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*Low particle Emissions and lOw Noise Tyres*



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## Revision History

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## Abbreviations and Units

BR	Butadiene Rubber
BSE	Back Scattered Electrons
CB	Carbon Black
DTG	Differential Thermogravimetry
EC	Elemental Carbon
EDX	Energy-dispersive X-ray spectroscopy
LN <sub>2</sub>	Liquid Nitrogen
NR	Natural Rubber
PM	Particulate Matter
SBR	Styrene Butadiene Rubber
SE	Secondary Electrons
SEM	Scanning Electron Microscope
SEM-EDX	Scanning Electron Microscopy with Energy-Dispersive X-ray spectroscopy
SLS	Static Light Scattering
SVOC	Semi-Volatile Organic Compounds
TGA	Thermogravimetric analysis
T(R)WP	Tyre (and Road) Wear Particles
TWP	Tyre Wear Particles

# 1 Introduction

Tyre and road wear particles (TRWPs) are an environmental contaminant of high concern due to their high abundance and wide environmental distribution (1–6). They are predicted to be the largest source of microplastics with annual global emissions estimated to be between 800–3300 kt (5, 6). TRWPs are a form of non-exhaust vehicle emissions (NEE) that have been recognised as a growing problem and are expected to be regulated in upcoming Euro 7 legislation. TRWPs are generally described as elongated particles with a heterogeneous composition of tyre tread with encrustations of traffic related particles such as brake and road wear (4, 7–10). Tyre tread consists for ~50% of synthetic and natural rubbers while the rest of the weight is due to softeners, vulcanisation agents, fillers and various other additives (4).

Several factors affect the environmental fate of TRWPs. Transport is affected by their size: small particles will be dispersed in the air, whilst large particles will deposit on or near the road surface after which they are further transported through resuspension and run-off to road side soils, sewers and surface waters (6, 11). According to Baensch-Baltruschat *et al.*, 66–76% of the coarse ‘non-airborne’ fraction is transported to soils and road banks near roads and 12–20% is released to surface waters (11). This is comparable with estimates in the Netherlands showing that 6% is directly ending up in surface waters, 15% in sewers and 67% in soil (12), and a Swiss study estimated that 74% is deposit in roadside soils and 22% in surface waters (13). As the most TRWPs are estimated to end up in roadside soils, understanding environmental fate of TRWPs in this compartment is of great importance. However limited information is available on the degradation of TRWP in soils (6, 11).

Often modelling studies are used to better understand the environmental fate and transport of TRWPs (14, 15). An important variable in these models is how fast TRWPs degrade in the different environmental compartments. Both photo- and biodegradation are relevant degradation paths to consider. Cadle and Williams studied the environmental degradation of T(R)WPs in soil including factors such as oxygen, heat, humidity, light and microorganisms in real-time, using an open set-up exposed to the elements (14). They found that degradation of tyre wear in soil had a rate of 0.15% per day and noted that the presence of microbes and type of tyre material (tread vs wear) play a big role in degradation. For unvulcanised rubber they conclude that oxidation processes are more significant than microbial degradation processes (14, 16). While some work has given mechanical insights that rubbers such as polystyrene-butadiene and poly-isoprene undergo photodegradation (17), the studies of Cadle and Williams remains the only one to provide a degradation rate. Recent lab-scale investigations into the effects of photodegradation also do not report degradation rates as these have focussed on the effects on leaching and transformation of tyre additives (18, 19).

The degradation rates used in the currently available modelling studies are mostly based on the research of Cadle and Williams from 1980 (14, 15). As Corella-Puertas *et al.* mention, this is the only experimental study to provide a half-life of tire wear particles in soil and there are no studies on the degradation of TRWP in the aquatic environment (20). As the composition of tyres has changed over the 40 years since the study, the results of Cadle and Williams may be less relevant now than when they were first published. Therefore, updated degradation rates for TRWP would result in a big improvement in the accuracy of modelling. Combining this with state of the art physicochemical characterisation of TRWPs as a result of ageing will further contribute towards understanding particle transport.

The aim of this research is to characterise the effect of UV-degradation on TRWP and provide updated degradation rates for TRWPs. To achieve this, samples have been aged following an accelerated UV-ageing procedure. To test the degradation of pure tyre tread, tyres have been cryomilled, which is recognized as an efficient method to reduce the particle size of rubber (18, 21). It has also been reported that the wear process that generates TRWPs under real conditions also causes significant chemical changes in the tread rubber, with studies showing that more rubber is unvulcanised so the material absorbs oxygen much faster than tread rubber which has been cryomilled to the same particle size (14). To investigate the effects of realistic wear, we use simulated TRWP generated at a road simulator. Thermogravimetric analysis (TGA) is used to investigate the degradation rate whilst static light scattering (SLS), electron microscopy (SEM-EDX) and infrared (FTIR) are used to characterise the physicochemical changes of the particles due to UV-ageing. A later study, attached as an addendum from page 20, investigated the biodegradation of a number of these materials.

## 2 Results and Discussion

In this research, four T(R)WP samples were used and these are summarised in Table 1. Two samples of tyre wear particles (TWPs) were prepared by cryogenic milling to investigate the effects of UV degradation purely on tyre material. A mix of light duty (car/van) tyres and heavy duty (truck) tyres were compared as the former contains more synthetic rubber and the latter more natural rubber. The other two samples were prepared by running tyres on a road simulator and collecting the TRWPs generated. This allows for investigation of more realistic particles that also include road wear encrustations. TRWPs were prepared from the same mix of light duty tyres, which were from the executive market segment, as the cryomilled car TWPs in order to investigate the influence of road wear encrustations and the wear process. TRWPs were also collected from a budget segment light-duty tyre, which contains different fillers and rubber formulations. Finally, all samples were sieved to a size fraction of 50 – 200 µm to be representative of T(R)WPs that are deposited in roadside soils, the environmental compartment with the most T(R)WPs.

**Table 1.** Summary of T(R)WPs samples used in this study.

Sample Name	Preparation Method	Tyre Details
Car TWPs	Cryomilling	Mix of four new executive segment tyres
Truck TWPs	Cryomilling	Mix of shredded end-of-life truck tyres from tyre recycler
Premium TRWPs	Road Simulator	Mix of four new executive segment tyres
Budget TRWPs	Road Simulator	One new budget segment tyre

T(R)WPs were subjected to accelerated photothermal ageing (for details see methods section; Appendix 5.1) with samples taken for characterisation at four time points (0, 160, 505 and 1000 h). For converting the accelerated exposure in the UV cabinet to environmentally relevant figures, the intensity of the UV light and the temperature need to be compared with natural conditions. Since we have only done UV ageing experiments at one temperature and one UV intensity, two assumptions were made: that thermal activation increases by a factor of 2 for every 10 °C and that UV-activation increases linearly. This leads to a total acceleration factor of 24. Given 1000 h of accelerated ageing and assuming an average sunshine exposure of 8 h per day in the environment, this leads to a simulated environmental ageing time of 8.2 years. This value has been used for calculating environmental degradation rates. As this has a large degree of error, degradation rates have been provided in brackets to show a range from 5-10 environmental years.

### 2.1 Degradation Rate of T(R)WPs

Thermogravimetric Analysis (TGA) was used to investigate the degradation rate of T(R)WPs. As different components of T(R)WP will be lost at specific temperatures it is possible to determine the composition of the different samples and how this changes with ageing.

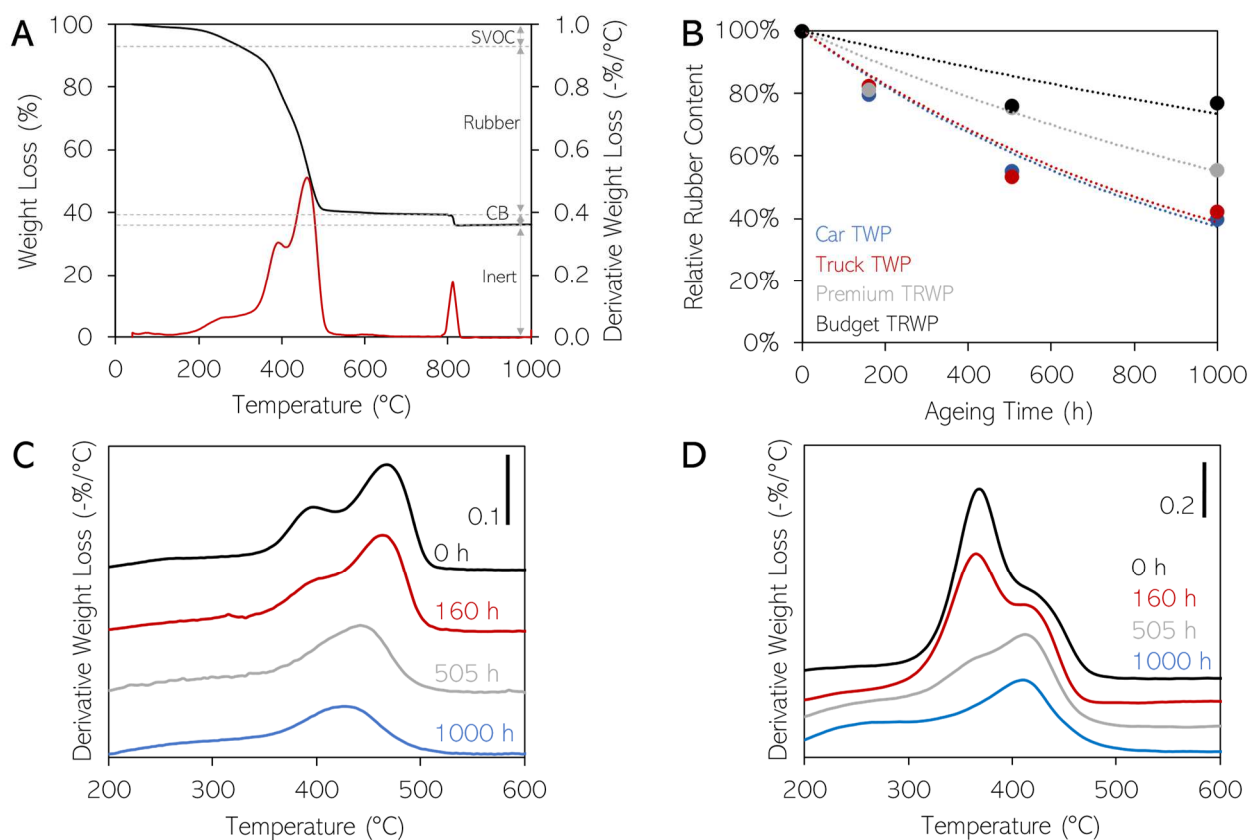
The composition of the four samples as determined by TGA is shown in **Error! Reference source not found.** The composition is expected for standard tyres, namely 40-60% rubber, 20-30% filler (SiO<sub>2</sub> or carbon black (CB)) and 12-15% oils (8). The car TWPs prepared by cryomilling have a much higher inert fraction compared to Truck TWPs. This is due to the inclusion of SiO<sub>2</sub> as a mineral filler in car tyres and for truck tyres CB is the main filler. The two TRWP samples have much higher inert fractions due to the presence of road wear particles which are mainly aluminium and silicon-rich minerals.

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**Table 2.** Composition of T(R)WP samples as determined by TGA.

	Volatiles	Rubber	Carbon Black	Inert
Car TWPs	6.5	52.8	4.1	36.6
Truck TWPs	4.6	60.8	29.0	5.6
Premium TRWPs	1.6	10.1	6.5	81.9
Budget TRWPs	1.5	7.6	5.7	85.2

Figure 1A shows the TGA trace of the car tyre sample in black with the different components annotated on the right-hand side (plots for the other samples are available in the Supplementary Information, Figure S1). There is a gradual weight loss until  $\sim 290^\circ\text{C}$ , which is due to the evaporation of semi-volatile organics present such as softeners, vulcanisation agents and antioxidants. From  $290 - 550^\circ\text{C}$  there is a sharp weight loss event due to pyrolysis of tyre rubber. At  $750^\circ\text{C}$ , the nitrogen environment is changed to air and this leads to a weight loss event at  $\sim 800^\circ\text{C}$  due to the oxidation of carbon black. The remaining weight is referred to as inert material or ash and this is mainly due to  $\text{SiO}_2$  filler.



**Figure 1.** A. TGA (black) and DTG (red) plots of the Car TWPs sample before ageing; B. Rubber concentration of the four T(R)WP samples as a function of ageing time; C. DTG peaks of rubber for the Car TWPs sample at the four ageing times; and D. DTG peaks of rubber for the Truck TWPs sample at the four ageing times.

The red line in Figure 1A shows the differential of the TGA trace (DTG) curve (black line). Using the differential, small changes in composition are elucidated. For example, the weight loss between  $290 - 550^\circ\text{C}$  due to rubber is shown to actually contain two events centred  $\sim 390^\circ\text{C}$  and  $\sim 480^\circ\text{C}$ . Previous work has shown that different types of rubber pyrolyse at different temperatures and these two peaks correspond to natural rubber (NR) and synthetic rubber (SBR+BR), respectively (22).

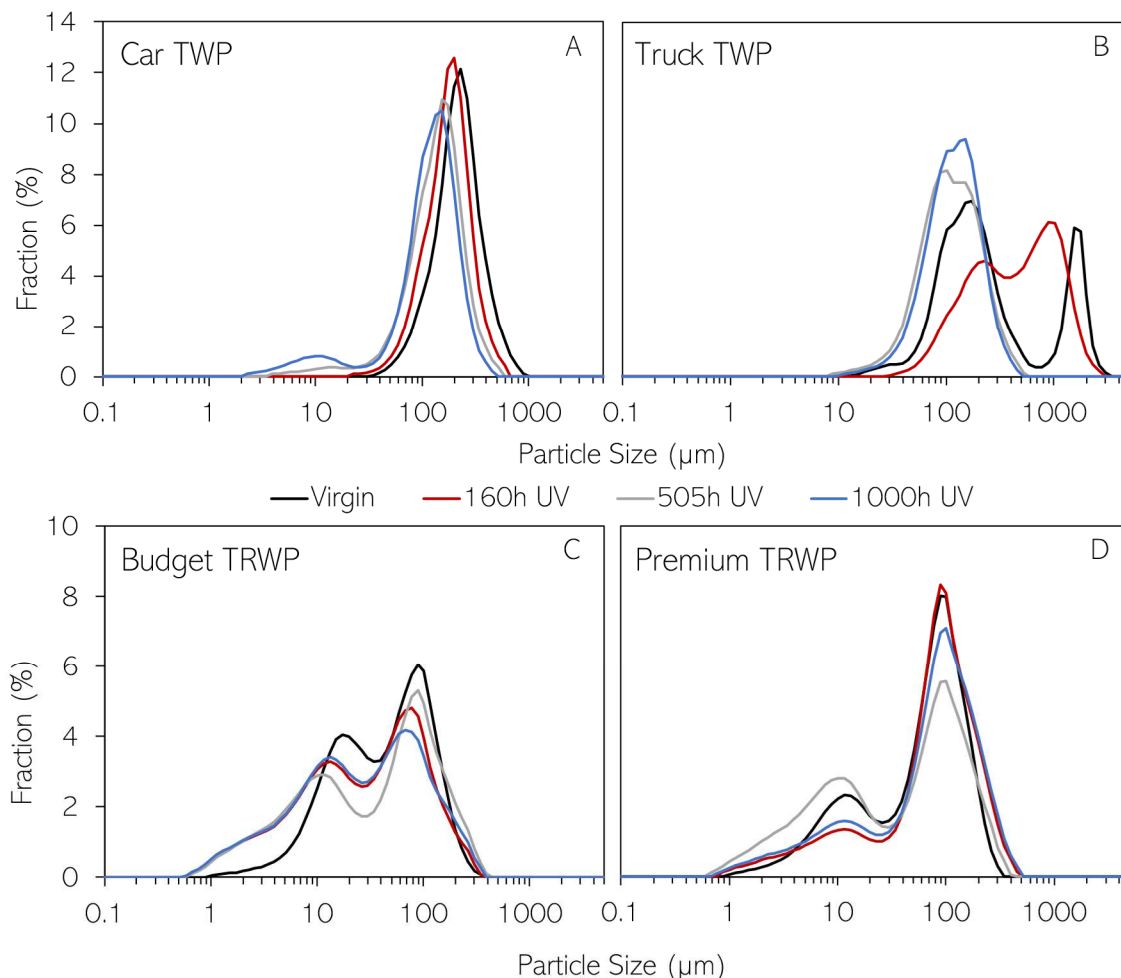
The rubber degradation of the four different samples is shown Figure 1B. The cryomilled car tyres and truck tyres appear to very similar degradation rates of 0.033 (0.027-0.054) and 0.032 (0.026-0.052) day<sup>-1</sup>, respectively. The samples from the road simulator had a much slower degradation rate. This is contrary to previous work that suggests that particles formed through wear absorb oxygen faster. The premium TRWPs which are the same tyres as the car TWP sample but run on the road simulator, had a degradation rate of 0.020 (0.016 – 0.032) day<sup>-1</sup>. Finally, the budget TRWPs show the slowest degradation of 0.011 (0.009 – 0.017) day<sup>-1</sup>. Cadle and Williams studied unaccelerated environmental degradation by exposing TWPs and TRWPs in soil and glass beads for a period of 16 months. The TWPs and TRWPs in glass beads are assumed to be representative of unaccelerated abiotic degradation and may be compared with the Car TWP and two TRWPs in this study. The TWP in the previous study showed no degradation, whilst in this study they show the fastest. TRWPs in glass showed a degradation rate of 0.09 day<sup>-1</sup> which is approximately 5-10x faster than the TRWP samples in this study. Cadle and Williams also showed that degradation in soil was roughly twice as fast (0.15 day<sup>-1</sup>) highlighting the important role biodegradation plays in the environmental fate of T(R)WPs.

The degradation rate is likely influenced by the type of rubber present in the tyres. As mentioned previously, using the DTG plot we can identify both natural and synthetic rubber. In Figure 1C, the rubber region of the DTG plot for cryomilled car tyres is shown with increasing ageing time. The fresh sample shows two peaks, *ca.* 390 °C and *ca.* 480 °C which have been shown to be due to NR and SBR+BR, respectively. It appears that the two types of rubber degrade at different rates, with the peak due to NR becoming indistinguishable after 500 h ageing. There also appears to be a shift of the synthetic rubber peak to lower temperatures with increased ageing, with the peak being centred around 430 °C after 1000 h ageing. This may be due to a chemical change in rubber occurring during ageing, such as devulcanization, that lowers the degradation temperature of the rubber. The aged truck tyres also show two peaks, as shown in Figure D, this time *ca.* 390 °C and *ca.* 435 °C. The shifted degradation temperature of the synthetic rubber with respect to that in the car tyres may be due to a different type or ratio of synthetic rubber(s) or, as these are end-of-life tyres, a different degree of vulcanisation. It is evident from the DTG that truck tyres contain a much larger proportion of natural rubber than synthetic rubber and this is again seen to degrade much quicker than the synthetic rubber.

## 2.2 Effect of Ageing on T(R)WP Size

It is also important to understand what influence degradation has on the physicochemical properties of T(R)WPs. This is of importance as a change in particle size will lead to different environmental transport behaviour, such as sedimentation rate in water and (re)suspension in air affecting long-range transport. It is also expected that smaller particles will be easier taken up by biota and present a potentially higher health effect. Static light scattering (SLS) was used to investigate changes in particle size with ageing and these are shown in Figure 2.





**Figure 2.** Volume distribution measured with Static Light Scattering (SLS) from **A.** Car TWP, **B.** Truck TWP, **C.** Budget TRWP and **D.** Premium TRWP. Each graph shows four lines that correspond to different ageing times: virgin (black), 160 h (red), 505 h (grey) and 1000 h (blue).

The modal particle size of the virgin car tire sample (Figure 2A) lies around 200 µm which is representative of the coarser TRWPs found in roadside soils. When looking at the samples that have been aged, the modal size of the TWPs clearly shifts to lower particle size with longer UV-exposure. From this we can calculate a particle size reduction rate of  $-0.03$  ( $0.02 - 0.05$ )  $\mu\text{m day}^{-1}$ . A peak is also seen emerging around 10 µm and this peak intensifies as the sample has been aged longer. This bimodal distribution suggests that both surface degradation and fragmentation play a role.

The modal particle size of the truck tire sample (B) is similar to the one of the car tire sample, around 175 µm. However, the virgin sample also seems to have a part of the fraction with particle size larger than 1 mm. This could indicate the formation of agglomerates in the sample, even though a surfactant was added before analysis to prevent this. The “stickiness” of natural rubber containing truck tyres has previously been reported as a limiting factor in the particle size analysis of truck tyre cryogrinds (18). The sample that has been aged for 160 hours also shows a bimodal distribution, however, the 505 and 1000 h samples have a monomodal distribution. This is in line with the differential thermogravimetry (DTG) data presented earlier which showed that NR was almost completely degraded after 505 h ageing and the sample is predominantly synthetic rubber. It could also be that the surface of the particles changes such that agglomerates are less likely to form after ageing by UV-light, for example through formation of carbonyl groups increasing hydrophilicity. Furthermore, after ageing more than 500 hours, the tail of the peak starts to shift to lower particle sizes, which indicates the formation of more particles around 10 µm.

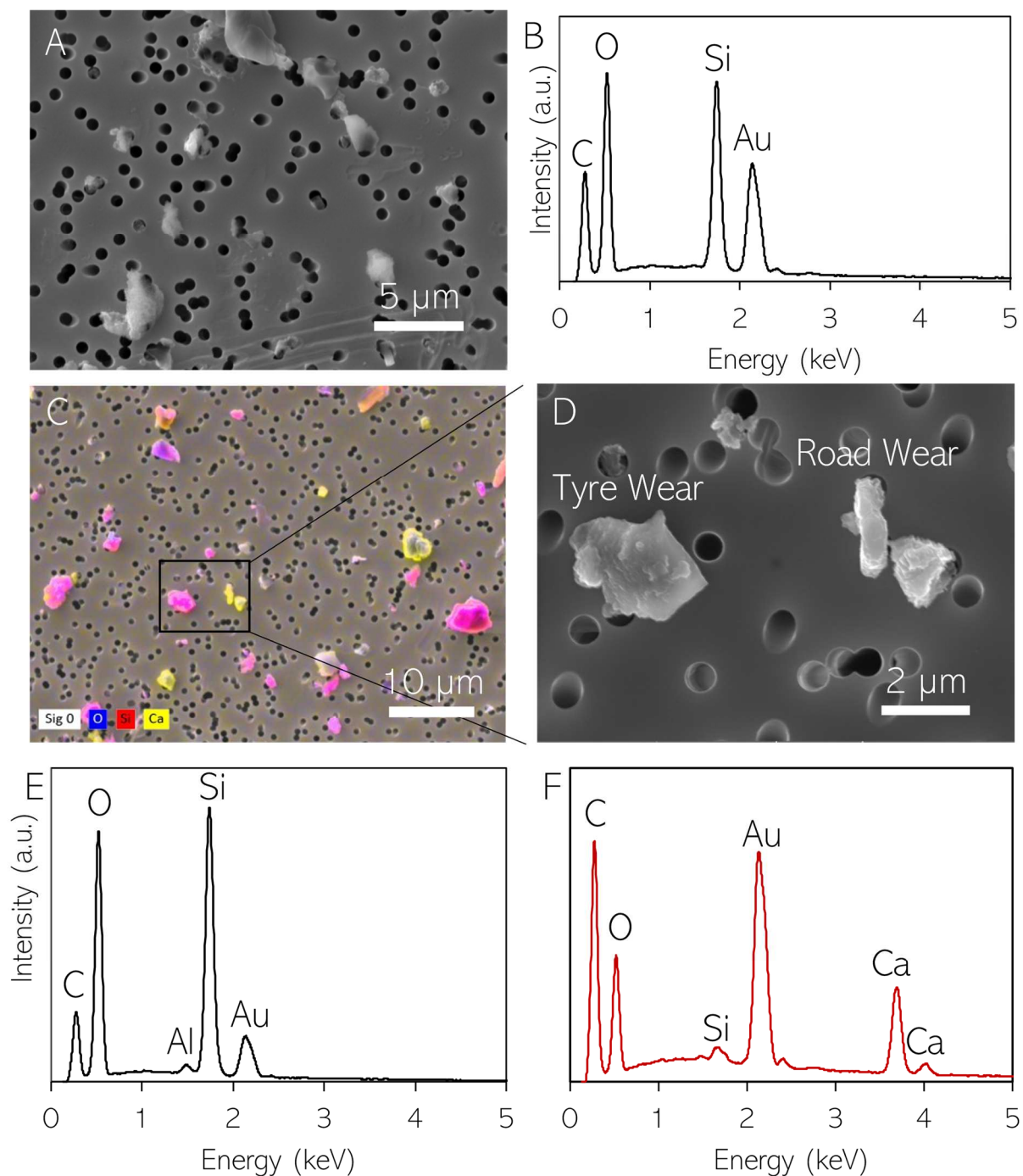
A difference between the milled TWP distributions and the budget (C) and premium (D) TRWP distributions, is the bimodal distribution of the unaged TRWP. Since the smaller particles are not present in the TWP samples, this could represent loose road wear in the sample. Whilst the samples were fractionated to between 53 – 200  $\mu\text{m}$  during preparation, ultrasonic treatment during dispersion for SLS measurement could liberate loosely bound road wear encrustations from the TRWPs. The budget TRWP sample (C) shows a shift of the distribution towards smaller particle sizes. The fraction of particles *ca.* 100  $\mu\text{m}$  is decreasing with longer degradation times, whilst the fraction of particles smaller than 10  $\mu\text{m}$  is increasing. Different from the milled tire samples, nanoparticles <1  $\mu\text{m}$  are also formed with longer UV exposure time. This increase in particles <1  $\mu\text{m}$  is also clearly visible in the premium TRWP sample (D), however, these distributions do not show a clear shift like the other samples.

## 2.3 Morphology and Elemental Composition of (Aged) T(R)WPs

In the SLS results we see in many samples the size fraction <10  $\mu\text{m}$  growing. For the car tire sample we can say with certainty that these particles originate from tire material, however for the TRWP samples it is not possible to say whether this fraction is due to rubber, road wear, or both. With SEM-EDX we can investigate both the morphology and elemental composition of individual particles, which may shed more light on this important smaller fraction.

In **Error! Reference source not found.**A particles from <10  $\mu\text{m}$  in the 1000 h aged car TWP sample are shown, together with their EDX spectrum in **Error! Reference source not found.**B. The SEM analysis confirms the SLS results that there are a considerable number of particles <10  $\mu\text{m}$  present in the sample. EDX analysis shows that these particles consist of a mixture of carbon (C), oxygen (O) and silicon (Si). The two Au peaks present are due to the gold-coated filters used to prepare the samples.

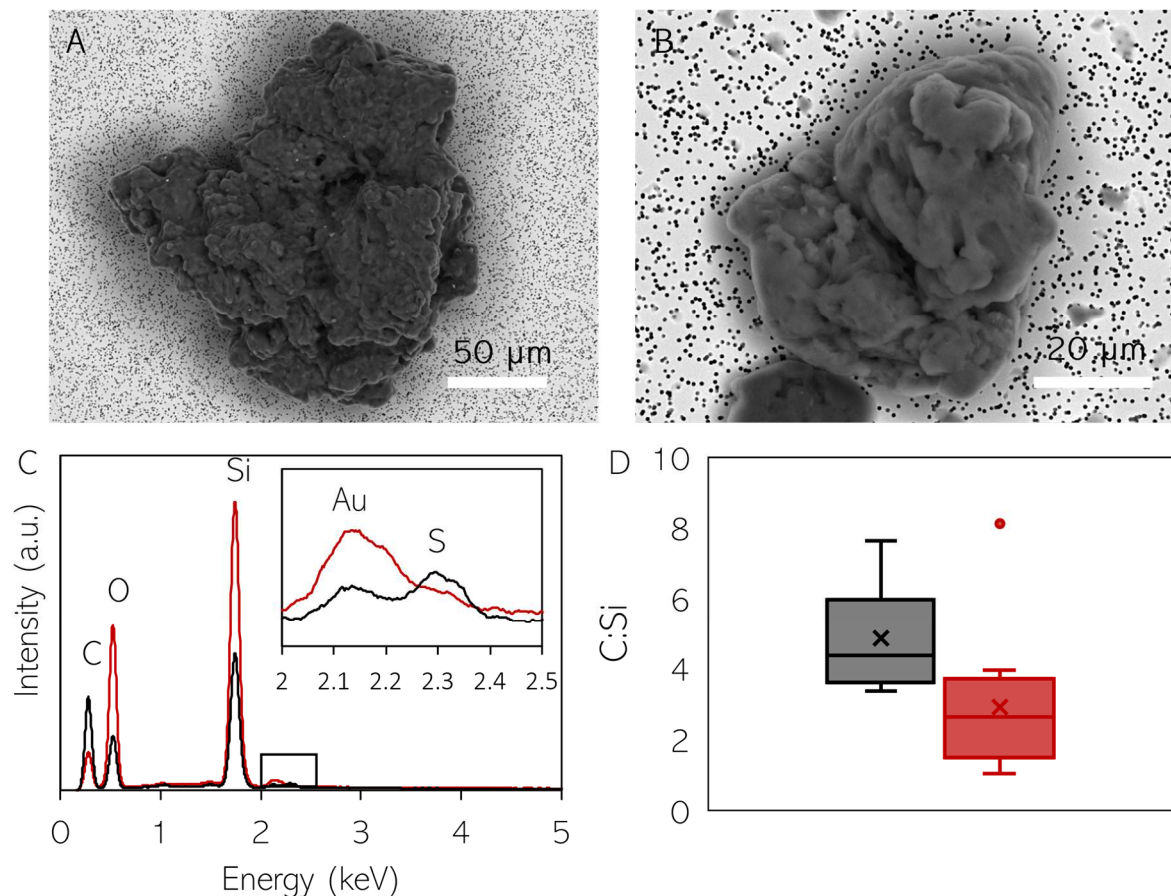
The SLS results show that the TRWP samples from the road simulator also contain particles <10  $\mu\text{m}$  which is again confirmed by SEM analysis. However, not all of these particles have the same elemental composition. **Error! Reference source not found.**C presents an elemental mapping of the particles in the premium TRWP sample after 1000 h ageing and it can be seen that this sample contains two types of particles <10  $\mu\text{m}$ : calcium rich particles (yellow) and particles without calcium (pink). A higher magnification image of a couple of these particles is shown in **Error! Reference source not found.**D. Here, the suspected tyre wear particle shows a similar morphology to the particles shown in **Error! Reference source not found.**A, whereas the road wear particles have a slightly rougher edge. EDX spectra of these particles are shown in **Error! Reference source not found.**E+F and here we also see that the suspected tyre wear particle has a similar elemental composition to that presented in **Error! Reference source not found.**B, namely C, O and Si. The presence of small amounts of Al may be due to contamination from the road surface of the simulator. The suspected road wear particles again show a high concentration of Ca which is to be expected from the road simulator which uses a cement running track. The presence of these two differing particle types and the similarities in both elemental composition and morphology of one of them with TWP material suggests that nanosized T(R)WPs could be formed through environmental degradation of larger particles.



**Figure 3.** A. SEM image of particles <10  $\mu\text{m}$  after 1000 h ageing of car TWPs; B. EDX spectrum of small car TWP particles. C. Elemental mapping of particles <10  $\mu\text{m}$  in the 1000 h aged Premium TRWP sample; D. SEM image of suspected tyre wear and road wear particles indicated by the box in A; E. EDX spectrum of the suspected tyre wear particle; F. EDX spectrum of the suspected road wear particle.

Next to the analysis of particles <10  $\mu\text{m}$ , SEM can also give an insight into the morphology and composition of the larger particles. When using the back scattered electron (BSE) detector, information on the composition of the particles is gained, with heavier elements appearing brighter in the image. Figure 4A+B show representative examples of a car TWP that has not been aged and a particle that has been aged for 1000 h, respectively. Looking at the morphology of the particles, it is noticeable that the aged particle is smoother than the virgin particle. This is also the case for the truck tire sample (Figure S2, Annex 5.3). When comparing the particles there is a clear difference in the

colour of the particles in the BSE images. The virgin particle is darker which means it contains more lighter atoms than the aged particle. This is also observed in the EDX spectra of the two particles, shown in Figure 4C. The virgin particle contains more carbon (C) than the aged particle, whereas the concentration of oxygen (O) and silicon (Si) are much higher in the degraded particle. The inset of Figure 4C also shows the area of the spectrum with S. S is present in low concentrations due to its use as a vulcanising agent and it remains present in cross-linking bonds of vulcanised rubber. The S seems to be present at much lower concentrations, if at all, in the 1000 h aged sample. This could suggest that UV ageing degrades cross-linkages in the rubber leading to devulcanization. This could explain the observations in Figure 1D where a shift in the decomposition temperature of synthetic rubber is observed. The C:Si ratio of fresh and 1000 h aged samples is further explored in Figure 4D.



**Figure 4.** A. BSE image of a virgin car TWP; B. BSE image of a car TWP after 1000 h ageing; C. EDX spectra of the virgin (black) and aged (red) car TWP particles; D. Box plot of C:Si ratio of virgin (black) and aged (red) car TWP particles determined using EDX of 10 particles from each sample.

## 3 Conclusions

Cryomilled tyre tread and TRWPs from a road simulator were subjected to accelerated UV-ageing to simulate abiotic environmental ageing. TGA analysis of the tyre composition as a function of ageing time showed that the average degradation rate was  $0.07 \text{ h}^{-1}$ , which when corrected for accelerated ageing corresponds to an environmental degradation rate of  $0.03$  ( $0.02 - 0.04$ )  $\text{day}^{-1}$ . Natural rubber was seen to degrade quicker than synthetic rubber with the consequence that heavy duty tyres, which often contain a higher percentage natural rubber, degrade quicker than light duty tyres. Particle size was also seen to be reduced during degradation with an environmental equivalent rate of  $0.03 \text{ µm}$  ( $0.02 - 0.05 \text{ µm}$ )  $\text{day}^{-1}$ . A fraction of small particles  $<10 \text{ µm}$  was also formed which was confirmed with

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SEM-EDX to occur for both the tyre tread and TRWP samples. This suggests that nanosized tyre wear particles may form in the environment due to degradation. The influence of biodegradation on these particles is investigated in an addendum to this deliverable from page 20.

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## 5 Appendix

### 5.1 Experimental methods and materials

#### *Materials*

Tween-20 (Sigma, Lot # SLCC6187) was used as received without further purification. All water is used was MilliQ filtered using a Millipore 0.22 µm filter. New light duty tyres were purchased from BandenConcurrent. Shredded end-of-life heavy duty tyres were provided by Rumal Tyre Recycling.

#### *Accelerated UV-Ageing*

The accelerated UV degradation was performed in an Atlas Suntester XXL, with three Xenon lamps (air cooled), a light intensity of 60 W/m<sup>2</sup> between 300 and 400 nm, relative humidity of 45% and a chamber temperature of 60 °C and a black standard temperature of 81 °C. The samples were sprayed every 12 hours for 1 minute with demi-water to remove any leachates from the rubbers, and thus mimic outdoor conditions. This sprayed water evaporated within one hour. Samples were taken after 160, 505 and 1000 h of ageing.

#### *Milling*

Small sections of tyre tread were removed from the running surface of the light duty tyres using cutting pliers. The shredded end-of-life truck tyres were used as received and may contain both running surface and sidewall rubber. These rubber chunks were then milled in a Ruhromag Retsch ZM-1 centrifugal mill with a 250 µm screen. Milling was performed under LN<sub>2</sub>.

#### *Fractionation*

Sieving was performed in water with a drop of Tween-20 using stainless steel sieves of 53 and 200 µm. The fraction between 53-200 µm was collected and dried at 60 °C.

#### *Static Light Scattering (SLS)*

SLS was performed using a Horiba LA-960S2 in a 10 ml fraction cell equipped with a magnetic stirrer to ensure particles maintain a well-dispersed suspension. Samples were measured in a 0.05% Tween-20 in MilliQ solution to reduce agglomeration and particle-cell interactions. Particle size distributions were calculated from the scattering results using a refractive index of 2.94-0.001 (23, 24).

#### *Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy*

SEM-EDX analyses were performed with a Tescan MAIA III Triglav field emission scanning electron microscope, equipped with Bruker XFlash Quantax 30 mm<sup>2</sup> silicon drift detectors for energy dispersive X-ray spectroscopy. SEM images were recorded in the secondary electron (SE) and backscattered electron (BSE) modes between 5 – 15 kV.

#### *Thermogravimetric Analysis (TGA)*

TGA was performed using a Mettler Toledo TGA 2 system following the method used by Son and Choi (3). The experiments were performed under nitrogen with a heating rate of 20 °C/min from 40 °C up to 750 °C before switching to air and continuing heating until 1000 °C. The mass loss with time and temperature is monitored.



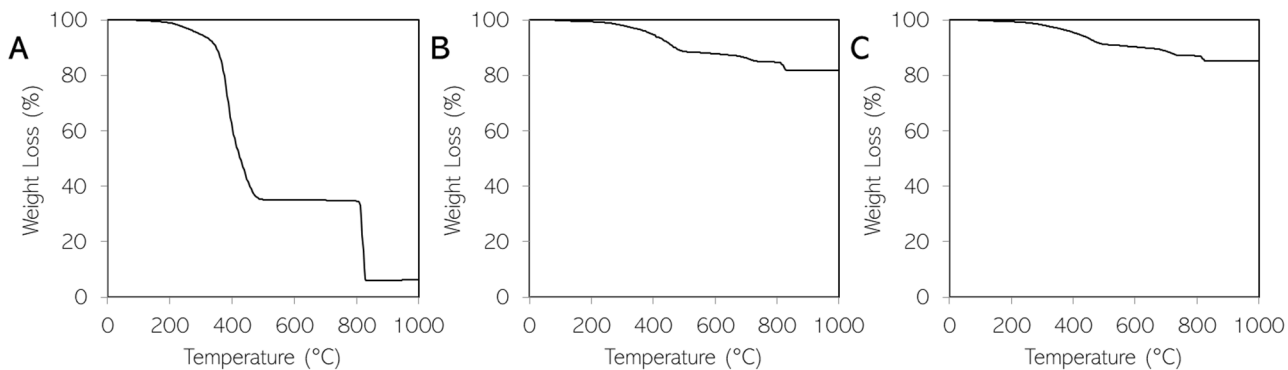
## 5.2 Supplementary information TGA

**Table S1.** Triplicate measurement virgin car tires

Sample Unit	T [°C]	Run 1 [%]	Run 2 [%]	Run 3 [%]	Average [%]	St. Dev
SVOC	40-290	6.3	7.0	6.2	6.5	0.4
Pyrolysis	290-550	53.4	53.1	51.9	52.8	0.8
EC	550-1000	4.1	4.3	4.0	4.1	0.2
Inert		36.2	35.6	38.0	36.6	1.3

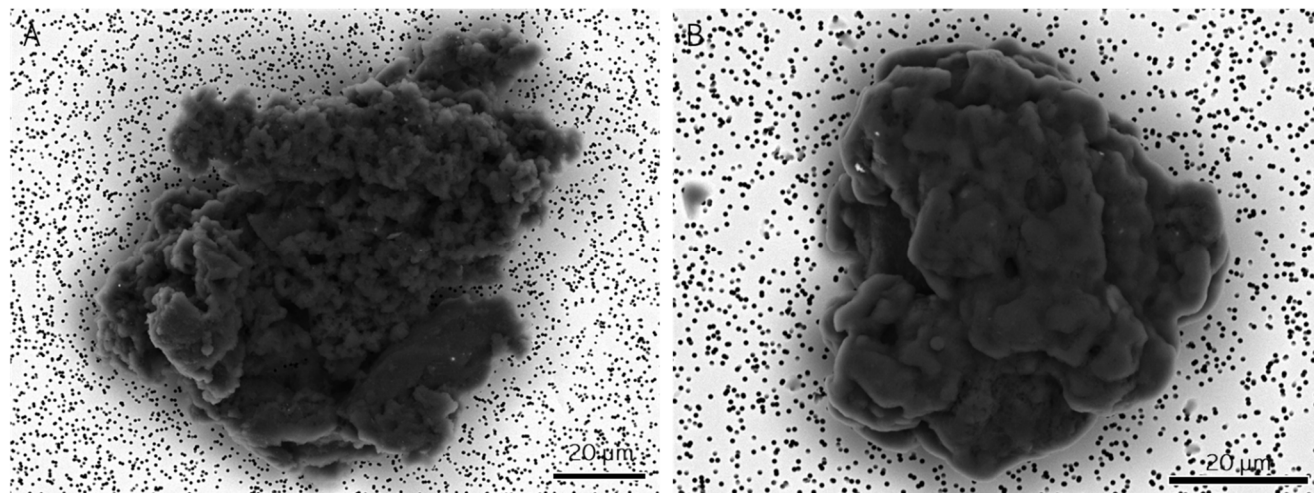
**Table S2.** Triplicate measurement virgin truck tires

Sample Unit	T [°C]	Run 1 [%]	Run 2 [%]	Run 3 [%]	Average [%]	St. Dev
SVOC	40-290	4.6	4.6	4.5	4.6	0.0
Pyrolysis	290-550	60.4	61.6	60.4	60.8	0.7
EC	550-1000	28.8	29.4	28.7	29.0	0.4
Inert		6.2	4.4	6.4	5.6	1.1



**Figure S1.** TGA traces of virgin **A.** Truck TWPs; **B.** Premium TRWPs; and **C.** Budget TRWPs.

### 5.3 Supplementary information SEM



**Figure S2.** A. BSE image of a virgin truck TWP; B. BSE image of a truck TWP after 1000 h ageing.

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*Low particle Emissions and IOw Noise Tyres*



Deliverable No.	D3.3 Addendum	
Deliverable Title	Addendum: Biodegradation and analysis of tyre wear particles	
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3	23/10/2024	Second TNO internal review	Jorien Strijk (TNO)

## Abbreviations

<b>4PCH</b>	4-phenylcyclohexene
<b>BOD</b>	Biological oxygen demand
<b>COD</b>	Chemical oxygen demand
<b>DP</b>	Dipentene
<b>DTG</b>	Differential thermogravimetry
<b>ISO</b>	International organisation for standardisation
<b>MRM</b>	Multiple reaction monitoring
<b>NR</b>	Natural rubber
<b>OECD</b>	Organisation for Economic Co-operation and Development
<b>SBR</b>	Styrene butadiene rubber
<b>T(R)WP</b>	Tyre (and road) wear particle
<b>TDU</b>	Thermal desorption unit
<b>TED-GCMS</b>	Thermal extraction and desorption gas chromatography mass spectrometry
<b>TGA</b>	Thermogravimetric analysis
<b>ThOD</b>	Theoretical oxygen demand
<b>UV</b>	Ultraviolet

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## 1 – Introduction

Understanding the degradation of tyre and road wear particles (TRWPs) in the environment, a complex interplay between abiotic processes such as UV and heat degradation and biotic degradation, is of great importance. Degradation plays a large role in the accumulation, fate and impact of these particles in the environment. In *Deliverable 3.3: UV-degradation and analysis of tyre wear particles*, we investigated the abiotic degradation of TRWPs through accelerated UV-ageing of car and truck tyres generated through cryomilling and road simulation tests. The average abiotic degradation rate for these particles was  $0.035 \text{ day}^{-1}$ (1). This was *ca.* 3x slower than the environmental degradation rate determined by Cadle and Williams in 1980, the most commonly used degradation rate in recent fate modelling studies(2). Some, or all, of this difference may be due to the biotic degradation of TRWPs.

Recent work has shown that biodegradation can occur in relatively short periods of time. Nielson *et al.* showed in 28 day tests with activated sludge inoculum that biodegradability of tyre tread particles was between 4.5 and 8.0%, dependent on rubber type and state of ageing, with UV-aged and natural rubber being more biodegradable(3). Klun *et al.* have shown that in freshwater environments biodegradability of ground tyre material reached a maximum of 3.8% after 39 days. Interestingly, this work also demonstrated the formation of a biofilm contributing up to 4.5 wt% of the sample mass which is much lower than biofilm formation of other microplastics. The authors observed leaching of both organic compounds and Zn and suggested that this may be inhibiting biofilm formation(4). Nielson *et al.* suggest in their work that removal of these toxic components via UV-ageing may be a reason for the subsequent increase in biodegradability(3).

This addendum to *Deliverable 3.3*, entitled *Biodegradation and analysis of tyre wear particles* focusses on the biodegradation of the materials used and generated in the main work. Following the experimental approach of the previously discussed papers, namely the oxygen demand during a 28 day incubation period, the cryomilled car and truck TWP samples from D3.3 were tested for biodegradability. This set of eight samples allows for in-depth investigation of both the rubber composition and effect of UV-ageing on biodegradability. Next to the oxygen demand measurements, thermogravimetric analysis (TGA) was used to confirm the extend of rubber degradation and thermal extraction and desorption gas chromatography mass spectrometry (TED-GCMS) was used to further quantify the different types of rubber in the samples. The results of this biotic degradation study will be combined with those of the previous abiotic degradation study in deliverable 3.5 to provide a better understanding of the environmental fate of TRWPs in soil and lead to up-to-date environmental degradation rates that may be used in subsequent fate modelling studies.

## 2 – Materials and methods

### 2.1 – Testing Materials

The test materials used for these studies are those used or generated through UV-ageing in *Deliverable 3.3: UV-degradation and analysis of tyre wear particles* and are summarised in Table 3. For further details on the production and ageing of these materials the reader is referred to Deliverable 3.3. These materials were chosen to investigate both the influence of rubber type (truck TWP contains mainly natural rubber and car TWP contains mainly synthetic rubber) and state of UV degradation on the biodegradability of tyre wear.

Table 3: Tyre wear materials used for biodegradability testing.

Sample Name	Tyre description	UV-ageing time	Biodegradation Test Round
CarTWP_0h	Mix of four new executive segment tyres	0 h	1
CarTWP_160h	Mix of four new executive segment tyres	160 h	1
CarTWP_505h	Mix of four new executive segment tyres	505 h	2
CarTWP_1000h	Mix of four new executive segment tyres	1000 h	2
TruckTWP_0h	End-of-life truck tyres from recycler	0 h	1
TruckTWP_160h	End-of-life truck tyres from recycler	160 h	1
TruckTWP_505h	End-of-life truck tyres from recycler	505 h	2
TruckTWP_1000h	End-of-life truck tyres from recycler	1000 h	2

### 2.2 – Biodegradation

The biodegradation assessment was carried out according to ISO 14851:2019 as respirometric tests with OxiTop® instrumentation, employing manometric monitoring of microbial respiration (biological oxygen demand, BOD) as a measure of substrate biodegradation. The measurement principle behind the Oxitop® instrumentation is based on pressure changes in a closed system as microorganisms consume oxygen. Each sample, containing microorganisms (i.e. activated sludge inoculum) is placed inside a sealed reaction bottle. This bottle also contains the test substance (i.e. the car tire particles or positive control). As the microorganisms metabolize the organic material in the sample, they consume oxygen from the air inside the sealed vessel, while producing CO<sub>2</sub> as a result of this reaction. The test bottles are fitted with a rubber stopper beneath the bottle lid. A CO<sub>2</sub> absorber, typically a sodium hydroxide (NaOH) pellet, is placed inside the rubber stopper. As oxygen is consumed and CO<sub>2</sub> is absorbed, the resulting pressure change inside the bottle is measured. This change in pressure is continuously logged by the device's measurement head and correlated to the amount of oxygen consumed i.e. BOD. The biodegradation evaluation is then based on the determined BOD relative to the theoretical oxygen demand (ThOD) of the car tire particles. According to ISO 14851:2019, the ThOD of a polymer test material can be calculated based on the chemical structure, if the elemental composition is known or can be determined by elemental analysis. This is not the case for the different car tire particles due to their complex composition and large content of additives. An alternative option is to estimate ThOD based on chemical oxygen demand (COD) measurements. Previous COD measurements for car tire particles have calculated values of 1.2 and 1.67 g<sub>COD</sub>/g<sub>TWP</sub>(3, 4). For the purpose of subsequent calculations, a value of 1.67 g<sub>COD</sub>/g<sub>TWP</sub> was used as this was calculated in previous work by this group(3). Individual measurements of COD for each sample could be carried out to refine the results further, however, this was not feasible within the timeframe of the current investigation.

The tests were conducted in 510 mL amber glass bottles, each bottle containing 365 mL of test media and 36.5 mg of test material, achieving a test concentration of 100 mg/L. The test media, in the first round, consisted of a 362 mL OECD mineral medium with an addition of 3.00 mL inoculum. In the second round, the test media consisted of a 363 mL OECD mineral medium with an addition of 2.45 mL inoculum. The microbial inoculum added corresponded to a total suspended solids (TSS) concentration of 25.0 mg TSS/L. This inoculum comprised activated sludge collected



from an aerated tank at the Mølleåværket wastewater treatment plant (WWTP) in Lundtofte. Subsequently, the inoculum underwent aeration in the laboratory until the experiment began three days after collection. Test bottles were sealed with rubber tops containing one sodium hydroxide (NaOH) pellet (for trapping microbially generated carbon dioxide (CO<sub>2</sub>)) and OxiTop lids. Bottles were incubated at 20 ± 2 °C and stirred on magnetic stirrers at 180 to 450 rpm. The BOD was periodically measured as a pressure decrease throughout the incubation period of 28 days.

After 28 days of incubation, 5 mg C/L sodium acetate (NaOAc) was added to all test bottles, to verify that the test materials did not have an inhibitory effect on the microbial activity. This inhibition test had a duration of approximately ten days. All experiments were carried out in triplicates. The experiments included blank samples (only containing test media) and positive controls (containing test media and NaOAc). The average BOD of the triplicate inoculum blanks was subtracted from the BOD values obtained in each test bottle to calculate the BOD related to the biodegradation of the added test material. The biodegradability was expressed as an average BOD percentage of ThOD [%ThOD]. Negative values were set to zero. The final biodegradation degrees on day 28 were calculated as an average of the final three measurement points for the triplicate samples. Two rounds of experiments were performed to cover the eight test materials. The first round included Car TWP 0h, Truck TWP 0h, Car TWP 160h, and Truck TWP 160h. The second round included Car TWP 505h, Truck TWP 505h, Car TWP 1000h and Truck TWP 1000h.

## 2.3 – Thermal Gravimetric Analysis (TGA)

To further quantify the loss of rubber due to biodegradation, all TWP samples were analysed with TGA before and after 28 days biodegradation. TGA was performed using a Mettler Toledo TGA 2 system following the method used by Son and Choi (5). The experiments were performed under nitrogen with a heating rate of 20 °C/min from 40 °C up to 750 °C before switching to air and continuing heating until 1000 °C. The mass loss with time and temperature was recorded. The composition of TWP was determined from the resulting TGA curve by calculating the weight loss between certain temperature ranges: Semi-volatile Organic Compounds (SVOC, 40 – 290 °C), Rubber (250 – 750 °C), Carbon Black (750 – 1000 °C), Inert (residual mass).

## 2.4 – Thermal Extraction and Desorption Gas Chromatography Mass Spectrometry (TED-GCMS)

All TWP samples were analysed with TED-GCMS before and after 28 days biodegradation to get more detailed information on rubber composition i.e. natural rubber (NR) vs synthetic rubber (SBR+BR) ratio. Samples were pyrolyzed with a thermogravimetric furnace TGA 2 with an auto sampler (Mettler Toledo GmbH, Germany) using 150 or 900 µL crucibles and a purge gas flow (N<sub>2</sub>) of 30 ml min<sup>-1</sup>. The following temperature program was used: 35 °C (2 min), 35-290 °C (20 °C/min), 290 °C (2 min), 290-550 °C (20 °C/min), 550 °C (2 min), 550-1000 °C (50 °C/min), 1000 °C (5 min). Between 35-290 °C the released gaseous products were discarded and only between 290-550 °C were the decomposition products sampled. Between 550-1000 °C the purge gas was synthetic air (50 mL/min) to burn residual carbon in the crucibles and to clean the TGA system.

The TGA is coupled to a thermal desorption unit (TDU 3.5, Gerstel, Germany) via a heated transfer line (250 °C), a heated coupling (130 °C) and a TAU interface that is cooled to 25 °C. Tenax TA 100 mg (Industry standard; length 3.5 in., diameter 1/4 in.) was used as solid-phase adsorbent agent. A 7000 GC/MS Triple Quad (Agilent Technologies, United States) was used to analyse the decomposition products. The GC was equipped with a cooled injection system (CIS 4, Gerstel) and a TDU 2 unit (Gerstel). The sample robot for the transfer of the thermal desorption tubes from the TGA to the TDU was designed with a MultiPurpose Sampler (MPS, Gerstel). The thermal desorption was carried out in solvent vent mode with a total He flow of 25 mL/min. The sample was thermally desorbed with a temperature program of 50 to 280 °C at a heating rate of 60 °C/min and hold time at 280 °C for 10 min. The compounds were trapped on Tenax TA in the CIS at -25°C, using a CCD2 system (Gerstel). For the chromatographic separation, a HP-5MS column (30 m, 0.25 mm i.d., df = 0.25 µm) with a flow rate of 1 mL/min He was used. The temperature program

### D3.3 Degradation and analysis of tyre and road wear particles (TRWP) - PU

for the GC was: 40 °C (2 min), 40-300 °C (10 °C/min), 300 °C (2 min). A temperature of 300 °C for the MS source was selected. The MS was used in MRM mode. DP and 4PCH were used as markers for qualification and quantification of NR and SBR. Identification and quantification of these markers was achieved via a comparison of retention times and MRM transitions with those of an external standard mixture. The following MRM transitions were used: quantifier (m/z) 93-77 (CE 15 eV) and qualifier (m/z) 67-41 (CE 15eV) for dipentene; quantifier (m/z) 104-78 (CE 15eV) and qualifier (m/z) 104 – 51 (CE 50eV) for 4PCH.

## 3 – Results

### 3.1 – Biodegradation Monitoring through Oxygen Demand

Biodegradability of the eight Truck and Car TWP samples are shown in Figure 1A and B, respectively. In general, only the non or lightly aged samples (0 and 160 h) exhibit significant biodegradation. Truck TWP showed higher biodegradability than car TWPs, with a maximum of 8% for the 160 h aged Truck TWPs compared to 6% for the unaged Car TWPs. There is a clear tendency for increased UV weathering time to decrease biodegradability, with the only exception being between the 0 and 160 h Truck TWP samples which exhibited ~1% more biodegradability after UV-ageing. This might seem counterintuitive, as UV irradiation could be hypothesized to modify the material towards being more susceptible to microbial interactions through the removal of toxic additives. However, it could be a result of more easily degradable compounds being removed through UV degradation, making the resulting substrates less prone to microbial attacks.

In the appendix, Figure 7 also shows the results of the positive controls of each test round and the degradation of NaOAc, added on day 28. The positive controls both reached approximately 70% ThOD within the test period. More specifically, 79% ThOD and 68 % ThOD for round 1 and 2, respectively. Both tests thus reaches the ‘pass level’ for the positive control of 60% ThOD within the 28 days test period. For test run 1 (with Car and Truck TWP with 0 and 160 h UV-ageing), this pass level is achieved within a 10 day window (10 day window begins when the degree of biodegradation has reached 10 %). However, for test run 2 (with Car and Truck TWP samples with 505 and 1000 h ageing), the pass level is only achieved 12-13 days after the degradation reached 10%. The positive controls showed that lack of biodegradation was unlikely to be caused by poor microbial fitness(3). The degradation of NaOAc added on day 28 confirmed the continued microbial activity of the inoculum at the end of the degradation period, and thus that the test materials did not cause microbial inhibition.

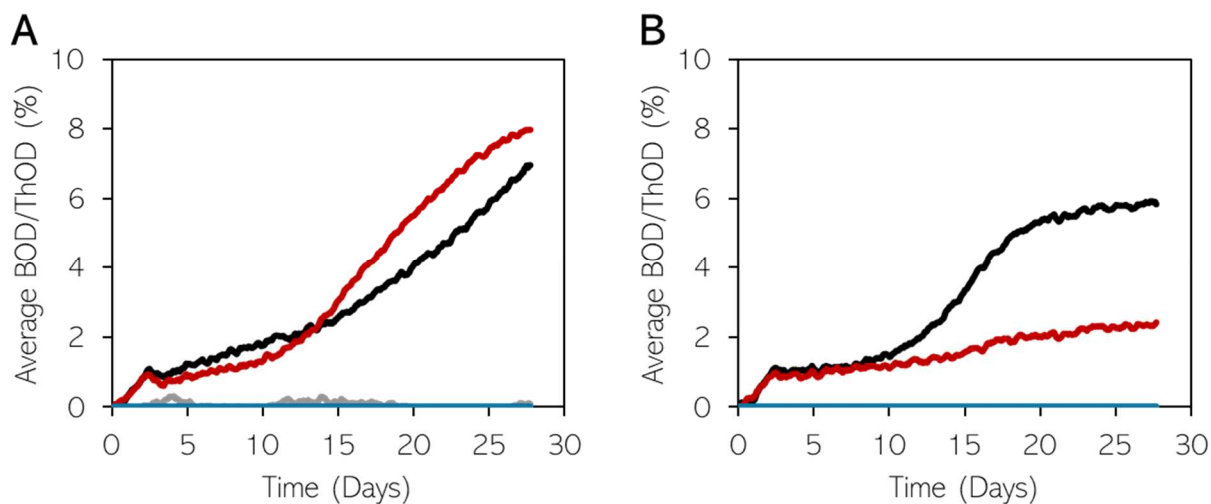


Figure 5: Biodegradability of Truck TWPs (A) and Car TWP (B) after 0 h (black), 160 h (red), 505 h (grey) and 1000 h (blue) UV-ageing. Results are the average of triplicate measurements.

### 3.2 – Influence of Rubber Composition on Biodegradation

As in the UV degradation study of deliverable 3.3, TGA was used to analyse the composition of TWP samples after biodegradation. The TGA results, presented in Table 4, are mainly in agreement with the oxygen demand measurements shown above. For both Car TWP and Truck TWP, the unaged samples (0 h) and shortly aged samples

### D3.3 Degradation and analysis of tyre and road wear particles (TRWP) - PU

(160 h) show large losses (2 – 6%) in rubber content similar to those presented in Figure 1, whilst the samples exposed to the longest UV-ageing (505 and 1000 h) show little change in rubber content. The Car and Truck TWP samples after 1000 h UV-ageing show a notable increase in rubber content after biodegradation. This may be due to the introduction of organic material with the inoculum that pyrolyses in the same temperature range as rubber but this needs to be further investigated.

**Table 4: Rubber content (%) of TWP samples before and after biodegradation as determined by TGA.**

Sample	CarTWP_0h	CarTWP_160h	CarTWP_505h	CarTWP_1000h	TruckTWP_0h	TruckTWP_160h	TruckTWP_505h	TruckTWP_1000h
<b>Rubber Before Biodeg.</b>	53.3%	48.0%	38.6%	31.5	61.0%	57.0%	44.5%	38.3%
<b>Rubber After Biodeg.</b>	48.1%	45.8%	38.9%	35.2	54.6%	52.5%	42.2%	40.8%

By taking the differential of the TGA curve (the DTG curve), detailed information about rubber composition (natural vs. synthetic) can be obtained. Figure 8 in the appendix shows the DTG curves of natural rubber (NR), butadiene rubber (BR) and styrene butadiene rubber (SBR). In all samples two peaks can be seen, a low temperature peak *ca.* 380 °C which is mainly due to natural rubber (NR) with small contributions from SBR+BR and a high temperature peak *ca.* 450 °C which is due to the synthetic rubbers (SBR+BR). The DTG curves of the Car and Truck 0 and 160 h samples are shown in Figure 2, as these samples showed the most biodegradation. We can see that the Truck TWP samples mainly contain NR whereas the Car TWP samples mainly contain SBR+BR. In Figure 2A+B, we see that although both rubber peaks are reduced, showing biodegradation of both types of rubber, the NR peak is reduced much quicker than the SBR+BR peak. This suggests that NR is more easily and quickly biodegraded than SBR+BR, although whether this is universally true or applies only for the microbial community we have used requires further investigation. This may also be the reason that Truck TWP samples exhibit higher degradation rates than Car TWP as demonstrated through TGA and oxygen demand measurements.

This preference for NR also explains why only unaged or “lightly” aged TWP are biodegraded. In the UV-ageing investigation of D3.3, we showed that a similar effect is observed for UV-ageing, with NR being more quickly degraded than SBR+BR. For most samples, the NR was not distinguishable after 505 h of UV-ageing and thus in these samples there is less biodegradable material available, or that the available material takes longer to biodegrade than the investigated timeframe.

In the car TWP samples, both peaks are also seen to be reduced as the rubber is biodegraded, but no difference between the two is discernible. This may be due to the SBR+BR contributions to the low temperature peak. It is possible that due to the lower NR content of the Car TWP, that the low temperature peak we see here is mainly due to SBR+BR and that this degrades at the same rate as the high temperature peak.

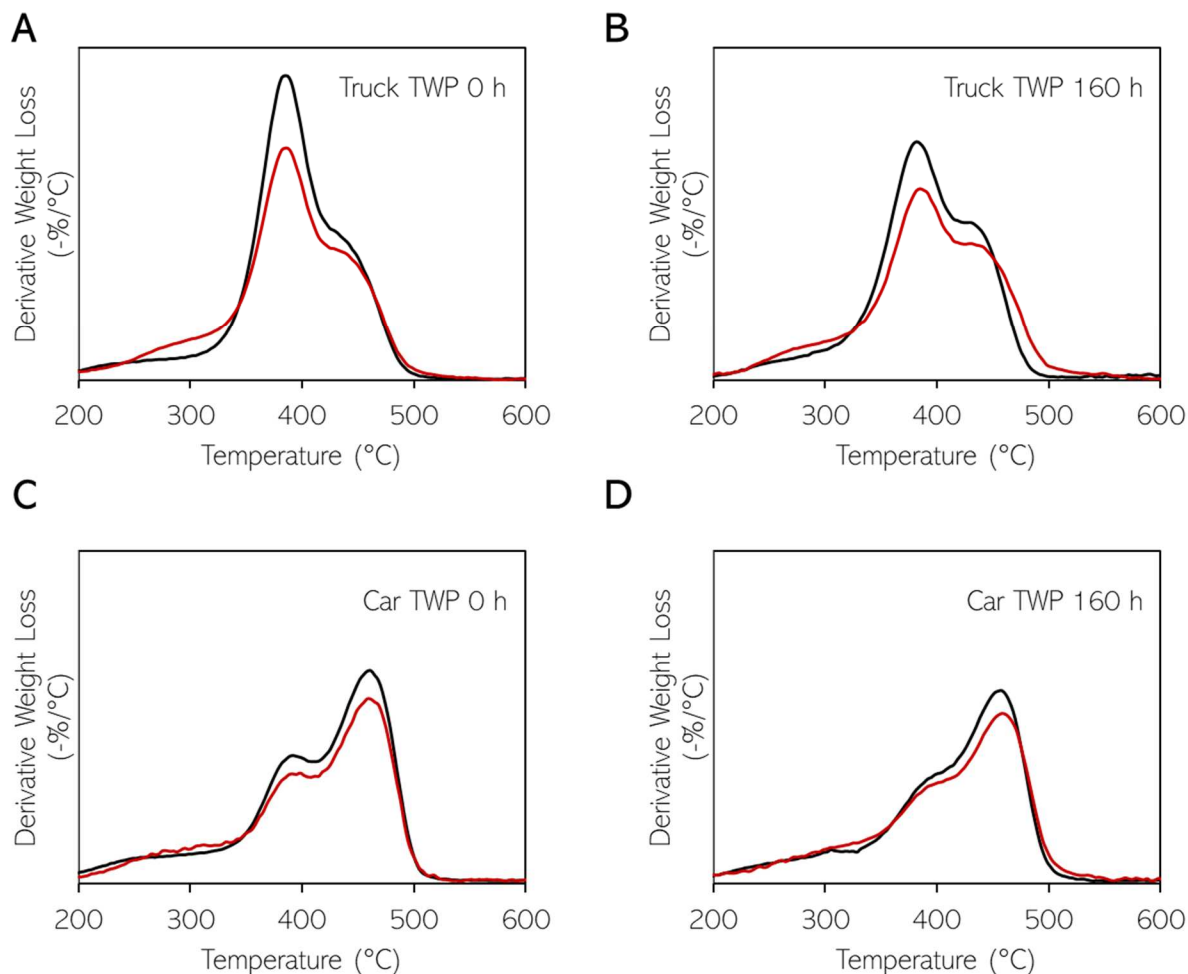


Figure 6: DTG curves for car and truck TWP before (black line) and after (red line) biodegradation.

To further elucidate the preferred biodegradability of NR vs. SBR, the two components were quantified using TED-GCMS and these results are shown in Table 5. As observed with DTG, the %NR decreases in both the Car and Truck TWP with prolonged UV exposure. We also observe that after biodegradation the %NR is reduced in all samples. This is in line with the TGA observations and further strengthens the hypothesis that NR is more easily and quickly biodegraded than SBR.

Table 5: Concentration of natural rubber as a percentage of total rubber in the samples as determined by TED-GCMS before and after biodegradation. The rest of the rubber content is due to synthetic rubber (SBR+BR).

Sample	CarTWP _0h	CarTWP _160h	CarTWP _505h	CarTWP _1000h	TruckTWP _0h	TruckTWP _160h	TruckTWP _505h	TruckTWP _1000h	
%NR in rubber fraction	Before Biodeg.	12%	11%	0.56%	0.13%	81%	84%	57%	8.5%
	After Biodeg.	8.6%	4.9%	0.38%	0.15%	65%	55%	42%	5.9%

## 4 – Discussion and Conclusions

The biodegradation of tyre wear particles was investigated using cryogenically milled tyre particles inoculated with microbes from sewage treatment plant sludge. Both car and truck tyres were used to investigate the influence of rubber type on biodegradation and the particles were subjected to varying degrees of UV-ageing before biodegradation testing to further elucidate the interplay of these two important processes that occur simultaneously in the environment.

The monitoring of biodegradation through oxygen demand showed that only non-aged or lightly UV-aged tyre wear particles showed noticeable degradation over the 28 day incubation period. Fresh truck tyres were the most biodegraded, exhibiting *ca.* 8% degradation after 28 days, whilst fresh car tyres exhibited *ca.* 6% biodegradation. UV-ageing of 160 h lowered this to *ca.* 6.5% and 2% for truck and car TWPs, respectively. TWPs that had been UV-aged for 505 and 1000 h showed negligible biodegradation. These results were confirmed through TGA of the samples before and after biodegradation. This is also in line with the previous work of Nielson *et al.* but in contrast with that of Klun *et al.*(3, 4).

DTG analysis suggested that natural rubber degrades faster than synthetic rubber due to both abiotic and biotic degradation and this was confirmed with TED-GCMS analysis of rubber composition. TED-GCMS showed that the natural rubber content of all tested samples decreased after biodegradation. This may explain why truck tyres degrade quicker, as they contain more natural rubber, and also why UV-aged particles degrade slower, as the natural rubber is also more susceptible to UV degradation. This is in line with previous work that has shown that only cis-1,4-isoprene containing rubbers such as natural and synthetic isoprene rubbers are susceptible to biodegradation and is sensitive to abiotic degradation whilst butadiene rubber is resistant to abiotic and biotic oxidation(6).

Future work should focus on how we can combine the insights learned through both the abiotic and biotic degradation parts of this deliverable into environmental degradation rates for use in fate modelling. This is challenging due to the different acceleration factors and timescales of the two degradation studies. Translating lab-scale biodegradation results into environmental rates is also especially challenging. Chamas *et al.* show in a literature study that the average accelerated and non-accelerated biodegradation rates can differ by up to an order of magnitude. They also show that the variation between different studies of non-accelerated degradation can vary up to an order of magnitude, showing that the exact microbial community and degradation conditions play a large role and any environmental value calculated from an accelerated lab-based test will have a large margin of error(7).

## Appendix

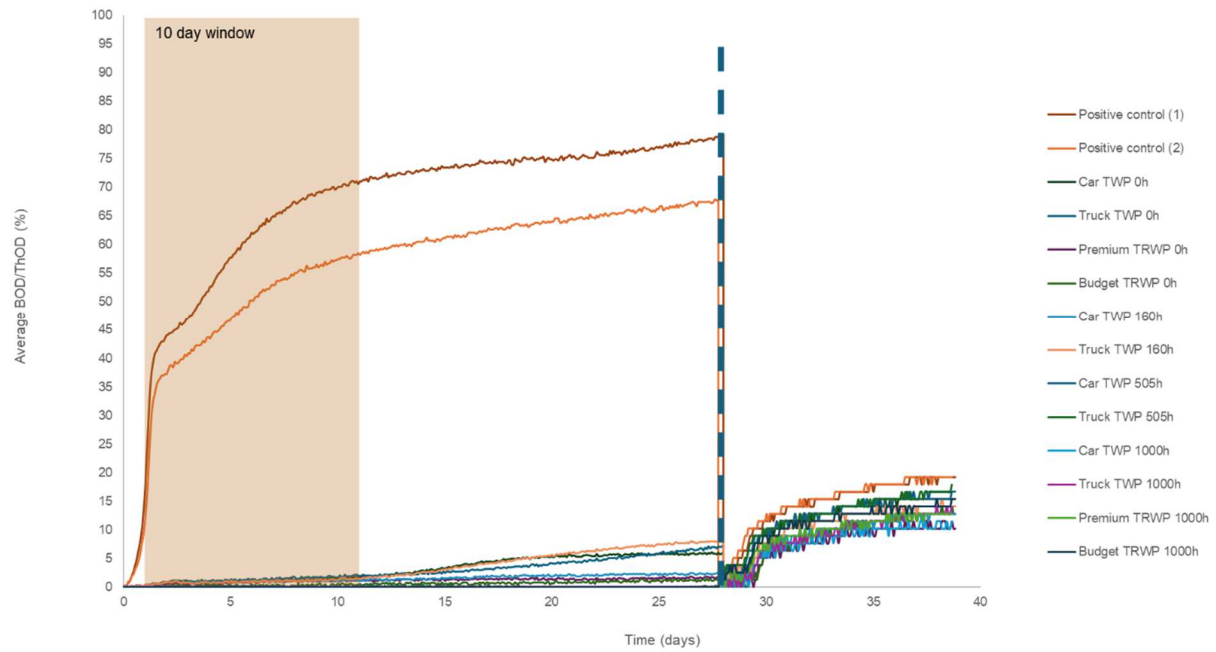


Figure 7: Biodegradability of all TWP samples and the positive control for both testing rounds. Based on triplicate samples. On day 28, the tests were restarted, and bottles were spiked with NaOAc to check for microbial activity; the dotted black line illustrates this.

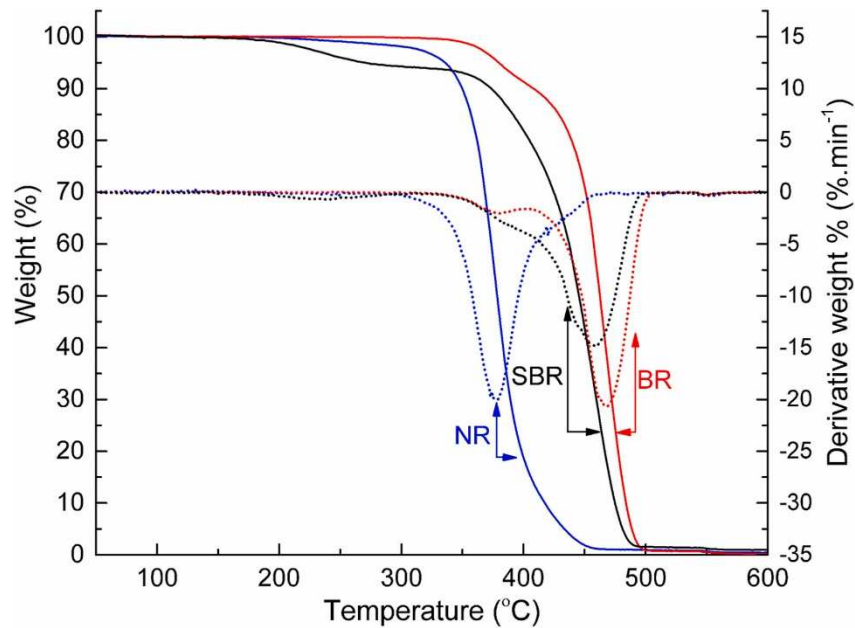


Figure 8: TGA and DTG curves for natural rubber (NR, blue), butadiene rubber (BR, red) and styrene butadiene rubber (SBR, black). Reproduced from ref (8).

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