al., 2018a; ⁷⁷Khan et al., 2019, ⁷⁸Present paper</p>
2020s	<p>Now that tire wear emissions are regarded as part of the microplastic designation, and current estimations put tire wear pollution to be one of the larger, if not largest, source of microplastic debris,</p>	<p>⁷⁹Hartmann et al., 2019; ⁸⁰Rochman et al., 2019; ⁸¹Frias and Nash, 2019; ⁸²ECHA, 2019</p>

the research within this area needs to be streamlined. Reviews and commentaries on microplastics are most relevant to the microrubber niche^{75, 79-81} but microrubber has disadvantages in analytical chemistry that needs attention⁸². Future needs include: lab protocols and standards for measuring tire wear particulates in different environmental compartments, comparing measurements in situ to models, ecotoxicology data on particulate effects and finally comparison of particulate and leachate toxicity effects and investigations on the biotic interactions possibly affected by tire wear exposure.

Table 2. Toxicity and effects of microrubber particles and leachate.

Relevant data on MR particulates and MR leachates are compared based on different groups of endpoints to provide an overview of the current available ecotoxicology data and pinpoint research gaps and differences in result dissemination and use of units. The endpoints are: short-term effects, ingestion and gut retention time, and long-term effects. Some research has been conducted on MR leachate in regard to short-term toxicity, compared to only two very recent studies on MR particles and short-term toxicity. Two, again very recent, papers provide photos of MR particle ingestion but more data is needed on ingestion and depuration rates along with gut retention times of different organisms. Lastly, few studies look at long-term toxicity of MR particles and MR leachate. When possible, leachate results have been re-calculated to be expressed in the same unit: g/L.

Short-term effects: acute toxicity, molecular markers and growth

MR Particulates	<i>Eisenia fetida</i> (red earthworm) demonstrated reduced survival time (16.2 %) during acute stress test when exposed to aged crumb rubber (Pochron et al., 2018).
	<i>Hyalella Azteca</i> LC ₅₀ value of 3426±172 particles/mL (~1 g/L) when exposed to tire wear particles in the aqueous phase from a worn tire abraded by grinding (Khan et al., 2019).
MR Leachates	<i>Oncorhynchus mykiss</i> (rainbow trout), <i>Daphnia magna</i> , <i>Pimephales promelas</i> (fathead minnow) and different bacteria were exposed to whole tires (worn, pristine and breakwater tires) submerged in groundwater with a 40 d leaching period. No toxicity was observed for <i>D. magna</i> or <i>P. promelas</i> . The 96-h LC ₅₀ for <i>O. mykiss</i> was determined at 11.8-19.3% v/v for old tire leachate, which was more toxic than leachate from new tires at 52.1-80.4% v/v (Day et al., 1993).
	<i>Cyprinodon variegatus</i> (sheepshead minnow) and <i>Palaemonetes pugio</i> (daggerblade grass shrimp) exposed to leachate produced from shredded scrap tires (crumb rubber) at salinities 5, 15 and 25 ‰ in 7 d static renewal tests showed decreasing toxicities with increasing salinities (Hartwell et al., 1998).
	<i>Allivibrio fischeri</i> (gram negative bioluminescent bacterium) exposed to shredded tire chips at a concentration of 50 g/L showed toxicity effects in all taxonomic orders and a correlation between decreasing toxicity and increasing salinity. The authors concluded that tire leachate is likely to be a greater threat to freshwater habitats than to estuarine or marine habitats (Hartwell et al., 2000).
	<i>O. mykiss</i> introduced to water containing whole tires, either containing highly aromatic compounds (HA) in the tread, or not, both showed increased EROD activity after 1 d exposure. Increased concentrations of glutathione S-transferase, glucose-6-phosphate dehydrogenase and glutathione reductase was measured in hepatic fish tissue after exposure from HA tire compared to HA-free tire (Stephensen et al., 2003).
	<i>Xenopus laevis</i> (African clawed frog) embryos exposed to 50 and 100 g/L of leached tire wear particles showed 80% and 27% mortality, respectively, and a TC ₅₀ of 38.3% and 70.6% at 120 h (Gualtieri et al., 2005b).

Daphnia magna exposed to 12 different tire wear leachates showed 48h-EC₅₀ in a range from 0.13-2.41 g/L. Leachates were made by grating new tires and equilibrating them for 72 h in a concentration range between 0.25 – 16 g/L (Wik and Dave, 2005).

D. magna exposed to 100 g/L of leached tire wear particles diluted at 1, 10, 50 and 100% leachate in media showed a 48 h LC₅₀ of 53.3 g/L (Gualtieri et al., 2005b).

Rahidocelis subcapita (green microalga) exposed to 50 and 100 g/L of tire wear particle leachate showed that a concentration of 50 g/L inhibited growth at 72 h better than 100 g/L (Gualtieri et al., 2005b).

O. mykiss displayed increased EROD and glutathione reductase activity after exposure to aquarium water provided by a rubber hose. Rubber chemicals: 2-mercaptobenzothiazole and diphenylamine and their metabolites were detected in the bile of the exposed fish (Stephensen et al., 2005).

D. magna exposed to diluted tire wear leachates from 25 different tires showed 48h-EC₅₀ in the range of 0.5-10 g/L. TWP used for leachate production was prepared by abrading a tire with a rasp and equilibrating TWP at 44°C for 72 h (Wik and Dave, 2006).

X. laevis studied with the embryo teratogenesis assay (FETAX) under exposure to tire leachate at concentrations ranging from 0.5-1.4g/L showed malformed larvae from 0.8 g/L. Tire leachate was produced by cryo fracturing tire scrap (Mantecca et al., 2007).

Ceriodaphnia dubia exposed to a series of diluted leachates from tire wear particles (3 different tires) showed 48-h EC₅₀ in the range of 0.55-5 g/L (Wik et al., 2009).

Rana sylvatica (wood frog) exposed to leachate from tire scraps aged in sediment (83.8 g/kg) showed significant differences in hatching success and increased time to reach metamorphosis, however, no significant difference in mortality was found (Camponelli et al., 2009).

Ulva lactuca (green alga) exposed to leachate of 0.25 g/L end-of-life tires abraded to crumb rubber with a steel file showed a significantly decreasing efficiency of photochemical conversion (F_v/F_m) with increasing percentage of leachate solution (Turner and Rice, 2010).

R. subcapita, *D. magna* and *P. promelas* exposed to sediment elutriate in a range of 0.1-10 g/L of tire tread created by road simulator showed no toxicity. Additional test on *D. magna* with high temperature leachate (leached at 44°C) gave an EC/LC₅₀ value of 5 g/L (Marwood et al., 2011).

Aedes albopictus and *Aedes triseriatus* (mosquitoes) exposed to a range of leachate concentrations showed significant effects on survivorship and population rate at concentrations ≥ 0.1 g/L. Also, a significant difference between the species tolerance was detected (Villena et al., 2017).

Ingestion and gut retention time

MR	<i>D. magna</i> observed ingesting tire wear particles (Wik et al., 2009).
Particulates	For <i>Gammarus pulex</i> and average of 2.5 and 4 tire wear particles were found in body and feces respectively after sediment exposure to 10% sediment dry weight tire wear particles (Redondo-Hasselerharm et al., 2018a).

	<i>H. azteca</i> ingestion and gut clearance time between 24-48 h after exposure to TWP in the aqueous phase (Khan et al., 2019).
	Pilot study (section 3.2) on ingestion and depuration of TWP in mysids resulted in a gut clearance time of >48 h exposed in water only.
MR Leachates	No research.
Long-term effects: reproduction and growth	
MR Particulates	<i>E. fetida</i> showed a 14 % reduction in growth after 33 d exposure to a 50:50 mix of soil to virgin crumb rubber (Pochron et al., 2017).
	No adverse effects on <i>G. pulex</i> , <i>Asellus aquaticus</i> (waterlouse), <i>Tubifex spp</i> (sludge worm), and <i>Lumbriculus variegatus</i> (blackworm) after 28 d exposure of up to 10 % sediment dry weight tire wear particles (Redondo-Hasselerharm et al., 2018a).
	<i>H. Azteca</i> neonate production and growth significantly reduced by exposure to 500 particles/mL (0.15 g/L) following 21 d exposure (Khan et al., 2019).
MR Leachates	<i>C. dubia</i> exposed to a series of diluted leachates from tire wear particles from 3 different tires showed 9-d EC ₅₀ in the range of 0.50-36 g/L and a 9-d EC ₅₀ on reproduction (corrected per female) at 0.10-18 g/L (Wik et al., 2009).
	<i>Chironomus dilutes</i> (lake flies), <i>H. Azteca</i> , <i>P. promelas</i> and <i>C. dubia</i> showed no significant effects or differences to control groups when exposed to TRWP leachate made from a concentration of up to 10g/kg sediment for 42 d. TRWP were derived from rubber particles generated using a road simulator (Panko et al., 2013).

Graphical abstract

Journal Pre-proof

Highlights

- Increased recognition on the importance of micronized tire rubber.
- The umbrella term 'microrubber' (MR) is proposed to describe elastomer particles ≤ 1 mm.
- MR is placed in the broader polymer landscape with microplastics.
- Focusing on chemical analysis of MR and its components is key to filling knowledge gaps.
- MR particulate and leachate effects need to be better distinguished and understood.

ANTHROPOGENIC POLYMER PARTICULATES

Man-made particles of high molecular weight chains formed either by the addition or condensation of numerous sub-units (monomers).

THERMOPLASTIC

Ability to soften when heated and harden when cooled.



PLASTICS

Containing a high molecular weight polymer, which at some stage can be shaped by flow. Elastomers are excluded from this definition, as they cannot be 'shaped by flow'.

PLASTIC DEBRIS / MICROPLASTICS

To gain a common ground for defining and interpreting the vast array of plastic debris a few recent papers discuss different criterions, possible pitfalls and future mitigation strategies; Based on these, the following traits that are relevant for describing plastic debris are; chemical composition, origin (primary or secondary), solubility, size, shape (morphology), colour and additives.

THERMOSET

Irreversibly hardened by curing, creating crosslinks in the polymer.



ELASTOMERS

The ISO defines elastomers as macromolecular materials, which after deformation can return rapidly to its initial dimensions.

NATURAL ELASTOMERS

Latex rubber, naturally occurring cis-1,4-polyisoprene, from *Hevea brasiliensis* in both liquid and cured form.

SYNTHETIC ELASTOMERS

Encompasses all elastomers that are produced synthetically (examples: styrene, butadiene and also synthetic cis-1,4-polyisoprene).

MICRORUBBER

Umbrella term for all micronized rubber particles.

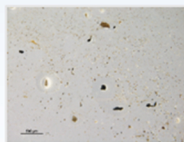
TWP

Tire wear particles abraded from the tread of tires. Most likely form to be generated for laboratory experiments by manual abrasion or by simulation. Unlikely to be sampled from the environment.



TRWP

Tire-road-wear particles, abraded from the tread of tires when driving on a road. Rubber particles found in combination with other car emissions and road dust. Most likely form to be sampled from the environment.



ELT

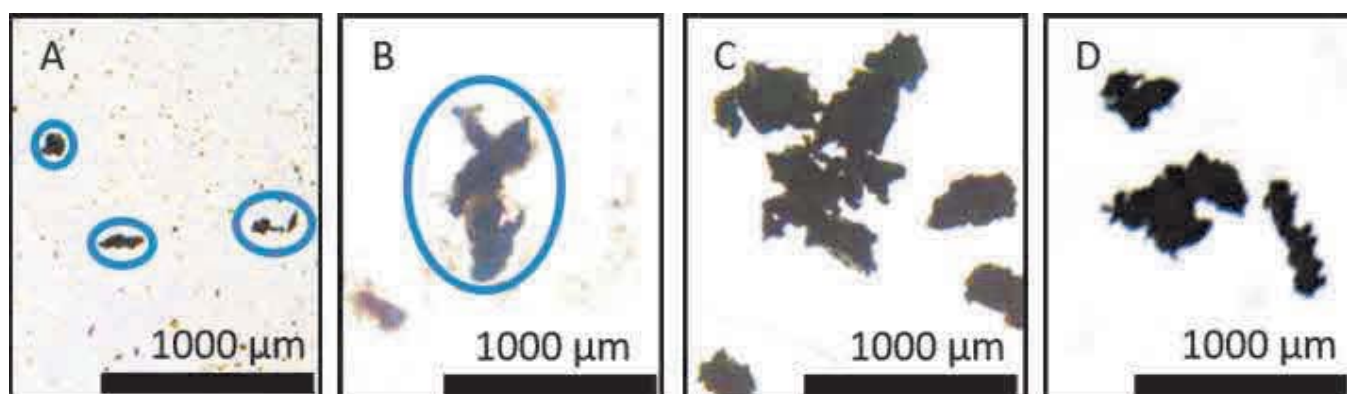
End-of-life-tires; Worn tires repurposed for alternative applications for example artificial turfs.

CRUMB RUBBER

Described by ISO as "elastomers in particulate form" Divided into size by ISO: Chip rubber (10-50 mm), Granulated rubber (1-10 mm), Ground vulcanized rubber (0.1 - 1 mm) and Fine powdered rubber (≤ 0.1 mm).

Figure 1

Figure_2
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Figure_3
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