UiT The Arctic University of Norway

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Investigating the Effect of Pressure and UV Radiation on Antioxidant and UV Stabilizing Additives in Different Tire Particles

An extraction study on the effect of laboratory simulated marine conditions on tire particles.

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This thesis document was typeset using the *UiT Thesis LaTeX Template*.

Finally, from so little sleeping and so much reading, his brain dried up and he went completely out of his mind.

“I need a holiday, a very long holiday, and I don’t expect I shall return. In fact,
I mean not to.”
–Bilbo Baggins

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Abstract

This thesis investigates the impact four different experimental marine conditions have on the extractability of tire additives meant to protect tires from UV radiation as well as some other common substances found in tires. The impact is evaluated between tire particle size and age to determine if the exposure affects them differently.

This thesis finds that the effect of marine conditions on extractability is more substance-dependent rather than particle size and age-dependent. In-depth evaluation of the development of extractability of five *p*-phenylenediamines (PPDs) was done and found that the most common among them (6PPD) had the least decrease in extractability over the course of the exposures. The same substance also had, by far, the largest percent content of them all, ranging from 64-97% of total extracted PPD content in samples. Its notorious transformation product 6PPD-Q was also detected in every single rubber sample with similar time-dependent extractability profiles as the rest of the PPDs.

Some effects of the experimental exposure on extractability were indicated. UV radiation exposure reduced the PPD extractability of the smallest particles, but no effect of UV radiation exposure could be determined on the larger particles. Experimental deep sea pressure conditions have no clear effect on the extractability of the substances. For each detected substance the latest time increment between the surface water and deep-sea samples within the same biotic influence groups was compared. It was counted that deep-sea pressure exposed samples more frequently had a higher extractability than samples exposed to surface water pressures. No concrete tendencies could be seen over time in the hyperbaric experiments, so there was little to no development toward this conclusion.

Abbreviations

6PPD	N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine
6PPD-Q	2-anilino-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione
77PD	N,N'-bis(1,4-dimethylpentyl)-*p*-phenylenediamine
A	Abiotic
B	Biotic
BTH	Benzothiazole
BT	Benzothiazole transformation product
CPPD	N-phenyl-N'-cyclohexyl-*p*-phenylenediamine
CRG	Crumb rubber granulate
CRU	Constant repeating unit
DC	Direct current
DNA	Deoxyribonucleic acid
DPPD	N,N'-diphenyl-*p*-phenylenediamine
DS	Deep-sea
DTPD	N,N'-di(*o*-tolyl)-*p*-phenylenediamine
EI	Electron ionization
EPA	Environmental protection agency
et al.	And others
FTIR	Fourier-transform infrared spectroscopy
GC	Gas chromatography
GC-HRMS	Gas chromatography high resolution mass spectrometry
GC-MS	Gas chromatography mass spectrometry
H.B.	Hyperbaric
He	Helium
HRMS	High resolution mass spectrometry
i.e.	That is
IPPD	N-isopropyl-N'-phenyl-*p*-phenylenediamine
ISTD	Internal standard
MS	Mass spectrometry
ND	Not detected
NILU	Norwegian Institute for Air Research
NR	Natural rubber
PAH	Polycyclic aromatic hydrocarbon

PB	Polybutadiene
PES	Polyethersulfone
PPD	*p*-phenylenediamine
PPD-Q	*p*-phenylenediamine-quinone
ppm	Parts per million
RF	Radio frequency
RNA	Ribonucleic acid
RT	Retention time
SBR	Styrene-butadiene rubber
SIM	Selective ion monitoring
SW	Surface water
TP	Transformation product
TPPD	N-phenyl-N'-tolyl-*p*-phenylenediamine
TRWP	Tire and road wear particles
TTMix	Tire tread mix
TWP	Tire wear particles
UV	Ultraviolet
UV-D	Not exposed
UV-L	UV exposed
VCR	Virgin crumb rubber
WCR	Weathered crumb rubber
Zn	Zinc

Contents

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Introduction

Mismanaged anthropological waste is turning into a larger and larger problem for the environment. New concerns regarding waste production and management, and how it is handled are continuously being made more clear to both researchers and the general public alike. Plastic waste and greenhouse gases are among the biggest topics concerning pollution and are part of numerous issues threatening the safety and habitability of our planet. The UN has defined the term 'planetary boundaries' which is a concept of nine boundaries that aim to state limits in which humanity can continue developing and prospering in the future.[1] The boundaries include widely known issues such as climate change, freshwater use, biodiversity loss, and ozone health. One of the boundaries is chemical pollution and the release of novel entities, such as plastics, into the environment.[1] This boundary is undoubtedly one of the more challenging to estimate due to the wide range of different chemicals that end up polluting the environment. The way chemicals are distributed is also one of the more challenging aspects to pinpoint, depending on many factors such as their chemical-physical properties, emission and transport routes, etc. The boundary is multiply defined as a combination of trends in production, trends of releases, and the unwanted impact on earth system processes. Both of the trends are increasing and the impact of novel entities is already reported to be negative. While there is no definitive quantitative boundary, an assessment in 2022 found that the earth is already operating outside the boundary due to the excessive production and release of novel entities. The assessment also concluded that even if production and emission were to be reduced to a sustainable level, the current presence of novel entities already poses a threat.[2]

While pollutants such as CO_2 are originating from fossil fuels, the presence of chemicals can arise from a larger variety of sources. Investigating every novel entity or chemical substance pollution is an impossible task, but the focus can be on products used in large volumes such as plastics or tires. Tires are used in an extreme volume, as nearly every land vehicle uses them. Recently, one of the most common additives in tires (N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine (6PPD)), was shown to have a derivative that was lethal to a certain species of coho salmon (Oncorhynchus kisutch).[3] This sparked an increased interest in the additives in tires. The field of tire additives is not as well documented as something such as microplastics or climate change, and it is in desperate need of further attention. Tires also have direct pathways into the environment as their intended usage produces particles that pollute the environment in the form of tire wear particles (TWP), which is also their main pathway into the environment. The magnitudes of TWP, their transport and pathways, the chemical composition of the tires, and the dangers those chemicals pose need to be further investigated.

1.1 Andromeda project

The Andromeda project is a collaborative research project between 15 European countries cooperating through a joint programming initiative called JPI Oceans. The project aims to develop efficient, advanced, and cost-effective techniques for *in situ* analysis and quantification of microplastics (including tire particles) and their degradation in marine environments.[4] This thesis is part of their 4th work package which aims to study microplastics (including tire particles) accelerated degradation in laboratory conditions. Specifically, the effect that microbiology, solar radiation, temperature, hyperbaric, and sediment environments have on the degradation of microplastics.[5]

1.2 Relevancy for a teacher

Doing a larger project within chemistry helps build knowledge of the scientific method and gives a first-hand insight into how scientists work. This knowledge could be useful in the teaching of younger pupils as the insight gained in doing a practical thesis is useful for knowing the limitations of time and knowledge for eventual student practical project work. It also helps build a repertoire of laboratory methods that are suitable for student work, and all lab work helps give the teacher more confidence in the laboratory, both with and without students.

Knowledge of environmental chemistry is also an important part of being a chemistry teacher of the future. As the curriculum involves more of an environmental focus, having first-hand knowledge of certain methods or fields could provide a unique opportunity for a teacher to get students more involved with real-world environmental chemistry. Sustainability has also recently been added as an interdisciplinary theme for all subjects in school,[6] which is the big-picture purpose that this thesis is part of.

1.3 Goal of the Thesis

The thesis aims to qualitatively and quantitatively assess what happens to selected ultraviolet (UV) stabilizing agents, some of their TPs, and a suspect list of substances frequently found in tire particles when exposed to four different laboratory experimental conditions simulating the marine environment. The tire particles were divided into three types depending on their size and weathering status. These particles include:

1. end-of-life particles (crumb rubber) that were used but not weathered (3±1 mm)

2. end-of-life particles (crumb rubber) that were used and then weathered in seawater for 12 months (3±1 mm)

3. new tire particles that were cryo-milled into fine dust (10-300 µm).

The objective was to investigate potential correlations between the behavior of the substances of interest once the particles were exposed to the experimental conditions, the age and size of the tire particles, and the exposure time. This investigation was done by doing an assessment of the extractability of the substances on samples both with and without exposure.

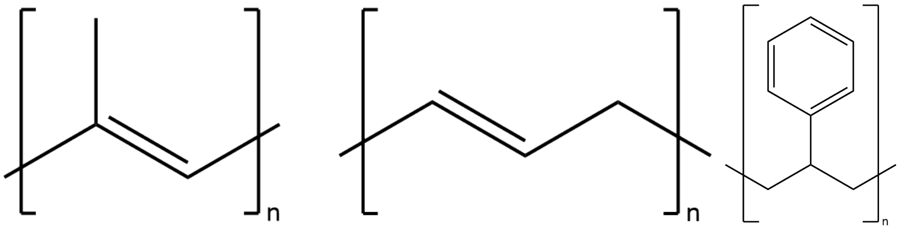
2

Background

2.1 Polymers

Polymers are chemical structures that are used in nearly every field of daily life, most often referred to as plastics. Plastics are mostly made through synthesis, but can also be naturally occurring. They can be made with many different properties such as toughness, elasticity, color, etc. Very frequently they are made with the express purpose of persistence, meaning that they are resistant to natural deformation and degradation such as weathering or bio-degradation.[7] The basic principle behind synthesizing polymers is linking together monomers in a process called polymerization.[7] The polymer's monomer or monomers are often referred to as its constant repeating unit (CRU) and is often the name given to the polymer to quickly describe which monomers it is made of. Monomers are molecules ranging from as simple as ethylene, to more complex structures such as the nucleotides of DNA and RNA. Polymers can of course then be as simple or as complex as the CRU chains that can be made. While there is not a clear definition of what a polymer is, it is commonly said to be when the molecular mass of the polymer is above 1000 amu.[7] A polymer's intrinsic abilities will depend on the molecular structure of the monomers, the total molecular weight, and the molecular structure of the polymer. Different structures in a polymer are divided into three main groups, linear polymers, branched polymers, and cross-linked polymers.[7]

Polymers can also be classified based on their chemical structures, properties, and other behaviors. Elastomers are one classification including materials with rubbery and/or elastic behavior.[7] Natural rubber is a naturally occurring elastomer stemming from trees.[8] The main elastomers in tire treads are usually blends of predominantly styrene-butadiene rubber (SBR) and polybutadiene (PB) and natural rubber (NR).[9] SBR consists of CRUs of mixtures of the monomers styrene and butadiene (Figures 2.1b and 2.1c). PB CRU is only the monomer butadiene (Figure 2.1b). NR's CRU is the monomer isoprene (Figure 2.1a).



(a) (b) (c)

Figure 2.1: The CRUs made by the monomers isoprene (a), butadiene (b) and styrene (c).

2.2 Additives in polymers

Despite polymers having intrinsic abilities based on the monomers that build long chains, it is still common to add other compounds called additives in order to have the best characteristics for their purpose. Additives are added to the polymer product either during vulcanization or mastication.[10] The duties of additives range from simply coloring the polymer, to stabilization, to protecting against the environment, to plasticizers.[11] Which additives a polymer has added to it depends on what the purpose of the product is and what it will be exposed to. For example, tires need protection against UV radiation, so UV stabilizers are added to the rubber.

Additives can be categorized into four main groups: functional additives, colorants, fillers, and reinforcements.[11] Functional additives perform duties such as softening the polymer, adding flame-resistant or heat-resistant properties, stabilizing against UV and oxidation, adding anti-static properties, curing agents, and more. Colorants are used if the product has a desirable color. Fillers are used to increase the volume at lower costs. Reinforcements are used to strengthen the polymer structure.[11] Sometimes reagents (i.e. vulcanization agents or catalyst neutralizers) are referred to as additives as well, but for the purposes of this thesis, additives will be referring to substances that manipulate the properties of the end product. Additives are not chemically bonded to the polymer but are present in the polymer matrix and are able to migrate within the polymer. This migration can happen by design such as for additives needed on the surface of the product, or by accident.[11]

While additives are an important part of polymer products, concerns need to be raised with every new additive substance as to its toxicity. In addition to the additives themselves, their derivatives need their toxicity evaluated as well, which increases the evaluation process substantially. Additives and their derivatives can leach out into the environment of whichever polymer product they are present in, including marine environments,[12–16] soil,[13,15,17] and investigations have started on direct leaching into organisms.[18] Accumulation of additives in aquatic environments should be a concern. Additives or their derivatives from tires have been reported to have adverse and even lethal effects on fish,[3,19] which has led to an increase in interest for tire additives.

2.3 Tires

Tires are almost ubiquitous when it comes to land vehicles. They provide a means of increasing friction between the vehicle and the ground, as well as cushioning for the terrain. Rubber tires consist of more than just the rubber elastomer, they are complex structures that also contain metal structures, ceramics, textiles, and additives.[20] The rubber part, called the tire tread, accounts for most of the tire with about 40-50% of the weight[20] and is the part of the tire meant to be in contact with the ground. The tread itself consists of predominantly elastomers, with almost half as much being fillers and reinforcements such as carbon black and silica.[16,21] Tire tread experiences severe friction in order to propel the car forward, and as a result, the rubber is expected to shed a significant amount of its weight as TWP.[16,22–26] These sheddings do not necessarily get processed as waste but instead can get transported into the surrounding the area and then further around the environment, often combining with road dust.[15,16,21] Tires are possible but difficult to recycle, and EU legislations are in place in order to keep them out of landfills, such as the directive ordering tires to be removed before demolition of cars.[20] Other attempts to reuse end-of-life tires are also made, such as making them into filler for artificial turfs.

In Norway, it is illegal to throw away car and trailer tires in the garbage. Instead, all tire retailers are required by law to also accept end-of-life tires, both with and without rims. The retailer is also required to ensure that the tires are recycled. By recycled the law means reusing, material recycling, or energy exploitation. The law also requires the retailers to be able to report where, and how the tires were recycled.[27] This includes exporting the tires out of Norway to be recycled.

2.3.1 Tire particles

TWP are the particles emitted from the tire tread due to friction between tires and the road they drive on. It is difficult to quantify the exact extent of TWP emitted into the environment, with issues such as difficulty to separate the particles stemming from the tire versus the road.[22] Kole *et al.* (2017) concluded that TWP emissions are between 0.23 to 1.9 kg/year per capita in 12 different countries, with the US being an outlier at 4.7 kg/year per capita.[23] Some claim that tire and road wear particles (TRWP), which includes particles from the road in addition to particles from tire tread, are the biggest source of microplastics in the environment.[24,25,28] The specific mechanisms of how TWP travel around the environment is also difficult to pinpoint, but some articles estimate 25% reach the hydrosphere[26] while others claim that less than 20% reach aquatic environments.[16] A major issue in investigating how much TWP is present in the environment is separating it from road wear particles, as the particles often mix and are transported together as TRWP.[29] Quantifying TWP from laboratory abrasion has been attempted, but these tests do not recreate accurate emissions from real-world driving.[16]

Determining the presence of tire tread also has challenges compared to the determination of microplastics. Microplastics are often determined from particulate using Fourier-transform infrared- (FTIR) and Raman spectroscopy, as it gives the chemical composition, identifying particles as microplastics.[30] FTIR and Raman spectroscopy cannot be used on tire particles however, due to the nature of the analyses methods depending on bond vibrations,[31,32] and elastomer bonds in rubber being vulcanized means the vibrations needed for the analysis are not available.[33] In addition, the carbon black content in the rubber makes it even more difficult to get valuable results from FTIR.[34] Instead, chemical markers are the main way of detecting TWP presence in an environment, by finding chemicals characteristic to tires leaching and searching for them instead of the tires themselves. The quantities of marker chemicals can then be used to make an estimate of the magnitudes of TWP abundance in that environment, but cannot precisely quantify them.[17]

Tire particles can also stem from sources other than tire tread abrasion on roads. Artificial turfs or playgrounds often use ground-up tires, called crumb rubber granulate (CRG), as the rubber infill of their fields as a way of repurposing end-of-life tires.[24,25,35–37] Estimating the release of CRG into the environment is not easy but some attempts have been made. Maintenance of football fields in Sweden has given an indication of how many tonnes of rubber granulate escapes from artificial football fields in Sweden, where they estimated 2-3 t/year per field of rubber granulate needs to be replaced, which equates to a total of 1640-2460 t/year in Sweden alone.[24] A study in Denmark estimated each athletic field using crumb rubber granulate loses 1.5-2-5 t/year, equating to 380-

640 t/year in Denmark.[36] The Norwegian EPA estimates 6% of athletic fields' CRG is transported off the fields each year, and that CRG together with plastic grass and the fall base of athletic fields pollutes 6000 t/year in Norway.[37] CRG pollution is without a doubt a significant contributor of tire particle pollution, which should be more easily addressed than TWP as the particles are generally bigger.

2.3.2 TWP transportation in the environment

TWP are predominantly (90-99.9%) in the form of non-airborne particles.[16] These particles are prone to combine with road dust and be transported by mechanical means such as road sweeping and water runoff.[15,37] The water runoff transports the TWP and eventually they can end up in the soil or marine environments, however, it is expected that the biggest particles remain on the side of the road.[16] Infrastructure around the road also has a significant impact on where the TWP are transported. If the nearest waterway leads to a wastewater treatment plant then there is the possibility of removing TWP from the waterway as can be done with microplastics, but this requires more effort as polymer density and particle sizes play an important role in removal.[16] Large rainfall events, or other reasons for floods can lead to TWP being transported from waters in sewers and into surface waters where they have a smaller chance of being removed or otherwise treated.[16] Sizes, shapes, and density of TWP also play an important role in how the particles get transported and ultimately end up as they can dictate whether or not the particles are easily transported along water runoff or if they are more prone to getting caught in drainage, canals, sediment, etc.[16]

CRG stemming from artificial turfs have several pathways into the environment, they can be mechanically moved into the surrounding soil or waterways,[25] or moved into snow, where the snow can be dumped directly into marine environments.[35] The particles can also be stuck to clothes and bags where they have several pathways, including into trash disposal through indoor vacuuming of dropped particles, or into sewer systems through the washing of the clothes with particles stuck to them.[25] Sewage systems and trash disposal then transport the particles into either marine environments or other places depending on the local trash disposal and sewage routines.

2.3.3 Additives in tires

Practically all tires have additives added to them.[16,23] Tires, like any other polymer products, have their own factors they are especially exposed to. As tires are mainly outdoor appliances, expected to be exposed to friction and high heat, as well as natural elements such as oxygen, ozone, sunlight, etc. Additives with the express purpose of increasing durability when exposed to exactly these elements are added. It is estimated that additives make up between 5 and 10% of tire tread weight,[16] but different brands and tire types contain different amounts. Among the additives, antioxidants (and UV stabilizers) roughly account for 1% of tire tread weight.[21] While tire manufacturers do not necessarily reveal their specific contents in their tires, several analyses indicate certain substances are present in nearly all tires. Generally, tire additives include[16,21]

- preservatives (halogenated cyanoalkanes)
- anti-oxidants (amines and phenols)
- desiccants (calcium oxides),
- plasticizers (aromatic and aliphatic esters)
- processing aids (mineral oils, peptisers), (these are closer to reactives than additives).

2.3.4 Transformation products of additives

Additives, like any other substance, can react into different substances that are called transformation products (TPs). Starting already at the vulcanization state when the additives are added, and continuing even after leaching, transformation happens throughout the entire life-cycle of the TWP and beyond. Transformation can happen either through degradation such as cleavages, or additive reactions. Any reactions leading to a TP introduce new possible pathways to even more TPs,[38] creating even more work in identifying TPs. TPs can have different solubilities than their parent compounds,[3] which then changes the leaching tendencies of the compounds. Transformation can happen in different ways depending on specific additives, for example, antioxidants can be oxidized.[3] Only screening for additives known to be directly added to tires and not their TPs will not give the entire picture of what can leach from the particle. Screening with the purpose of identifying all substances in a particle should include a non-target screening (NTS) in order to identify as many substances as possible. Once recurring substances are identified a suspect list can be made, which should then include both additives and TPs. Screening with quantitative purposes should then use the suspect list to get a better picture of the additives and their TPs.

2.3.5 Antioxidant additives

Antioxidant additives in rubber protect against oxidation through heat, light radiation, and just general oxidation through O_2 and O_3.[10,11,39] Oxidation of polymers is a general expression that describes the process in which the polymer chain is radicalized through some external factors such as heat, radiation, or mechanical stress. It then follows that the radical part of the polymer can react with oxygen either in the form of O_2 or O_3 or it can cross-link the polymer through peroxide bridges or directly.[12,40] Once the radicals have been made and cross-linking has begun it leads to a chain reaction leading to more cross-linking and oxidation further disruption of the polymer chain, which leads to hardening and cracking of, for example, tires.[10–12,19,40] The implication is that by stopping the radical reaction the antioxidants slow down the aging by preventing further chain-reaction degradation.

Antioxidants, sometimes called antiozonants, are present in all tires and accounts for approximately 1% of tire tread mass, meaning any tires or tire particles in the environment are a source of both antioxidants and their transformation products.[21] They are added during the mastication of rubber, both synthetic and natural.[10] Antioxidants can be sorted into types based on their molecular formula. There are amine, phenolic, heterocyclic, and phosphite antioxidants, used in different areas, but amine is the most common.[10] While antioxidants are meant to protect against aging they are also at risk of being transformed into TPs.[3,10,11,19,21,26,38,41,42] The compound that sparked interest into tire additives and their TPs is the transformation of the amine antioxidant 6PPD into 6PPD-quinone (2-anilino-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione) (6PPD-Q) through oxidation by ozone.[3,19] Other *p*-phenylenediamine (PPD) antioxidants are also common, and each molecule of PPD is able to scavenge at least four O_3 molecules.[39]

2.3.6 Ultraviolet stabilizers

UV radiation of polymers is an initial step for the oxidation and rapid degradation of the polymer.[40] Light radiation has been mentioned earlier as a reason for the oxidation of polymers, and that was mostly in reference to UV radiation. While antioxidants aim to neutralize the radicals formed in polymers to prevent further degradation, UV stabilizers aim to absorb UV radiation preventing the formation of the radicals.[40] They do this by absorbing the radiation and either dissipating the energy as heat or fluorescence.[40] Some can also work in a similar way to antioxidants by neutralizing polymers if they enter an excited state.[40]

Some types of UV stabilizers include titanium oxide, carbon black, benzophenone, triazoles, and recently, for research purposes, Schiff bases and organometallic complexes.[40] UV stabilizers have been used as markers for tire pollution, along with some inorganic metals such as zinc (Zn), but Zn is not suited for tire-specific pollution as it can stem from other sources such as breaks and oil.[43] The amount of UV stabilizers added to a tire is not disclosed by tire manufacturers, but due to their effectiveness, even in as low quantities as 0.5% of tire tread weight have significant effects on the photostability of polymers.[40]

2.4 Specific substances of interest

2.4.1 6PPD and its derivatives

Antioxidants are the main additives in rubber, accounting for close to 40% of rubber additives consumed globally.[10] Of which 6PPD (Figure 2.2a), an antioxidant (and antiozonant), accounts for 55% of production in China, with a production of 189 500-208 600 t/year.[10] China accounting for more than 70% of amine antioxidant production annually.[10] 6PPD is ubiquitous in tires around the world and accounts for somewhere between 0.4-2% of tire weight.[3] It has been found in several bodies of water both close to and far away from urban areas.[3,14,16] Macro-movement of 6PPD is connected to storm events, which implies it is present and easily movable in all aquatic bodies of water.[14] In addition, the presence of TWP seems to be the main anthropogenic source of 6PPD in aquatic environments.[16]

6PPD has been evaluated and deemed a good marker for tire-wear particles in the environment, even more stable than benzothiazoles (BTH) which have previously been used as markers.[16] In addition, 6PPD seems to have a lower leaching tendency into the aquatic environment with a $\log K_{OW}$ over 4[16] (4.68 according to KOWWIN™calculations[44]), which increases the time 6PPD can be used to determine TWP presence in an environment.

Seiwert *et al.* (2022) identified 38 transformation products of 6PPD, each of these possibly toxic for the environment.[38] Kinetics of the degradation of 6PPD in tire particles found that UV radiation played the most important role, leading to a significantly faster degradation (half-life of days vs months) in both cryo-milled and TRWP particles in laboratory aged and natural aging experiments respectively.[45] Recently a notable transformation product is the quinone 6PPD-Q (Figure 2.2b), a transformation product made when 6PPD is in the presence of ozone, has been found to be lethal for a certain species of coho salmon (Oncorhynchus kisutch)[3] as well as zebrafish larvae

2.4.2 6PPD-Q

Since its discovery and subsequent discovery to be lethally toxic for coho salmon,[3] 6PPD-Q has been under scrutiny.[3,13,14,19,38,45–47] The quinone has caused a great deal of concern and is now suspected as a possible explanation for urban stormwater mortality syndrome[48] in coho salmon. 6PPD-Q was found to be a transformation product of 6PPD when in the presence of ozone.[3] Experiments on TWP comparing aging with UV radiation to aging without radiation showed that the concentration of 6PPD-Q increased in the first 5 days of UV radiation exposure, before decreasing.[45] Indicating that the UV radiation initially accelerates the process in which 6PPD is transformed into 6PPD-Q before the rate at which 6PPD-Q is degraded overtakes the rate at which it is generated. Meanwhile in natural thermal experiments, the concentration of 6PPD-Q in TWP slowly but steadily increased over the 120 days, while the concentration of 6PPD slowly decreased.[45]

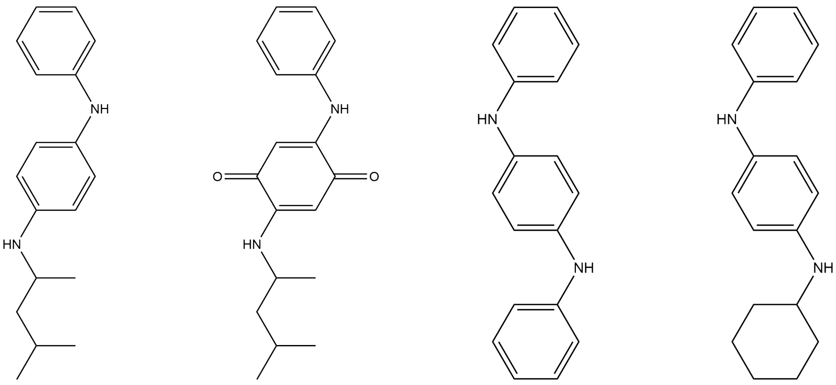
Concerns are also raised as the slow leaching of organic chemicals from microplastics and TWP means the leakage of potentially toxic substances can continue for years. 6PPD-Q leaching into aqueous phases is slow,[17,49] leaching at a rate of 5.2 μg/g over a period of 6 hours of flow-through conditions.[49] However several studies have identified it in stormwater runoff,[14,38] so leaching into aqueous environments happens. The predicted octanol-water partition coefficient $\log K_{OW}$ is somewhere between 3.2 and 5.5[3,17] (4.30 according to Hu *et al.* (2023)[49]), and water solubility of 24 mg/L[17] (38±10 μg/L according to Hu *et al.* (2023)[49]) which is higher than that of 6PPD.[44] Investigations for 6PPD-Q leaching from TWP have also found significant leaching into soil[13,17] and even uptake into lettuce.[47]

2.4.3 Other PPD substances

Along with 6PPD, there are several other widespread PPDs being used as antioxidants in tires[46] including

- N-isopropyl-N’-phenyl-*p*-phenylenediamine (IPPD)
- N,N’-bis(1,4-dimethylpentyl)-*p*-phenylenediamine (77PD)
- N,N’-diphenyl-*p*-phenylenediamine (DPPD)
- N-phenyl-N’-cyclohexyl-*p*-phenylenediamine (CPPD)
- and N,N’-di(*o*-tolyl)-*p*-phenylenediamine (DTPD)

whose quinones have also been investigated previously.[46,50] The previously mentioned PPDs are also sometimes used in other elastomer products such as belts, hoses, and cables.[50] TWP seem to be the main pollutant of PPDs in the environment.[46] Both DPPD (Figure 2.2c) and CPPD (Figure 2.2d) are among the targeted substances in this thesis.



(a) **(b)** **(c)** **(d)**

Figure 2.2: Molecular structures of 6PPD (a), 6PPD-Q (b), DPPD (c), and CPPD (d).

PPD and *p*-phenylenediamine quinones (PPD-Q) frequencies in different matrices (water runoff, air particles, roadside soils) in Hong Kong has been investigated.[50] 6PPD and 6PPD-Q was present in all samples. DPPD was present in 56%, 81%, and 100% of samples depending on matrix, water runoff being the lowest, then air, and soil. Surprisingly DPPD-Q was detected in 100% of samples across matrices, and in air particles made up the bulk of PPD-Qs, beating even 6PPD-Q whose presence is the highest out of all PPD-Qs in water runoff and soil samples. CPPD was detected in 75% (air particles), 100% (water runoff), and 92% (soil) of samples, while the quinone was detected in 69%, 94%, and 72% of samples respectively.[50]

2.4.4 Benzothiazole and its derivatives

Benzothiazole (BTH) and its TPs are vulcanization agents in rubber production[51,52] as well as corrosion inhibitors.[52] The chemical and its TPs (BTs) have found their way into several matrices. These matrices include TWP, tap water, sewage, sediments, road dust, indoor air, indoor dust, and clothing textiles.[51] BTH has also been detected in biological matrices such as breath, urine, and fat tissue.[51] A problem concerning these BTs are their high solubility in water, frequently finding their way into aquatic environments.[51] This presence in aquatic environments raises concerns as to the BTs toxicity. BTs have been found to be ubiquitous in tires,[51] including CRG used in playgrounds,[53] and have been used as markers for TWP in the environment.[16] Although this is not without challenges as BTs are found in way smaller quantities than were added before curing.[16] Nevertheless, TWP remain another pathway for BTs into marine environments due to their ubiquitous presence in tires and as leaching is fast due to their high solubility in water. BTH, just like 6PPD, is faster degraded into its derivatives by UV radiation.[45,52]

2.4.5 Other substances (polycyclic aromatic hydrocarbons)

Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds that contains at least two benzene rings fused together.[54] Several are known to be toxic,[55] carcinogenic,[55–57] and mutagenic.[55] They are present in tires, either due to being added in the form of mineral oils as plasticizers or due to contamination in carbon black.[54] Generally, PAHs are made from incomplete combustion,[58] which explains why they are sometimes contaminating carbon black. SBR has also been investigated and found to be able to cleave its benzene rings and react into several different PAH at higher temperatures (>320°C).[58] While PAHs are made naturally in forest fires, volcanic eruptions, and other high-temperature events,[56] a significant amount can be expected to be polluted through car exhaust and TWP.[59] Tire tread is estimated to have a significant amount of PAH content, but studies show that even within the same manufacturers this varies significantly,[60] but PAHs are expected to be ubiquitous in some shape or form in tires.

Pyrenes exist as different structures where benzene rings are fused to the core four benzene rings (Figure 2.3a), such as benzo-pyrenes and dibenzo-pyrenes. Toxicity and carcinogenicity are individual to each of them.[60] Pyrenes are one of the most predominant PAHs in tire tread, with a varying abundance of 23.9-49.1% of PAH content in the literature.[60] Fluoranthenes (Figure 2.3b), like the pyrenes, can have benzene rings fused in different places, leading to different abundances, toxicity, and carcinogenicity.[61] Fluoranthene and pyrene are isomers of each other, so one has to rely on different retention times (RTs) to separate them. In a hearing from the European Commission Committee on Toxicity, Ecotoxicity and the Environment they determined that 8 PAHs are without a doubt carcinogenic, where three of them were benzo-fluoranthenes, and two of them were benzo-pyrenes.[62] Pure pyrene and fluoranthene were also among the suspected carcinogens.[62]

(a) Pyrene

(b) Fluoranthene.

Figure 2.3: Molecular structures of pyrene (a) and fluoranthene (b).

2.5 Mechanisms of leaching and sorption

2.5.1 Octanol-water partition coefficient

In order to describe the distribution of pollutants in the environment a two-phase partition coefficient can be used. This is used to describe the ratio of concentrations of a chemical between two phases in equilibrium at a specific temperature.[63] For example, the coefficient between the vapor and liquid phases of any chemical is described by Henry's law.[64] The same principle can be applied to any chemical able to distribute or change between any two phases. Often used in environmental chemistry is the partition coefficient between octanol and water, called K_{OW}, given in equation 2.1.[63]

$$K_{OW} = \frac{concentration\ in\ octanol}{concentration\ in\ water}\ (at\ equilibrium) \tag{2.1}$$

Determining the K_{OW} for a chemical is useful for gaining insight into how it interacts with environments. Marine environments are primarily water, so a lower K_{OW} indicates more accumulation into the marine environment. Soil and biota, however, contain weakly polar organic matter and lipid tissue, which means a higher K_{OW} indicates higher tendencies to accumulate in soil and biota.[63] The coefficient is advantageous as it can be measured in laboratories.[63] In theory, a partition coefficient could be made between any two phases, such as between a tire particle and water, but this is often not practical, and as such the K_{OW} is often used as it already describes the equilibrium between a weakly polar organic phase and water. Polymers can be considered a weakly polar organic phase for additives since they do not have a covalent bond to the polymer.[11] Furthermore, sometimes LogK_{OW} is used instead of K_{OW} due to the large differences in concentration at equilibrium.

2.5.2 Fugacity

The concept of fugacity is relevant to understanding how chemicals distribute in the environment. Essentially, fugacity refers to the natural movement of chemicals from regions with high chemical potential to regions with lower potential, until equilibrium is formed.[63] While this is most often used in relation to pressures of ideal gasses or dissolved materials in liquid, it can also be applied to the movement of any substance between phases in an environment.[63] For example movement of additives between a polymer particle and its environment.

It is important to note that fugcaity differences between phases is not the same as the two phases having different concentrations. While the fugacity in a phase is proportional to the concentration, the solubility of the substance in the given phase has to be considered as well.[63] An organic phase solves organic substances better, and thus have to have a higher concentration before reaching a fugacity equilibrium than the same substance dissolved in a water phase.

2.5.3 Leaching and sorption of additives in tires

In the evaluation of leaching and sorption of additives, polymers particles are evaluated as its own phase, while whichever environment the pollutant particle is in is the other. Generally, the fugacity of organic chemicals in pollutant polymers is higher in the polymer phase than in the seawater phase.[65] This means organic pollutants have high enough concentrations in a polymer that will force them to leach into the water despite having a K_{OW} indicating an equilibrium where the substance would predominantly be in the polymer phase. The seawater being likely free from or having a concentration in minuscule amounts of the additives found in TWP has to be considered (Figure 2.4). The opposite is also possible, as there are substances in the oceans that are not present in TWP, and as such can be absorbed into tire particles.[66]

Investigation into leaching tendencies has to be done for individual compounds or classes of compounds and their TPs. In addition, TWP size, time spent in the environment, and specific environments have to be considered. The impact the particle size of TWP has on leaching is unclear.[12] Cryomilled particles were compared to TRWP in terms of kinetics of degradation and were found to be a good proxy as the detected substances follow roughly the same models.[45] However, some issues in studying this arise as the TRWP matrix contain road particles as well, and separating them from each other is not feasible. Thus the study of leaching from TRWP contains substances from roads and not just the ones found in TWP.[45] Using microplastics as a vector for the leaching tendencies of TWP, then the rate-limiting process governing the transfer of additives between plastic and water is the diffusion within the particles.[67] This means that the movement of the additives through the polymer phase and to the surface is the most important step in the leaching process. In addition, the extraction of additives is dependent on substance size, smaller molecules with lower boiling points will migrate faster.[11]

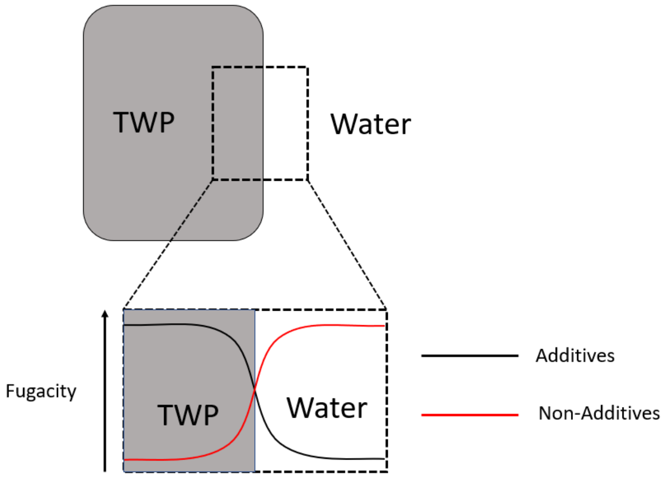


Figure 2.4: Fugacity explanation of substances between TWP and water phases. Adopted from Kwon *et al.* (2017).[67]

2.6 Analysis method

The analysis method for this thesis is gas chromatography coupled with high resolution mass spectrometry (GC-HRMS), which is one of the most common methods used in analytical chemistry. The method excels at both targeted and untargeted analyses. For untargeted analyses, it works well for separating the unknown substances from each other and subsequently obtaining their mass spectrum for possible identification. For targeted analysis, it works excellently for separating the analytes from each other in matrices for even better identification, and quantification by getting even better signal-to-noise ratios. The combination of techniques eliminates difficulties of the two techniques alone, such as poor selectivity in spectra obtained by mass spectrometry (MS) in a matrix of many molecules, or the ambiguity of identification of substances in gas chromatography (GC).[68] A limitation of the technique is that the matrix has to be either in a gaseous phase or volatile with relatively stable target substances of interest.

2.6.1 Gas Chromatography

Gas chromatography (GC) is a chromatographic separation technique in which the mobile phase is gaseous.[69] This means the analyte needs to be either gaseous, a volatile liquid, or pyrolysis has to be utilized in some cases in order to transform the analyte into gas. The stationary phase of the column can be either liquid-phase, solid-phase particles supporting a liquid phase, or just solid-phase particles. An open tubular column is the most common version of a gas chromatogram separator, and its length can range from 15 to 100 m, with a usual length of 30 m. The inner diameter of the column is the distance between the inner walls of the stationary phase and can range from 0.10 to 0.53 mm. A more narrow inner diameter requires higher pressure in order to run, but increases the resolution of the data from the column. The stationary phase which coats the walls ranges from 0.1 to 5 µm with 0.25 µm being standard.[69]

In order to push the analyte continually through the column a carrier gas is utilized. The carrier gas is ideally an inert gas that does not react with the analyte, usually He, N_2, or H_2. The three different gases have different advantages such as H_2 being able to give the fastest separation, and N_2 gives a lower detection limit than He.[70]

When a GC program is run it usually has pre-programmed pressure and temperature over the full course of the chromatography. Increasing the temperature over the course of the chromatography increases the sharpness of the peaks in addition to decreasing the RT of later-eluting peaks. For compounds that do not tolerate higher temperatures, changes in pressure throughout the GS can help reduce RT, also decreasing the total time it takes to run the column.[71]

2.6.2 Mass spectrometry

MS is an analysis method for determining the ionic, atomic, or molecular mass by measuring a mass-to-charge ratio (m/z). In addition, the relative abundances between the peaks of mass-to-charge ratios are counted by the mass detector. The relative abundance is shown in terms of the ratio of peak area compared to the most abundant peak area. In order for the mass spectrometer to obtain the mass of a molecule or atom it needs to be charged in some way, and one must choose whether to run the mass spectrometer in positive charge or negative charge mode.[72] In order to detect otherwise uncharged molecules, several methods exist, but one of the most common, when your analyte is gaseous, is electron ionization (EI), where a beam of electrons is fired from a hot filament. The electrons are accelerated by a voltage in the filament, usually 70 eV, and interact with the molecules creating mostly positively charged molecular ions.[73,74] These, often radical, ions are higher in internal energy and, as a result, are also prone to fragment.[74]

Fragmentation is the breaking apart of molecules or ions into smaller pieces, and it happens all the time in MS.[74] Knowledge about fragmentation is vital to be able to use MS, as it explains why there are signals at m/z ratios of molecules or ions that are not injected into the spectrometer. It is also sometimes vital for identifying parent substances that would otherwise be indistinguishable with only the molecular mass. If the mass spectrometer does not have a high enough resolving power to distinguish two molecules with the same nominal mass, or even if it has, isomers will still appear similar in molecular mass but can produce different fragment m/z signals.[74] The fragments' signal intensities will depend on the energy used to ionize the molecules with,[74] and the eventual energy used to intentionally fragment molecules in collision-induced dissociation.[75] Interactions with higher energy in the mass spectrometer are expected to increase the intensity of the fragment ions while decreasing the molecular ion peak. The EI voltage most commonly used (70 eV) creates almost similar fragmentation patterns across machines and instrument vendors, which is why it has become the standard method and energy used to ionize molecules in GC-MS.[74] Fragmentation profiles can then be cataloged and shared in software. These profiles can be stored in databases such as MassBank, mzCloud, and NIST20 Mass Spectral Library, and can be used for the identification of compounds in analyses.[76]

2.6.3 Q Exactive Orbitrap mass spectrometer

The Orbitrap mass spectrometer can perform high resolution mass spectrometry (HRMS). Modern resolving powers are generally in the range of 140 000-480 000 at m/z 100.[77] The resolution is m/z dependent which means the resolution decrease the farther away from optimal m/z ratio and not be the same everywhere in the mass spectrum. The Orbitrap is able to differentiate between ions with an accuracy of 1-5 parts per million (ppm) using external calibration. With proper internal calibrations, it is able to see differences down to the part per millions of m/z.[77] Which is more than able to distinguish between molecules with the same nominal mass but different molecular formulas.

Before the ions reach the Orbitrap they can be passed through a quadrupole mass filter. A quadrupole mass filter only lets ions with m/z values of interest pass.[78,79] The filter consists of four parallel rods that are operated using direct current (DC) and radio frequency (RF) potentials. Rods diagonally opposite to each other operate with the same magnitudes and types of potential but with opposite signs. The DC and RF potentials, along with the frequency of the RF, dictate which ions are allowed to pass through the filter and into a tiny slit at the end of the filter. It does this by constantly switching potentials making the ions oscillate back and forth. Heavy ions respond more to the DC potentials, while lighter ions respond to the RF potential as well. Ions with the wrong m/z values collide into the rods and will not reach the slit to pass through. The two rods utilizing the DC potential work as a filter for the heavier slower ions, and the two rods using RF potentials filter the lighter and faster ions.[78,79] In a Q Exactive Orbitrap, there is a quadrupole mass filter before the Orbitrap mass analyzer.

In an Orbitrap analysis, the ions are injected in a packet.[77] This injection is performed by the C-trap, which highly compresses and injects them quickly (>300 ns) into the Orbitrap mass analyzer.[80] The ions are directed by electromagnetic fields inside the Orbitrap which makes them oscillate around an electrode. The frequency of the oscillations is mass-dependent which means it can be measured and the m/z ratio can be extrapolated with very high accuracy.[80]

2.6.4 Combining GC with MS

Gas chromatography with tandem mass spectrometry (GC-MS) allows the user to both identify and quantify more complicated matrices. The analyte matrix is continually sent through the gas chromatograph and subsequently into the mass spectrometer.[68,81] Ideally, the gas chromatograph separates the matrix content so that the MS analyzes fewer substances at any given time, giving better MS results. Between the two machines, there needs to be a reduction in pressure as the mass spectrometer operates in a near vacuum, while the gas chromatogram operates under pressure. This reduction in pressure is done by a differential pump as the gas inlet enters the ionization chamber.[68] Knowing the RT of when a substance elutes allows the mass spectrum to focus on specific windows of m/z values when the analyte is expected to elute from the GC, this technique of only focusing on specific m/z values is called selective ion monitoring (SIM). SIM allows increased time spent focusing on specific m/z values, which gives a better signal-to-noise ratio.[82] A quadrupole mass filter is well suited for performing SIM as it can filter entire intervals of m/z values at a time.

Once the analysis is done, the total detected MS current can be plotted against time in software in order to recreate a total ion chromatogram. From here a specific m/z ratio can be selected to simplify the chromatogram to only show signals for that m/z ratio. A substance can then be confirmed if it matches both the RT and the m/z ratio, and subsequently its area integrated for quantification.[83]

2.6.5 Isotopic dilution method

An internal standard (ISTD) is a known mixture of substances added to the analyte, and is used for precise quantification. It is expected that some analyte is lost both during sample preparation and during the analysis. To compensate for this an ISTD is added to the analyte as early as possible to expose the ISTD to as much of the same preparation and experimental methods as the analyte in order to get as accurate data from the ISTD as possible. From the analysis there will be signals from both analyte and from the ISTD, and a comparison between the ISTD signal data and the initial concentration of ISTD added can be done. By finding the ratio of the ISTD signal to initial ISTD concentration, the assumption can be made that that the analyte also has the same ratio between the measured analyte signal and the analyte concentration before preparatory work and analysis. The original analyte concentration can thus be extrapolated from the signals.[84]

It is important that the ISTD is not the exact same isotopic substance as our analyte, but should ideally have the same or similar chemical properties as the analyte.[84] For measuring using mass spectrometry the best ISTD are the same chemical substance as the analyte but with different isotopes, most commonly with one or more protons replaced by deuterium, or carbon atoms exchanged with ^{13}C. This would produce a completely different mass signal, while still retaining identical chemical properties and interactions within the matrix and machine.[82]

2.6.6 Quantification standard, external calibration, and linear calibration curve

A quantification standard should be used in order to quantify the compounds detected in any analysis since the data recorded is just arbitrary numbers without it. By doing analyses on samples spiked with known concentrations the data recorded can be compared with ones where the data is correlated to a concentration. The samples with known concentrations are called quantification standards, and should also be similar in chemical properties, or ideally the same compound, as the analyte. Analyzing ideally 6 or more quantification standards at different concentrations, ranging from below to above the analyte concentrations, allows for a calibration curve to be made by plotting the analysis responses against concentrations. These curves are made assuming there is a linear relationship between analyte concentration and signal in the analysis, such that a linear curve can be made. The concentration range where there is a linear relationship is called the linear range, and outside this range the curve flattens and quantification becomes unreliable.[85] Ideally, there is an internal standard in our spiked calibration samples as well, so the ratios between the spiked analyte responses and ISTD responses are used for the calibration curve.[82] The ratio values for the calibration curve can only be used if there also is ISTD in the samples.

/3

Methods

Three different types of rubber samples were used for both the hyperbaric and the UV experiments, the three types of rubber samples were pre-exposed in their respective ways. Exposure in this thesis will be referring to the specific ways in which the three sample types were treated with pressure and UV radiation. The first type is virgin crumb rubber (VCR), which is CRG taken straight from sports fields in Tromsø to be exposed. Weathered crumb rubber (WCR) is CRG taken from the same sports turf in Tromsø and sat in seawater for 12 months to simulate marine weathering. Finally, tire thread mix (TTMix) combines 20 different tire treads of new tires which have been cryo-milled to a very fine mixture of particles. The list of tire types and their size distribution is in Appendix F.

3.1 Hyperbaric experiment

Four different sample types were prepared for this part of the experiment, the three rubber sample types (VCR, WCR, and TTMix) and filter-only samples. Each of these four types was further divided in two by whether or not they were filtered for biotic material denoted as biotic (B) or abiotic (A). Furthermore, these samples were divided into deep sea (DS) and surface water (SW) samples, for a total of 16 different sample types. Not all of these samples were available for this thesis (Table 3.1). Filter-only samples were the same Polyethersulfone (PES) filters as were used in the samples containing rubber exposed in the same way as the rubber particles without any rubber particles present in the sample.

Table 3.1: Tire particle samples prepared for the hyperbaric laboratory weathering experiment. The four different boxes denote the different sample types of the extraction experiments, either only PES filters or either of the three different rubber sample types. The rubber particles include virgin crumb rubber (VCR), weathered crumb rubber (WCR) and finely milled particles from new tires (TTMix). Deep-sea denotes samples that were exposed to seawater and pressures of simulated deep-sea conditions. Surface water samples were exposed to seawater and pressure simulating surface conditions. Abiotic denotations mean the sample had $HgCl_2$ added during exposure, while biotic samples did not. Time-increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) indicate for how long the sample was in exposure.

	Time				
	t_0 (0 h)	t_1 (6 h)	t_2 (24 h)	t_3 (1 w)	t_4 (2 w)
Filter-only:					
Deep-sea biotic	Yes	-	-	-	-
Deep-sea abiotic	Yes	-	-	Yes	
Surface water biotic	Yes	-	-	-	-
Surface water abiotic	Yes	-	-	-	Yes
VCR:	Yes				
Deep-sea biotic		Yes	Yes	Yes	
Deep-sea abiotic		Yes	Yes	Yes	Yes
Surface water biotic		Yes	Yes	Yes	-
Surface water abiotic		Yes	Yes	Yes	Yes
WCR:	Yes				
Deep-sea biotic		Yes	Yes	Yes	-
Deep-sea abiotic		-	-	-	Yes
Surface water biotic		Yes	Yes	Yes	-
Surface water abiotic		-	-	-	Yes
TTMix:	Yes				
Deep-sea biotic		Yes	Yes	-	-
Deep-sea abiotic		Yes	Yes	Yes	Yes
Surface water biotic		Yes	Yes	Yes	-
Surface water abiotic		Yes	Yes	Yes	Yes

Water samples for exposure were collected from the Mediterranean Sea, about 30 km south of Toulon in France. The area has a depth of 2400 m, allowing for deep sea water to be collected. Surface water was collected at 0.5 m depth, while deep seawater was collected at 2000 m depth. The seawater was filtered onboard the vessel using microfiber filters (0.7 µm, WHATMAN™ GF/F filter).

Once filtered the water samples were transferred straight into 100 mL SCHOTT glass bottles containing the test samples. The bottles were filled completely to avoid air bubbles, which equated to around 130 mL of seawater. The bottles were separated into biotic and abiotic test samples, and to the abiotic bottles, $HgCl_2$ was added resulting in 10 mg/L concentration to disinfect the waters from any microbial interference.

Surface water samples were kept in the dark at atmospheric pressure, while the deep sea samples were kept in the dark at a pressure of 200 bar in hyperbaric pressure bottles. All samples were kept at a controlled 13±1°C which is meant to represent in situ water conditions. Samples were taken in triplicate. Rubber and control filter sampl es were taken out of exposure at the time intervals: 6 h (t_1), 24 h (t_2), 1 w (t_3), and 2 w (t_4). When taken, the rubber samples were filtered out using a 0.22 µm PES filter and, along with the filter, coated in burnt aluminum foil and stored at 4°C before being sent to NILU for analysis. This was done prior to the start of this thesis.

3.2 UV exposure experiment

The same three pre-exposed rubber types were used in this experiment (VCR, WCR, and TTMix). Rubber material (50 mg) in 50 mL surface seawater was kept at 22°C and exposed to a Suntest CPS+ system (ATLAS) with a daylight filter. The power of the radiation was set at 300 W/m^2 to simulate the mean solar radiation in Sanary-sur-Mer in France in July (measured mean = 298 W/m^2). Using an equation from Gewert et al.[86] the exposure to the UV-radiation is meant to simulate a longer exposure to natural sunlight. Three different time lengths were tested, 66 h (2.75 d (T_1)) exposure to UV radiation equating to 6 days in the environment, 158 h (6.58 d (T_2)) exposure equating to 14.4 days in the environment, and 336 h (14 d (T_3)) exposure equating to 30.6 days in the environment. Additionally, four samples were prepared of only PES filters in seawater. Samples kept in the dark were kept in seawater for the same amount of real-time as the UV exposed samples were exposed to UV. Both hyperbaric and UV experiments containing rubber share initial unexposed samples (t_0). This exposure was also done prior to the thesis.

Table 3.2: UV-experiment samples extracted in this thesis. The four different sample types include the three types of rubber as well as samples containing only the PES filters. The rubber particles include virgin crumb rubber (VCR), weathered crumb rubber (WCR) and finely milled particles from new tires (TTMix). Light denotes samples that were exposed to UV radiation, while dark denotes samples that were kept in the dark throughout the exposure. Time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) indicate for how long the sample was in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

	Time			
	t_0 (0 h)	T_1 (2.75 d)	T_2 (6.58 d)	T_3 (2 w)
Filter only:	Yes x 3	-	Yes	-
VCR:				
Light:	Yes	Yes	Yes	Yes
Dark:		Yes	Yes	Yes
WCR:				
Light:	Yes	Yes	Yes	Yes
Dark:		Yes	Yes	Yes
TTMix:				
Light:	Yes	Yes	Yes	Yes
Dark:		Yes	Yes	Yes

3.3 Solid-liquid Extraction

3.3.1 Hyperbaric experiment

The rubber particles were added to 7 mL vials. Along with them was the filter they were filtered with, rolled up so that it stood up in the vial. The vials were then left overnight. Hexane was added to the vials until the filter was completely submerged, which ended up being 7-8 mL n-hexane. For samples where the filter was otherwise folded, broken, or not present, the approximate same amount of n-hexane was added. Blanks were also made by adding hexane into 7 mL vials. Into each vial, 50 µL ISTD was added (Appendix G). The vials were then prepared in batches by first vortexing swiftly until the rubber particles and filter could be observed to swirl, before being put in a sonicator for 20 minutes. Once sonicated for 20 minutes the vials were taken out and vortexed once again before being put back into sonication for another 20 minutes, this was repeated twice for a total of 60 minutes in sonication for every vial. The vials were then left for two days. From the top of the vials, hexane was taken and transferred to GC-MS vials using Pasteur pipettes. For the TTMix samples, a piece of Kimtech Science™ Kimwipes™ was added to the tip of the pipette to filter out any potential particles of rubber from entering the GC-MS vial.

3.3.2 UV exposure experiment

Rubber particles were collected in vials of different sizes ranging from 4 - 40 mL (Appendix D). They were covered in various amounts n-hexane depending on vial size such that the filter was completely submerged, and 50 µL ISTD was added. They were left for approximately two days before they were sonicated for 20 minutes and vortexed, not repeating. Hexane from the top of the vials was extracted and transferred into GC-MS vials using Pasteur pipettes. No precautions were taken for TTmix samples as all the particles were visible at the bottom of the vial. Approximate volumes based on vial size were noted down.

3.4 GC-MS specification, external standards, and calibration curves

Specifications for the GC and MS analyses can be found in Appendix H. Calibration curve analyses were done on the same GC-MS instruments and programs as the samples previously by NILU.

3.5 Calibration curves and concentration calculations

No signal from the ISTD could be detected in samples containing rubber (Section 5.9.2). It could, however, be detected in blank-, and filter-only samples. As a result, only external standards were used for quantification.

Three-point calibration curves for 6PPD (1.004-100.4 pg/µL, R^2 = 0.9999), 6PPD-Q (1.000-100.0 pg/µL, R^2 = 1.0000), CPPD (0.999-99.9 pg/µL, R^2 = 0.9997), and DPPD (1.014-101.4 pg/µL, R^2 = 0.9992) were made for sample quantification (Figure 3.1).

The mentioned substances were quantified using their respective external calibration curves and Equation 3.1, where C_E is the concentration in the hexane extracts, A_S is the sample area, "Const." is the constant of the linear curve, and "Slope" is the slope of the linear curve. The rest of the detected substances including the PAHs were quantified using the 6PPD calibration curve with the exception of BTH.

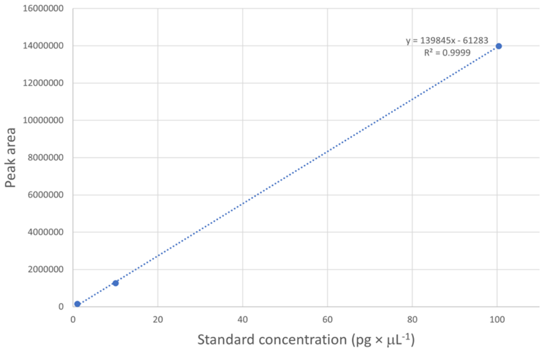
BTH was quantified using single-point external calibration. External standard (200 pg/µL) was run and related retention time and peak area was obtained. Extracted hexane concentrations for BTH (pg/µL) were calculated using Equation 3.2, where C_C is the external calibration standard concentration, A_S is the sample area, and A_C is the external calibration standard area.

All quantities were then converted from concentration in hexane (Appendix B) into concentrations relative to rubber sample weight (Table 4.1 & 4.2) by Equation 3.3, where C_S is the rubber-specific concentration in µg additive per g rubber, V_S is the sample volume, and m_S is the weight of the rubber.

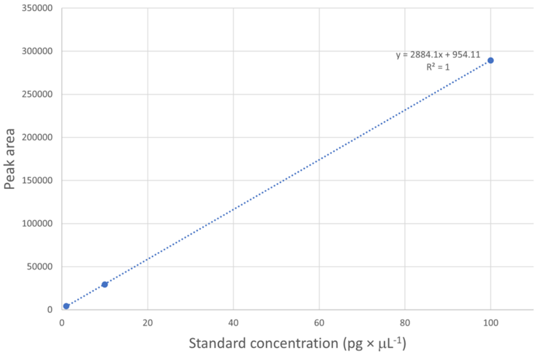
$$C_E = \frac{A_S - Const.}{Slope} \tag{3.1}$$

$$C_E = \frac{A_S}{A_C} \times C_C \tag{3.2}$$

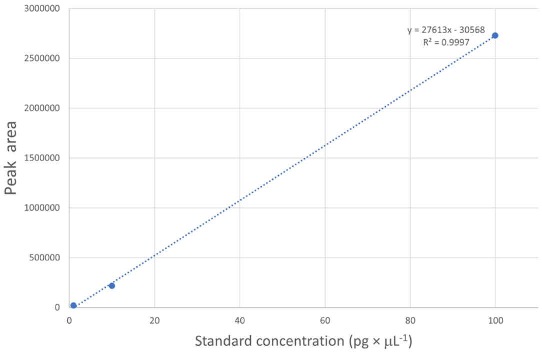
$$C_S = \frac{V_S}{m_S} \times C_E \tag{3.3}$$



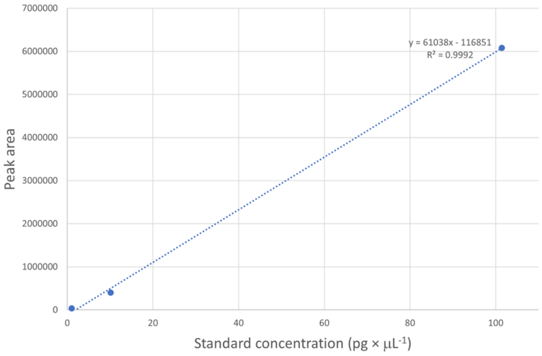
(a) 6PPD



(b) 6PPD-Q



(c) CPPD



(d) DPPD

Figure 3.1: External calibration curves for 6PPD (a), 6PPD-Q (b), CPPD (c), and DPPD (d) relating mass spectral signal to sample concentration.

3.6 Targeted and suspect list screening

Certain substances are targeted in the analysis as isotope-labeled versions of these were added through the internal standard. Specifically 6PPD and 6PPD-Q, as well as four other UV stabilizers. The list of substances in the internal standard is in Appendix G. As UV additives have already been on the agenda at NILU for a year or so, there was also a suspect list of substances extracted from tire particles available for analysis. Although not every substance was identified, their RT in a hexane matrix and mass spectra are cataloged. As such the analysis was able to utilize this suspect list. The full list of substances screened for is found in Appendix A.

3.7 Attempted identification of unknown substances

The molecular formula was determined by researchers at NILU by comparing mass spectra with previously obtained mass spectra. From there the molecular formulas were searched for in SciFinder® in attempts to gauge which substances it could be with assistance from senior researchers at NILU. Substances with a higher number of references were considered more likely to be the substance.

4

Results

4.1 Hyperbaric experiment

4.1.1 Known compounds results

Results for the known compounds analysis (Table 4.1). All 35 rubber samples detected 6PPD and all of them also exceeded the concentrations for the calibration curve for 6PPD, meaning all samples' hexane extracts had more than 100.4 pg/µL. Four out of the five filter-only samples (Filter-DS-B-t_0, Filter-DS-A-t_0, Filter-DS-A-t_4, Filter-SW-B-t_0) also detected 6PPD. 6PPD-Q was ubiquitous in the rubber-containing samples, but not present in any filter-only, in addition, 11 samples exceeded the linear calibration concentration of 100 pg/µL. DPPD was detected in every rubber-containing sample but no filter-only samples and 10 samples were above the linear calibration curve concentration of 101.4 pg/µL. None of the samples were outside the linear concentrations of CPPD and it was detected in 13 samples, those all being the TTMix samples. The linear calibration curves were used for all the quantification under the assumption that the linear trend covered all concentrations.

N-phenyl-N'-tolyl-*p*-phenylenediamine (TPPD) and DTPD (Figure 4.1) were also detected in every rubber-containing and no filter-only samples. They were also quantified using the 6PPD linear calibration curve.

Fluoranthene was detected in every rubber-containing sample tested and no filter-only samples. Pyrene was detected in two VCR, one WCR, six TTMix samples, and no filter-only samples. BTH was detected in every single sample including filter-only samples, although some of the signals produced were lower in intensity than those of some blanks. There has not been set a limit of quantification, and as such every sample is shown containing some concentration of BTH (Table 4.1).

(a) **(b)**

Figure 4.1: Molecular structures of DTPD (a) and TPPD (b).

Table 4.1: Hyperbaric experiment results for 6-, D- and C-, T- PPD, DTPD, 6PPD-Q, BTH, fluoranthene, and pyrene (µg substance extracted per g rubber). Samples outside the linear calibration concentrations in hexane extracts are marked (*). Samples where the substance was not detected are noted as not detected (ND). The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into deep-sea (DS) which were exposed to high pressure, and surface water (SW) with were exposed to atmospheric pressure. And further divided into abiotic (A) which had $HgCl_2$ added during exposure, biotic (B) which did not. Time-increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) indicate for how long the sample was in exposure.

Substance		VCR					WCR					TTMix				
		t_0	t_1	t_2	t_3	t_4	t_0	t_1	t_2	t_3	t_4	t_0	t_1	t_2	t_3	t_4
6PPD	DS-B	177*	60.1*	45.7*	36.1*	-	102	69.9*	55.6*	76.6*	-	1150	142*	996*	-	-
	DS-A		51.9*	37.9*	68.5*	62.5*		-	-	-	144.0*		1040*	815*	504*	879*
	SW-B		59.9*	60.5*	67.1*	-		64.6*	49.4*	55.9*	-		912*	835*	684*	-
	SW-A		51.9*	46.3*	42.2*	38.5*		-	-	-	66.3*		688*	839*	819*	1080*
6PPD-Q	DS-B	10.5	9.07*	4.86	6.11*	-	4.04	0.370	1.44	0.813	-	9.31	1.92	12.8*	-	-
	DS-A		5.38	4.68	9.91*	7.35*		-	-	-	3.74		13.4*	14.0*	5.91	17.5*
	SW-B		9.70*	3.24	6.73	-		1.60	1.14	1.32	-		11.8	11.4	9.64	-
	SW-A		5.38*	5.79*	4.26	4.78		-	-	-	1.34		6.83	12.0	11.0	14.1
DPPD	DS-B	36.9	2.06	3.45	1.99	-	25.9*	3.36	1.82	3.08	-	91.0*	1.61	9.09*	-	-
	DS-A		5.93*	2.25	6.90*	5.90*		-	-	-	4.98		8.56	8.39	4.20	10.1
	SW-B		6.18*	18.0*	6.70*	-		1.62	1.61	1.54	-		7.74	5.68	5.46	-
	SW-A		5.93*	1.27	2.55	4.79		-	-	-	1.80		4.92	7.09	6.84	9.27
CPPD	DS-B	ND	ND	ND	ND	-	ND	ND	ND	ND	-	9.12	1.02	6.03	-	-
	DS-A		ND	ND	ND	ND		-	-	-	ND		6.38	4.50	2.65	5.53
	SW-B		ND	ND	ND	-		ND	ND	ND	-		5.07	4.98	3.68	-
	SW-A		ND	ND	ND	ND		-	-	-	ND		3.70	4.59	4.39	6.48
TPPD	DS-B	14.2	2.83	2.45	2.11	-	9.29	3.52	1.61	3.39	-	28.0	2.10	12.3	-	-
	DS-A		6.10	2.00	4.22	6.58		-	-	-	7.03		9.82	9.80	4.60	11.5
	SW-B		4.80	11.1	3.85	-		1.98	1.66	1.49	-		7.47	6.90	7.28	-
	SW-A		7.06	2.49	1.82	2.30		-	-	-	2.16		4.23	6.75	8.30	11.3
DTPD	DS-B	30.5	14.6	9.10	8.43	-	19.7	22.8	9.75	18.3	-	46.2	9.96	62.3	-	-
	DS-A		21.4	8.86	12.30	30.4		-	-	-	43.4		52.9	55.1	25.3	67.3
	SW-B		16.8	33.2	13.0	-		11.1	11.6	8.34	-		39.1	40.6	38.2	-
	SW-A		21.4	12.5	8.05	6.74		-	-	-	14.0		23.3	39.3	45.6	58.6
BTH	DS-B	20.0	10.8	17.8	18.0	-	11.1	2.92	2.03	4.26	-	5.29	0.002	0.003	-	-
	DS-A		0.016	14.9	9.79	0.008		-	-	-	3.36		0.904	5.31	10.7	0.010
	SW-B		19.5	16.1	15.3	-		0.008	8.08	4.63	-		5.87	6.99	6.32	-
	SW-A		0.016	0.010	0.110	0.070		-	-	-	13.4		6.22	6.20	6.00	16.2
Fluoranthene	DS-B	0.614	0.306	0.520	0.404	-	0.188	0.515	0.453	0.428		9.25	4.31	15.4	-	-
	DS-A		0.517	0.330	0.307	0.457		-	-	-	0.747		14.8	13.8	9.87	17.4
	SW-B		0.412	0.515	0.702	-		0.228	0.346	0.522	-		15.4	13.2	12.2	-
	SW-A		0.517	0.432	0.533	0.390		-	-	-	0.425		11.2	16.0	14.9	18.3
Pyrene	DS-B	ND	ND	0.520	ND	-	ND	ND	ND	ND	-	ND	5.27	15.4	-	-
	DS-A		ND	ND	ND	0.460		-	-	-	ND		0.910	0.670	ND	1.07
	SW-B		ND	ND	ND	-		ND	ND	ND	-		ND	12.8	ND	-
	SW-A		ND	ND	ND	ND		-	-	-	0.43		ND	ND	ND	ND

4.1.2 Unidentified compounds results

Suspect list screening detected five different unidentified substances: $C_{14}H_{23}$ at RT 17.14 and RT 17.82, $C_{21}H_{26}N_2$, $C_{23}H_{26}N_2$, and $C_{27}H_{34}N_2$ which were quantified using the 6PPD linear calibration curve (Table 4.2). The $C_{14}H_{23}$ substance at RT 17.14 was ubiquitous in the rubber-containing samples, while the $C_{14}H_{23}$ substance at RT 17.82 was detected in 24 out of the 35 rubber-containing samples. Neither of the $C_{14}H_{23}$ substances were detected in any filter-only samples or blanks. The substances $C_{21}H_{26}N_2$, and $C_{23}H_{26}N_2$ were ubiquitous in the rubber samples. $C_{21}H_{26}N_2$ was also detected in the same four out of the five filter-only samples that 6PPD were detected in (Filter-DS-B-t_0, Filter-DS-A-t_0, Filter-DS-A-t_4, Filter-SW-B-t_0). $C_{23}H_{26}N_2$ was also detected in one of the five filter-only samples (Filter-DS-A-t_4). $C_{27}H_{34}N_2$ was detected in only one sample overall (WCR-DS-B-t_4).

Table 4.2: Hyperbaric experiment results for unidentified substances (µg additive per g rubber). Samples were quantified using 6PPD linear calibration curve. $C_{14}H_{23}$ concentrations are the sums of both signals for the same molecular formula at RT 17.14 and 17.82. The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into deep-sea (DS) which were exposed to high pressure, and surface water (SW) with were exposed to atmospheric pressure. And further divided into abiotic (A) which had $HgCl_2$ added during exposure, biotic (B) which did not. Time-increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) indicate for how long the sample was in exposure.

Substance		VCR					WCR					TTMix				
		t_0	t_1	t_2	t_3	t_4	t_0	t_1	t_2	t_3	t_4	t_0	t_1	t_2	t_3	t_4
$C_{21}H_{26}N_2$	DS-B	9.01	7.53	5.55	5.59	-	2.69	0.530	0.453	0.372	-	7.86	2.13	11.4	-	-
	DS-A		7.53	6.02	7.11	7.05		-	-	-	0.692		11.6	10.8	7.04	11.3
	SW-B		6.69	7.20	7.47	-		0.484	0.797	0.494	-		13.1	10.3	9.17	-
	SW-A		8.06	7.71	5.83	5.56		-	-	-	0.277		9.08	12.5	10.9	14.5
$C_{23}H_{26}N_2$	DS-B	7.37	7.08	9.33	5.43	-	3.46	4.48	5.96	4.11	-	10.3	3.75	19.5	-	-
	DS-A		9.16	7.55	10.4	9.85		-	-	-	9.76		18.5	18.7	11.8	26.4
	SW-B		15.5	11.3	15.8	-		3.84	5.69	4.10	-		22.3	18.0	14.7	-
	SW-A		11.2	11.7	5.99	8.14		-	-	-	3.03		11.2	18.7	16.3	23.0
$C_{14}H_{23}$	DS-B	1.10	0.22	1.07	1.13	-	0.27	0.56	0.99	1.01	-	1.35	0.82	5.06	-	-
	DS-A		1.63	0.62	1.41	1.77		-	-	-	2.96		2.42	2.13	1.12	7.63
	SW-B		2.13	2.14	1.92	-		1.05	0.47	0.78	-		2.16	1.61	1.69	-
	SW-A		1.63	0.89	0.98	1.02		-	-	-	0.90		1.17	5.68	4.36	2.42

4.2 UV experiment

4.2.1 Known compounds results

The targeted and suspect list analysis in the UV experiment detected all the same substances as the hyperbaric experiment did (Table 4.3). Every rubber-containing sample detected 6PPD, but no filter-only samples detected it. Out of 21 samples that detected 6PPD, 18 samples exceeded the upper limit of concentration in the linear calibration curve for 6PPD. DPPD was detected in all but 3 rubber-containing samples, and 3 exceeded the linear calibration curve upper concentration. CPPD was only detected in 4 TTMix samples and did not exceed the linear calibration curve concentration. DTPD was ubiquitous in the rubber-containing samples, and TPPD was detected in 18 out of 21 rubber-containing samples. Fluoranthene was detected in every sample including the filter-only samples. Pyrene was only detected in 5 samples, all being TTMix. BTH was detected in every sample including filter-only and blank samples as well.

The samples exceeding the linear calibration curve concentrations were still quantified using the linear calibration curves, and as a result, could, in reality, have a lower concentration than those calculated.

Table 4.3: UV experiment results for 6-, D- and C- PPD, 6PPD-Q, and BTH (µg additive per g rubber). Samples exceeding the linear calibration area for concentration in hexane extracts are marked (*). The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into UV radiation-exposed (L) and not exposed (D). Samples where the substance was not detected are noted as not detected (ND). Time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) indicate for how long the sample was in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

Substance		VCR				WCR				TTMix			
		t_0	T_1	T_2	T_3	t_0	T_1	T_2	T_3	t_0	T_1	T_2	T_3
6PPD	L	177*	104*	51.6*	11.0*	101	92.6*	143*	39.1*	1150*	49.6	26.1	51.3*
	D		81.8*	26.7*	55.8*		88.3*	71.4*	47.2*		231*	287*	253*
6PPD-Q	L	10.5	3.44	ND	ND	4.04	0.797	0.981	ND	9.31	ND	ND	0.780
	D		2.01	0.252	ND		1.11	ND	0.288		ND	0.773	0.729
DPPD	L	36.9	1.63	0.784	0.634	25.9*	0.871	17.7	0.627	91.0*	ND	ND	ND
	D		0.869	0.457	0.809		4.66	0.977	0.378		1.38	1.67	1.23
CPPD	L	ND	ND	ND	ND	ND	ND	ND	ND	9.12	ND	ND	ND
	D		ND	ND	ND		ND	ND	ND		0.989	1.21	1.03
TPPD	L	14.2	1.57	0.629	0.195	9.29	0.810	6.39	0.337	28.0	ND	ND	0.470
	D		1.21	0.209	0.606		2.31	0.651	0.373		0.763	1.10	1.23
DTPD	L	30.5	12.1	4.36	0.543	19.7	8.81	19.9	2.65	46.2	1.79	1.38	3.61
	D		8.62	1.59	4.58		11.0	5.76	3.87		4.22	8.97	10.2
BTH	L	20.0	13.0	4.74	1.57	11.1	4.48	3.81	2.69	5.29	3.29	1.43	1.66
	D		9.87	5.88	5.76		3.38	1.72	1.96		2.34	3.71	3.83
Fluoranthene	L	0.614	0.393	0.370	0.197	0.188	ND	1.37	0.224	9.25	8.68	8.61	9.67
	D		9.87	5.88	5.76		ND	0.312	0.199		13.3	12.0	11.8
Pyrene	L	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.24	9.74	11.3
	D		ND	ND	ND		ND	ND	ND		0.911	ND	11.5

4.2.2 Unidentified compounds results

Four of the same five unidentified substances from the hyperbaric experiment were detected in the UV experiments as well. Here, the compounds $C_{14}H_{23}$ at RT 17.14 and RT 17.82, $C_{21}H_{26}N_2$, and $C_{23}H_{26}N_2$ were detected and quantified using the 6PPD linear calibration curve (Table 4.4). The $C_{14}H_{23}$ substance at RT 17.14 was detected in all but one (TTMix-t_0) rubber-containing samples, while $C_{14}H_{23}$ at RT 17.82 was detected in 11 out of 22 rubber-containing samples. Neither of the two substances were detected in any filter-only samples. $C_{21}H_{26}N_2$, and $C_{23}H_{26}N_2$ were ubiquitous in the rubber-containing samples but were not detected in any filter-only samples.

Table 4.4: UV experiment results for the unidentified substances (µg additive per g rubber). Concentrations for $C_{14}H_{23}$ are the sums of two separate signals with the same molecular formula at RT 17.14 and 17.82. The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into UV radiation-exposed (L) and not exposed (D). Time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) indicate for how long the sample was in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

Substance		VCR				WCR				TTMix			
		t_0	T_1	T_2	T_3	t_0	T_1	T_2	T_3	t_0	T_1	T_2	T_3
$C_{21}H_{26}N_2$	L	9.01	4.95	3.19	2.37	2.69	0.257	0.392	0.203	7.86	3.65	0.508	0.904
	D		5.24	5.53	5.43		0.290	0.287	0.190		8.75	10.5	9.42
$C_{23}H_{26}N_2$	L	7.37	4.07	4.65	4.17	3.46	1.86	2.75	1.93	10.3	6.06	2.77	3.81
	D		4.60	8.16	5.68		1.63	2.75	1.91		11.5	13.5	12.1
$C_{14}H_{23}$	L	1.10	1.17	0.805	0.545	0.276	0.424	0.807	0.397	1.35	3.14	2.8	3.34
	D		0.618	0.859	1.22		0.409	0.565	0.484		4.18	1.71	4.18

4.3 Possible structures of unidentified compounds

4.3.1 $C_{14}H_{23}$

Two different ions were detected with the mass $C_{14}H_{23}$ at two different RTs (17.14 and 17.82). Searching for this formula yielded few good results, so another search adding a methyl group making the chemical formula $C_{15}H_{26}$ produced that the two substances are maybe some sort of terpenoids (Figure 4.2). While these results were not the top results, they seemed reasonable suggestions considering the monomers typically used in tires (Figure 2.1). It is clear when looking at the molecules for $C_{15}H_{26}$ that through degradation or fragmentation losing one methyl group is reasonable in order to get a signal for $C_{14}H_{23}$. As the identification of these compounds is dubious at most, the results and evaluation of them are going to be of their combined concentrations.

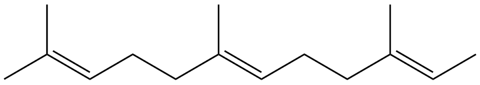
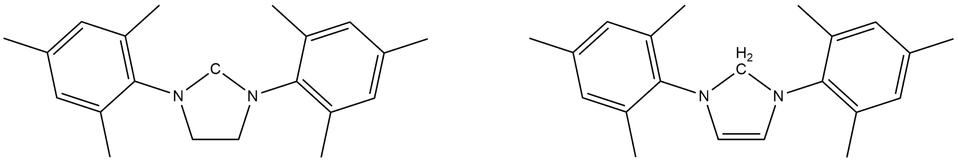


Figure 4.2: Molecular formula $C_{15}H_{26}$ suggested terpenoid molecule. More variants with different combinations of E and Z configurations at the double bonds exist.

4.3.2 $C_{21}H_{26}N_2$

The substance eluted at RT 13.49 was determined to have the chemical formula $C_{21}H_{26}N_2$. As this substance was detected in almost every sample with the exception of four filter-only samples, it is of particular interest as its presence in tires seems to be ubiquitous. The search in SciFinder® yielded 1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene (Figure 4.3a), a carbene, as the most referenced (300 at the time of search) substance. Another result with only 8 references at the time of the search showed a similar structure, but not a carbene (Figure 4.3b).

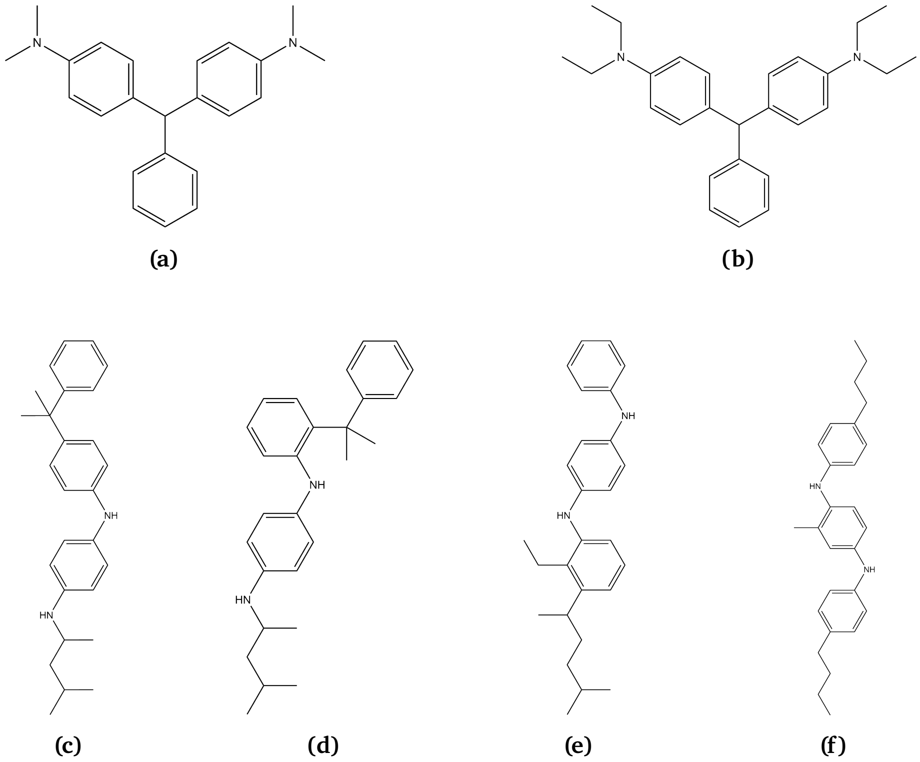


(a) (b)

Figure 4.3: Top referenced SciFinder® search result for $C_{21}H_{26}N_2$ (a), and similar result without reactive carbene center (b).

4.3.3 $C_{23}H_{26}N_2$ and $C_{27}H_{34}N_2$

$C_{23}H_{26}N_2$ at RT 15.03 was ubiquitous in rubber samples which makes it especially interesting. The search on SciFinder® suggested that it was leucomalachite green (Figure 4.4a), a colorless derivative of malachite green. $C_{23}H_{26}N_2$ was only detected in one sample, and the search indicated that it could be leucobrilliant green (Figure 4.4b). While leucobrilliant green had 81 references at the time of the search, there were also some results with 12, 5, 2, and 2 references out of the top 50 that indicated it could be derivatives of some PPDs (Figure 4.4c-4.4f). This is purely speculation and needs to be investigated compared with standards and mass spectral libraries.



(a) (b)

(c) (d) (e) (f)

Figure 4.4: Suggested molecular structures for $C_{23}H_{26}N_2$ called leucomalachite green (a), and $C_{27}H_{34}N_2$ as either leucobrilliant green (b) or PPDs (c-f).

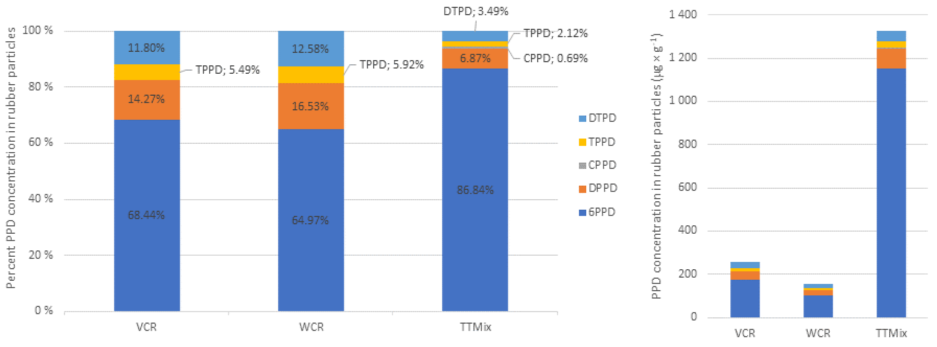
5

Discussion

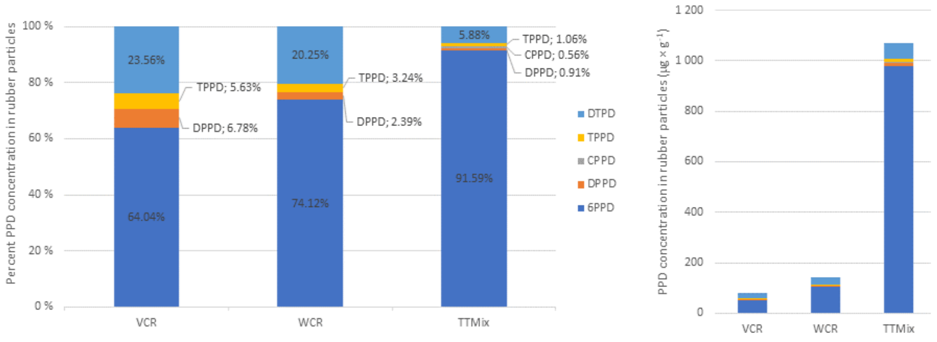
5.1 Differences between size and age of particles

The initial difference in extractability of additives from smaller particles (TTMix) versus the CRG particles (VCR and WCR) is highly substance-dependent and can be seen in the initial extracted concentrations (t_0). Starting the evaluation between the PPDs. Their extractability is a lot higher in TTMix samples versus VCR and WCR (Figure 5.1a). There is also a small difference between VCR and WCR, where WCR has less PPD concentration extracted than VCR, indicating a lower extractability of PPDs for older CRG particles.

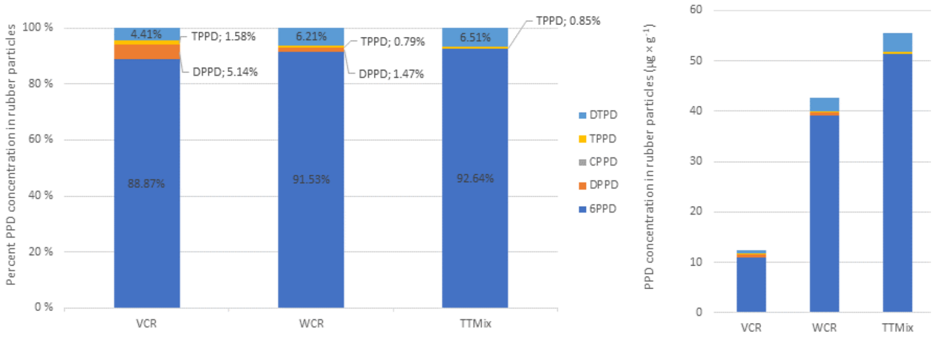
The effect that 2 weeks in water has on the different sample types can be seen between the start and end points of the hyperbaric experiments where possible (Figure 5.1a and 5.1b). As every rubber type only had 2 t_4 samples for the hyperbaric experiment the averages of them were used, those being the deep sea and surface water biotic samples. More in-depth evaluation between deep sea and surface water pressures will be examined later. The total extractable PPD content for TTMix and WCR decreases relatively little in relation to starting concentrations (1320 to 1070 µg/g and 157 to 142 µg/g respectively), while for VCR it decreased from 259 to 78.8 µg/g, which is a substantially higher relative content. While the TTMix difference in extracted concentration is higher (250 µg/g), the relative decrease in extracted concentration is a lot smaller (19% for TTMix versus 69% for VCR).



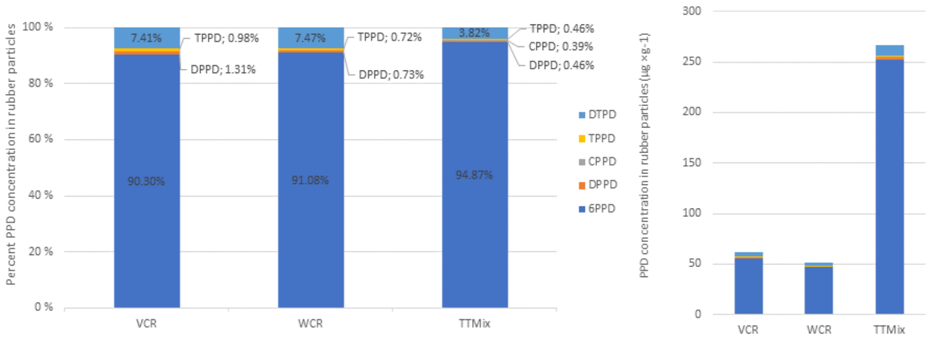
(a) Both experiments t_0



(b) Average of hyperbaric experiment t_4.



(c) UV experiment UV exposed T_3



(d) UV experiment not UV exposed T_3

Figure 5.1: Graphs showing the percentage each extracted PPD accounts for (left) and the total concentration of PPDs extracted (right). Showing the concentrations at t_0 (0 h) (a), the average of hyperbaric experiment at t_4 (2 w) (b), and UV experiment radiation exposed (c) and not exposed (d) at T_3 (2 w). The three graph bars represent the three different rubber sample types, virgin crumb rubber (VCR), weathered crumb rubber (WCR), and finely milled new tire tread particles (TTMix).

For the UV exposed samples, the effect of 2 weeks in water can be seen in PPD extraction concentrations (Figure 5.1a and 5.1c). It is seen that the TTMix and VCR samples are more affected than WCR. The total decrease in extracted PPD content is 1260 µg/g (96%) for TTMix, 247 µg/g (95%) for VCR, and 114 µg/g (73%) for WCR. The weathered particles' PPD extractability is less affected by exposure than those of virgin particles. Comparing the results of TTMix and VCR then the effect of exposure seems to be more similar between particle sizes.

For the samples not exposed to UV radiation, the difference in PPD extracted concentrations from start to end (Figure 5.1a and 5.1d) are still not similar to those of the hyperbaric experiment, but less dissimilar than the UV exposed samples. TTMix PPD extract concentrations decrease with 1054 µg/g (80%), VCR decreases with 197 µg/g (76%), and WCR decreases with 105 µg/g (67%). Once again it seems that TTMix is affected the most, followed by VCR and then WCR. Although not as much as in the UV exposed experiment.

Comparing the same things for the non-PPD concentrations (both targeted and suspect list substances) produces very individual results based on substance and it is tough to see any correlations between age and size of particles (Figures 5.5 and 5.6).

5.2 Hyperbaric and biotic influence effect on extractability

An end-point comparison was done at the latest time where both surface water and deep sea pressures were both present within a sample type and within either biotic or abiotic sampling. For all three sample types, biotic samples had samples for both deep sea and surface water at t_4 . For abiotic samples, comparisons within TTMix were done at t_2 , while VCR and WCR comparisons were done at t_3 . Substances where either of the two samples being compared did not detect the substance were not counted.

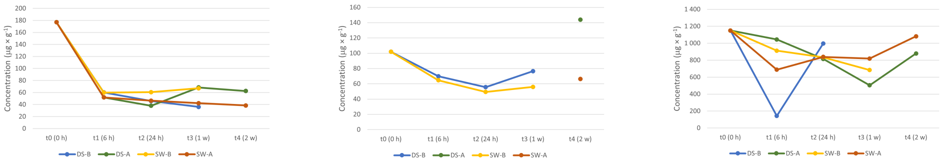
For the PPDs including 6PPD-Q: 5 VCR samples, 9 WCR samples, and 9 TTMix samples have a higher PPD extractability when exposed to deep-sea pressure. In comparison 5 VCR, 1 WCR, and 2 TTMix samples had a higher PPD extractability when exposed to surface water pressure (Figure 5.2). There seems to be a slight tendency for deep-sea pressure to increase extractability for PPDs, but it does not seem to favor any particular rubber sample type.

Comparing the non-PPD substances in the same way (Figure 5.5) then 5 VCR, 5 WCR, and 7 TTMix extracted concentrations were higher in the deep-sea samples. Comparatively 4 VCR, 4 WCR, and 3 TTMix samples had a higher concentration after surface water pressure exposure. Once again a slight tendency of increased exposure when exposed to deep-sea pressure.

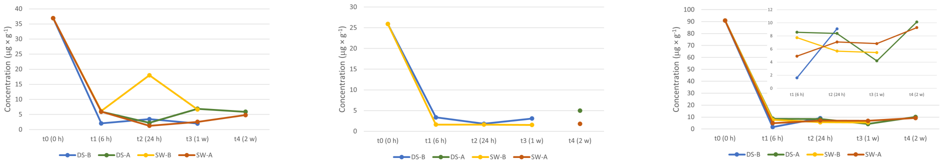
There seems to be no pattern over time that all deep-sea pressure or surface water pressure-exposed samples follow. Neither does there seem to be any pattern over time in extractability between biotic and abiotic samples (Figure 5.2 and 5.5).

There are a few outliers in terms of patterns that raise concerns as to the samples' validity. Such as TTMix-DS-B-t_1 seems to be spiking downwards for every nearly substance, save for Pyrene, BTH, and $C_{14}H_{23}$, before increasing or continuing stable over to TTMix-DS-B-t_2 (Figure 5.2, 5.5, and 5.4c). Such random differences in sampling are not expected to have such an impact on the TTMix samples, as they should be more predictable due to the particle mixture being nearly identical across samples. The different TTMix samples should also have similar additive contents and extractabilities. This is, of course, unless there is a change in extractability happening after 6 hours of exposure that is reversed in time for 12 hours.

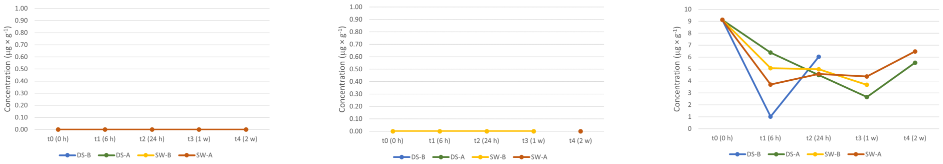
Looking at the PPDs concentration development in the hyperbaric experiment (Figure 5.2) there are some patterns. DPPD and TPPD showcase clear patterns across sample types, where it initially decreases by a lot before stabilizing around a lower concentration for the duration of the experiment. 6PPD also has this feature to a lesser degree, with only the VCR samples matching the pattern almost perfectly, WCR and TTMix samples look like they have a slight decrease but not nearly as steep as VCR. This could also be a discrepancy in the t_0 samples of VCR where the concentration is disproportionally high causing it to look like there is a steep decrease before concentrations stabilizing.



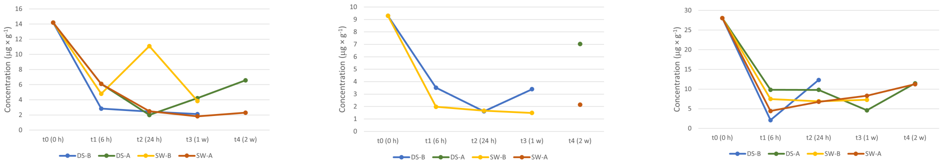
(a) 6PPD - VCR **(b)** 6PPD - WCR **(c)** 6PPD - TTMix



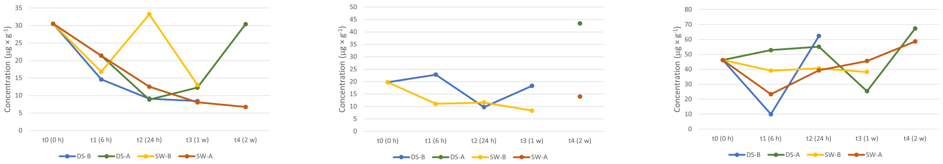
(d) DPPD - VCR **(e)** DPPD - WCR **(f)** DPPD - TTMix



(g) CPPD - VCR **(h)** CPPD - WCR **(i)** CPPD - TTMix



(j) TPPD - VCR **(k)** TPPD - WCR **(l)** TPPD - TTMix



(m) DTPD - VCR **(n)** DTPD - WCR **(o)** DTPD - TTMix

Figure 5.2: Hyperbaric experiment extracted PPD concentrations over time. The left column shows virgin crumb rubber (VCR) samples, the middle column shows weathered crmb rubber (WCR) samples, and the right column shows finely milled new tire tread samples (TTMix). The order of substances from top to bottom is 6PPD (a-c), DPPD (d-f), CPPD (g-i), TPPD (j-l), and DTPD (m-o). The y-axes show extracted concentrations from the tire particles (µg/g), and the x-axes are the sample time increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) which indicate for how long the samples were in exposure.

5.3 UV effect on extractability

Throughout the UV experiments; all PPDs feature a decrease over time, both in UV radiation exposed and not-exposed samples (Figure 5.3). Most noticeable is the initial decrease in extracted concentration from t_0 to T_1 in all the TTMix samples as well as some of the PPDs in VCR and WCR samples.

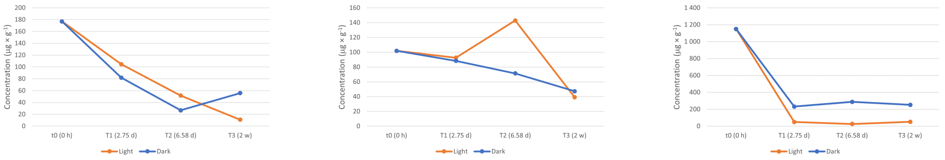
There are indications in the TTMix samples that the samples exposed to UV radiation have less extractable PPD content compared to the samples not exposed to UV radiation, although this is also largely because PPD content was not detected at all in a lot of the UV exposed TTMix samples (Figure 5.3). The observed difference between light and dark is not apparent in VCR and WCR samples.

BTH alternates between having higher extracted concentrations between UV exposed and not-exposed samples over time in both VCR and TTMix samples but ends up being higher in concentration in the not-exposed samples (Figure 5.6a and 5.6c). The WCR samples follow a pattern showing that the UV-radiation exposed samples are consistently higher in concentration (Figure 5.6b.

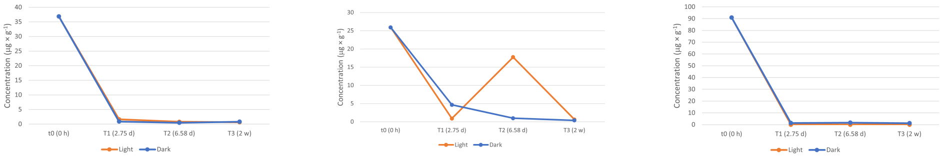
Fluoranthene concentrations are separated well already at T_1 between UV exposed and not exposed in TTMix samples and remain stable in concentration throughout (Figure 5.6f). VCR sample concentrations between UV exposed and not exposed interchange at different times but end up being almost identical at the end of the experiment. WCR samples are also similar in concentration between UV exposed and not exposed except at T_2, but ends up at almost identical concentrations at T_3. Pyrene was only detected in TTMix samples, and the UV exposed samples are stable throughout, and higher than the non-exposed counterparts. The non-exposed samples have near-zero concentrations until T_3 where it spikes and matches the concentration of the UV-radiation exposed sample.

The difference in extracted concentration for $C_{21}H_{26}N_2$ and $C_{23}H_{26}N_2$ seems to be separated between samples exposed to UV radiation and those not exposed (Figure 5.6). It is most apparent in TTMix and VCR samples, where it even seems that the difference increases over time. WCR concentrations were very similar between them and as such it does not seem that there is a difference between UV exposed and non-exposed samples.

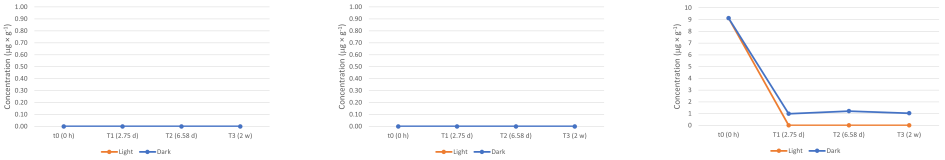
WCR-Light-T_2 seems to have a spike in concentration for all detected substances (Figure 5.3), even the suspect-list concentrations (Figure 5.6), the only exception is BTH. The spike in concentration could be due to the sample containing specific CRG particles with elevated levels, or the T_1 concentration could be unusually low. VCR and TTMix samples do not have any obvious patterns, such as spikes upwards or downwards in a single sample, that make it seem like there are any deviations.



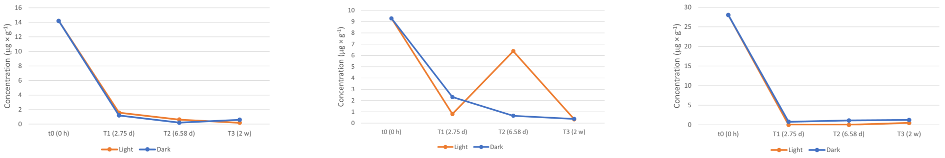
(a) 6PPD - VCR **(b)** 6PPD - WCR **(c)** 6PPD - TTMix



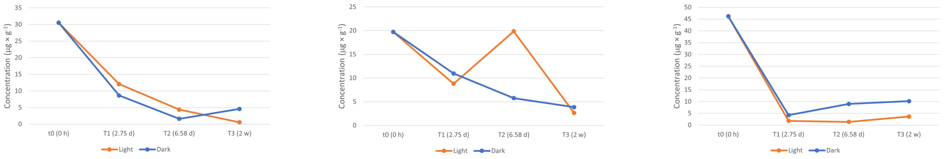
(d) DPPD - VCR **(e)** DPPD - WCR **(f)** DPPD - TTMix



(g) CPPD - VCR **(h)** CPPD - WCR **(i)** CPPD - TTMix



(j) TPPD - VCR **(k)** TPPD - WCR **(l)** TPPD - TTMix



(m) DTPD - VCR **(n)** DTPD - WCR **(o)** DTPD - TTMix

Figure 5.3: UV experiment extracted PPD concentration over time. The left column shows virgin crumb rubber (VCR) samples, the middle column shows weathered crmb rubber (WCR) samples, and the right column shows finely milled new tire tread samples (TTMix). The order of substances from top to bottom is 6PPD (a-c), DPPD (d-f), CPPD (g-i), TPPD (j-l), and DTPD (m-o). The y-axes show extracted concentrations from the tire particles (μg/g), and the x-axes are the time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) which indicate for how long the samples were in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

5.4 PPDs

For all PPD concentrations, there is a clear difference between VCR, WCR, and TTMix. TTMix has a higher initial (t_0) concentration of every single PPD (Figure 5.1a). For 6PPD, TTMix has a larger initial 6PPD concentration over VCR by a factor of 6.5 and over WCR by a factor of 11. The trend continues with factors of 2.5 and 3.5 for DPPD, 1.9 and 3.0 for TPPD, and 1.5 and 2.3 for DTPD. TTMix is also the only sample type where CPPD was detected. Either CPPD is difficult to detect, harder to extract, or in lower abundances in the CRG particles tested.

Comparing PPD content with each other it is easily seen that 6PPD makes up the bulk of PPDs in every single sample (Figure 5.1), the rest of the charts are found in Appendix C. It ranges from 64.0% to 96.9% of detected PPD content. After 6PPD, DPPD has a higher percentage concentration in t_0 samples by a small margin over DTPD (Figure 5.1a), but is overtaken by DTPD in samples already at t_1 and T_1, and remains a higher percent content throughout the rest of the experiments.

6PPD is by far the most prevalent PPD in our samples. This is consistent with 6PPD being the most produced and used antioxidant for tires. It keeps a consistently higher percentage of PPD content in TTMix samples as opposed to VCR and WCR (Figure 5.1), with it being slightly more equal in the UV samples compared to hyperbaric samples.

DPPD was not detected in any of the TTMix light samples, which could be due to those samples having the lowest weight while still having a larger volume of hexane extracting them. Otherwise, the DPPD content follows the general pattern of having a sharp decrease in concentration in the initial timeframe, before either slowly decreasing or keeping stable at a concentration (Figure 5.3d - 5.3f).

CPPD was detected in the lowest number of samples as well as in the smallest quantities. Within TTMix samples, which was the only rubber type where it was detected, it only dips too low to be detected only for samples exposed to UV radiation (Figure 5.3i). This could again be due to the TTMix UV exposed samples having a lower weight and higher extraction volumes, bringing the hexane extract concentration low enough that it can no longer be detected without extracted concentration relative to rubber necessarily being lower, or it could indicate UV exposure has an effect on extractable amounts.

DTPD percent content remains relatively equal in TTMix hyperbaric experiments compared to UV experiments, but for VCR and WCR there is a difference between the hyperbaric and UV experiments. The hyperbaric experiments

(t_1 -t_4) contain a larger percentage of DTPD with values ranging from 14.6% to 23.6% while the UV experiments (T_1-T_3) break the 10% mark only thrice, ranging from 1.77% to 10.62%. TTMix samples' DTPD extracted concentration remains similar across UV and hyperbaric experiments remaining around 2-6% of PPD content. DTPD concentration decrease mostly happens in the UV experiments, but is not as prevalent as in the hyperbaric experiments, as seen when comparing Figures 5.2m, 5.2n, and 5.2o to Figures 5.3m, 5.3n, and 5.3o.

TPPD is detected in all but two samples with a relatively low percent content of the PPDs. Like DTPD, the hyperbaric percent concentrations are higher than those of the UV experiment for VCR and WCR sample types, but by a smaller margin than DTPD. It is most apparent in VCR samples where the percent concentration of TPPD ranges from 4.20-6.07% in the hyperbaric samples, but only 0.72-1.58% in the UV samples.

Comparing the UV and the hyperbaric experiment for the PPDs shows an indication that the not exposed UV samples, generally, end up at a lower concentration than the hyperbaric experiments regardless of pressure and time in seawater (Figure 5.1b and 5.1d). One possible explanation, other than random differences, could be that the UV experiment samples were exposed to warmer water throughout the experiment (22°C vs 13±1°C).

There are higher PPD concentrations in TTMix for almost all samples of interest with the exception of TTMix-Light-T_1 and TTMix-Light-T_2. These two samples are also the only UV radiation-exposed samples where only 6PPD and DTPD were detected, and even then 6PPD was detected at a low concentration of only 49.6 and 26.1 µg/g respectively. These two samples could be outliers as the extracted concentrations are higher both before and after Light-T_1 and Light-T_2, but this cannot be confirmed.

Looking at the final exposure times (hyperbaric experiment t_4 and UV experiment T_3) and comparing the total PPD content between sample types, there are still large differences between total PPD content. The Light-T_3 samples have closer concentration than the rest of the end-of-experiment samples (Figure 5.1c). All the Light-T_3 samples are way lower in concentration than the average of the hyperbaric-t_4 samples, even TTMix, being the highest concentration in Light-T_3 at 55.4 µg/g is below the average PPD content of all hyperbaric samples, the lowest being 78.8 µg/g. Indeed, at no point does any average PPD content for any hyperbaric exposure time ever come below that of Light-T_3 (Appendix C).

Evaluating the **initial decrease** in extracted concentration, meaning the difference between t_0 and t_1 , or t_0 and T_1, is of special interest as these time-frames have the largest differences for the PPDs. This was done by calculating the decrease in rubber concentration divided by initial concentration, and for averages excluding those where the initial concentration decreased to below detectable (Table 5.1). It can also be seen between the first and second points in Figures 5.2 and 5.3 in every graph.

As expected from the switch in the second highest percentage content, DPPD has the highest initial decrease in detected PPD concentration with an average decrease of 92.6% from the initial concentration. Comparatively, 2nd place is TPPD with an initial average decrease of 82.3%, and in last place is 6PPD with an initial average decrease of only 48.3%. It is important to note that their initial decreases are extremely sample type- and experiment-dependent. Implications of these figures are that DPPD has the largest tendency for reduction in extraction overall, and 6PPD has the lowest. Since it seems that the initial decrease is responsible for most of the decrease in PPD concentration, at least in the UV experiments, it would be reasonable that the rest of the PPDs also pseudo-follows this order (Table 5.1).

Table 5.1: Initial decrease of PPD concentration in both experiments. Average decreases were also calculated for each sample type, not including the 100% decrease figures. The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Hyperbaric (H.B.) percentages are the decrease from t_0 to the average of samples at t_1. UV experiment exposed (UV-L) and not exposed (UV-D) are their respective decreases to the sample data taken at T_1.

	VCR			WCR			TTMix		
PPD:	UV-L	UV-D	H.B.	UV-L	UV-D	H.B.	UV-L	UV-D	H.B.
6PPD	40.9%	53.8%	68.4%	9.2%	13.3%	34.0%	95.7%	79.9%	39.4%
DPPD	95.6%	97.6%	86.4%	96.6%	82.0%	90.4%	100%	98.5%	93.7%
CPPD	N/A	N/A	N/A	N/A	N/A	N/A	100%	89.2%	55.7%
TPPD	88.9%	91.5%	65.1%	91.3%	75.1%	70.4%	100%	97.3%	78.8%
DTPD	60.5%	71.8%	39.2%	55.4%	44.4%	14.2%	96.1%	90.9%	32.2%
6PPD-Q	67.2%	80.8%	29.7%	80.3%	72.4%	75.6%	100%	100%	9.0%
Average decreases across rubber types (excl. 100% samples)									
6PPD	54.3%			18.8%			71.7%		
DPPD	93.2%			89.7%			96.1%		
CPPD	N/A			N/A			72.4%		
TPPD	81.8%			78.9%			88.0%		
DTPD	57.1%			38.0%			73.0%		
6PPD-Q	59.2%			76.1%			9.0%		

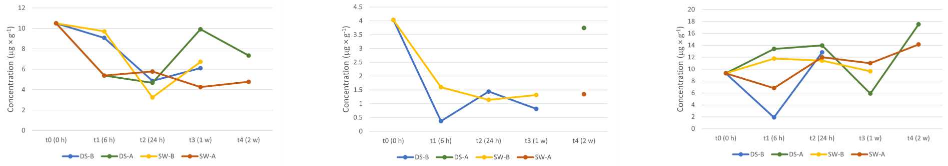
5.5 6PPD-Q

6PPD-Q is present in all samples, with an initial concentration of 9.3 µg/g for TTMix, 10.5 for VCR, while WCR is significantly lower at 4.0 µg/g. In the

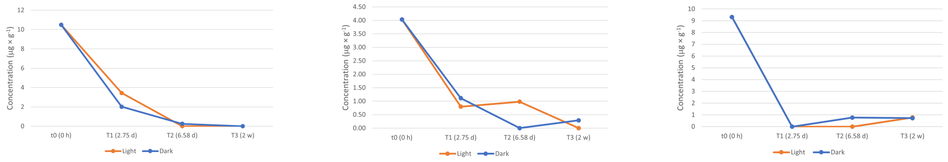
UV experiment samples the tendencies for the decrease in concentration are consistent with a major decrease in concentration in the initial time period (Figure 5.4), similar to those of the rest of the PPDs. Two of the initial hyperbaric experiments have an increase in concentration, both being in the TTMix sample type, those being TTMix-DS-A and TTMix-SW-B (Figure 5.4c). The two other TTMix samples feature an increase in concentration in the next timespace (t_1 -t_2).

Once again there seems to be a difference between UV and hyperbaric experiments. UV experiments feature more defined decreases of 6PPD-Q compared to the hyperbaric experiments. Even the dark experiments which should be more similar to the hyperbaric experiments feature a larger decrease in concentration across the three sample types. The UV experiment samples end up with a 6PPD-Q concentration of <1 µg/g already at T_2 of the experiments (Figure 5.4d, 5.4e, and 5.4f). The only ones comparable from the hyperbaric experiment are two WCR samples (Figure 5.4b).

Although 6PPD-Q formation is accelerated by exposure to UV radiation, there is no indication of this here since there is no clear increase in extracted 6PPD-Q concentration or any indication that formation plays a role in keeping the extracted concentration from decreasing compared to the not-exposed samples from the UV experiment (Figure 5.4d - 5.4f).



(a) Hyperbaric - VCR **(b)** Hyperbaric - WCR **(c)** Hyperbaric - TTMix



(d) UV - VCR **(e)** UV - WCR **(f)** UV - TTMix

Figure 5.4: Extracted 6PPD-Q concentration development over time. The three columns correspond the three rubber sample types, virgin crumb rubber granulate (VCR) on the left, weathered crumb rubber granulate (WCR) in the middle, and finely milled new tire tread particles (TTMix) on the right. The top row (a-c) shows the results from the hyperbaric experiment, and the bottom row (d-f) shows the results from the UV experiment. The y-axes show extracted concentration (µg/g), and the x-axes are the time-increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) for the hyperbaric experiment, and t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) for the UV experiment. The time increments indicate for how long the sample was in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

5.6 BTH

Benzothiazole was detected in every single sample, including blanks and filter-only samples. Two out of four blank samples were significantly higher in BTH concentration, 24 and 25 pg/µL contra 0.03 and 0.07 pg/µL for the other two blank samples in the hexane extracts. Filter-only samples had 0.04-0.1 pg/µL in their hexane extracts. Comparatively, the highest hexane extract concentration for samples containing rubber was at, 338 pg/µL. Exact quantification is not possible with a single-point calibration, but the conclusion that detected BTH is predominantly from the tire particles is still valid.

The initial extract concentrations of BTH are found in the VCR samples (20.0 µg/g), followed by WCR (11.1 µg/g), and finally TTMix (5.29 µg/g). BTH concentrations do have a decreasing trend in concentration in the UV samples over time similar to those of the PPDs (Figure 5.6a - 5.6c). In the hyperbaric experiment, BTH concentrations are sporadic, and little can be gauged from them (Figure 5.5a - 5.5c).

5.7 PAH

Fluoranthene concentrations are higher in TTMix samples than both VCR and WCR throughout all the UV and hyperbaric experiments, with an initial factor of 49 and a factor of at least 6.3 (Light-T_2). In addition, it seems that the TTmix samples not exposed to UV radiation have a higher concentration compared to the samples exposed to UV radiation. The extracted concentrations remain stable throughout the experiment for TTMix but are a lot more sporadic in VCR and WCR (Figure 5.6d - 5.6f). Due to this, it is difficult to draw any conclusions. As for the hyperbaric results (Figure 5.5d - 5.5f), they are overall stable over time. VCR has a slight downward trend, while WCR and TTMix seem to have more upwards trends.

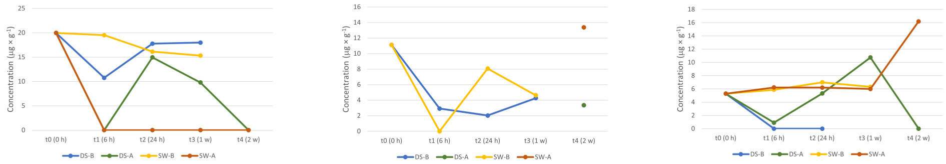
Pyrene is sporadic in samples and nothing can be gauged from the results (Figures 5.5g - 5.5i and 5.6g - 5.6i).

5.8 Screened for substances

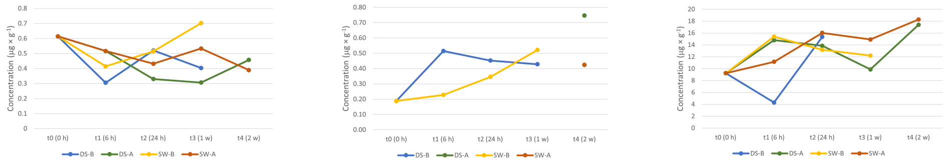
$C_{21}H_{26}N_2$ was detected in every sample. It has some similar time development traits as the PPDs in the WCR samples (Figure 5.5n and 5.6n). There is also a large difference between UV radiation exposed and not-exposed samples in the TTMix samples (Figure 5.6o). The initial concentrations for this substance are highest in VCR (9.01 µg/g), followed by TTMix (7.86 µg/g), and WCR (2.69 µg/g).

$C_{23}H_{26}N_2$ was detected in all samples. Initial concentration is highest in TTMix (10.3 µg/g), followed by VCR (7.37 µg/g), and WCR (3.46 µg/g). Extraction does not favor weathered particles. The substance has a clear difference between not-exposed and UV radiation exposed samples in TTMix, similar to that of $C_{21}H_{26}N_2$ (Figure 5.6r).

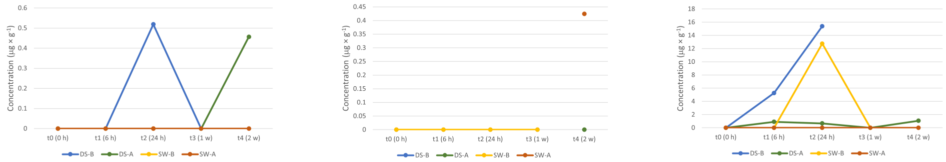
$C_{14}H_{23}$ has no discernible patterns either as the two separate signals or as the combined signals (Figure 5.5j-5.5l). The only thing to gauge here is that initially, TTMix has the highest extracted concentration (1.35 µg/g), followed by VCR (1.10 µg/g), and WCR (0.27 µg/g).



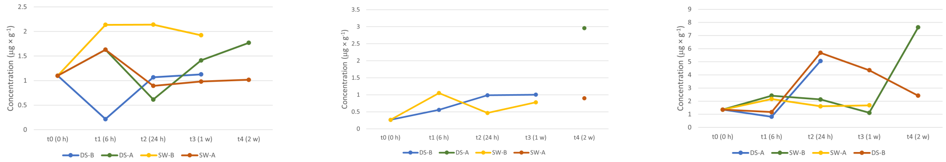
(a) Benzothiazole - VCR **(b)** Benzothiazole - WCR **(c)** Benzothiazole - TTMix



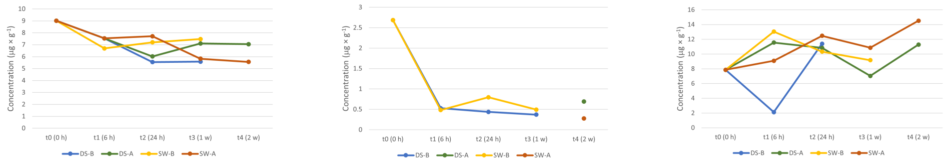
(d) Fluoranthene - VCR **(e)** Fluoranthene - WCR **(f)** Fluoranthene - TTMix



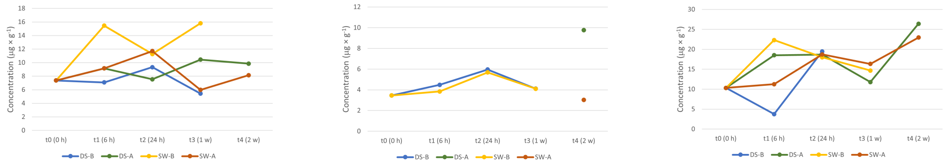
(g) Pyrene - VCR **(h)** Pyrene - WCR **(i)** Pyrene - TTMix



(j) $C_{14}H_{23}$ - VCR **(k)** $C_{14}H_{23}$ - WCR **(l)** $C_{14}H_{23}$ - TTMix

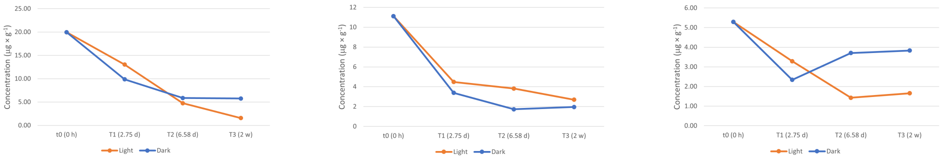


(m) $C_{21}H_{26}N_2$ - VCR **(n)** $C_{21}H_{26}N_2$ - WCR **(o)** $C_{21}H_{26}N_2$ - TTMix

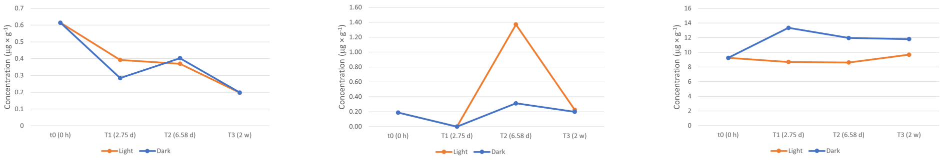


(p) $C_{23}H_{26}N_2$ - VCR **(q)** $C_{23}H_{26}N_2$ - WCR **(r)** $C_{23}H_{26}N_2$ - TTMix

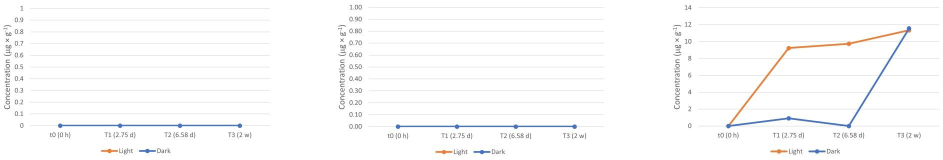
Figure 5.5: Hyperbaric experiment non-PPD extracted concentrations. The left column shows virgin crumb rubber (VCR) samples, the middle column shows weathered crmb rubber (WCR) samples, and the right column shows finely milled new tire tread samples (TTMix). The order of substances from top to bottom is BTH (a-c), fluoranthene (d-f), pyrene (g-i), $C_{14}H_{23}$ combined concentrations (j-l), $C_{21}H_{26}N_2$ (m-o), and $C_{23}H_{26}N_2$ (p-r). The y-axes show extracted concentration (µg/g), and the x-axes are the sample time increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) which indicate for how long the samples were in exposure.



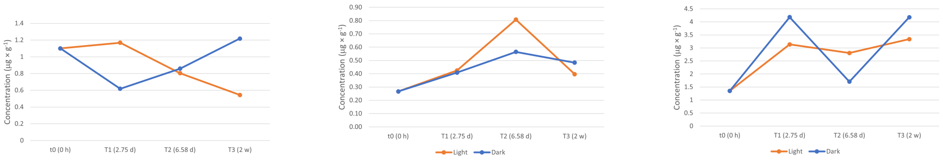
(a) Benzothiazole - VCR **(b)** Benzothiazole - WCR **(c)** Benzothiazole - TTMix



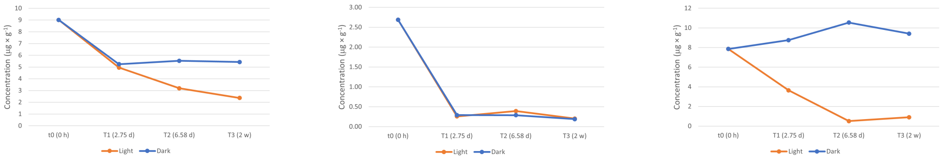
(d) Fluoranthene - VCR **(e)** Fluoranthene - WCR **(f)** Fluoranthene - TTMix



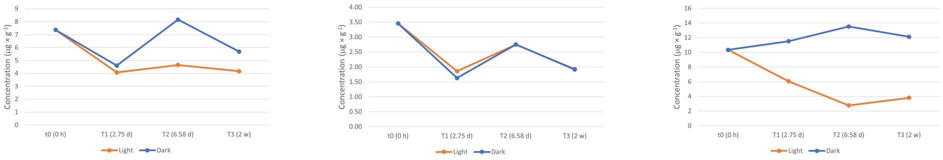
(g) Pyrene - VCR **(h)** Pyrene - WCR **(i)** Pyrene - TTMix



(j) $C_{14}H_{23}$ (combined) - VCR **(k)** $C_{14}H_{23}$ (combined) - WCR **(l)** $C_{14}H_{23}$ (combined) - TTMix



(m) $C_{21}H_{26}N_2$ - VCR **(n)** $C_{21}H_{26}N_2$ - WCR **(o)** $C_{21}H_{26}N_2$ - TTMix



(p) $C_{23}H_{26}N_2$ - VCR **(q)** $C_{23}H_{26}N_2$ - WCR **(r)** $C_{23}H_{26}N_2$ - TTMix

Figure 5.6: UV experiment non-PPD extracted concentrations. The left column shows virgin crumb rubber (VCR) samples, the middle column shows weathered crmb rubber (WCR) samples, and the right column shows finely milled new tire tread samples (TTMix). The order of substances from top to bottom is BTH (a-c), fluoranthene (d-f), pyrene (g-i), $C_{14}H_{23}$ combined concentrations (j-l), $C_{21}H_{26}N_2$ (m-o), and $C_{23}H_{26}N_2$ (p-r). The y-axes show extracted concentrations from the tire particles (µg/g), and the x-axes are the time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) which indicate for how long the samples were in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

5.9 Evaluation of the study

It is important to note that the results are not the absolute concentrations of the additives in the rubber samples. The concentrations are just extracts representing what is able to be extracted from the surface of the particles with hexane. Concentrations are given in µg/g but that does not mean this is the entire concentration of the additives within the rubber particles at the times. This is only a means of comparing the results of their extractability to each other, as is possible since they were extracted in the same way, and the extracts were analyzed the same way.

External calibrations are not optimal without some internal standard to compare to. As mentioned the ideal situation for a GC-MS analysis is having an ISTD with isotopic marks in each sample, and while there was such an ISTD for both 6PPD and 6PPD-Q, these were not detected in the analysis of any rubber samples. In addition, the external calibrations were done using only 3 points, and although the R^2 values were very close to 1, indicating a good linear relationship between concentration and analysis response. Many of the analyte concentrations were also above the concentrations used for the calibration curves. This could mean the concentrations have been overestimated, as the curves have a tendency of flattening above the linear range.[85] However, because the R^2 values were so close to 1, it is possible that the linearity continues until the concentration of even the highest concentration samples (9580 pg/µL). In order to ensure that future samples analyzed are not outside the linear range external calibration curves need to include concentration towards 100 times the current highest concentration. Of course, any future calibrations should also be done in tandem with ISTD and their ratios for a higher precision concentration.

The 6PPD linear calibration curve was also used for many of the other substances in quantification, which means the concentrations cannot be evaluated in any other means other than relative to the same substance. The other substances could also have had other interactions in the sample prep and analysis leading to different linearity between area and concentration compared to the one that was applied. Ideally, an external calibration for all the PPDs individually would be available, but using the 6PPD standard for all of them is not a useless solution as they share similar chemical properties. This could particularly affect the relative PPD percentage concentrations (Figure 5.1). For the PAHs and other suspect list substances, however, there is not any basis for the chemical similarity between 6PPD and them. It is impossible to gauge if they would act in similar ways depending on their respective response factor in the MS, or if they would interact differently than 6PPD giving completely different signals depending on concentration. In essence, it can only be told if the different concentrations are above or below each other, but not by how much.

A single-point calibration for the BTH concentrations should not be accepted as valid for quantification by any sources. However, since an absolute quantification is not being done, the concentrations are viable for some comparative purposes of extractability.

VCR and WCR samples have an extra element of randomness compared to TTMix samples, as the particles in each CRG sample are in lower quantities and are collected from an unknown mixture of tires. The number of particles is around 10-20 for each sample in VCR and WCR. Random differences in the composition of tires are possible, for example, one sample can contain a particle of a particular brand of tire that uses an increased amount of one additive, leading to that one sample having a significantly increased extracted concentration. This is further exaggerated by the low number of particles per sample. Meanwhile, the TTMix samples are made of a known composition of known tires and include a number of particles in the tens of thousands, which makes them more consistent mixtures. Multiple parallels would be preferable in order to reduce the element of randomness that the VCR and WCR samples have.

5.9.1 Quality assurance

Precautions were taken in the analysis to minimize contamination of the samples from other substances. As the analysis deals with quantities in very low amounts, any pollution could interfere severely with the analysis. All the glassware used was burnt in an oven before using at 450°C for 6 hours. For the extraction GC grade hexane was used to keep a high purity throughout.

During sonication, water was exchanged in the sonicator between sonications to keep the temperature lower. The temperatures were between 17-33°C throughout sonications.

The analysis methods used for both external calibration and the samples were the exact same, to ensure an as accurate treatment of external calibration as possible. In addition, the method is an already established method for analyzing UV-stabilizing additives at NILU.

5.9.2 The disappearing internal standard

Isotope-marked ISTD for 6PPD and 6PPD-Q was added to all samples, but none was detected in any samples containing rubber particles. This leads to a suspicion that the rubber adsorbed the ISTD in the process of creating an equilibrium between polymer and hexane. The ISTD only added 5000 pg of substance to the samples, which would give a concentration of only 0.66 pg/µL in the hyperbaric hexane extracts. Comparatively, the lowest 6PPD hexane extract concentration detected was in the TTMix-t_0 sample, which only detected 0.94 pg/µL, in a 10500 µL volume sample. In the same particular sample the ISTD concentration would be 0.47 pg/µL. The median rubber-containing sample had a hexane extract concentration of 1040 pg/µL. This can be addressed in a future study by adding ISTD after extracting the hexane from the particles directly into the GC-MS vials, or by having a higher concentration of ISTD, either through more ISTD or a lower volume of hexane. Adding ISTD after extraction decreases how well it represents the preparatory conditions that the substances extracted from the rubber particles are exposed to. A recovery standard can then be used address this.

5.10 Future prospects

Standards for the NTS screening suspected substances can confirm or rule out suspicions. Currently, there are plans of acquiring the suspected $C_{23}H_{26}N_2$ molecule (leucomalachite green) for confirmation purposes. If the other non-identified substances keep being detected, then it could be of interest to test the standards of those as well. Even the commercial standard for 6PPD-Q is relatively new (2022),[3] so more investigation should be on the agenda for experiments involving tire additives and especially the PPD-Qs.

A study with more frequent time points and potentially over longer times could be of interest. Especially more sampling points for the initial decrease in extractability could be interesting as it could give more insight into how quickly the extractability changes. Continuing the study for longer could also give insight into whether or not the PPDs show any trends over longer time.

The volume of hexane can still be optimized for analysis. The volume used needs to be enough to extract as much additive as possible while at the same time being low enough that amounts are detectable. This could also be done through upconcentration by various means but adds another step to the analysis which increases costs and efficiency. Since the difference in concentration of the PPDs vary so much between 6PPD and the rest, it could be of interest to focus on the ones that are not 6PPD in analyses, which would require higher concentrations to ensure detectability of them all throughout.

The Andromeda Project also has two other parallels of the same types as in this thesis which are being analyzed by different laboratories. Comparing results with these could help give a better picture of the effect of UV radiation, hyperbaric pressure, and biotic influence on the different age and size of particles.

Comparing the results with the water extract portion of the project could also be one of the most important ways of determining if the substances have leached out of the tire particles or of there are other factors impacting the extractability of additives. It could also help with evaluating which VCR and WCR samples had random differences due to sampling by comparing concentrations in the water with extracted concentrations in the rubber particles.

6

Conclusions

The extractability and effect of exposure on extractability of additives in tires is more substance-dependent rather than particle-property dependant. Some tendencies can be seen between the size and age of particles in extractability such as a higher extractability of PPDs in finer particulate. There is a higher PPD extractability on virgin particles compared to weathered ones. Over longer times in the hyperbaric experiment, the extractability of PPDs is relatively more reduced in virgin particles contra both smaller and weathered particles. However, the opposite is true in the UV experiment, where the smaller particles' additive extractability was most decreased throughout the experiment, both exposed and not exposed to UV radiation. The virgin particles extractability were still more reduced than the weathered particles throughout the experiment.

There were more indications that the extractability on particles exposed to deep sea pressure conditions was increased rather than deep sea pressures at the end points of the experiments. Smaller particles had this tendency more frequently than CRG. This entire tendency is not investigated enough to draw any concrete conclusions, as the patterns for extractability throughout the exposure did not indicate any unanimous results.

There are strong indications that UV radiation exposure reduces PPD extractability in smaller particles compared to non-exposed particles, but is inconclusive in CRG. Two of the unidentified substances ($C_{21}H_{26}N_2$ and $C_{23}H_{26}N_2$) showed tendencies of having their extractability reduced after being exposed to UV radiation. This last tendency was most apparent in TTMix, less apparent in

VCR, and not apparent in WCR.

There are still unknown substances that are commonly found in tires, and further studies have to be done in order to identify and quantify them. From there their sources need to be evaluated, determining if they are additives or TPs, as well as determining their ecotoxicity. From there, studies should investigate their behavior over time in all sorts of conditions such as submerged in deep sea, and surface waters, with and without biotic influence, and both with and without UV radiation.

For small particles most of the PPD decrease in extractable concentration seems to happen in the earlier timeframes of tire particles reaching aquatic environments. The trend is not apparent, but not ruled out from larger particles. It was also only clearly definable in the UV experiments. Just how early they happen is not possible to say from this study, but within the first 6 hours, there is a significant decrease in extracted PPD content from tire particles. What specifically happens to the PPD content that is no longer available for extraction is not possible to say from these results.

6PPD-Q was detected in all the samples tested, meaning that even after being used in sports fields, as well as being weathered in seawater for an entire year, it is still possible to extract. There are no grounds to say whether or not exposure to UV radiation has any effect on abundance from this study. It was not screened for any other PPD-Qs, which could also be of interest, but their concentrations would likely be too low. The other PPD-Qs are likely also being formed in the tire particles, but since 6PPD is in such high abundance comparatively the 6PPD-Q concentrations would also be a lot higher than the other PPD-Qs.

An important piece of information for future studies on tire particles is the fact that the particles seem to act as sponges for the ISTD. This information needs to be kept in mind for future analyses of TWP additive content. There are several ways of addressing this, and more testing is needed for an optimal method. Projects utilizing recovery standards should be performed in order to further develop extraction methods.

In the large scale of things this thesis is but a small part of understanding the spread of tire additives in the environment. The thesis has given some insight into what can be expected in the extractability of some suspected substances in differently aged, and sized tire particles after they are exposed to UV radiation and the differences between deep sea and surface water pressures. Still, countless more research has to be done in order to fully understand the scale of additive pollution from tires. UV stabilizers and antioxidants are only some of the additives added. Identification of any detected unknown substances is an entire thesis of its own, and the attempts made in this thesis should be taken very lightly. For a complete and undoubted identification, standards need to be run so the RT as well as the mass fragmentation profile can be used to identify the correct substance.

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A

List of substances screened for

- 6PPD (targeted)
- CPPD
- DPPD
- TPPD
- DTPD
- 6PPD-Q (targeted)
- Hexadocanoic Acid
- Octadecanoic Acid
- Bisnorbietatriene
- Fluoranthene
- Pyrene

- Naphthylaniline
- BTH
- Octocrylene
- $C_{13}H_9NS$
- $C_{14}H_{23}$ at RT 17.14
- $C_{14}H_{23}$ at RT 17.82
- $C_{19}H_{24}N_2O$
- $C_{21}H_{26}N_2$
- $C_{23}H_{26}N_2$
- $C_{24}H_{36}N_2$
- $C_{25}H_{30}N_6O_6$
- $C_{27}H_{34}N_2$

B

Hexane extract concentrations

Table B.1: Concentrations of all substances detected in hexane extracts (pg/µL) from the hyperbaric experiment. Samples where the substance was not detected are noted as not detected (ND). The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into deep-sea (DS) which were exposed to high pressure, and surface water (SW) with were exposed to atmospheric pressure. And further divided into abiotic (A) which had $HgCl_2$ added during exposure, biotic (B) which did not. Time-increments t_0 (0 h), t_1 (6 h), t_2 (24 h), t_3 (1 w), and t_4 (2 w) indicate for how long the sample was in exposure.

Hyperbaric experiment																
Substance		VCR					WCR					TTMix				
		t0	t1	t2	t3	t4	t0	t1	t2	t3	t4	t0	t1	t2	t3	t4
6PPD	DS-B	1590	1040	793	625	-	948	1200	964	1330	-	2710	1850	11900	-	-
	DS-A		894	658	1190	1080		-	-	-	2500		9580	8910	3300	6340
	SW-B		1040	1050	1130	-		1120	857	969	-		4100	5040	3300	-
	SW-A		894	803	709	625		-	-	-	1150		2560	3930	4120	2840
6PPD-Q	DS-B	94.3	157	84.3	106	-	37.6	6.35	25	14.1	-	22.0	24.9	153	-	-
	DS-A		92.6	81.2	172	127		-	-	-	64.9		123	153	38.7	126
	SW-B		168	56.2	113	-		27.7	19.8	22.8	-		52.9	69.1	46.6	-
	SW-A		92.6	100	71.5	77.6		-	-	-	23.3		25.4	56.2	55.3	37.1
DPPD	DS-B	331	35.6	59.7	34.6	-	241	57.5	31.5	53.3	-	215	20.9	109	-	-
	DS-A		102	38.9	120	102		-	-	-	86.4		78.5	91.7	27.5	73.2
	SW-B		107	312	113	-		28.0	28.0	26.8	-		34.8	34.3	26.4	-
	SW-A		102	22.1	42.9	77.9	-	-	-	ND	31.2		18.3	33.2	34.4	24.3
CPPD	DS-B	ND	ND	ND	ND	-	ND	ND	ND	ND	-	21.5	13.3	72.1	-	-
	DS-A		ND	ND	ND	ND		-	-	-	ND		58.5	49.2	17.4	39.9
	SW-B		ND	ND	ND	-		ND	ND	ND	-		22.8	30.1	17.8	-
	SW-A		ND	ND	ND	ND		-	-	-	ND		13.7	21.5	22.1	17.0
BTH	DS-B	179	186	308	312	-	103	50.0	35.1	73.9		12.5	0.028	0.0376	-	-
	DS-A		0.278	259	170	0.14		-	-	-	58.3		8.29	58.1	70.3	0.0755
	SW-B		338	280	258	-		0.134	140	80.3	-		26.4	42.2	30.5	-
	SW-A		0.278	0.184	0.186	0.114		-	-	-	232		23.1	29.1	30.2	42.5
Fluoranthene	DS-B	5.52	5.3	9.02	7.00	-	1.75	8.83	7.85	7.42	-	21.8	56.0	184	-	-
	DS-A		8.90	5.73	5.32	7.93		-	-	-	13.0		136	151	64.6	126
	SW-B		7.19	8.92	11.8	-		3.95	5.99	9.04	-		69.2	79.6	58.9	-
	SW-A		8.90	7.49	8.94	6.33		-	-	-	7.36		41.4	75.1	75.1	48.0
Pyrene	DS-B	ND	ND	9.00	ND	-	ND	ND	ND	ND	-	ND	68.5	184	-	-
	DS-A		ND	ND	ND	7.92		-	-	-	ND		8.34	7.32	ND	7.71
	SW-B		ND	ND	ND	-		ND	ND	ND	-		ND	77.0	ND	-
	SW-A		ND	ND	ND	ND		-	-	-	7.36		ND	ND	ND	ND
$C_{21}H_{26}N_2$	DS-B	81.0	131	96.1	96.9	-	25.0	9.08	7.58	6.44	-	18.5	27.7	137	-	-
	DS-A		130	104	123	122		-	-	-	12.0		106	119	46.1	81.5
	SW-B		116	125	126	-		8.39	13.8	8.57	-		58.6	62.4	44.3	-
	SW-A		130	134	97.8	90.4		-	-	-	4.81		33.7	58.5	54.7	38.1
$C_{19}H_{18}N_2$	DS-B	128	49.1	42.4	36.6	-	86.4	60.3	28.0	58.8	-	66.1	27.3	147	-	-
	DS-A		105	34.6	73.2	114		-	-	-	122		90.0	107	30.1	82.6
	SW-B		83.2	192	64.9	-		34.3	28.8	25.8	-		33.5	41.7	35.2	-
	SW-A		105	43.2	30.5	37.3	-	-	-	-	37.4		16.4	31.7	41.8	29.5
$C_{23}H_{26}N_2$	DS-B	66.2	123	162	94.2	-	32.2	76.9	103	71.2	-	24.4	48.8	233	-	-
	DS-A		158	131	181	171		-	-	-	169		170	204	77.2	191
	SW-B		268	195	267	-		66.6	98.6	71.1	-		100	109	71.0	-
	SW-A		158	203	100	132		-	-	-	52.5		41.8	87.8	82.2	60.3
$C_{20}H_{20}N_2$	DS-B	274	253	158	146	-	184	391	169	317	-	109	129	745	-	-
	DS-A		369	154	213	527		-	-	-	753		485	602	166	485
	SW-B		291	576	220	-		192	201	145	-		176	245	184	-
	SW-A		369	217	135	110		-	-	-	243		86.7	184	229	154
$C_{14}H_{23}$ RT 17.14	DS-B	3.33	3.77	8.73	8.81	-	2.48	9.59	5.61	7.46	-	3.19	1.51	21.0	-	-
	DS-A		11.9	10.7	10.0	13.7		-	-	-	23.2		20.8	23.3	7.32	25.0
	SW-B		17.4	14.8	12.0	-		7.96	8.10	5.37	-		9.72	9.73	8.15	-
	SW-A		11.9	3.41	6.57	7.90		-	-	-	7.22		4.34	10.0	7.89	6.35
$C_{14}H_{23}$ RT 17.82	DS-B	6.56	ND	9.76	10.7	-	ND	ND	11.5	9.95	-	ND	9.10	39.5	-	-
	DS-A		16.1	ND	14.5	17.0		-	-	-	28.1		1.45	ND	ND	30.1
	SW-B		19.6	22.2	20.4	-		10.3	ND	8.17	-		ND	ND	ND	-
	SW-A		16.1	12.1	9.92	8.64		-	-	-	8.41		ND	16.7	14.0	ND

Table B.2: Concentrations of all substances in hexane extracts (pg/µL) from the UV experiment. Samples where the substance was not detected are noted as not detected (ND). The three columns indicate the three rubber sample types, virgin crumb rubber granulate (VCR), weathered crumb rubber granulate (WCR), and finely milled new tire tread particles (TTMix). Samples are further divided into UV radiation-exposed (L) and not exposed (D). Time-increments t_0 (0 h), T_1 (2.75 d), T_2 (6.58 d), and T_3 (2 w) indicate for how long the sample was in exposure. Initial time increment (t_0) was neither exposed to nor kept from UV radiation.

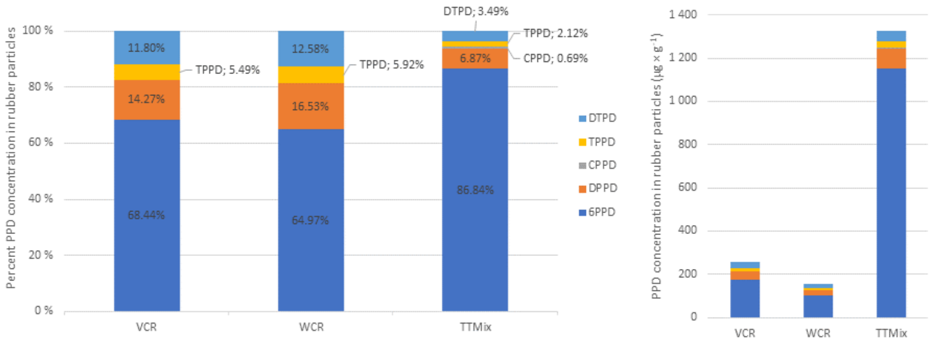
		UV Experiment											
Substance		VCR				WCR				TTMix			
		to	T1	T2	T3	to	T1	T2	T3	to	T1	T2	T3
6PPD	L	1590	419	258	64.4	948	370	715	230	2710	76.7	81.3	114
	D		409	347	765		442	939	656		2170	2130	1710
6PPD-Q	L	94.3	13.8	ND	ND	37.6	3.19	4.91	ND	22.0	ND	ND	1.73
	D		10.1	3.28	ND		5.57	ND	4.00		ND	5.74	4.92
DPPD	L	331	6.5	3.92	3.73	241	3.48	88.7	3.69	215	ND	ND	ND
	D		4.34	5.93	11.1		23.3	12.9	5.25		13.0	12.4	8.31
CPPD	L	ND	ND	ND	ND	ND	ND	ND	ND	21.5	ND	ND	ND
	D		ND	ND	ND		ND	ND	ND		9.29	9.01	6.96
BTH	L	179	52.1	23.7	9.24	103	17.9	19.1	15.9	12.5	5.09	4.45	3.67
	D		49.4	76.3	78.8		16.9	22.7	27.2		22.0	27.5	25.9
Fluoranthene	L	5.52	1.57	1.85	1.16	1.75	ND	6.85	1.32	21.8	13.4	26.8	21.4
	D		1.42	5.24	2.71		ND	4.11	2.76		125	88.8	79.7
Pyrene	L	ND	ND	ND	ND	ND	ND	ND	ND	ND	14.3	30.3	25.1
	D		ND	ND	ND		ND	ND	ND		8.55	ND	78.0
$C_{21}H_{26}N_2$	L	81.0	19.8	16.0	13.9	25.0	1.03	1.96	1.20	18.5	5.64	1.58	2.00
	D		26.2	71.9	74.4		1.45	3.78	2.64		82.2	78.3	63.6
$C_{19}H_{18}N_2$	L	128	6.28	3.14	1.15	86.4	3.24	31.9	1.98	66.1	ND	ND	1.04
	D		6.03	2.72	8.3		11.6	8.57	5.18		7.16	8.19	8.31
$C_{23}H_{26}N_2$	L	66.2	16.3	23.3	24.5	32.2	7.44	13.8	11.3	24.4	9.38	8.63	8.43
	D		23.0	106	77.8		8.15	36.2	26.6		108	100	81.8
$C_{20}H_{20}N_2$	L	274	48.3	21.8	3.20	184	35.2	99.3	15.6	109	2.77	4.3	7.99
	D		43.1	20.6	62.8		54.8	75.8	53.8		39.6	66.6	68.7
$C_{14}H_{23}$ RT 17.14	L	3.33	2.28	1.16	1.49	2.48	1.70	4.04	2.33	3.19	2.09	3.19	2.55
	D		3.09	11.2	8.56		2.04	7.43	5.45		14.4	12.7	9.67
$C_{14}H_{23}$ RT 17.82	L	6.56	2.40	2.86	1.72	ND	ND	ND	ND	ND	2.77	5.55	4.84
	D		ND	ND	8.13		ND	ND	1.27		24.8	ND	18.6

C

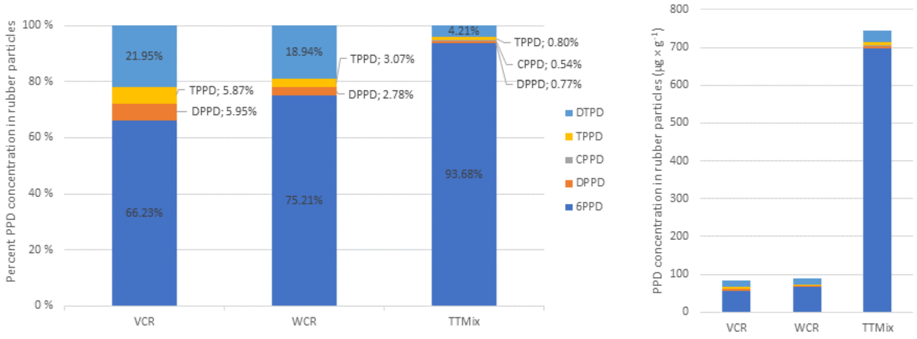
Complete PPD percentage and total content in samples.

Table C.1: Percent content of each PPD out of total concentration PPDs extracted along with total concentration PPD extracted from the samples. Hyperbaric data is the average of the 2-4 samples at each timepoint.

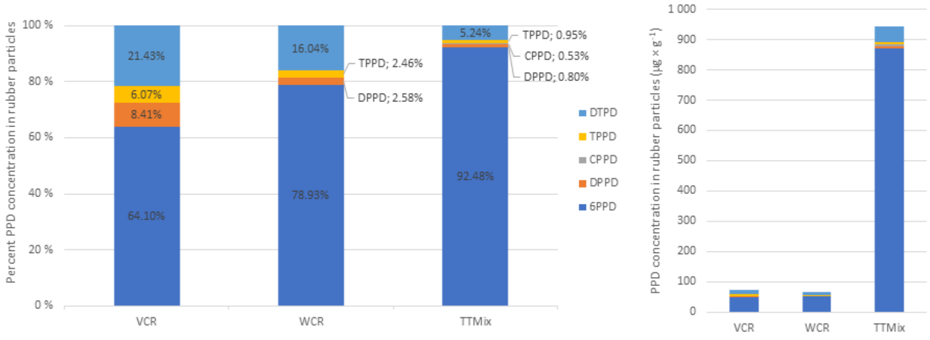
				VCR							
	t_0	t_1 (avg of 4)	t_2 (avg of 4)	t_3 (avg of 4)	t_4 (avg of 2)	L-T_1	D-T_1	L-T_2	D-T_2	L-T_3	D-T_3
6PPD	68.44%	66.23%	64.10%	74.83%	64.04%	87.27%	88.44%	89.94%	92.22%	88.87%	90.30%
DPPD	14.27%	5.95%	8.41%	6.35%	6.78%	1.36%	0.94%	1.37%	1.58%	5.14%	1.31%
CPPD	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TPPD	5.49%	5.87%	6.07%	4.20%	5.63%	1.31%	1.30%	1.10%	0.72%	1.58%	0.98%
DTPD	11.80%	21.95%	21.43%	14.62%	23.56%	10.06%	9.31%	7.59%	5.48%	4.41%	7.41%
Sum (µg/g):	259	84.5	74.3	71.5	78.8	120	92.5	57.4	29.0	12.3	61.8
				WCR							
	t_0	t_1 (avg of 2)	t_2 (avg of 2)	t_3 (avg of 2)	t_4 (avg of 2)	L-T_1	D-T_1	L-T_2	D-T_2	L-T_3	D-T_3
6PPD	64.97%	75.21%	78.93%	76.95%	74.12%	89.82%	83.12%	76.47%	90.62%	91.53%	91.08%
DPPD	16.53%	2.78%	2.58%	4.77%	2.39%	0.84%	4.38%	9.49%	1.24%	1.47%	0.73%
CPPD	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TPPD	5.92%	3.07%	2.46%	2.83%	3.24%	0.79%	2.18%	3.42%	0.83%	0.79%	0.72%
DTPD	12.58%	18.94%	16.04%	15.45%	20.25%	8.54%	10.32%	10.62%	7.32%	6.21%	7.47%
Sum (µg/g):	157	89.4	66.6	86.1	142	103	106	187	78.8	42.7	51.9
				TTMix							
	t_0	t_1 (avg of 4)	t_2 (avg of 4)	t_3 (avg of 3)	t_4 (avg of 2)	L-T_1	D-T_1	L-T_2	D-T_2	L-T_3	D-T_3
6PPD	86.84%	93.68%	92.48%	92.77%	91.59%	96.51%	96.92%	94.97%	95.68%	92.64%	94.87%
DPPD	6.87%	0.77%	0.80%	0.76%	0.91%	0%	0.58%	0%	0.56%	0%	0.46%
CPPD	0.69%	0.54%	0.53%	0.50%	0.56%	0%	0.41%	0%	0.40%	0%	0.39%
TPPD	2.12%	0.80%	0.95%	0.93%	1.06%	0%	0.32%	0%	0.37%	0.85%	0.46%
DTPD	3.49%	4.21%	5.24%	5.04%	5.88%	3.49%	1.77%	5.03%	2.99%	6.51%	3.82%
Sum (µg/g):	1 320	744	942	721	1 070	51.4	239	27.5	300	55.4	266



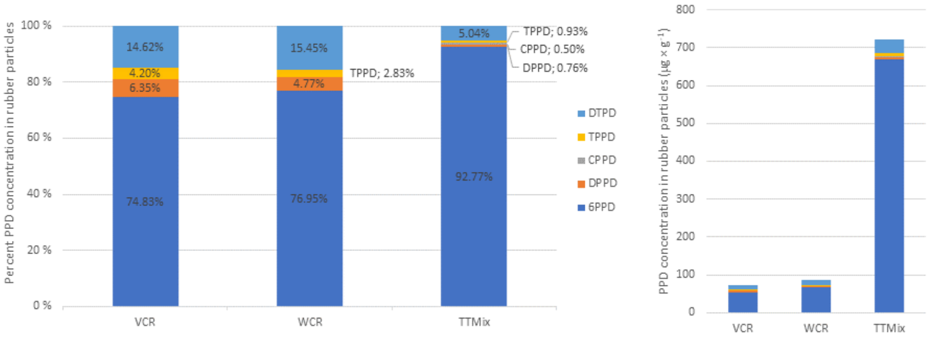
(a) Both experiments t_0



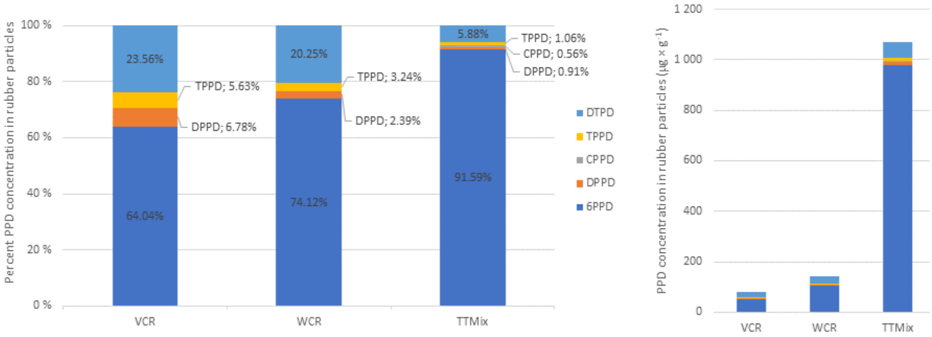
(b) Average of hyperbaric experiment t_1.



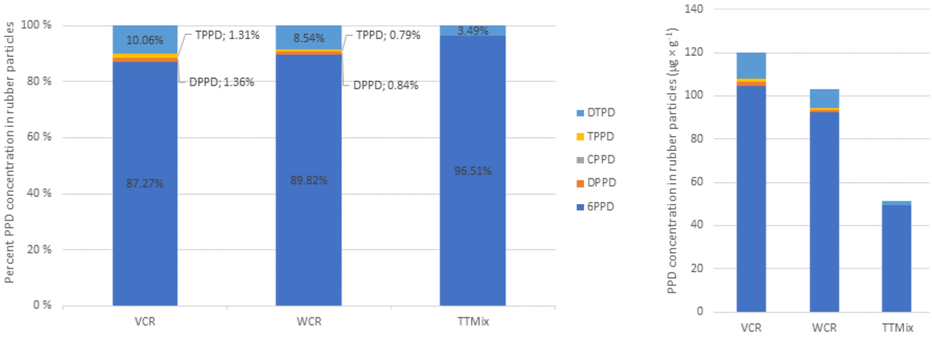
(c) Average of hyperbaric experiment t_2.



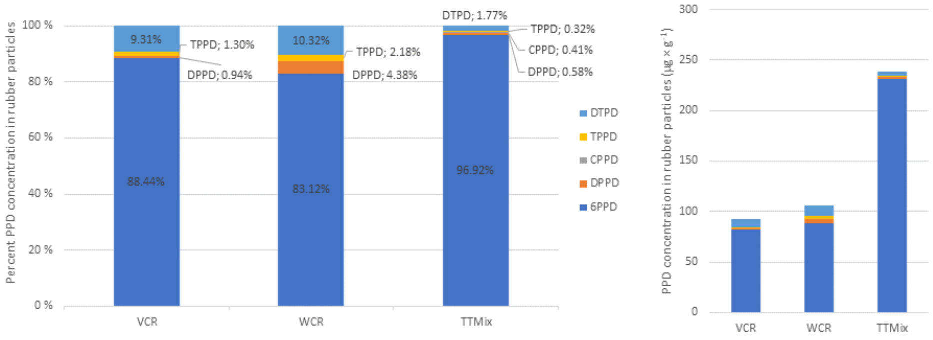
(d) Average of hyperbaric experiment t_3.



(e) Average of hyperbaric experiment t_4.

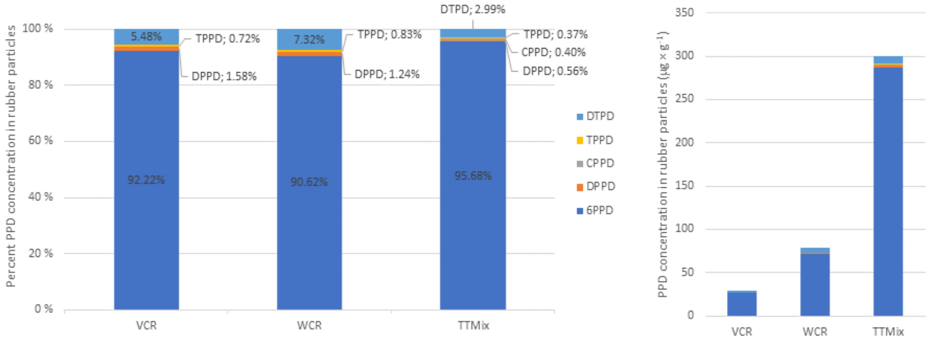


(f) UV experiment UV exposed T_1

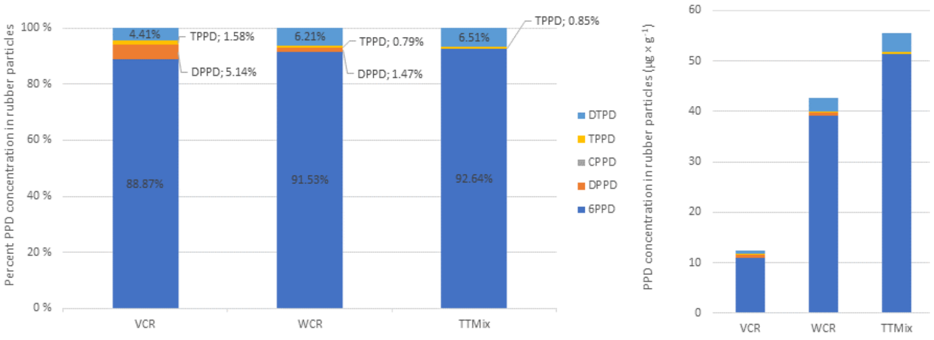


(g) UV experiment not UV exposed T_1

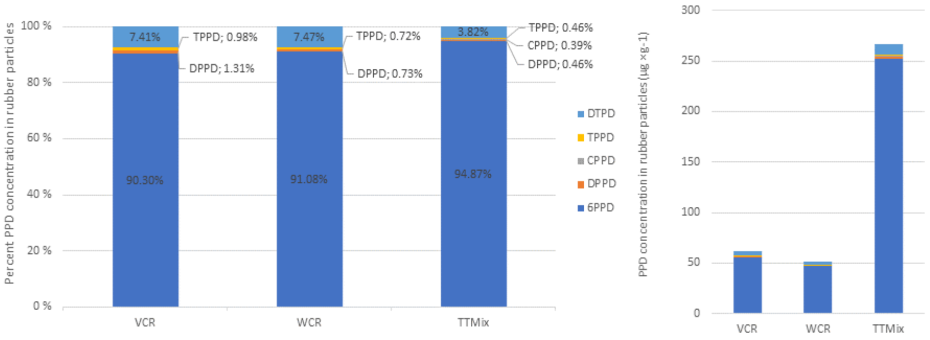
(h) UV experiment UV exposed T_2



(i) UV experiment not UV exposed T_2



(j) UV experiment UV exposed T_3



(k) UV experiment not UV exposed T_3

Figure C.1: Graphs showing the percentage each extracted PPD accounts for (left) and the total amount of PPD extracted (right). Showing the results for every time increment. The three graph bars represent the three different rubber sample types, virgin crumb rubber (VCR), weathered crumb rubber (WCR), and finely milled new tire tread particles (TTMix).

/D

UV experiment sample bottles

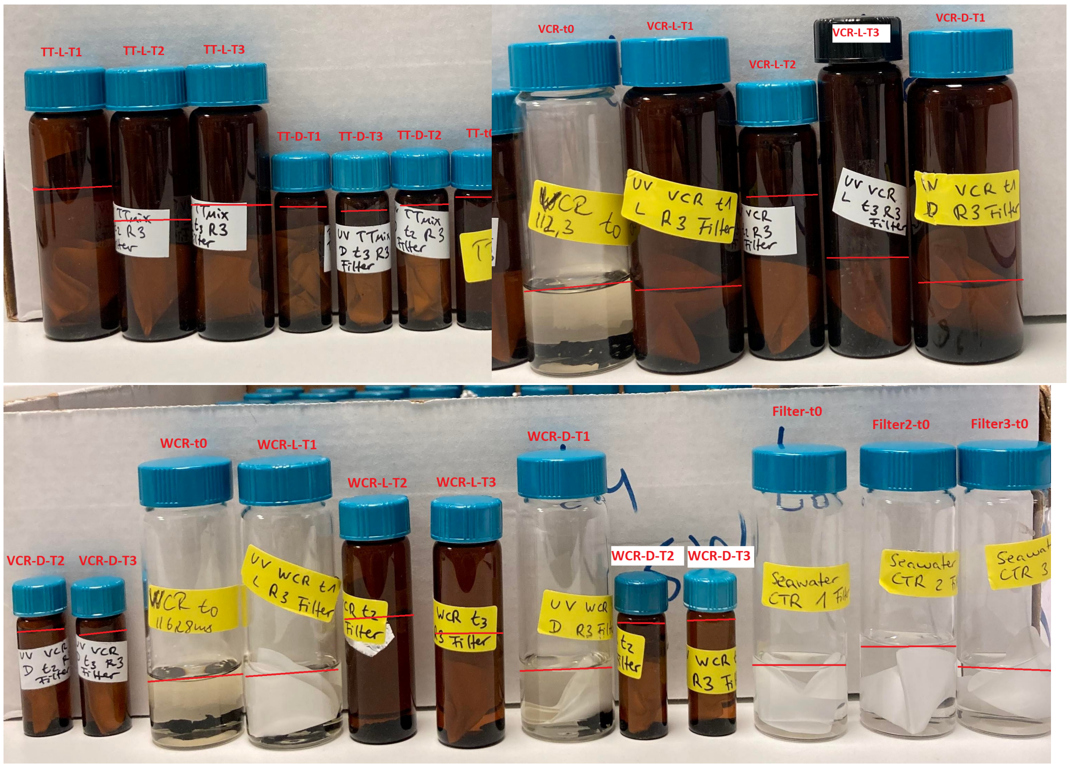


Figure D.1: Overview of hexane content in UV experiment bottles. Red lines indicate where the meniscus is and where the approximation of hexane volume was based on. Bottle sizes are variations between 15 mL, 4 mL, 22 mL, and 40 mL.

E

Sample weights and volumes

Table E.1: UV experiment sample weights and volumes.

Sample:	Volume (µL)	Weight (g)
WCR-t0	12500	0.11628
WCR-Light-T1	12500	0.05
WCR-Light-T2	10000	0.05
WCR-Light-T3	8500	0.05
WCR-Dark-T1	10000	0.05
WCR-Dark-T2	3800	0.05
WCR-Dark-T3	3600	0.05
VCR-t0	12500	0.1123
VCR-Light-T1	12500	0.05
VCR-Light-T2	10000	0.05
VCR-Light-T3	8500	0.05
VCR-Dark-T1	10000	0.05
VCR-Dark-T2	3850	0.05
VCR-Dark-T3	3650	0.05
TTMix-t0	4000	0.00944
TTMix-Light-T1	10000	0.01547
TTMix-Light-T2	8000	0.02493
TTMix-Light-T3	9000	0.01992
TTMix-Dark-T1	4000	0.03756
TTMix-Dark-T2	3700	0.02746
TTMix-Dark-T3	3900	0.02633
UV-Filter-T2	10500	0.09528
Blank 1	7500	0
Blank 2	7500	0
Blank 3	7500	0
Blank 4	7500	0

Table E.2: Hyperbaric experiment sample weights and volumes.

Sample:	Volume (μL)	Weight (g)
TTMix-DS-B-t1	7500	0.09750
TTMix-DS-B-t2	7500	0.08974
TTMix-DS-A-t1	7500	0.06878
TTMix-DS-A-t2	7500	0.08201
TTMix-DS-A-t3	7500	0.04908
TTMix-DS-A-t4	7500	0.05412
TTMix-SW-B-t1	7500	0.03368
TTMix-SW-B-t2	7500	0.04529
TTMix-SW-B-t3	7500	0.03623
TTMix-SW-A-t1	7500	0.02785
TTMix-SW-A-t2	7500	0.03516
TTMix-SW-A-t3	7500	0.03773
TTMix-SW-A-t4	7500	0.01968
WCR-DS-B-t1	7500	0.12863
WCR-DS-B-t2	7500	0.13
WCR-DS-B-t3	7500	0.13
WCR-DS-A-t4	7500	0.13
WCR-SW-B-t1	7500	0.13
WCR-SW-B-t2	7500	0.13
WCR-SW-B-t3	7500	0.13
WCR-SW-A-t4	7500	0.13
VCR-DS-B-t1	7500	0.13
VCR-DS-B-t2	7500	0.13
VCR-DS-B-t3	7500	0.13
VCR-DS-A-t1	7500	0.12919
VCR-DS-A-t2	7500	0.13
VCR-DS-A-t3	7500	0.13
VCR-DS-A-t4	7500	0.13
VCR-SW-B-t1	7500	0.13
VCR-SW-B-t2	7500	0.13
VCR-SW-B-t3	7500	0.12639
VCR-SW-A-t1	7500	0.13
VCR-SW-A-t2	7500	0.13
VCR-SW-A-t3	7500	0.12582
VCR-SW-A-t4	7500	0.12184
Filter-Only-DS-B-t0	7500	0.10055
Filter-Only-DS-A-t0	7500	0.09520
Filter-Only-DS-A-t4	7500	0.09035
Filter-Only-SW-B-t0	7500	0.12254
Filter-Only-SW-A-t4	7500	0.09607

Tire Tread particles Mixture (TTmix)

20 different types of Tire Wear Particles:

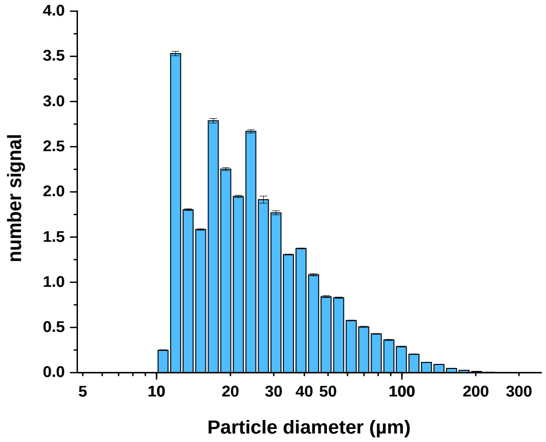
Sample	Season	Details
TP1	all-season	Falken outside Euroall Season 225/50 R17 98 V
TP2	winter	WINTEC PN150 165/65 R15 91T M+S
TP3	winter	Goodyear Vector 5+ M+S 185/65 R15 88T
TP4	winter	Fulda Kristall Montero 2 M+S 195/60 R15 88T
TP5	winter	Continental ContiWinter Contact TS830 205/55 R16
TP6	summer	tire rubber summer (typical mixture)
TP7	winter	tire rubber winter (typical mixture)
TP8	not specified	Continental Germany
TP9	not specified	Triangle, China
TP10	not specified	Wanlitire, China
TP11	not specified	Cheng Shin, China
TP12	summer	Bridgestone DriveGuard 225/40R18 92Y DRGSFZ 67854 VRT7
TP13	winter	Pirelli Sottozero 3 225/40 R18 92Y M+S extra load studless tubeless
TP14	winter	Fulda Kristall Montero 3 205/65 R15 94T M+S
TP15	all-season	Continental VancoFourSeason 2 235/65 R16 C
TP16	summer	Dunlop SP Sport Maxx GT 235/65 R17
TP17	summer	Sava intensa uhp 225/50 R16 92W
TP18	summer	Continental ContiSportContact 5 235/45 R17 94W
TP19	summer	Hankook VentusPrime 3 205/55R16 91V
TP20	summer	Semperit Speed-Life 195/50 R15 82H alpine proven

All the samples were mixed together and homogenized with the cryo-milled.

Particle size of TTmix

The particle size was determined with the *EyeTech Combi (ambiValue)*.

	Volume (µm)								Number (µm)							
	Video				**Laser**				**Video**				**Laser**			
Replies	**Mean**	**St.D**	**D50**	**D90**	**Mean**	**St.D**	**D50**	**D90**	**Mean**	**St.D**	**D50**	**D90**	**Mean**	**St.D**	**D50**	**D90**
TTmix_1	175.09	90.73	157.53	299.31	127.03	55.12	122.86	201.80	54.64	42.70	40.74	111.12	43.96	34.27	33.48	92.68
Ttmix_2	190.03	84.16	177.93	316.65	129.36	52.28	125.30	201.99	62.04	49.45	45.84	128.97	45.27	35.78	33.51	95.05
Ttmix_3	169.69	92.05	148.86	296.25	130.19	54.14	123.85	204.99	58.81	41.97	46.86	114.18	47.25	35.95	35.75	94.87
Average	**178.27 ± 88.98**		**161.44**	**304.07**	**128.86 ± 53.85**		**124.00**	**202.93**	**58.50 ± 44.71**		**44.48**	**118.09**	**45.49 ± 35.33**		**34.25**	**94.20**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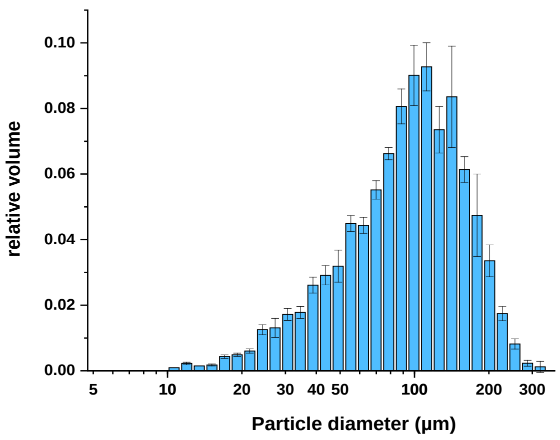
1.0
1.5
2.0
2.5
3.0
3.5
4.0
5
10
20
30
40
50
100
200
300
Particle diameter (µm)


relative volume
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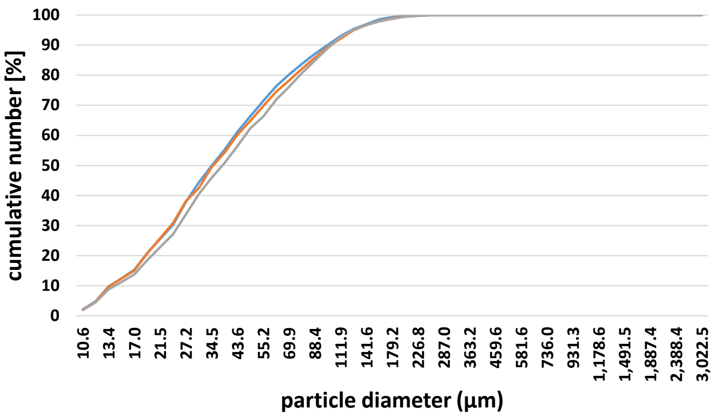
0.02
0.04
0.06
0.08
0.10
5
10
20
30
40
50
100
200
300
Particle diameter (µm)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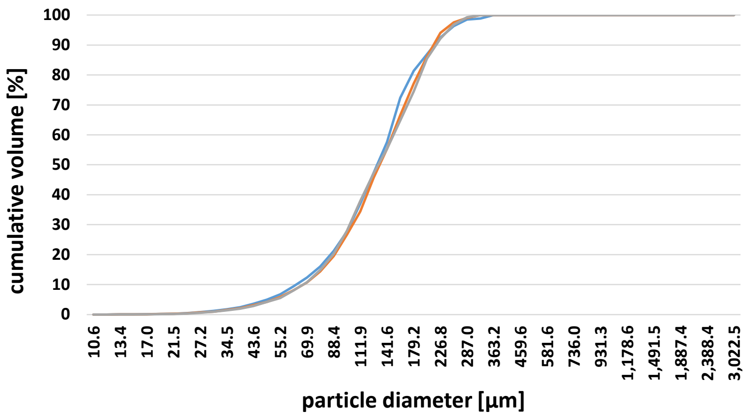
20
30
40
50
60
70
80
90
100
10.6
13.4
17.0
21.5
27.2
34.5
43.6
55.2
69.9
88.4
111.9
141.6
179.2
226.8
287.0
363.2
459.6
581.6
736.0
931.3
1,178.6
1,491.5
1,887.4
2,388.4
3,022.5
particle diameter (µm)


cumulative volume [%]
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10
20
30
40
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60
70
80
90
100
10.6
13.4
17.0
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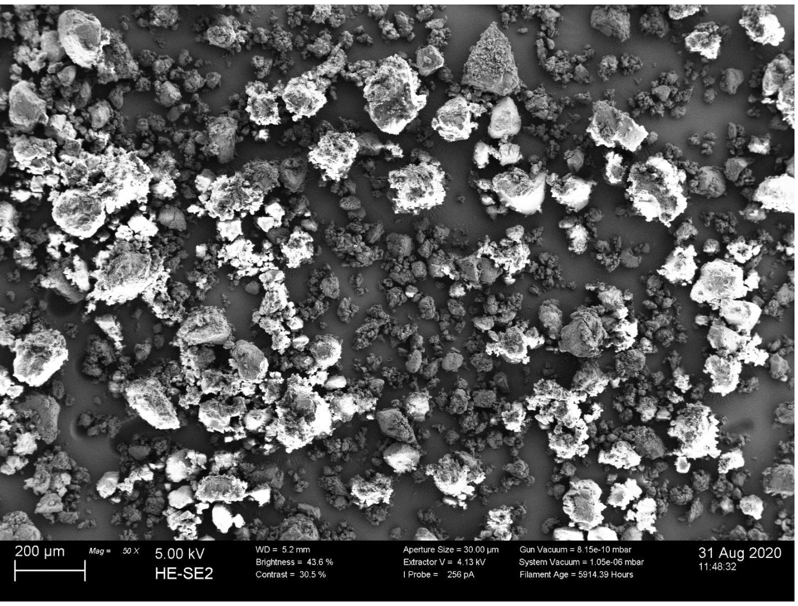
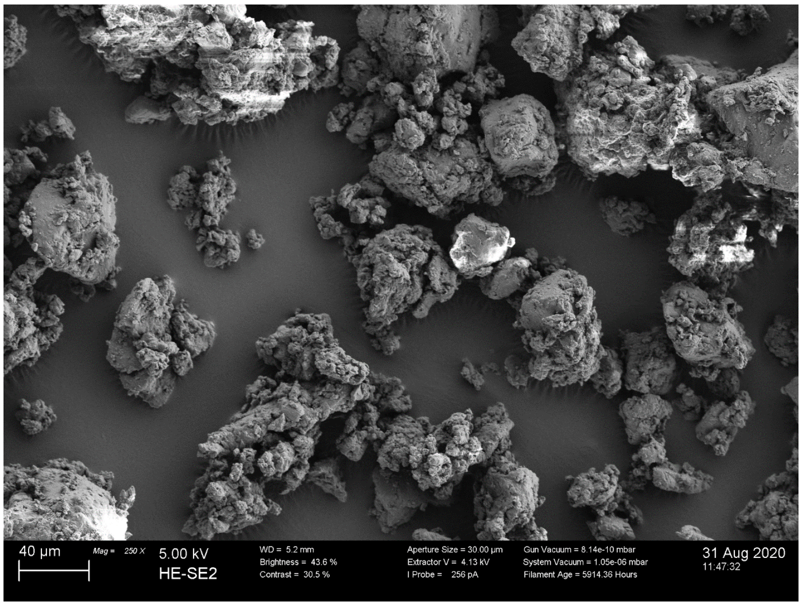
34.5
43.6
55.2
69.9
88.4
111.9
141.6
179.2
226.8
287.0
363.2
459.6
581.6
736.0
931.3
1,178.6
1,491.5
1,887.4
2,388.4
3,022.5
particle diameter [µm]

Particle shape of TTmix

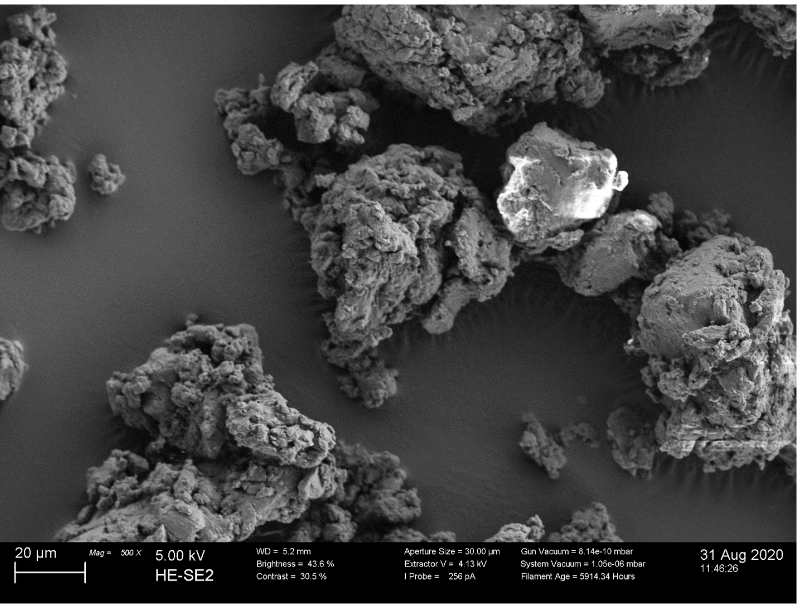
The images were taken by Zeiss Merlin VP compact SEM

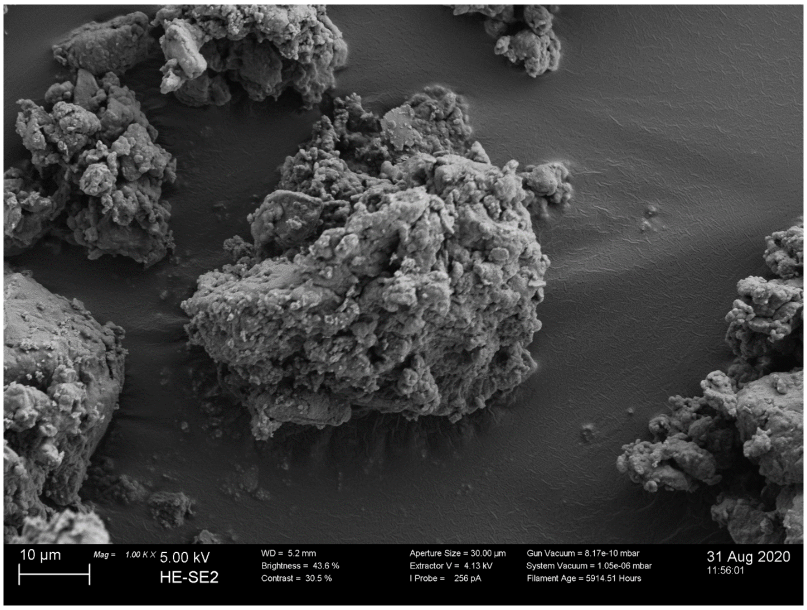




Particle shape of TTmix

The images were taken by Zeiss Merlin VP compact SEM





G

Chemicals and Standards

Table G.1: Chemicals used.

Substance:	Quality:	CAS Number:	Supplier:
Hexane	SupraSolv	110-54-3	Merck
Acetone	Suprasolv	67-64-1	Merck
Helium (g)	5.0	7440-59-7	Nippon gases (Praxair)

Table G.2: Internal standard mixture used for every sample.

Name:	Compound:	Amount:
ISTD UV 320, 6, 7, 8, 6PPD, 6PPD-Q DEUTERATED	UV-320-d4	250 ng
	UV-326-d3	250 ng
	UV-327-d3	250 ng
	UV-328-d4	250 ng
	6PPD-d5	250 ng
	6PPD-Q-d5	250 ng
	Acetone	2500 µL

/H

GC-MS Programming

Table H.1: TRACE 1310 Gas Chromatograph Settings

Column:	TG-5SILMS (30 m × 0.25 mm ID × 0.25 µm) (P/N 26096-1425)
Injection volume:	1 µL
Liner:	Thermo Scientific™LinerGOLD™GC Liner (P/N 453A1345-UI)
Inlet temperature:	250°C (S/SL)
Carrier gas:	He at 1.2 mL/min
Oven temperature programming	
Initial: Hold time:	40°C 1.34 min
Temperature 1: Rate: Hold time:	240°C 30°C/min 0 min
Temperature 2: Rate: Hold time:	255°C 5°C/min 0 min
Temperature 3: Rate: Hold time:	270°C 3°C/min 0 min
Temperature 4: Rate: Hold time:	300°C 30°C/min 7 min

Table H.2: Q Exactive GC Orbitrap Mass Spectrometer Settings

Transfer line:	280°C
Ionization type:	Electron Ionization (EI)
Ion Source:	250°C
Electron energy:	70 eV
Polarity:	Positive
Acquisition mode:	Fullscan - SIM
Scan range:	50-750 m/z
Resolution:	120 000 (FWHM at m/z 200)