

Ecotoxic potential of road-associated microplastic particles (RAMP)

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Summary

Road-related microplastic particles (RAMP) is a group of particles in the microscale size range 0.1-1000 μm with plastic compounds (polymers) in them, which is present in road runoff. Tire-wear particles (TWP) are estimated as the largest single source of microplastic particles in Norway, contributing up to 5000 tons of microplastic per year of a total of 8400 tons of microplastics per year. RAMP also includes road-wear particles from polymer-modified bitumen (RWP_{PMB}) and road-wear particles from road marking (RWP_{RM}). RAMP is a diverse particle group both when it comes to particle properties and chemical compounds. Several studies have confirmed toxicity effects in experiments using TWP leachates at environmentally relevant concentrations according to known concentrations. However, more research is needed on the concentrations in the environment, uptake in biota for all three types of RAMP and the toxicity effects from these.

Sammendrag

Økotoksikologisk potensiale i mikroplastpartikler fra vei (RAMP). Vegrelatert mikroplastpartikler (RAMP) er en gruppe partikler i størrelsesordenen 0.1-1000 μm som inneholder plast (polymerer) og er tilstede i vegavrenning. Slitasjepartikler fra dekk (TWP) er estimert som den største enkeltkilden til mikroplast i Norge, med et bidrag på

opp mot 5000 tonn av det totale utslippet på 8400 tonn. RAMP inkluderer også slitasjepartikler fra vegbanen, både fra polymer-modifisert bitumen (RWP_{PMB}) og fra vegmerking (RWP_{RM}). RAMP er en variert gruppe partikler med ulike egenskaper og kjemisk sammensetning. Flere studier har bekreftet toksiske effekter i eksponeringsforsøk med TWP i konsentrasjoner som er tilsvarende de konsentrasjoner som er funnet i naturen. Det er likevel behov for mer forskning både når det gjelder hvilke konsentrasjoner av RAMP som finnes ut i naturen, på opptak i biota og hvilke toksiske effekter dette kan ha.

Introduction

The focus of this review is to give an overview of the ecotoxic potential of microplastic particles originating from road and tire wear. Although road-related pollutants have been studied for many decades, it is only recently that the term “microplastic” has been associated with them. The total release of microplastic particles in Norway is estimated at 8400 tons per year (Sundt et al., 2014), and of that 4500-5300 tons comes from tires (Sundt et al., 2014; Sundt et al., 2016; Vogelsang et al., 2018).

Microplastic particles

Plastic is a synthetic or semisynthetic organic material, made from either one or several polymers and additives. Plastics can be divided

into different size ranges: macroplastic >25 mm, mesoplastic 1-25 mm, microplastic 0.1-1000 µm and nanoplastic <0.1 µm (Bowmer and Kershaw (2010). Microplastics and nanoplastics are in the size ranges that can potentially be taken up by organisms, humans included, and thereby pose a great environmental threat, although the severity of this threat is still debated (Burton, 2017; Backhaus and Wagner, 2018).

Microplastics have been found in all environmental matrices and from urban to remote areas, including remote tropical islands, the Arctic, the Antarctic, and deep-sea sediments (GESAMP 2016). Studies have also shown that microplastics are taken up by different trophic levels (Wright et al. 2013, Su et al. 2018, Bråte 2017, Lusher et al. 2017). Microplastic particles may be mistaken as food and fill up the organisms' stomach with indigestible particles, causing illness and death due to malnutrition. Microplastic particles may work as a vector and transfer different environmental toxins to organisms, either from the additives to the polymers in production or by attracting pollutants to the

particle surface and thereby transport them to the organisms (Koelmans et al. 2016, Frias et al. 2010) and pathogens (Kirstein et al. 2016, Oberbeckmann et al. 2015). Studies have shown that this is possible for some pollutants, like zinc (Zn) and copper (Cu) (Brennecke et al., 2016) and polycyclic aromatic hydrocarbons (PAH) (Endo et al., 2005; Mato et al., 2001; Rios et al., 2007; Bowmer and Kershaw, 2010), which also may cause harmful effects on both lower and higher trophic levels.

However, microplastic research is still in its infancy and there are several knowledge gaps that need to be filled for us to be able to grasp the consequences of microplastic particles spreading into the environment.

Road-associated microplastic particles (RAMP)

Overview

Road runoff has a complex transport route from the road to the environment (Figure 1), with a potential to spread to many different matrices. Road runoff is typically linked to high levels of

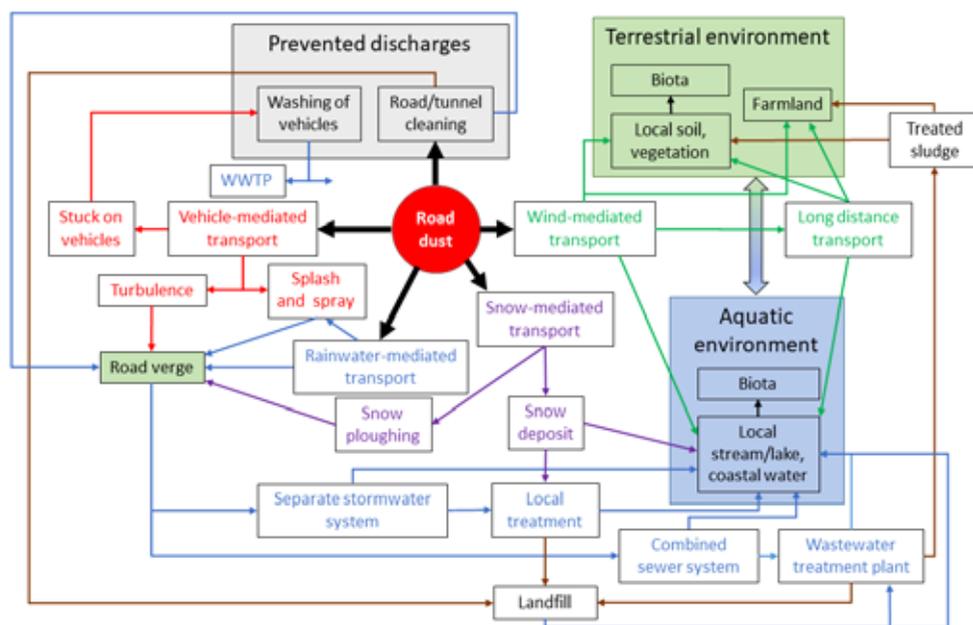


Figure 1: Potential main pathways for microplastics in road dust to reach aquatic environments (blue background) and terrestrial environments (green background). Some pathways may prevent discharges to the environment (grey background). Vogelsang et al., 2018. Reprinted with permission from NIVA and Miljødirektoratet.

particles and heavy metals such as zinc, copper, cadmium and nickel (Hallberg et al., 2014; Meland, 2010; Roseth and Meland, 2006) and organic micro pollutants such as polycyclic aromatic hydrocarbons (PAH), organophosphates, octylfenoles and phtalates (Grung et al., 2016; Meland, 2010; Meland, 2012; Åstebøl et al., 2011). Release of runoff from tunnels, accumulated between tunnel wash events, have been found to be both chronic and acute toxic to aquatic organisms (Meland, 2010; Meland et al., 2010b; Meland 2012a). Heavy metals and PAHs are especially correlated to the concentration of particles in the runoff, and measures such as sedimentation ponds and filter treatment has been proven to be quite effective (Åstebøl and Coward, 2005; Roseth et al., 2012). Even though particles from road runoff have been a research area for many a long time, it has received more attention the last few years because it also includes particles with polymer components, making road runoff one of the largest sources of microplastic particles to the environment. In fact, particles created by the wear and tear of car tires is estimated as the single largest source of microplastics in Norway (Sundt et al., 2014).

This review is focusing on the toxic potential of road-associated microplastic particles (RAMP), which includes tire-wear particles (TWP), road-wear particles from polymer-modified bitumen (RWP_{PMB}) and road-wear particles from road marking (RWP_{RM}). One of the essential questions regarding RAMP is if these types of microplastic particles will behave like other types of microplastic in the environment or completely different? This will affect both the threat it poses to the environment and to organisms, as well as it may affect the success of our analytical methods and the detection of the RAMP in environmental samples. It should also be mentioned that microplastic particles originating from sources like plastic bags or plastic wrappings also is expected to be found alongside roads, but these are not included in the definition of RAMP and therefore not a topic in this study.

Characteristics of RAMP

Size, shape and density

The bioavailability of RAMP may be affected by its size, shape and density. A summary of the particle properties of RAMP is given in Table 1. In general, particles are being taken up by organisms all the time. Some organisms feed on sediments or filter particles in the water column to find nutrients. These organisms are highly adapted to sorting out the inorganic particles, like minerals, silt, clay or other compounds that have no nutritional value and keeping the organic, nutrient particles. The inorganic particles are either stopped in a sorting mechanism before being taken up, for instance in the gills, or they are taken up and excreted afterwards by the digestive system.

Size and the shape of the particles may impact the probability of them being taken up by organisms, both as a physical barrier if they are too large or in a shape that is not easily taken up passively or actively by the organisms. Another aspect is how the size and shape impact the transport of these particles from the road and to the aquatic environment. In general, it is expected that large particles from road runoff will accumulate in the roadsides or in gully-pots, and only the smaller fractions will be able to transport with the runoff (Vogelsang et al., 2018). In general, the trap-efficiency for gully-pots is very low for particles $<50 \mu\text{m}$. However, this is based on assumption made from road runoff particles in general and further research on this subject is needed. TWP is expected to be in the size range of $350\text{--}50 \mu\text{m}$ (85%) and $<50 \mu\text{m}$ (15%) (Kreider et al. 2010; Broeke et al. 2008). RWP_{PMB} is expected to have a similar size range as TWP (Vogelsang et al., 2018). RWP_{RM} is expected to be in the size ranges $50\text{--}4000 \mu\text{m}$ (Vogelsang et al., 2018). When it comes to shape, both TWP and RWP_{PMB} have been found as elongated, sausage-like particles (Figure 2). Often associated with minerals and other road-related particles. RWP_{RM} differs from the other two by being more square-like fragments.

The density of the particles also affects the bioavailability, as particles with densities lower

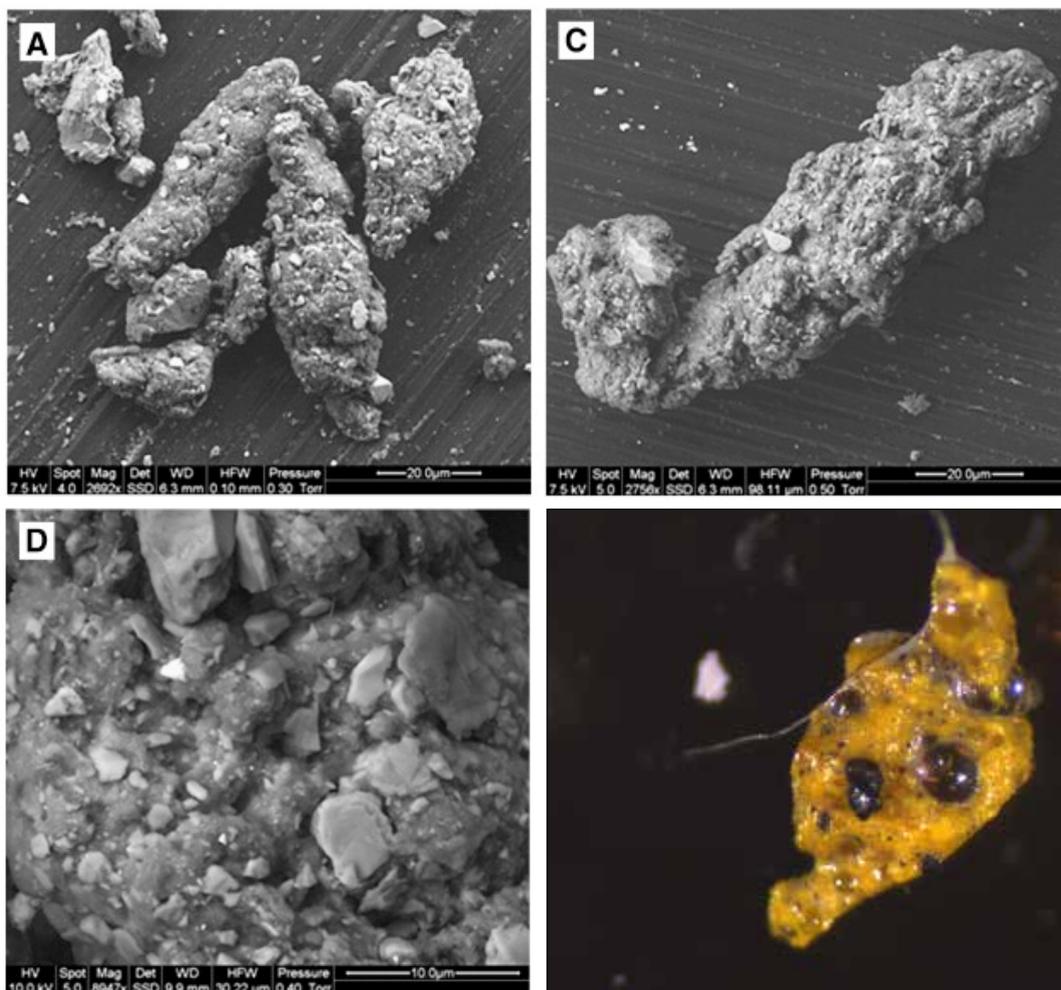


Figure 2. Examples of the shape and form of RWPPMB particle (A), a TWP particle (C, D) and a RWPRM particle (lower right corner). Photo: A, C and D is adapted from Kreider et al., 2010 and reprinted with permission from Elsevier. The RWPRM particle is a photo by Elisabeth Rødland, NIVA.

or close that of freshwater (1 g/cm^3) will float and easily available for all organisms that live in the surface layer. Sea water have higher densities, depending on the salt concentration ($1.02\text{-}1.03 \text{ g/cm}^3$). Particles with densities higher than the surrounding medium, will sink to the bottom. However, the time it uses to sink will be influenced by the turbulence in the medium. Asphalt and minerals such as quartz and limestone, have high densities (2.36 g/cm^3 , 2.65 g/cm^3 , 2.0 g/cm^3 , respectively) and will fall to the sediments in both freshwater and saltwater recipients. The density of TWP range between $1.7\text{-}2.1 \text{ g/m}^3$

(Kayhanian et al. 2012, Snilsberg 2008). RWP_{PMB} is expected to have a similar range as TWP (Vogelsang et al., 2018). RWP_{RM} on the other hand is expected to have lower densities ($>1.2 \text{ g/m}^3$) than both TWP and RWP_{PMB} , depending on the amount of glass beads in each particle.

Chemical composition

Tire-wear particles (TWP)

The different tire producers have their own recipes for different types of tires, and the exact recipes are confidential information. Overall, they all have 40-60 % of polymers (SBR, isoprene,

other polymers), 20-35 % carbon black (reinforcer), mineral oils, ZnO, S, Se and additives (amines, phenols, aromatic and aliphatic esters, others). Especially the use of different additives may differ a lot between different tire manufacturers and different tire types, and some of these may pose an ecotoxic threat. For instance, Peter et al. (2018) studied leachates from TWP and road runoff using nontarget screenings, and found several compounds linked to mortality of coho salmon (*Oncorhynchus kistuch*). A (methoxymethyl)melamine compound group detected in both TWP and road runoff, as well as in urban creeks suggests that tire leachates is more important as a toxic source in road runoff than previously recognized. They also found significant concentrations of long-chained glycols and ethoxylates (PEGs, OEPOs and PPGs) and bicyclic amines. In Marwood et al. (2011) they found the organic compound aniline and N,N'-bis(1,4-dimethyl(pentyl)-p-phenylenediamine. Aniline has previously been found to be acute toxic to *D. magna* and these were all lower concentrations than those found the study of Marwood et al. (2011).

Road wear particles from road marking (RWP_{RM})

Two types of road marking are used in Norway, thermoplastic markings and water-based polymer

paint (Sundt et al., 2014). In the thermoplastic type the polymer content is 1-5 % (Sundt et al., 2014). According to one of the producers of road markings in Norway, the binding agent constitutes 20%. 2% of this is polymers and the rest is made of natural or synthetic resins and oils (Geveko, 2018). The polymers used are either Styrene Isoprene Styrene (SIS) or ethylene-vinyl acetate copolymer resin (EVA) in the white markings, and SIS or polyamid in the yellow markings (Geveko, 2018). Further the markings have 5-7% pigments (Ti, organic pigment), 30-35% fillers (dolomites, quartz), 40% glass beads made from recycled glass (old glass windows).

Road wear particles from polymer-modified bitumen (RWP_{PMB})

Road pavement in Norway is made from asphalt. Asphalt is 94-95% minerals and 5-6 % bitumen. Some types of asphalt have polymer-modified bitumen (PMB). In PMB, polymers are added to it to enhance the performance in area with large impact from heavy traffic. In PMB asphalt, 3-10% of the bitumen is polymer. The most common used polymer in PMB in Norway is Styrene Butadiene Styrene (SBS). Typically, 5% of the bitumen is SBS on Norwegian PMB roads (Statens vegvesen, 2014).

Table 1: Summary of the known size, shape, density and chemical composition of RAMP.

	TWP	RWP _{PMB}	RWP _{RM}
Size	85% 50-350 µm 15% <50 µm	Assumed similar to TWP	50-4000 µm
Shape	Elongated, sausage-like particles	Assumed similar to TWP	Squared-like flakes
Density	1.7-2.1 g/m ³	Assumed similar to TWP	>1.2 g/cm ³
Chemical composition	40-60 % of polymers (SBR, isoprene, other polymers), 20-35 % carbon black (reinforcer), mineral oils, ZnO, S, Se and additives (amines, phenols, aromatic and aliphatic esters, others).	94-95% minerals and 5-6 % bitumen (3-10%: SBS)	20% binding agent (2% polymer: SIS, EVA, 18% natural or synthetic resins), 5-7% pigments (Ti, organic pigment), 30-35% fillers (dolomites, quartz), 40% glass beads
References	Kreider et al., 2010; Broeke et al., 2008; Wang et al., 2017; Kayhanian et al., 2012; Snilsberg, 2008.	Statens vegvesen, 2014; Vogelsang et al., 2018.	Vogelsang et al., 2018; Geveko, 2018.

Concentrations of RAMP in the environment

Knowing the concentrations of RAMP in the environment is important for many reasons, both to assess the scale of the potential problem, to measure the transport from the source and to the environment and to evaluate the different measures we can install to stop RAMP from impacting the environment. For toxicity assessments we also need to know what the relevant environmental concentrations are. Current estimates based on emission factors indicate a yearly emission of 8400 tons microplastic in Norway (Sundt et al. 2014), with 4668 – 5348 tonnes in total coming from RAMP (Sundt et al., 2014; 2016; Vogelsang et al., 2018).

TWP - estimates

The yearly estimated emission of microplastic particles from tires is 4250-5000 tons (Sundt et al. 2016, Vogelsang et al. 2018). The TWP emission is although higher, depending on how much polymers we assume are in the tires. Most literature have described the polymer content in tires as 40-60%, which means that the total TWP emission for Norway is between 7 083 – 10 625 tons per year using the estimates of Sundt et al., or 2016 or 8 333 – 12 500 tons per year using the estimates of Vogelsang et al., 2018. Similar estimation studies from other countries have also concluded that tires are the main source of microplastic particles from roads (Sundt et al. 2014, Sherrington et al. 2016). For German highways, Wagner et al. (2018) also estimated the mass flow from highways to the environment in different scenarios. In these models, only between 6% and 23% of the total TWP reached water bodies. The expectation is that the smaller TWP particles are more easily transported from the roadsides than the larger ones. This will also affect how well low-cost measures such as gully-pots and sedimentation ponds work when it comes to removing TWP from road runoff (Loganathan et al., 2013). Many of the larger roads in Norway has gully-pots to retain particles and particle-bound pollution from road runoff. About 20-50% of

the total solid matter that reaches the gully-pots are retained (Deletic et al., 2000; Pitt and Field, 2004). Using estimated efficiency of the gully-pots, the highest retention (50-60%) is assumed for particles in the size 200-250 µm during low water flow events (5 L/s). For particles 50-200 µm, the retention is estimated to be much lower (10-50% at 5 L/s and 0-20% at >15 L/s). Goonetilleke et al. (2017) also shows that both the accumulation and transport of TWP will be affected by factors such as rain intensity and infrastructure. As most roads in Norway have little (gully pots) or no water treatment systems, potentially all RAMP will be deposited alongside the roads and the smaller fractions will be transported to rivers, ponds, lakes and marine waters through the local runoff processes.

TWP – measured environmental concentrations

There are a limited number of studies that have measured the concentration of TWP in the environment. Nearly all of them have used tracers to estimate the concentration of TWP, such as different benzothiazoles and sink (Zn) that are present in tires, and some has measured the concentrations of tire-related polymers (SBR/NR). The most studied matrices are road dust from the road surfaces, roadside soil, sediments from sedimentation basins and river sediments. These studies are all from different countries all over the world and represent different traffic volumes, as well as being measured with different analytical approaches and the comparison between them should therefore be done with caution. However, they represent the current knowledge of TWP concentrations in the environment and is therefore relevant to use when discussing the ecotoxic potential of RAMP.

Figure 3 summarizes the findings of Wik and Dave, 2009; Unice et al., 2013; Klöckner et al., 2019 and Bye & Johnson, 2019. In Wik and Dave (2009) the current knowledge on concentrations in the environment was presented and discussed, using several sited studies. The concentrations of TWP in road dust differed between road surfaces outside tunnels (700 – 72000

mg TWP/kg) and inside tunnels (2700 – 210 000 mg TWP/kg) (Wik and Dave, 2009). One recent study (Bye & Johnson, 2019), the only study from Norway included here, looked at the concentrations of TWP in a tunnel with high traffic volumes (Smestad tunnel in Oslo, annual average daily traffic, AADT, 66 322). Bye & Johnson (2019) found 4083 mg TWP/L in the tunnel wash water. The corresponds to the accumulation of TWP since the last tunnel wash, a period of 60 days, and a production of nearly 3 kg of TWP per day.

The concentrations found for road runoff is found between 12 and 179 mg TWP/kg (Wik and Dave, 2009). One study has looked at roadside snow and found 563 mg TWP/L (Wik and Dave, 2009). In roadside soil, the highest concentrations were found closest to the road, ranging between 600 – 117 000 mg TWP/kg at 0m, with considerably lower concentrations from 0-30 m from the road (0-1000 mg TWP/kg) (Wik and Dave, 2009). In Eisentraut et al.

(2018) they used a Thermal extraction desorption gas chromatography–mass spectrometry (TED-GC-MS) to measure the amount of SBR from sediments in a road runoff treatment. They found 3.9 – 9.3 mg/g of SBR in the samples. The approximate concentration of SBR in a tire is 11.3% (Eisentraut et al., 2018), although this probably varies a lot between different tire brands and types of tires (i.e. summer- and winter tires, studded and non-studded). Using the approximate value of SBR, calculated concentration of TWP in the sample is between 34.5 – 82.0 g TWP/kg runoff sediments.

In road sedimentation basins, the highest concentrations were found in the sediments, 350 -130 000 mg TWP/kg, (Klößner et al., 2019; Wik and Dave, 2009) and the lowest concentrations found in water, 2,3 mg TWP/L (Wik and Dave, 2009). One study has also looked at the retention of TWP in soil retention filters and found 150 000 mg TWP/kg accumulating in this filter (Klößner et al., 2019).

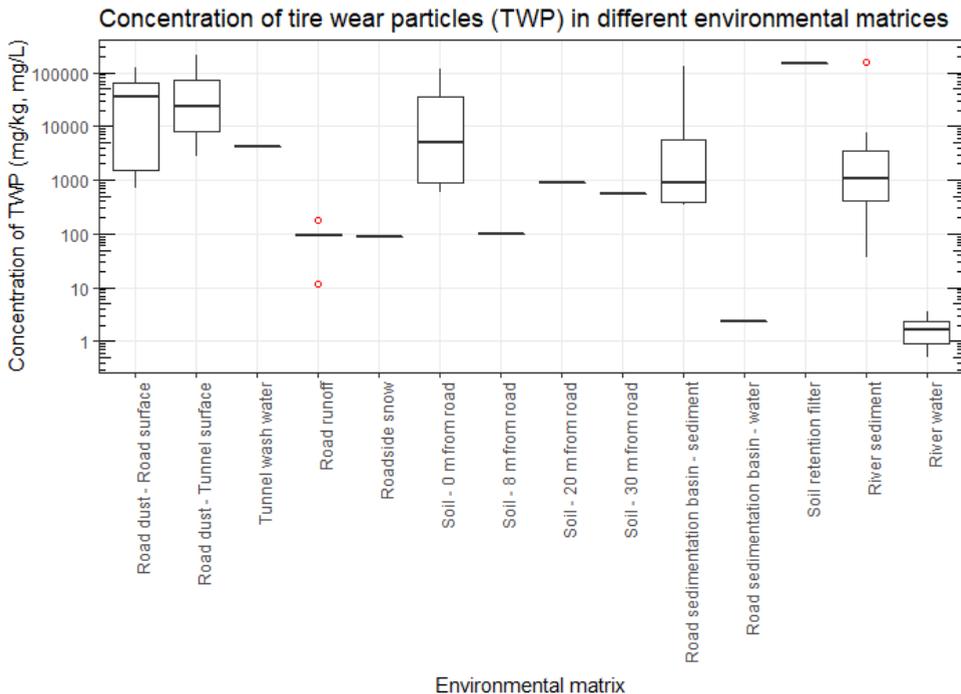


Figure 3: The figure shows a boxplot of TWP concentrations in the environment. Red dots represent outliers. Each data entry is a mean value, and the figure summarizes a number of different studies from 1974 – 2019. The figure is based on data from Wik and Dave, 2009; Unice et al., 2013; Klößner et al., 2019 and Bye & Johnson, 2019.

For river sediments, the concentrations found vary a lot, between 36 to 155 000 mg TWP/kg (Wik and Dave, 2009; Unice et al., 2013). For river water, the concentrations are much lower, 1.6-36 mg TWP/L. Comparing the concentrations found in river water and water from sedimentation ponds to river sediments and sediments from sedimentation ponds, the current data provide clear indications that TWP will accumulate in the sediments.

RWP_{RM}

Road markings are estimated as the second largest source of microplastics from roads, with a yearly emission of 90-320 tons of polymers in Norway (Sundt et al., 2014; Vogelsang et al., 2018). Road markings have also been studied in other European countries, although found to be considerably lower compared to Norway (Sherrington et al., 2015). This difference may be linked to the difference in climate between Norway and the rest of Europe, in which road paint is worn away a lot faster by both weather, road maintenance and the use of studded tires. Some of the road paint will also be removed together with removal of old asphalt, so not all the plastic in road paint will turn into microplastic particles. There is also uncertainty to how the traffic (Average Annual Daily Traffic, AADT), the use of studded tires and other factors affects the deterioration rate (Sundt et al. 2016). When it comes to measuring concentrations of RWP_{RM} in the environment, Horton et al. (2016) was the first study to report these particles in environmental samples. They found road marking fragments from sampling the river Thames in the United Kingdom. From Norway there are currently no published data on the concentrations, although RWP_{RM} has been found in roadside samples (Vogelsang et al., 2018) and in tunnel wash water (unpublished data from Rødland, 2019).

RWP_{PMB}

PMB is estimated as the third and the smallest source of microplastics from roads, with a yearly emission of 28 tonnes of polymer (SBS) (Vogel-

sang et al. 2018). This estimate is based on the total length of roads in Norway with PMB (2770 km of county and state roads; Statens vegvesen, 2018) and the abrasion by studded tires. The estimates are based on several assumptions (Vogelsang et al., 2018), as both the amount of SBS and the percentage of studded tires is difficult to assess accurately.

Uptake of RAMP and possible effects in organisms

When assessing the ecotoxic potential of RAMP, we need to both assess the toxicity of the particles as well as the probability of exposure to organisms. Several studies have documented uptake of microplastic particles in different organisms of different trophic levels (Wright et al. 2013, Su et al. 2018, Bråte 2017, Lusher et al. 2017). However, there are no studies yet proving that RAMP is taken up by organisms in the environment. In Lusher et al. (2017), they found black rubbery particles in blue mussels, which fits the size and shape characteristics of tire particles (Figure 4). In this study they did not have the analytical tools to determine the polymer content of these particles because of the high content of carbon black. The site where it was found is close to Oslo centre and receives a high input of urban runoff, so the presence of tire particles here is probable.

However, there are several studies that have done toxicity experiments with TWP on orga-

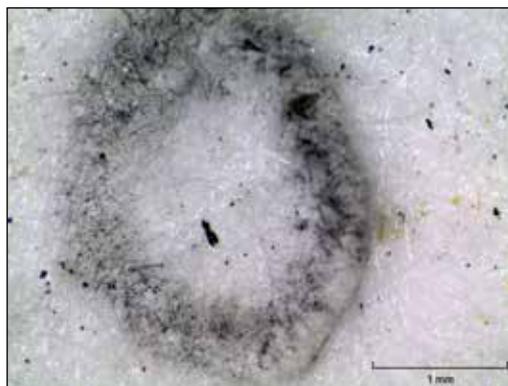
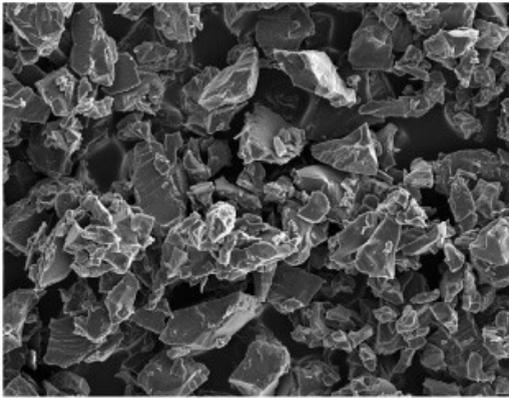
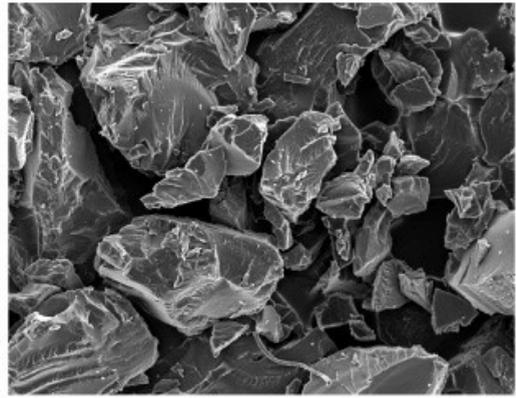


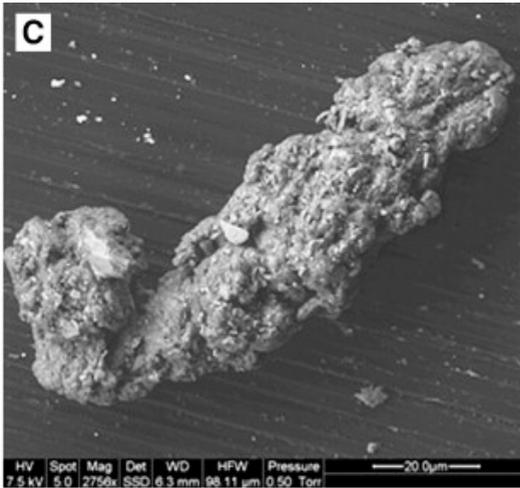
Figure 4: One of the possible tire particles found in blue mussels off Akershus Festning. Photo: Inger Lise N. Bråte, NIVA.



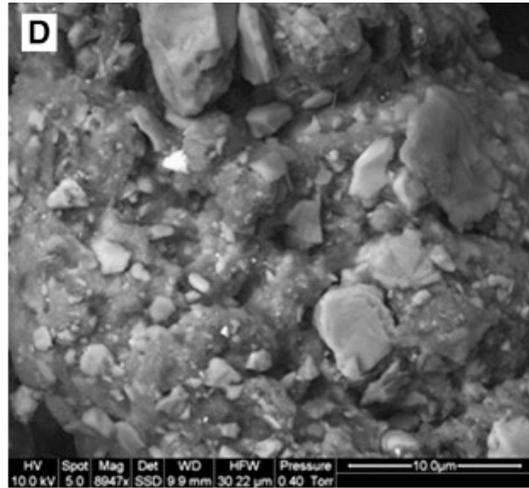
(a)



(b)



C



D

Figure 5. (a, b) Tire particles made by shredding. Pictures taken in electron microscope. Adapted from Specht et al., 2007. (C, D) Tire particles made by road simulator. D) showing the mineral encrustation on the particle surface. Adapted from Kreider et al., 2010 and reprinted with permission from Elsevier.

nisms, confirming that uptake of hazardous compounds from tires are possible. These are summarized in Table 2. No such studies have been found performed for RWP_{RM} or RWP_{PMB} .

Due to difficulties in finding the TWP in the environment, many studies have used lab-made tire particles (TP) in their toxicity tests. These can be made in different ways; grounded tires or tire scrap (granulates: TP_{GR}), cryo-fractured particles (TP_{CF}), particles abraded from the tires with different rasps or steel files (TP_{AB}) or made with road simulators (TWP_{RS}).

Research has found that the morphologies of shredded tire particles, TWP_{RS} and TWP found

in road runoff differs quite a lot (Figure 5) (Wagner et al., 2018; Specht et al., 2007; Kreider et al., 2010; Wang et al., 2017; Camatini et al., 2001). This shows that there are several processes that affects the TWP in real road conditions. These processes might alter both the size, shape and chemical composition of the TWP, as well as create conglomerates of the TWP together with minerals and other road particles (Panko et al., 2013; Adachi and Tainosho, 2004). This makes it harder to replicate samples in the lab or by road simulators. Still, using a road simulator with asphalt pavement to create the particles would be the method closest to the way TWP is

made in the environment, giving particles both similar in shape and size, and possibly also with the encrustment of minerals and bitumen from the asphalt, giving these particles similar density as real TWP.

Tire leachate

Tire leachates is a liquid sample where TP have been present, but then removed before using it further in the toxicity tests. Using TP_{GR} from old tires, Villena et al., 2017 exposed two species of mosquitos (*Aedes albopictus* and *Aedes triseriatus*) to concentration of 100 000 mg/L, in dilutions of 0.1-100%. The two species responded differently, one species showed rapid decline in growth and survival at exposure of 100 mg/L, and the other species at 10 000 mg/L. Using TP_{CF} from tire scrap materials, Gualtieri et al. (2005a), exposed embryos of *Xenopus laevis* (frog) to concentrations of 50 000 mg/L and 100 000 mg/L TP_{CF} in series of dilution from 0-100%. Undiluted tire leachate with 50 000 mg/L TP_{CF} caused 80% mortality in the embryos, as well as teratogenic effects using both concentrations. EC50¹ was found at 50 000 mg/L at 40.2% dilution and for 100 000 mg/L at 73.3% dilution. Mantecca et al. (2007) also exposed *Xenopus laevis* to TP_{CF} from tire scrap materials at the concentrations of 50-1400 mg/L TP_{CF}. 50 mg/L was found to be the non-observable effect concentration (NOEC²). Mortality effects were seen at 80 mg/L and above. The LC50³ was found at 145 mg/L.

In Wik and Dave (2005), they used the 250-16 000 mg/L TP_{AB} concentrations made from 12 different new tires in their tests with the water flea *Daphnia magna* (daphnid). All tests showed toxicity with *D. magna* after both 24hour (24h) exposures and 48hour (48h) exposures. The EC50 for 24h varied between 290 to 32 000 mg/L and the EC50 for 48h varied between 62.5 to 2100 mg/L. All tires were found to be toxic

and all showed a higher toxicity after 48h compared to 24h exposures. In Wik and Dave (2006), they tested 24 separate leachates made from 24 new tires on *D. magna* (900 mg TP_{AB}/90ml). The mixture was heated to 44°C for 72 hours, representing a possible “worst case scenario” from a hot summer day. Immobilisation of *D. magna* varied a lot between the different tire types. The toxicity increased in all samples with exposure time, and the 24h EC50 ratios ranged from 1400 to >10 000 mg/L and the 48h EC50 ranged from 500 to >10 000 mg/L. For the 48h EC50 the variation was up to 25%. In Wik et al. (2009), leachates were tested on *Daphnia magna*, *Ceriodaphnia dubia* (daphnid), *Pseudokirchnerella subcapitata* (algae), eggs from *Danio rerio* (zebra fish) at concentrations of 10-10 000 mg TP_{AB}/L for periods of 5, 9, 20, 7, 5, and 11 days (leaching 1-6). The results differed between species and tire types. The tests showed that there was a significant decrease in toxicity from the number of days leaching, in which leaching 6 had significantly lower toxicity than leaching 1. For all test organisms the EC50 after leaching 6 was >100 mg/L. In the same study they also did toxicity identification to characterise the compounds found in the tire leachates and found that the toxicity was mainly caused by zinc and lipophilic organic compounds. Turner and Rice (2010) used leachates from 20 different used tires at concentrations of 500 mg TP_{AB}/L (<500 µm) on *Ulva lactuca* (macroalgae), and the test observed toxicity as reduced photochemical conversion at 25mg/L concentrations.

Three studies used TP_{RS} to make the leachates. Gualtieri et al. (2005) used particles from 10-80 µm in the concentration 50 000 – 100 000 mg/L. In *D. magna*, all juveniles exposed to 10% concentration of tire leachate died after only 5 days. They also found strong teratogenic effects in *Xenopus laevis* (frog), and undiluted leachate caused mortality in 80% of the organisms. In

¹ EC50: (median effective concentration) is the concentration of the test substance that causes 50% reduction in the effect parameter (Walker et al., 2012)

² NOEC: (No Observed Adverse Effect Concentration) is the highest exposure concentration at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control (https://www.chemsafetypro.com/Topics/CRA/Toxicology_Dose_Descriptors.html)

³ LC50: a statistically derived dose at which 50% of the animals will be expected to die (https://www.chemsafetypro.com/Topics/CRA/Toxicology_Dose_Descriptors.html)

Marwood et al. (2011), particles below 150 µm was used and leachates was made with 625 to 5000 mg/L concentrations and elutriates made with spiked sediment (100 - 10 000 mg/L). On the elutriates, 4 different temperature regimes were used. They used three organisms, *Pseudokirchneriella subcapita*, *Daphnia magna* and *Pimephales promelas* (fish). The only observed effect was in *D. magna* exposed to elutriates that had been kept at 44°C (NOEAC: 1.250 g/L). The leachates kept at room temperature did not yield a similar response and the NOEAC for this was above 10 g/L. There was no observed response in the other test organisms. In Panko et al. (2013), they used TP_{RS} below 150 µm and used spiked sediment to create elutriates, which was further used in the toxicity tests. The test organisms used was *Ceriodaphnia dubia* (daphnid) and *P. promelas* (fish). They found no significant effects of the tests.

Spiked sediments

Camponelli et al. (2009) used TP_{GR} to spike sediments with a concentration of 83 800 mg/kg and exposed the matrix to *Rana sylvatica* (frog). The presence of TP_{GR} in the sediment had an impact on both hatching success and hatching time used, but no mortality was observed. Marwood et al. (2011), spiked sediments with TP_{RS} at concentrations 100, 500, 1000 and 10 000 mg/L. The size of the particles were below 150 µm. The organisms used were *Pseudokirchneriella subcapita*, *D. magna*, and *P. promelas*. No acute toxicity was found for any of the organisms at the tested concentrations and the NOEAC was established at >10 000 mg/L. Panko et al. (2013) also used sediment spiked with 10 000 mg/kg TP_{RS}. The test organisms used *Chironomus dilutes* (lake fly) and *Hyalella azteca* (amphipode). Only for *C. dilutes* some growth inhibition was observed (-20%), however, this was not significant.

Road runoff sediments

In the study of Wik et al. (2008), sediments were collected from road runoff treatment systems and used in acute toxicity tests. The concentra-

tion of TWP was found by the organic zinc content of the sediment and found to be between <150 to 10 800 mg/kg dry sediment. They used *Hyalella azteca* for the test on the whole sediment sample and also used sediment to make elutriates, which was tested on *Daphnia magna* and *Ceriodaphnia dubia*. Even though they found contaminants in the sediment that exceeded the values in which they expected to find toxic effects (high levels of zinc (Zn) and copper (Cu) as well as PAHs), they found little or no correlation with the toxic tests.

Direct exposure to TP compared to tire leachates

In the study of Khan et al. (2019), they made TPs by abrading a used tire with a grind stone and sieved it with a 500 µm steel mesh. Then they used the particles in two types of tests, one with particles dispersed in freshwater medium and one with leachates of the TPs. The test organism was *Hyalella azteca*. The TP exposure was with 0-15 000 TP/mL and the tire leachate exposure was made with 0-125 000 TP/mL. The transit of TPs through the digestive tract of *H. azteca* was photographed with a stereomicroscope camera, and all test organisms ingested the TPs. Gut retention time was 24-48 hours. In the acute tests, the study found differences in the response between the organisms exposed to dispersed particles compared to those exposed to tire leachates. For the tests with TPs, the LC50 was found at 3426 ± 172 TP/mL, but using the tire leachates, the exposure did not follow a sigmoidal concentration-response pattern so the LC50 could not be determined. At low numbers of particles, it was reported that the tire leachates are more toxic than the particles, but at higher numbers of particles this shifted and the presence of TPs seem to cause more toxic effects than the leachates. As suggested in the study, this may be explained by the ingestion of the particles by the animals, causing a higher uptake of the harmful chemicals in the tire particles than what is achieved directly from the water. It could also be caused by physical effects from the particles themselves. Khan et al. (2019) also

Table 2: Summary of the toxicity test studies relevant for assessing the toxic potential of RAMP

Test matrix	Size	Concentration	Organisms	Outcome	Reference
Tire leachate, TP _{RS}	10-80 µm	50 000 – 100 000 mg/L 10%	<i>Daphnia magna</i>	100% mortality in juveniles	Gualtieri et al. 2005b
		50 000 – 100 000 mg/L 100%	<i>Xenopus laevis</i>	80% mortality + teratogenic effects	
Tire leachate, TP _{RS}	<150 µm	625-5000 mg/L	<i>Pseudokirchneriella subcapita</i>	No observed effects	Marwood et al. 2011
		625-5000 mg/L	<i>Daphnia magna</i>	No observed effects	
		625-5000 mg/L	<i>Pimphales promelas</i>	No observed effects	
Elutriates from spiked sediment with TP _{RS}	<150 µm	100-10 000 mg/L all temp.	<i>Pseudokirchneriella subcapita</i>	NOEAC > 10 000 mg/L	Marwood et al. 2011
		100-10 000 mg/L all temp.	<i>Pimphales promelas</i>	NOEAC > 10 000 mg/L	
		100-10 000 mg/L room temp	<i>Daphnia magna</i>	NOEAC > 10 000 mg/L	
		100-10 000 mg/L high temp.	<i>Daphnia magna</i>	EC/LC50 5000 mg/L, NOEAC 1250 mg/L	
Elutriates from spiked sediment with TP _{RS}	<150 µm	10 000 mg/kg	<i>Ceriodaphnia dubia</i>	No observed effects	Panko et al. 2013
		10 000 mg/kg	<i>Pimphales promelas</i>	No observed effects	
Tire leachate, TP _{GR}	<590 µm	100 000 mg/L, 0.1-100% diluted	<i>Aedes albopictus</i>	Zero growth and survival > 10 000 mg/L	Villena et al. 2017
		100 000 mg/L, 0.1-100% diluted	<i>Aedes triseriatus</i>	Zero growth and survival > 100 mg/L, declined growth and survival < 100 mg/L	
Tire leachate, TP _{CF}	na	50 000-100 000 mg/L, 0-100% dilution	<i>Xenopus laevis</i>	50 000 mg/L 80% mortality. EC50 (50 000 mg/L): 40.2%, EC50 100 000 mg/L 73.3%	Gualtieri et al. 2005a
Tire leachate, TP _{CF}	na	50 - 1400 mg/L	<i>Xenopus laevis</i>	NOEAC < 50 mg/L. Mortality effects > 80 mg/L. LC50 145 mg/L.	Mantecca et al., 2007
Tire leachate, TP _{AB}		250 - 16 000 mg/L	<i>Daphnia magna</i>	Toxicity effects in all tests. 24h EC50 290-320µm/L, 48h EC50 125-2410 mg/L	Wik and Dave, 2005
Tire leachate, TP _{AB}	na	900 mg/90mL water, 44C for 72h	<i>Daphnia magna</i>	24h EC50 1400 -> 10 000 mg/L, 48h EC50 500 -> 10 000 mg/L.	Wik and Dave, 2006
Tire leachate, TP _{AB}	na	10 000, 1000, 100, 10 mg/L water. Leaching 5-11 days (leaching 1-6).	<i>Daphnia magna</i>	EC50 370 mg/L (2nd leaching, tire A) to 7450 mg/L (2nd leaching, tire B)	Wik et al., 2009
			<i>Ceriodaphnia dubia</i>	EC50 10 mg/L (2nd leaching, tire A) to 3590 mg/L (6th leaching, tire C)	
			<i>Danio rerio</i>	No consistent toxicity found	
			<i>Pseudokirchneriella subcapita</i>	EC50 50 mg/L (1st leaching, tire A) to 2840 mg/L (6th leaching, tire B)	
Tire leachate, TP _{AB}	<500 µm	500 mg/L seawater	<i>Ulva lactuca</i>	Toxicity effects 25mg/L	Turner and Rice, 2010
Spiked sediments, TP _{GR}	na	83 800 mg/kg sediment	<i>Rana sylvatica</i>	Delayed hatching, no mortality observed	Camponelli et al., 2009
Spiked sediments, TP _{RS}	<150 µm	100-10 000 mg/L	<i>Pseudokirchneriella subcapita</i>	NOEAC > 10 000 mg/L	Marwood et al. 2011
		100-10 000 mg/L	<i>Pimphales promelas</i>	NOEAC > 10 000 mg/L	
		100-10 000 mg/L	<i>Daphnia magna</i>	NOEAC > 10 000 mg/L	
Spiked sediments, TP _{RS}	<150 µm	10 000 mg/kg	<i>Chironomus dilutes</i>	20% growth inhibition, not significant	Panko et al. 2013
		10 000 mg/kg	<i>Hyalella azteca</i>	No observed effects	
Runoff sediment	na	<150 -10 800 mg/kg dry sediment	<i>Hyalella azteca</i>	No observed effects	Wik et al. 2008
Elutriates from runoff sediment	na	<150 -10 800 mg/kg dry sediment	<i>Ceriodaphnia dubia</i>	No observed effects	
		<150 -10 800 mg/kg dry sediment	<i>Daphnia magna</i>	No observed effects	
Direct exposure TP _{AB}	<500 µm	0-15 000 particles/mL	<i>Hyalella azteca</i>	LC50 3426 ± 172 particles mL, zero survival at 2000 TP/mL for 21 days	Khan et al., 2019
Tire leachate, TP _{AB}	<500 µm	0-125 000 particles/mL	<i>Hyalella azteca</i>	No LC50 established	

performed a long-term exposure of 0-2000 TP/mL for 21 days, and the *H. Azteca* had zero survival when exposed to 2000 TP/mL for 21 days and negative effects on reproduction and growth was found at the lower concentrations. As suggested by the study, more research is needed on this subject to understand what the actual effects in the environment will be.

Discussion

RAMP is not a homogenous plastic compound, but rather a complex particle matrix with different chemicals and particle properties. Several of the chemical compounds found in RAMP particles can be acute or chronically toxic to different organisms at different concentrations by themselves. For TWP, especially zinc (Zn) has been pointed out as one of the toxic compounds to aquatic organisms, as it can be both acute toxic as well as lead to sublethal effects (in gills, liver and reproduction of fish) (Giardina et al., 2009; Nelson et al., 2004). The organic compounds (methoxymethyl)melanine (Peter et al., 2018) and aniline (Marwood et al., 2011) found in tires has also been linked to toxic effects. However, as there exist several different tire brands as well as a range of different types of tyres within each brand, and they all keep the recipes for their tires secret, it is difficult to know which potential toxic compounds are in each tire. In Wik and Dave (2006) they tested 24 different unused tires and found a large variation in the toxicity from each tire. They measured a large variation in the concentration of Zn from the tires in the leachates, between 110 to 590 g/L⁻¹. These findings were supported by other tests done by Wik and Dave (2005) and Wik et al. (2009). This makes toxicity assessments of tires rather difficult. Even so, several studies have found toxic effects in toxicity tests with tire leachates made from both new and old tires. This indicates that there are toxic compounds in tires that can potentially harm organisms. It also shows that TWP might be toxic to organisms even if particles are not being taken up, as harmful chemical substances are being leaked from the particles to the environment. Toxicity tests using

concentration levels of TWP currently found in river sediments as well as road runoff and tunnel wash water have confirmed toxicity effects in organisms, as well as tests using higher concentrations of TWP indicates even stronger negative effects. Even though the concentrations used in some of these toxicity tests are well above the values found in river water, they may realistic to rivers and water bodies receiving untreated road runoff and tunnel wash water. As there are only a few studies available on the concentrations of TWP in the environment so far, we do not actually know what the relevant environmental concentrations of TWP is. As with road runoff in general, the concentration of TWP might be related to several factors, such as speed and acceleration, which causes higher friction and increases the wear and tear of each tires.

When it comes to RWP_{RM} and RWP_{PMB}, there are currently less knowledge on both the relevant environmental conditions and the toxicity compared to TWP. According to estimates, the concentrations of both are expected to be far lower than for TWP. However, the densities of RWP_{RM} is expected to be lower than for TWP, which might cause it to float more easily and thus be more bioavailable to aquatic organisms than TWP. When it comes to the toxicity, most of the road paint is made of quartz and dolomites and glass beads (70-75%), but it does also include TiO₂, which have been found to cause different adverse effects, for instance cell damage, genotoxicity, inflammation etc. (Skokaj et al., 2011). Also, the glass beads that makes up about 40% of the road marking, is mainly made from recycled glass, usually old windows. Many old windows have toxic elements in them (Pb, As, Sb) (Santos et al. 2013). The RWP_{PMB} is mainly made of minerals and with a small amount of bitumen (5-6%), in which the polymer is added. The bitumen itself is made from saturated hydrocarbons, aromatics, resin and asphaltene (Strausz et al., 2010). Naphtene acids coming from bitumen have been found to be both cytotoxic and cause endocrine disruption and found to cause adverse effects in both fish and mammals (Headley and McMartin, 2004).

The size of RAMP is also important to consider when looking at the toxic potential, as different organisms will be affected by particles of different sizes. The smaller the particles are, the easier they will also pass to critical parts of an organisms, such as blood stream or over cell walls. So far, most of the RAMP that have been observed in the environment is found to be larger than 50 μm , which means they are in the size range like sand and above silt and clay. RAMP is therefore expected to mainly be exposed to organisms either as leachates or taken up as possible nutrients by filter-feeding organisms. The shape of RAMP differs between TWP and RWP_{PMB} on one side as elongated, round shapes and RWP_{RM} on the other hand as square-like larger flakes. There are so far no studies confirming actual uptake of RAMP in the environment except some possible uptake of TWP in blue mussels (Lusher et al., 2017). However, it is possible that TWP and RWP_{PMB} resembles other sediment particles, and they might therefore be more likely taken up by organisms that look for food in sediments. On the other hand, if organisms feed on sediments, they may not distinguish between the RAMP particles in the sediment at all. In that case, it might be more likely that RWP_{RM} are the ones that get retained in the digestive system because of their irregular shape. As there are no actual studies published on this, this is just speculations and more research is needed on this subject.

In the environment, RAMP will always be present together with other road-related substances, which also has possible toxic effects, such as zinc (non-tyre related), cadmium, nickel and organic pollutants such as PAH and PCB. It may be difficult to distinguish between the toxicity of TWP, RWP_{RM} , RWP_{PMB} and road runoff in general when doing field studies. Also, distinguishing between road-related microplastics, meaning the particles with polymers in them, and other road-related particles, might well be an artificial and less optimal way of studying road runoff. As seen by the available toxicity studies on TWP, it is probably the amount of Zn and different organic compounds in the tires

that causes toxicity, and not the polymers themselves.

Conclusions

Toxicity studies on RAMP is only available for TWP, and current studies vary a lot between exposure conditions and set up. Some studies confirm toxicity effects at relevant concentrations, thus showing that TWP has a potential to be toxic to organisms. However, most studies so far have used either concentration well above what is expected in the environment or environment conditions that are harsher than in real-life (e.g. high temperatures to create leachates). There is still an urgent research need to perform toxicity tests under realistic conditions, using real TWP, RWP_{RM} and RWP_{PMB} and standardized tests so the studies can be compared. In order to do this, we still need to have more information about the concentrations of RAMP in the environment, at different areas (roadsides, sediment, water phase etc.). The difference in toxicity between different tires must be considered when preparing new toxicity tests. It should also be a discussion on why the tires are so different and if there are any compounds in the tires that can be limited to lower the toxicity of tire leachate to the environment.

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