Synthesis Report: Py-Harmony

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Synthesis Report: Py-Harmony

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Contents

Summary

This synthesis report brings together the knowledge and experience that TNO has built over many years of working with pyrolysis-based methods to study plastics. The aim is to highlight the main challenges we have seen across different projects and show how these challenges align with the key insights from the first Py-Harmony roundtable.

The main challenges identified are:

- Different laboratories use different methods, which makes results hard to compare.
- Results can be influenced by common materials in samples, such as organic material, cotton or detergents.
- There is a lack of shared standards, which limits the wider use and acceptance of data.

Conclusion:
By documenting these challenges in this synthesis report, TNO provides a knowledge base that supports the move toward harmonized methods. Aligning approaches across labs will make results more consistent and trustworthy, helping science, industry, and policymakers address the issue of microplastics more effectively.

Introduction

In the original Py-Harmony proposal, validation tests were planned to be performed to fill the identified knowledge gaps highlighted during the round table. However, it was decided to slightly deviate from this original plan, due to the readily available data present within TNO that can be used to address the knowledge gaps mentioned during this Round table meeting. These data originate from previous performed projects on for example the quantification of a range of microplastic (MP) compounds, including tire ware particles (TWP), but also projects in which many challenges within thermoanalytical analysis were investigated, such as matrix effects, internal standard, the use of suitable markers, etc. Therefore, it is believed that this information can be useful for labs using thermoanalytical analysis, which can use these findings for validation testing or for developing their own procedures for the quantification of MPs. Thus, in this synthesis report knowledge gained from past projects performed within TNO will be described to assess the main gaps in knowledge regarding Pyrolysis GC-MS analysis of MPs.

1. Matrix effects arising from organic materials

Influence of Pine needles and Oak-leaves on the PE markers

From both the literature, as well as from in-house experiments performed, it is known that organic material influences the TED-GC-MS analysis of suspended matter. These organic materials cause matrix effects, which influence the GC peaks of different types of plastics. Therefore, the removal of the organic matrix in the suspended matter is important for not only a reliable but also a simpler analysis. Pine needles and oak leaves are organic materials that contain organic compounds such as cellulose and lignin. The pyrolysis of lignin produces markers that contribute to certain polyethylene (PE) markers, which results in the overestimation of this plastic during quantification. In the Guerilla 2.0 project, the contribution to the PE concentration of markers originating from oak leaves and pine needles was investigated. Firstly, potential cellulose and lignin markers were identified by the TED-GC-MS analysis of pine needles and oak leaves in scan modus, in which a significant correlation was observed between the PE marker 1,11-dodecadiene, and the lignin markers 2-methoxyphenol (2-mp) and 2-methoxy-4-methylphenol (2-m-4-mp) (see Figure 1.1). Subsequently, a 43 % and 45 % theoretical contribution of these two lignin markers (2-mp and 2-m-4-mp respectively) to the signal of the PE marker 1,11-dodecadiene was calculated, based on the amount of these lignin markers in the analyzed samples. It should be noted that this theoretical value was calculated assuming these two markers are completely originating from lignin in pine needles and oak leaves. However, in reality, this number would be expected to be lower, as there are additional organic compounds present containing lignin, which would contribute to the concentration of these markers. Thus, this theoretical contribution should be regarded as an upper limit. To limit the contribution of these markers to the concentration of PE, sample pre-treatment for cleanup of these types of samples should be performed, to obtain reliable data.

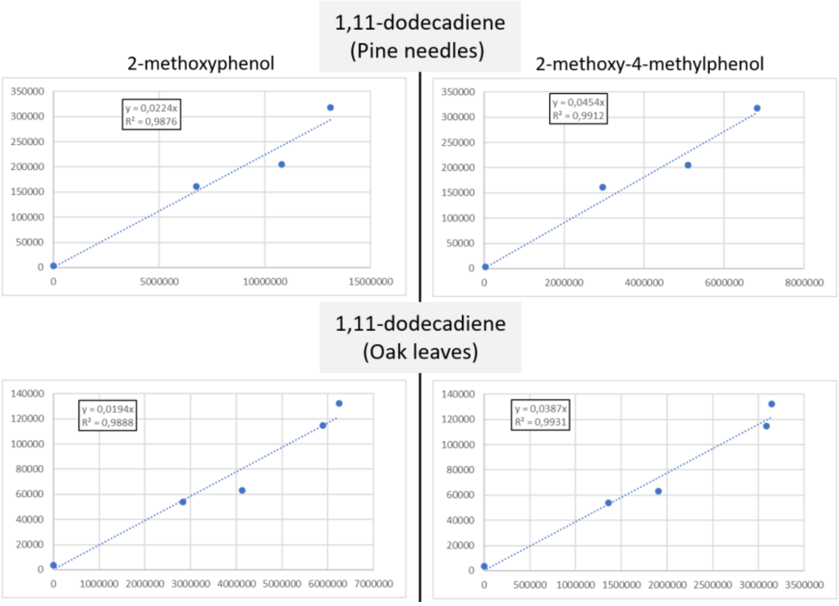


Figure 1.1: Correlation between the lignin pyrolysis compounds 2-methoxyphenol (left) and 2-methoxy-4-methylphenol (right) with 1,11-dodedecadiene originating from Pine needles (top) and Oak leaves (bottom). The x-axis highlights the response of 1,11-dodedecadiene, while the y-axis illustrates the response of 2-mp (left) and 2-m-4-mp (right).

Fenton's digestion for the removal of organic materials

Fenton's digestion was regarded as a potentially suitable pre-treatment technique for the removal of lignin from oak leaves and pine needles. In the Guerilla 2.0 project, the removal efficiency of this technique of organic material origination from pine needles and oak leaves was investigated. In this study the pyrolysis markers of PE, styrene-butadiene rubber (SBR), natural rubber (NR), polypropylene (PP), polystyrene (PS), polyamide (PA), polyethylene terephthalate (PET), and polymethyl methacrylate (PMMA) were measured in oak leaves and pine needles before and after Fenton's digestion of 1 hour reaction time (see Figure 1.2). The markers for SBR and NR were completely removed from the samples, while for the remaining plastics markers, a reduction of 35 – 85 % was observed (with a 40 % removal of the PE marker). Moreover, it was also seen that almost 80 % of the total mass of oak leaves and pine needles were removed by Fenton's digestion after 1 hour of reaction time. These results gave a first indication that this pre-treatment technique would be suitable for the removal of organic contents in real samples that might contribute to the concentrations of various plastic polymers.

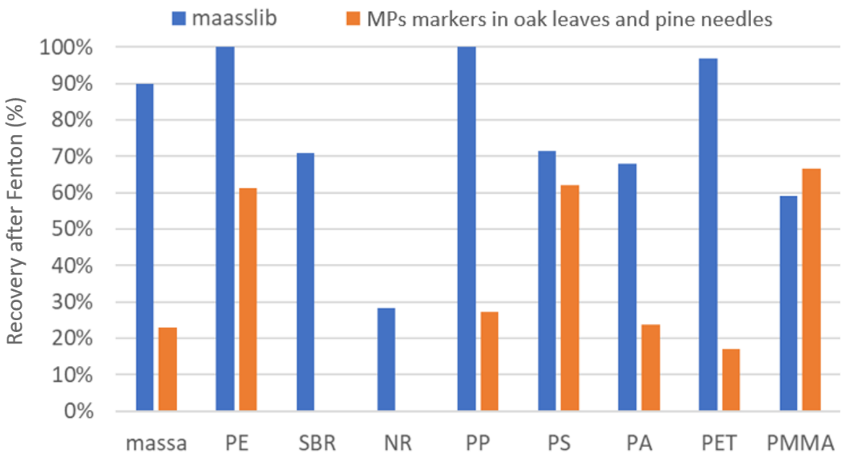


Figure 1.2: Recovery of the MPs markers for various polymers in oak leaves and pine needles (highlighted in orange) after Fenton's digestion.

The influence of Fenton's digestion on the markers of PE, SBR and NR was further investigated within the Guerilla 1.0 project. In this study different Fenton's digestion reaction times, namely 0.5, 1, 2, 4, 8, and 16 hours were performed and compared, to determine a optimal reaction time in which the a minimal removal of the polymer markers is observed. Based on the results obtained from this project, it was observed that the PE particles remained unaffected by the Fenton's digestion procedure for up to 4 hours of reaction time, and the SBR particles for up to 30 minutes, while the NR particles were almost immediately digested by the method (see Figure 1.3). These results clearly highlight the influence of the reaction time in the removal of polymer markers. Therefore, when opting to use digestion methods for the cleanup of samples, the reaction time should be taken into account, as not only undesired organic contamination will be removed, but also desired polymers in the samples.

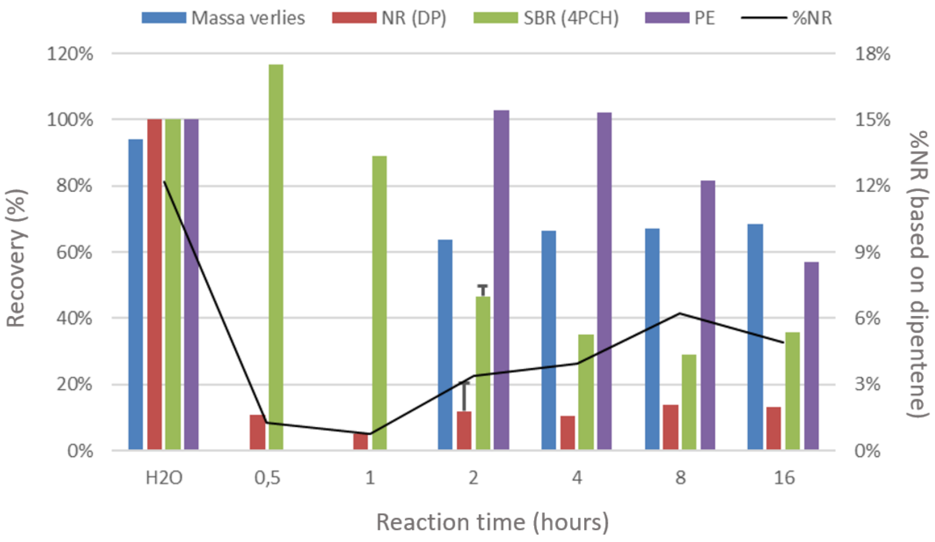


Figure 1.3: The influence of the reaction time of Fenton's digestion on the PE, SBR and NR concentration in river sediment samples.

2. Instrumental Setup

Quantification of TWP using three widely used Py-GC-MS methods
The quantification of TWP is not as straightforward as the quantification of various polymers, due to the complex composition of these particles. Tires are made from an intricate blend of materials, including different synthetic rubbers, natural rubber, and a range of fillers and additives, all of which vary by tire type and brand. Therefore, the markers used for the quantification of the rubbers can have a major effect on the calculated TWP concentrations. Moreover, quantification efforts are further complicated by the evolving formulations of tires over time, which make it challenging to establish consistent baselines for measurement. In the project LEON-t an approach was made to accurately quantify TWP, by factoring in differences between car and truck tire compositions as well as shifts in tire makeup over time. This approach's main strength is its ability to directly quantify TWP using real world marker conversion factors, derived from a large dataset of market-representative tires. On the contrary, this approach lacks the ability to differentiate between various rubber types, such as NR, SBR, and BR. Car tires are mainly composed of SBR and BR (44% assumption based on the ISO technical specifications), while truck tires mostly consist of NR (45%). Subsequently, 4-vinylcyclohexene (4VCH) is a marker used to quantify SBR and BR, while dipentene (DP) is used for the quantification of NR.

As stated earlier, the concentration of the tire wear markers measured through Py-GC-MS analysis is impacted by the diverse compositions of tires and the constantly shifting mix of tires on the market. Therefore, the database of 30 tires created by Tromp et al. was re-analyzed to investigate this hypothesis.[1] These databases were compared to the database of Rauert et al.[2] The results depicted in Figure 2.1 of this comparison shows a high variability in the calculated concentrations based on 4VCH (RSD of 54% [Rauert 2021], 52% [TNO 2012] and 33% [TNO 2022]), which is most likely due to the wide variation in tire compositions. Subsequently, no significant difference ($p > 0.1$) in 4VCH concentrations was observed in the tires in a span of 10 years, as the calculated concentrations differed from 0.98 ±0.51 mg/g in 2012, to 1.08 ±0.58 mg/g in 2020 and 1.25 ±0.42 mg/g in 2022. As mentioned earlier, it is widely believed that 4VCH is a marker for synthetic rubbers SBR and BR, which is typically absent in truck tires, as truck tires primarily consist of natural rubber. However, the findings also reveal that 4VCH is detected in every truck tire examined, with concentrations similar to those in car tires ($p > 0.1$). Next, cyclohexenylbenzene (SB) is widely used as a marker for SBR to this marker producing similar concentrations across different tire compositions. The tire dataset results are illustrated in Figure 2.1 shows a contrary result, as the SB concentrations display a wide variation with a RSD of 70%, making it far less reliable then the calculated concentrations of 4VCH. But, similar to 4VCH, these results also illustrate no significant differences in calculated SB concentrations in car and truck tires (0.17 ±0.09 mg/g and 0.16 ±0.13 mg/g, respectively), which indicates that SB might not be an as reliable indicator of car tires as previously thought. Lastly, the NR-specific marker dipentene typically appears in higher concentrations in truck tires compared to car tires. The tire dataset comparison (Figure 2.1) show a significantly higher concentration ($p < 0.01$) in truck tires (4.9 ± 2.2 mg/g) than in car tires (1.01 ± 0.97 mg/g), despite the high variability for both car tires (RSD = 97%) and truck tires (RSD = 43%).

Using the results of these tire datasets, as well as a 6% correction for truck tire proportions, which is made using official transport data with current estimates showing freight traffic contributes around 6% of TWP in the Netherlands, conversion factors were determined to be 1.0 (±0.4), 0.17 (±0.09) and 1.2 (±1.0) mg/g for 4VCH, SB, and DP, respectively.

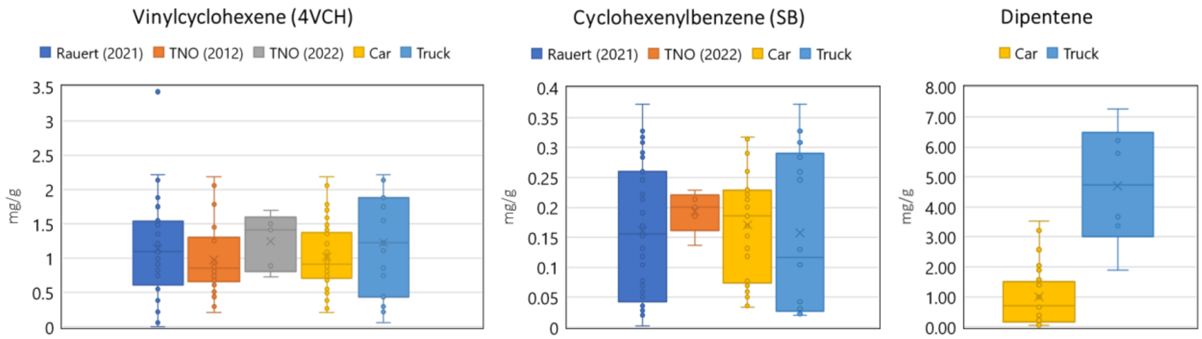


Figure 2.1: Concentration of common TRWP markers from different tire datasets. Each dot represents a tire, the cross represents the arithmetic mean, the line represents the median value, the box represents the 25th and 75th percentiles and the error bars indicate the lower and upper values. Dots outside the error bars are outliers.

In the LEON-t project three commonly referenced methods from existing studies were compared, along with TNO's current measurement technique, which are described in detail in Table 2.1. These methods involve two distinct thermo-analytical approaches: TED-GC-MS, where pyrolysis products are initially trapped on an absorbent and later released via thermal desorption, and Py-GC-MS, which channels pyrolysis products that are directly injected into the GC-MS system for analysis. The quantification methods ISO/TS 21396/20593 published by the International Organization for Standardization (ISO), use Py-GC-MS for analysis in which the sample is rapidly heated to 670 °C, with 4-vinylcyclohexene (4VCH) as the marker for quantifying SBR and butadiene rubber (BR), and dipentene as the marker for NR.[3,4] The Bundesanstalt für Materialforschung und -prüfung (BAM) method employs TGA-TED-GC-MS, meaning that the samples are heated from 25 to 600 °C under nitrogen, allowing for both the mass loss data and analysis of the pyrolysis products.[5] This method uses SB as the primary marker to quantify SBR, while phenyl-[4.4.0]bicyclodecene (SBB) serves as a qualifying marker. Subsequently, contrary to the ISO methods, the marker 4-vinylcyclohexene (4VCH) was deemed too unspecific as it can be used for the quantification of both SBR and BR rubber. For NR, while certain isoprene dimers, trimers, and tetramers (iso2/iso3/iso4) were tested, they were found unsuitable for environmental samples due to interference from plant matter. As a result, the BAM method quantifies only SBR, using a conversion factor of 8.9 to estimate TWP mass from SBR concentrations. Lastly, the TNO method developed by Tromp et al. consists of a TED-GCMS method that uses a tube furnace to pyrolyze samples at 550 °C for 15 minutes, with nitrogen flowing over the sample to collect decomposition products on Tenax-TA.[1] These are later analyzed via automated thermal desorption (ATD) GCMS. The working principle of this setup is described in further detail in the next section. TWP mass was quantified using the markers 4VCH and SB.

Table 2.1: Comparison of the two most commonly used literature methods, and the TNO TED-GCMS and TGA-TED-GCMS methods used in the LEON-t project.

Description	TNO (2012)[1]	ISO/TS 21396/20593[3,4]	Bundesanstalt für Materialforschung und -prüfung (BAM)[5]	TNO (2021)[1]
Analysis technique	Pyrolysis + TD-GC-MS	Py-GC-MS	TGA-TED-GC-MS	TGA-TED-GC-MS
Pyrolysis conditions	550 °C, 15 min, N_2	670 °C, 5 sec, H_2, induction time <0.2 sec	25-600 °C , 10 °C/min, N_2	290-550 °C, 20 °C/min, N_2
Pyrolysis system	tube furnace sample with stainless steel tube	micro-furnace with quartz tube, Curie-point with holder, Pt-filament with holder	TGA with 150 µL alumina crucibles	TGA furnace with Tenax tubes
Adsorption medium	Tenax-TA	-	SPE Sorbstar / PDMS Twister	Tenax-TA
Sample pre-treatment	-	-	pre-heating 25-290 °C (optional)	-
Pyrolysis markers :				
Styrene (SBR)	qualifier	qualifier	-	qualifier
Isoprene (NR)	-	qualifier	-	-
Butadiene (SBR+BR)	-	qualifier	-	-
Dipentene (NR)	qualifier/quantifier	quantifier	qualifier	qualifier/quantifier
Iso3/Isi4 (NR)	-	-	qualifier/quantifier	-
DMVCH (NR)	-	-	-	-
4VCH (SBR+BR)	quantifier	quantifier	-	quantifier
SB (SBR)	quantifier	-	quantifier	quantifier
SBB (SBR)	-	-	qualifier	-
Pyrolysis internal standard	4-fluorostyrene (dissolved in DCM)	d-PI and d-PB (dissolved in chloroform)	d-PS (dissolved in DCM)	4-fluorostyrene (dissolved in DCM)
Injection internal standard	toluene-d8 / ethylbenzene-d10	-	-	-

Quantification method	external 5 points calibration with decomposition products spiked on Tenax-TA	matrix-free external 5 points calibration by pyrolysis of IR2200 and SBR1500 (dissolved in chloroform)	standard addition with SBR / matrix-free external 6 points calibration by pyrolysis of SBR (dissolved in toluene)	External 5 points calibration through the TGA pyrolysis of pure standards
Quantification result	TWP	SBR+BR and NR	SBR	TWP
Conversion T(R)WP	average concentration of pyrolysis markers in car- and trucks, with correction for freight traffic (6% in NL); conversion factors VCH, PCH and dipentene are *ca.* 1.0, 0.17 and 1.2 mg/g TWP; no conversion to TRWP	rubber content (SBR+BR+NR) in tyre tread is *ca.* 50% (SBR in passenger tread is 44%, NR in truck tread is 45%); correction factor of 0.9 for styrene content: styrene content in SBR+BR in tyre tread is 15% (reflecting 65% SBR and 35% BR) and styrene content in SBR1500 is 23.5%; content tyre wear in TRWP is 50%	average content SBR in tyre tread is ca. 11.3%; content tyre wear in TRWP is 50%	average concentration of pyrolysis markers in car- and truck s, with correction for freight traffic (6% in NL); conversion factors VCH, PCH and dipentene are *ca.* 1.0, 0.17 and 1.2 mg/g TWP; no conversion to TRWP

Subsequently, roadside soil samples were collected and analyzed with these three methods (TNO old, BAM, and ISO methods). The calculated TWP concentrations are compared in Figure 2.2. Similar calculated TWP concentrations were observed based on 4VCH, between the BAM and TNO methods. On the other hand, the TWP concentration determined with SB is higher in the BAM method than in the TNO. This can be explained by the assumption made in the BAM method, which assumes only 11.3% SBR in tire tread, compared to the 44% SBR assumed in car tires for the ISO and TNO methods. When TWP concentrations are recalculated using the 44% SBR assumption, they decrease by roughly a factor of 4, aligning closely with TWP concentrations based on SB in the TNO method, as shown in Figure 2.2 (SB ISO% SBR). These results illustrate that the assumed NR and SBR content in tire tread, along with the markers used for quantification, have a major effect on the calculated TWP concentrations, making these estimations crucial for reliable quantification of TWP in environmental samples. Moreover, these results also highlight that similar results can be obtained with the right assumptions and marker choice, in terms of TWP concentrations using different methods.

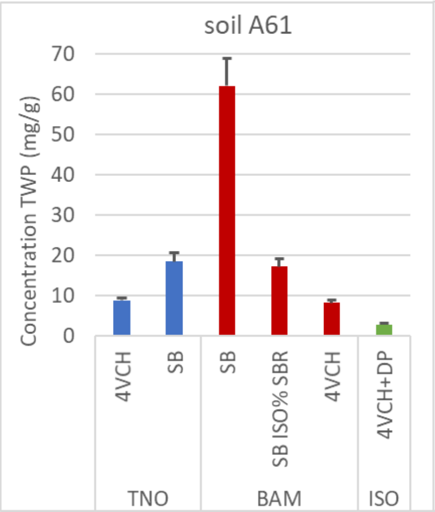


Figure 2.2: TWP concentration in soil samples (Germany, A61) as determined by the TNO, BAM and ISO methods using 4VCH, SB and 4VCH+DP markers.

Comparison of the old and new pyrolysis-GC-MS setups (Pyrolysis-GC-MS vs TGA-GC-MS)

Within TNO two different pyrolysis-GC-MS setups have been used for the quantification of tire-ware particles (TWP). In the LEON-t project quantification comparison of TWP using both these setups was conducted. The first setup used a separate table furnace in which samples were heated at 550°C for a period of 15 minutes (pyrolysis + TD-GC-MS). A nitrogen flow ensures that the produced pyrolysis products are captured on Tenax-TA tubes. After sampling these tubes are transferred to an automatic thermal desorption (ATD) GC-MS where they are analyzed. The second setup uses a TGA furnace, in which samples are heated from 40 – 1000°C for 48 min. Sampling occurs in the temperature segment 290-550°C. During this time the pyrolysis products are captured onto Tenax-TA tubes by the nitrogen flow present. After sampling the Tenax-TA tubes are automatically transferred to the thermal Desorber by the GERSTEL MultiPurpose Sampler (MPS). Subsequently, the released analytes are analyzed by an Agilent 7000 GC-MS/MS triple quad instrument. See **Table 2.1** for a detailed summarization of the two setups used for the quantification of TWPs in the Leon-T project.

The quantitative comparison of the TWP was conducted by the analysis of roadside soil collected at highway A61 in Germany. Quantification of the TWP concentration was performed with calibration curves of the markers 4VCH, SB and DP (Figure 2.3). The results illustrate no significant differences in the calculated TWP concentrations using the three markers between the two instrumental setups. Thus, again these results indicate the importance of using the appropriate markers for quantification, combined with the correct assumptions of the SBR and NR%, which can result in obtaining similar TWP concentrations between different instrumental setups. Therefore, it is also beneficial to upgrade manual setups, for example, the table furnace setup that consists of many labor-intensive steps (TNO 2012), to automated setups comparable to the TGA-TED-GC-MS setup that possesses an autosampler, which drastically decreases the amount of manual labor necessary for each measurement.

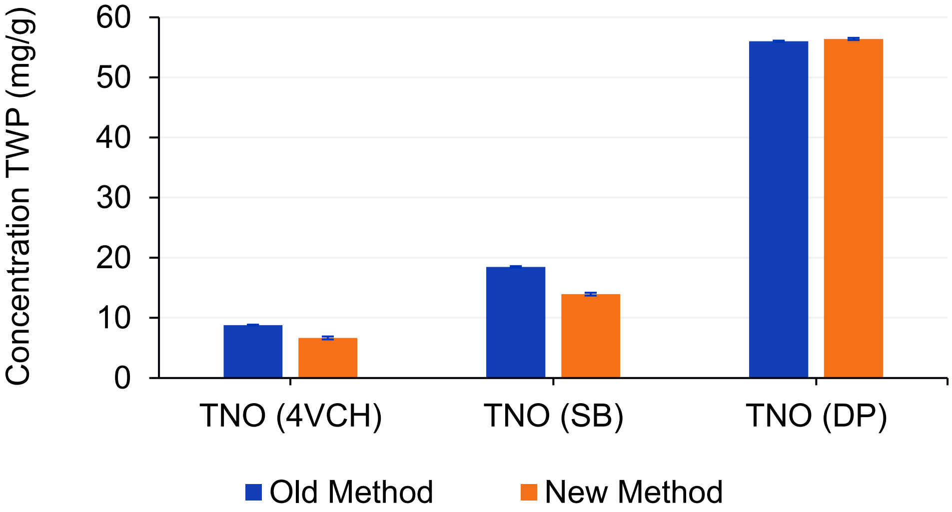


Figure 2.3: T The TWP concentration measured with pyrolysis tube furnace TD-GCMS (old method) compared to that measured with the TGA-TED-GCMS (new method), based on the markers 4-vinylcyclohexene (4VCH), cyclohexenylbenzene (SB) and dipentene (DP).

3. Standards

In TED-GC-MS analysis, quantification is typically performed using an external standard. The response factor of the chosen quantifier for this standard forms the basis for calculating the final result. However, comparing absolute concentrations across different studies can be challenging when different standards are used. A lot of factors can influence accurate quantification of polymers. Standardizing the analysis with traceable, consistent standards would improve the reliability and comparability of results. In recent years, commercial MPs standards have become available. For instance, the Frontier Labs Microplastics Standard provides a selection of microplastics in either a silica or calcium carbonate matrix. Both matrices are also available separately for sample dilution and addition, and the standard is offered in two concentration levels.

Weighing precise amounts of solid microplastic standards can be difficult since the desired quantity often falls below the range of standard (micro) balances. The Frontier Labs Microplastics Standard addresses this by combining various microplastic types in a low concentration (0.2–15 µg per 4 mg) within a ceramic powder matrix. This mix allows for easier weighing of smaller amounts. However, particle size differences can affect the homogeneity of the solid mixture, potentially introducing variation across analyses. While batch-to-batch variation in microplastics concentration exists, it should in principle not pose a major issue as concentrations are well documented for each batch.

Interlab study by Rijkswaterstaat using TNO's standard

A small interlab experiment was performed by Rijkswaterstaat using TNO's standard. RWS measured the TNO Frontier mix standard and compared it with the RWS standard (which contains ~10 times higher concentrations). In the comparison series, alternately the TNO and RWS standards were measured in five replicates and weighed a ~10x higher concentration for the TNO standard. Values were corrected with an internal standard (PFS dimer, m/z 244). The response factor was determined by dividing the IS-corrected response by the corresponding plastic concentration. Recovery was calculated by quantifying the TNO standard using the RWS standard and then dividing it by the amount of plastic weighed in the TNO standard (**Table 3.1**).

Table 3.1: Comparison of different batches of a standard

Name	Quantifier	Average Response factor TNO Fmix (n=5)	RSD (n=5)	Average Response factor RWS Fmix (n=5)	RSD (n=5)	Average recovery (n=5) of TNO standard, quantified with RWS	RSD recovery (n=5)
PA6/66 mz 113 Caprolactam	x	1,7E+04	26%	7,4E+03	16%	233%	27%
PE mz 81 1.13-Tetradecadiene	x	6,7E+01	30%	4,3E+01	16%	156%	29%
PE mz 81 1.14-Pentadecadiene	x	3,4E+01	31%	2,4E+01	17%	148%	33%
PE mz 81 1.15-Hexadecadiene	x	1,9E+01	32%	1,3E+01	16%	148%	34%
PET mz 150 Ethylbenzoate	x	2,1E+06	9%	1,2E+06	31%	190%	39%
PMMA mz 100 Methylmethacrylate	x	1,0E+08	5%	8,2E+07	6%	126%	8%
PP mz 111 2.4.6.8-Tetramethylundec-10-ene	x	1,1E+03	29%	9,7E+02	20%	119%	36%
PP mz 111 2.4.6.8-Tetramethylundec-10-ene	x	3,9E+02	29%	3,5E+02	19%	116%	35%
PS mz 208 2.4-Diphenyl-1-butene	x	9,7E+02	20%	4,7E+02	24%	217%	28%
SBR mz 104 Cyclohexenylbenzene	x	2,2E+03	41%	1,2E+03	21%	199%	38%

These results indicate that there will be a visible deviation in results depending on which batch of Frontier mix is used. This was also evident in a previous test we conducted with two different batches of the high-concentration Frontier mix (Table 3.2). The deviation varies depending on the type of plastic (for PP and PMMA, for example, it is minor—less than a factor of 1.3; for PE, a factor of 1.5; and for PA, PS, and SBR, approximately a factor of 2).

Here, there were also differences, but they were much smaller than in the comparison between a high and low batch of the "old Fmix". These results from the recent inter-laboratory comparison highlighted significant variation in response factors and recoveries when different batches of Frontier Labs standards were used, affecting different plastic types to varying degrees (e.g., deviations up to a factor of 2 for PA, PS, and SBR). Using traceable, consistent standards could reduce this batch-to-batch variability and improve comparability between studies. The recent availability of commercial standards is a step forward, yet the data underscores the importance of standardization across batches and laboratories for reliable quantification.

Table 3.2: Results comparison of two different batches of solid microplastics standard mixes

Compound:	Average Recovery of new Fmix, quantified with old Fmix (n=7)	RSD (n=7)
PA6/66 mz 113 Caprolactam	142%	8%
PE mz 81 1.13-Tetradecadiene	115%	8%
PE mz 81 1.14-Pentadecadiene	116%	6%
PE mz 81 1.15-Hexadecadiene	118%	5%
PET mz 150 Ethylbenzoate	126%	26%
PMMA mz 69 Methylmethacrylate	92%	23%
PMMA mz 100 Methylmethacrylate	92%	24%
PP mz 111 2.4.6.8-Tetramethylundec-10-ene	108%	8%
PP mz 111 2.4.6.8-Tetramethylundec-10-ene	108%	8%
PS mz 208 2.4-Diphenyl-1-butene	127%	9%
SBR mz 104 Cyclohexenylbenzene	117%	7%

4. Quantification of cotton in Washing machine effluent

In TNO, a method to quantify manmade microfibers in effluent from washing machines was developed. The study was funded by the Ministry of I&W and was executed in collaboration with Hanzehogeschool Enschede and TKT. The main focus was on polyester (polyethylene terephthalate) and polyamide (PA 66). These are the manmade fibers that are used the most. As it was also of interest to know the amount of cotton, we also looked into the quantification of cotton. The markers used for quantifying cotton were selected based on the previously described publications and were analyzed in triplicate.[6,7] These markers are depicted in Table 4.1.

Table 4.1: Selected markers for the identification and quantification of cotton.[6,7]

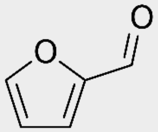
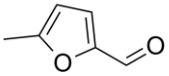
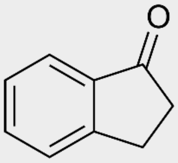
Marker	Afkorting	CAS	Structuur
Furfural	FUR	98-01-1	
5-methylfurfural	5MF	620-02-0	
1-Indanone	IND	83-33-0	

Table 4.2 presents the results of the blank waters. No textiles were washed in these samples. These procedural blanks provide an indication of the background concentration of PET, PA, and cotton. In all blanks, a quantity of PET, PA, and cotton was found. The samples were prepared in a laboratory at Saxion University of Applied Sciences, where textile fibers are handled. The sample "tap water, detergent, and SBL" contains an average of 28 mg/L of cotton.

Table 4.2: Results of the blank washing measurements performed.

	Tapwater		Tapwater		Tapwater	
	-		detergent		detergent / SBL	
	Average (mg/L)	**(+/-)**	**Average (mg/L)**	**(+/-)**	**Average (mg/L)**	**(+/-)**
Polyamide (cyclopentanone)	0,04	0,04	0,05	0,01	0,14	0,02
PET (vinylbenzoate)	0,01	0,004	0,31	0,06	0,86	0,07
Katoen (furfural)	0,04	0,01	2,27	0,23	*28,17*	*5,10*

The results of the test waters with cotton can be found in Table 4.3. During these experiments textile samples (cotton, polyamide, and PET) were washed with water, to which detergent was added (4g/L) and one sheet of standard ballast load (SBL, 20x20 cm in 10 L), which material to simulate. This material consists of a mixture of olive oil, lipstick, make-up, cooking fat, fruit juice, tea, curry, milk, chocolate, gravy, grass juice, red wine, chocolate ice cream, and clay. The measurements were carried out in triplicate. The results show that a comparable amount of cotton was found in the "Cotton + water" sample as in the sample where detergent was also added. The sample with added SBL (Soil Ballast Load) contains more than twice as much cotton.

Table 4.3: Results of the cotton washing experiments.

	Cotton		Cotton		Cotton	
	Water		Water/Detergent		Water / Detergent / SBL	
	Average (mg/L)	**(+/-)**	**Average (mg/L)**	**(+/-)**	**Average (mg/L)**	**(+/-)**
Polyamide (cyclopentanone)	0,02	0,0001	0,05	0,01	0,13	0,01
PET (vinylbenzoate)	0,06	0,01	0,29	0,05	0,84	0,01
Katoen (furfural)	11,66	2,86	10,47	0,41	25,34	3,10

From the test water measurements, it is noticeable that the markers for cotton are present in blanks containing detergent. From the composition of the model detergent used, it appears that it contains 1.3% carboxymethylcellulose. CMC contains a building block similar to cotton (glucose, a ring structure of six carbon atoms, and an aldehyde group). Therefore, it is possible, and very likely, that the pyrolysis products of CMC are (partially) the same as those of cotton. To rule out that the signals of the cotton markers in the wash water blanks do not come from cotton-related contamination, an additional blank was made containing the detergent (4 g/L) that was supplied with the dry samples. This was made using MilliQ water. This analysis also showed an increase in the cotton markers. The blank with tap water, detergent, and SBL also contains a high level of cotton, i.e., 28 mg/L. This concentration cannot be explained solely by the presence of CMC. The dirt added to the test waters, Soil Ballast Load (SBL), also turns out to contain cotton. These are sheets with a standard amount of 'dirt' that can be added to a wash cycle. They are mainly used for testing washing processes. The basis of these sheets is 100% cotton. This is a logical explanation for the high concentration of cotton measured in samples where SBL was used. The fact that substances are present in the wash water matrix that form the same pyrolysis markers makes it impossible to accurately quantify the cotton content. These values are at most indicative.

5. Influence of matrix on fluorinated Polystyrene response

Poly(4-fluorostyrene) (PSF) is often used as an internal standard in pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) for the analysis of MPs. PSF produces distinct pyrolysis markers, primarily fluorinated styrene-based compounds, which are easily distinguishable from the pyrolysis products of other polymers like polystyrene. This chemical distinctness allows PSF's markers to be isolated from target compounds in a complex matrix, aiding in more accurate quantification.

Recently, a paper was published on the influence of matrix on poly 4-fluorostyrene recovery.[8] This study investigates the role of poly(4-fluorostyrene) (PSF) as an internal standard to address matrix effects in the Py-GC-MS analysis of polystyrene (PS) microplastics. Literature indicates that varying soil matrices, particularly those differing in organic carbon content (Corg, 1.0–13.6%), alongside PS molecular weight (Mw), can significantly impact the detection of PS pyrolysis markers. The addition of PSF as an internal standard aims to normalize responses for key pyrolysis products—styrene, styrene-dimer, and styrene-trimer—and mitigate matrix-related interference. Results show that higher organic carbon in soils correlates with increased trimer formation, leading to a decreased dimer-to-trimer ratio and highlighting a strong matrix effect on calibration curve slopes, with variations of up to 8-fold. This matrix effect appears closely linked to Corg levels, yet normalization by PSF marker areas effectively reduces calibration variability, supporting PSF's utility as a stabilizing internal standard. Differences in PS molecular weight also impact marker formation: lower Mw PS (Mw 35,000) generates reduced amounts of all markers, with a pronounced decrease in styrene-dimer compared to high Mw PS (Mw 400,000). These findings emphasize that the choice of pyrolysis marker significantly influences calibration curves, underlining the importance of marker selection in accurately quantifying microplastics within complex soil matrices through Py-GC-MS methods.

Similar observations were reported by Rijkswaterstaat. The PFS response varies significantly between the same sample without or with standard addition. The cause of this is not yet understood. It seems to occurs more often directly after maintenance of the TGA-TDU component. After two 'conditioning' series, the differences become smaller again. In the light of the findings above: At TNO, the Poly (4-fluorostyrene) was purchased from Polymer Source as the powder. The average is Mw 3,200. It might be of interest to investigate the influence of different molecular weights of PSF on the quantification of polymers in environmental matrices.

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Signature

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