Analysis of recycled rubber: Development of an analytical method and determination of polycyclic aromatic hydrocarbons and heterocyclic aromatic compounds in rubber matrices

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HIGHLIGHTS

- The presented method gave the best results for analysis of a wide suite of aromatic compounds present in crumb rubber.
- Identification of 21 heterocyclic compounds, two aromatic amines and several methyl-PAHs together with the 16 EPA PAHs.
- Mats purchased in Dutch shops exceed the EU limits for articles placed on the market for use by the public.
- The obtained results stress the need for expanding the list of target compounds analyzed in crumb rubber.

ARTICLE INFO

ABSTRACT

Recycled crumb rubber (CR) is rich in compounds with unrecognized toxic potency; this study aims at the development of an analytical method that would allow identification and quantification of a very wide range of organic compounds extractable from the complex rubber matrix.

The analytical set-up includes target analysis of polycyclic aromatic hydrocarbons (PAHs) and methyl-PAHs and suspect screening of raw extracts to tentatively identify primary organic compounds present, but not included in the standard target analysis of recycled rubber, followed by analytical method development and target analysis of identified compounds. Analyzed samples included weathered and new CR originating from football turf granulates, rubber mats, and end-of-life car tires (ELTs).

The developed analytical method involves sonication extraction, followed by solid phase extraction (SPE) fractionation that enables simple and efficient separation of analytes with broad polarity scale. The application of SPE fractionation resolves coelution problems and simplifies the chromatograms. This analytical approach allowed to identify and quantify 46 sample specific compounds, including several heterocyclic PAHs like 2-methylthiobenzothiazole, benzonapthothiophenes, benzonaphthofuranes and aromatic amines like diphenylamine and N-phenyl-2-naphthylamine, which to our knowledge were not determined before. The PAHs concentrations determined in CR tiles purchased in Dutch and Spanish shops exceed the EU limits for articles marketed for use by the public. Furthermore, sets of methylated PAHs, dibenzothiazoles and aromatic amines were identified and quantified, and several other compounds were tentatively identified. The obtained results stress the need for expanding the list of target compounds analyzed in CR and the need for longitudinal studies on weathering processes taking place in CR.

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1. Introduction

Problems with the disposal and recycling of end-life tires (ELTs) have led to the use of crumb rubber (CR), originating from recycled ELTs, in consumer products. According to the United States Car Tire Manufactures Association (How a Tire is Made) a new car tire can

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last as long as 60,000 km before it is worn out. The tire wear, created during a tire's life time, is a major component of urban runoff (Wik and Dave, 2009). Because car tires are not biodegradable, the toxicological and environmental risks associated with them do not disappear but rather increase when they have to be disposed. The traditional method of waste tires management like stockpiling, landfilling or illegal dumping, can be a source of possible pollution to groundwaters (El-Fadel et al., 1997; Ishchenko, 2019; Kamata et al., 2011).

Car tires consist of a blend of natural rubber and synthetic polymers like butadiene-, styrene butadiene- and halogenated polyisobutylene-rubber (40−60%); fillers like carbon black and amorphous silica (20−35%); extender oils (15−20%), often containing polycyclic aromatic hydrocarbons (PAHs); stearic acid (1%); zinc oxide (1.5%); sulfur (1%); accelerators and anti-degradants (2.2−2.5%) (Wik and Dave, 2009; European Chemicals Agency (ECHA), 2017). This last group includes inter alia vulcanization additives like different benzothiazoles (e.g. 2-mercaptobenzothiazole and 2-2′-dithiobisbenzothiazole) or sulphonamides (e.g. N-cyclohexylthiophthalimide, 2-morpholinothiobenzothiazole), antioxidants (e.g. hindered phenols and secondary aromatic amines), anti-ozonants like *para*-phenylene diamines and plasticizers, tackifiers and softeners (e.g. phthalates). Car tires also contain many other unidentified compounds, that can be created during vulcanization and manufacturing processes, like different heterocyclic and alkylated PAHs, nitrosamines, or other hydrophobic compounds that may possibly be adsorbed during their use time.

As stated by the European Tire and Rubber Manufacturers Association (Tyre and Manufacturers Association, 2011), in 1996, 49% of the end-of-life tires in Europe ended up in landfills. Since 1996 more than 24 million tons of ELTs have been recovered, either through energy or material recovery, and by 2015 between 5% and 29% (in 15 "old" and 13 "new" EU member states, respectively) were discarded in landfills (European Comission:E, 2020). In 2010 about 1.3 million tons of ELTs went into material recovery, involving reuse of whole tires in civil engineering applications (erosion barriers, artificial reefs, breakwaters, insulation, etc.) or were shredded or reduced to granular or powder rubber. Granulated and powdered rubber is used as flooring for playgrounds and sports stadiums, as shock absorbing mats for schools and stables, as paving blocks or tiles for patios and swimming pools and as roofing materials. Granulated rubber is also widely used in the construction of artificial turf on football fields, and recently this last application has raised many questions about the potential health risks of hazardous substances present in recycled rubber (European Chemicals Agency (ECHA), 2017; Rijksinstituut voor Volks, 2017). The hypothesis that playing on synthetic turf can cause cancer, particularly lymphoma and leukemia and primary by goal-keepers, was raised after football coach Amy Griffin started collecting names of sick players in 2009 (Rappleye, 1736).

According to the European Chemical Agency (ECHA) report from 2017[6], there were over 13,000 synthetic turf football fields and 47,000 mini-pitches used for football in 2017 in the EU. It is estimated that by 2020 21,000 full-size pitches and ca. 72,000 mini-pitches will exist in the EU. These numbers do not include children playgrounds, small practice fields, and indoor recreation facilities.

During the last decade several studies performed worldwide have investigated the composition of CR (Brandsma et al., 2019; Celeiro et al., 2014, 2018), environmental and health risks directly associated to CR (Perkins et al., 2019; Peterson et al., 2018; Bleyer and Keegan, 2018; Pronk et al., 2018; Watterson, 2017) and bio-accessibility of the compounds determined in the CR (Coppola,

2014; Zanetti et al., 2014; Pavilonis et al., 2014; Li et al., 2010). Most of the studies investigating the composition of CR, or the chemicals available for exposure through release into air or water, concentrate only on "standard" pollutants like heavy metals, PAHs confined to the 16 EPA-PAHs (US Environmental Protection Agency) or 8 carcinogenic PAHs (as defined in entry 50 to Annex XVII to REACH (European Chemicals Agency, 2012)), phthalates, bisphenol-A and benzothiazole (BTZ). CR research generally does not include compounds belonging to the group of additives and vulcanization agents. Vulcanization accelerators, like inter alia 2-methylthiobenzothiazole and 2-morpholinobenzothiazole, benzothiophene, dibenzothiophenes and other sulfur containing PAHs (PASHs), have been detected in urban runoff (Hüffer et al., 2019; Kumata et al., 2002; Zeng et al., 2004) but were not analyzed in CR. Also other rubber-specific compounds like sulfenamides (Ema et al., 2007), aromatic amines (Bolognesi and Moretto, 2014) and other toxic rubber additives have until now not been listed among the target organic compounds analyzed in CR. Recently, Schneider et al. (2020) analyzed a group of „substances suggested by tire manufacturers based on sector-specific knowledge" and Celeiro et al. (2020) analyzed a large group of plasticizers, antioxidants and vulcanization agents in CR. Previously, Perkins et al. (2019) reported 306 chemicals identified in CR, from which 197 met the carcinogenicity criteria set in their computational toxicology analysis.

In 2017, two risk assessment reports prepared by ECHA (European Chemicals Agency (ECHA), 2017) (based on a literature study, published in 2017) and by the Dutch National Institute for Public Health and the Environment (RIVM) (Rijksinstituut voor Volks, 2017) (based on the analysis of rubber granulate sampled from 100 Dutch football pitches and published in 2017) concluded that there is no significant health risk from exposure to CR. According to these reports, the risk of cancer after a lifetime's exposure to the PAHs measured in the European sports fields is negligible. In addition, the concentrations of PAHs, phthalates, methyl isobutyl ketone and bisphenol-A are below the legal limits set in the EU for recycled rubber granules. It should be pointed out, that these legal limits are still under discussion. CR on football pitches is classified by the European REACH regulation as a mixture (European Chemicals Agency (ECHA), 2017) and the maximum allowable concentration limits for mixtures are set at 100 mg/kg for BaP and DahA (all abbreviations are listed in Table 1), and 1000 mg/kg for the other six, out of the eight carcinogenic ECHA-PAHs PAHs; BeP, BaA, CHR, BbF, BjF, BkF. The restriction proposed by the European Commission on September 2019 lowers the total concentration limit of eight PAHs to 20 mg/kg (European Chemical Agency., 2020). Other maximum tolerance limits apply to shock absorbing mats, used e.g. on children playgrounds, in nurseries or sport schools. They fall under the category of rubber consumer products and therefore, the applied maximum concentration limit is 1 mg/kg for each of eight carcinogenic PAHs (Rijksinstituut voor Volks, 2016).

A rubber matrix is very complex and therefore a robust and reliable analytical method is needed to determine organic compounds present within it. The aim of this study was to develop a method, that could enable the analysis of a much wider set of potentially harmful chemicals in CR, than the until now available standardized methods for PAHs. The CR samples analyzed in this study consist of rubber granulates collected from artificial football fields in the area of Amsterdam (weathered CR) and rubber mats purchased from different home improvement stores in the Netherlands and in Spain (new CR). Additionally, also three ELTs samples were analyzed to obtain information about the levels of analyzed compounds in unprocessed ELTs. These CR and ELTs samples were recently analyzed for chlorinated paraffins by

Table 1
Studied compounds, CAS nos. And mass spectrometric information.

No.	Name	Code	CAS	Quantification ion (in bold) and identification ions (m/z)	LOD (ppm)	Linear range (ppm)	Precision (intraday) RSD
1	Acridine	AC	260-94-6	**179**, 178, 152	0.01	0.4−0.05	n.c.
2	Carbazole	CA	86-74-8	**167**, 166, 139	0.01	0.4−0.05	n.c.
3	Dibenzofuran	DF	132-64-9	**168**, 139	0.002	0.3−0.006	0.8
4	Benzo[b]naphtho[2,1-d]furan	BN21dF	239-30-5	**218**, 189, 219	0.01	0.3−0.05	0.3
5	Benzo[b]naphtho[1,2-d]furan	BN12dF	205-39-0	**218**, 189, 219	0.01	0.3−0.05	1.7
6	Benzo[b]naphtho[2,3-d]furan	BN23dF	243-42-5	**218**, 189, 219	0.02	0.3−0.05	1.4
7	Benzothiazole	BTZ	95-16-9	**135**, 108	0.002	0.3−0.06	0.2
8	2-Methylthiobenzothiazole	MTBTZ	615-22-5	**181**, 148	0.004	0.4−0.01	6.6
9	2-Phenylbenzothiazole	PBTZ	883-93-2	**211**, 210, 108	0.02	0.3−0.05	0.4
10	2-Mercaptobenzothiazole	MBTZ	149-30-4	**167**, 109	1	n.c.	n.c.
11	Dibenzothiophene	DBT	132-65-0	**184**, 185	0.0006	0.3−0.01	0.6
12	4-Methyldibenzothiophene	4MDBT	7372-88-5	**198**, 197	0.002	0.3−0.01	1.2
13	2-Methyldibenzothiophene	2MDBT	20,928-02-3	**198**, 197	0.003	0.3−0.01	n.c.
14	3-Methyldibenzothiophene	3MDBT	16,587-52-3	**198**, 197	0.004	0.3−0.01	n.c.
15	1-Methyldibenzothiophene	1MDBT	7372-88-5	**198**, 197	0.003	0.3−0.01	n.c.
16	4,6-Dimethyldibenzothiophene	46DMDBT	31,613-04-4	**212**, 211, 197	0.002	0.3−0.01	0.5
17	3,6-Dimethyldibenzothiophene	36DMDBT	89,816-75-1	**212**, 211, 197	0.01	0.3−0.01	n.c.
18	2,6-Dimethyldibenzothiophene	26DMDBT	1207-12-1	**212**, 211, 197	0.003	0.3−0.01	n.c.
19	Benzo[b]naphtho[2,1-d]thiophene	BN21dT	239-35-0	**234**, 235, 117	0.009	0.3−0.01	0.3
20	Benzo[b]naphtho[1,2-d]thiophene	BN12dT	205-43-6	**234**, 235, 117	0.005	0.3−0.01	1.7
21	Benzo[b]naphtho[2,3-d]thiophene	BN23dT	243-46-9	**234**, 235, 117	0.009	0.3−0.01	1.4
22	N-Phenyl-2-naphthylamine	PNA	135-88-6	**219**, 218, 217	0.01	28−0.2	n.c.
23	Diphenylamine	DPA	122-39-4	**169**, 168, 167	0.02	28−0.2	n.c.
24	Naphthalene	NAP	91-20-3	**128**, 108	0.001	2.0−0.01	3.3
25	Acenaphthylene	ACY	208-96-8	**152**, 151	0.001	2.0−0.01	2.3
26	Acenaphthene	ACE	83-32-9	**154**, 153	0.001	2.0−0.01	6.8
27	Fluorene	FLU	86-73-7	**166**, 165	0.001	2.0−0.01	4.1
28	Phenanthrene	PHN	85-01-8	**178**, 176	0.001	2.0−0.01	2.5
29	Anthracene	ANC	120-12-7	**178**, 176	0.002	2.0−0.01	2.5
30	2-Methylphenanthrene	2MPHN	2531-84-2	**192**, 191, 189	0.003	2.0−0.01	5.0
31	Fluoranthene	FLA	206-44-0	**202**, 203	0.001	2.0−0.01	5.3
32	Pyrene	PYR	129-00-0	**202**, 203	0.001	2.0−0.01	3.5
33	1-Methylpyrene	1MPYR	2381-21-7	**216**, 215, 213	0.002	2.0−0.01	4.7
34	Benzo[a]anthracene	BaA	56-55-3	**228**, 226	0.01	2.0−0.01	5.1
35	Triphenylene	TPH	217-59-4	**228**, 226	0.008	2.0−0.01	n.c
36	Chrysene	CHY	218-01-9	**228**, 226	0.008	2.0−0.01	5.1
37	5-Methylchrysene	5MCHY	3697-24-3	**242**, 241, 239	0.008	2.0−0.01	n.c.
38	Benzo[b]fluoranthene	BbF	205-99-2	**252**, 253	0.003	2.0−0.01	1.4
39	Benzo[k]fluoranthene	BkF	207-08-9	**252**, 253	0.004	2.0−0.01	2.3
40	Benzo[j]fluoranthene	BjF	205-82-3	**252**, 253	0.003	2.0−0.01	n.c
41	Benzo[e]pyrene	BeP	192-97-2	**252**, 253	0.002	2.0−0.01	n.c.
42	Benzo[a]pyrene	BaP	50-32-8	**252**, 253	0.0005	2.0−0.01	6.1
43	Perylene	PER	198-55-0	**252**, 253	0.005	2.0−0.01	n.c
44	Dibenzo[a,h]anthracene	DaA	53-70-3	**278**, 279	0.02	2.0−0.01	6.7
45	Indeno[1,2,3-cd]pyrene	IND	193-39-5	*276*, 277	0.01	2.0−0.01	4.8
46	Benzo[ghi]perylene	BghiP	191-24-2	**276**, 277	0.006	2.0−0.01	5.8

Nos.1−23: target compounds first tentatively identified with suspected target screening and non-target screening (underlined); nos. 24−46: target PAHs. LOD: limit of detection; RSD: relative standard deviation; n.c: not calculated.

Brandsma et al. (2019).

The analytical set up of this study includes three steps:

1. Screening of raw extracts of CR in order to obtain a broad „chemical picture" of CR and to set up a list of tentatively identified organic compounds for the following target analysis. The screening is based on the list of the suspected compounds, including inter alia vulcanization accelerators, antioxidants and other compounds used in rubber production. This list was set using previous studies (Perkins et al., 2019; US Environmental Protection Agency (US EPA); Centers for Disease Control and Prevention(CDC), 2019) and technical information (ulcanization & Ac, 1177).
2. Development and optimization of an analytical method involving extraction of rubber matrix and clean-up of raw extracts.
3. Target analysis of the compounds selected on the basis on the aforementioned screening using the developed method.

In this "known unknown" approach we have concentrated on aromatic compounds like heterocyclic PAHs, methyl PAHs and other compounds (e.g. aromatic amines) that to our knowledge were until now not included in the regular analysis of CR. Additionally, we have determined the concentrations of target analytes including twenty parent PAHs and three methyl PAHs; 5-methylchrysene, 1-methylpyrene and 2-methylphenanthrene (5MCHY, 1MPYR, 2MPHN). 5MCHY is classified by ECHA as suspected of causing cancer and 1MPYR and 2MPHN are likely to meet criteria for CMR (carcinogenic, mutagenic and reprotoxic) category 1A or 1B.

2. Materials and methods

2.1. Reagents and materials

Information about standards, chemicals and suppliers is provided in the Supporting Information of this manuscript. The list of the studied compounds, their chemical names and abbreviations, is

presented in Table 1. The calibration curves were linear over the entire range of standards. The standard solutions were prepared in isooctane and the specific range for each compound is shown in Table 1. In all cases, the method showed a good linearity, with co-efficients of determination (R^2) higher than 0.99.

2.2. Sample collection

In total 21 samples were included in this study: ten rubber granulates collected from ten artificial football fields in the area of Amsterdam, the Netherlands (samples coded F1−F10), three ELTs collected from a garage in Postbauer-Heng, Germany (samples coded C1−C3), five new rubber mats (four purchased in the Netherlands and coded T1-T4 and one in Spain; the Spanish mat consisted of two types of crumbs analyzed separately and coded S1 and S2) and one weathered rubber mat collected from a school playground in Amsterdam, the Netherlands (coded T5). The four rubber mats (T1-T4) were purchased from different Dutch shops; mat T1 was intended for roofing and the other mats were floor mats. The Spanish mat (S1, S2) was purchased in a store from an international company. It was intended for indoor and outdoor flooring. More information about the samples is provided in Table S1 in the Supporting Information.

Samples coded F1−F9 and C1−C3 were also analyzed for the presence of chlorinated paraffins by Brandsma et al. (2019) and details regarding sampling procedure can be found there.

Car tires and rubber mat samples were cut with a surgical blade into small particles (<3 mm). All rubber samples were rinsed with mili-Q® water and dried at the room temperature prior analysis to remove dust and soil.

2.3. Suspect screening

Raw extracts of rubber granulates originating from three artificial football fields were analyzed by comprehensive two-dimensional gas chromatography with Time-of-Flight mass spectrometry (Pegasus GCxGC-Tof MS from Leco, Geleen, the Netherlands), with the nominal mass resolution of 1000 for $m/z = 1000$ (at the 50% of the peak height). The separation was achieved using a RTX-Cl column (30 m/0.25 mm/0.25 μm) in the first dimension combined with a BPX50 column (10 m/0.1 mm/0.1 μm) in the second dimension. The GC-MS analytical details are provided in the Supporting Information of this manuscript. The extracts were obtained according to the procedure described below.

GCxGC chromatograms were analyzed using AMDIS software (Automated Mass spectral Deconvolution and Identification System, NIST, version 2.73). Chromatograms were deconvoluted using NIST Mass spectral search Program and NIST mass spectral database (NIST/EPA/NIH Mass Spectral Library Version 2.0, April 2011).

For suspect screening a list of compounds was selected prior the analysis: see Table 2. These chemicals were reported in previous tire crumb rubber studies (European Chemicals Agency (ECHA), 2017; Perkins et al., 2019; US Environmental Protection Agency (US EPA); Centers for Disease Control and Prevention(CDC), 2019).

Furthermore, several additional compounds, represented by the abundant peaks resolved in second dimension, could be tentatively identified with NIST library. The tentatively identified compounds were recognized based on a NIST library match greater than 90%. The list of target compounds was set up by selecting tentatively identified heterocyclic PAHs, benzothiazoles, methyl PAHs and aromatic amines and injecting available standards to confirm their presence. The list of positively identified compounds analyzed in this study is given in Table 1. The positively identified "non-target" compounds (not included in the suspect-screening target list) are underlined in Table 1.

Table 2
Target Compounds selected for Suspect Screening Analysis.

Name	CAS
Carbazole	86-74-8
Benzothiazole	95-16-9
2-Phenylbenzothiazole	883-93-2
2-Hydroxybenzothiazole	934-34-9
2-Aminobenzothiazole	136-95-8
2-Methylbenzothiazole	120-75-2
2-Mercaptobenzothiazole	149-30-4
2-Methoxybenzothiazole	63,321-86-8
2-Morpholinothiobenzothiazole	102-77-2
2,2-Dithiobis benzothiazole	120-78-5
Dibenzofuran	132-64-9
Benzothiophene	95-15-8
Dibenzothiophene	132-65-0
Diphenylamine	122-39-4
N-Cyclohexyl-2-benzothiazolamine (NCBA)	28,291-75-0
N-Cyclohexyl-2-benzothiazolesulfenamide (CBS)	95-33-0
1,2-Dihydro-2,2,4-trimethylquinoline	147-47-7
N,N′-Diphenyl-p-phenylenediamine (DPPD)	2350-01-8
N,N-Dicyclohexyl-2-benzothiazolesulfenamide (DCBS)	4979-32-2
N-tert-Butyl-2-benzothiazolesulfenamide (TBBS)	95-31-8
N-Isopropyl-N′-phenyl-p-phenylenediamine (IPPD)	101-72-4
1,3-Diphenylguanidine	102-06-7
1,3-Di-o-tolylguanidine (DOTG)	97-39-2

2.4. Extraction and fractionation procedure

The extraction method was based on the method of Menichini et al. (2011) with few modifications; the volumes of extraction solvents were adjusted for smaller samples and hexane, the second extraction solvent used by Menichini et al. was replaced here by ethyl acetate (EtAc). All extractions were performed in triplicate. Ca. 0.1 g of rubber was spiked with a mixture of deuterated PAHs and a mixture of deuterated acridine, benzothiazole and dibenzothiophene. Subsequently, the samples were ultrasonically extracted with three portions of 2 mL dichloromethane (DCM) and with one portion 2 mL (EtAc). All four extracts were combined, spiked with 1 mL of isooctane and 10% of the resulting solution was concentrated under a light N_2 flow (final volume ca. 0.5 mL) and used for further clean up. After evaporation, the concentrated extracts were fractionated on SPE cartridges (Bond Elut SI Normal Phase and Bakerbond™ SPE Octadecyl 7020−06). The clean-up procedure was based on the method described by Ionas et al. (Ionas and Covaci, 2013) with minor modifications. The SPE cartridges were first conditioned with 6 mL of EtAc and 6 mL of n-hexane. Organic solvents with increasing polarity were used to elute different compounds groups. The elution scheme was as follows: (1) 6 mL n-hexane, (2) 6 mL n-butyl chloride, (3) 6 mL DCM and (4) 6 mL EtAc/methanol (1:1 v/v).

The four fractions were spiked with 1 mL isooctane. Fractions 1 and 4 were centrifuged for 15 min at 17,000 rpm in a Heraeus Biofuge Stratos Centrifuge. The supernatant was concentrated under a light N_2 flow till the final volume of ca. 0.2 mL. Fractions 2 and 3 were concentrated under a light N_2 flow, without the centrifugation step. The four resulting fractions were analyzed with the GC-MS.

The SPE method was compared with clean-up procedures based on open column fractionation with the following stationary phases: (1) 15 g alumina with 8% H_2O, (2) 15 g Florisil, (3) 7 g activated silica and (4) 2 g silica with 1.5% H_2O. The applied elution patterns were as follow: (1 and 2) 170 mL pentane, 50 mL DCM; (3) 50 mL pentane, 50 mL DCM, 50 mL EtAc; (4) 14 mL n-hexane, 10 mL n-hexane with 15% diethyl ether, 5 mL diethyl ether.

To check the background levels, procedure blanks were performed with every batch of samples.

2.5. GC-MS analysis

Analyses were performed on an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973 Mass Selective Detector. The separation was carried out on a SLB-PAHms Supelco column (30 m × 0.25 mm ID x 0.25 μm) purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). All injections were performed at 275 °C in the pulsed splitless mode (pulse pressure: 1.50 bar, pulse time: 0.85 min). The injection volume was 1 μL and the oven temperature program was set as follows: isothermal at 90 °C for 2 min, then with 5 °C/min to 325 °C and kept isothermal for 10 min. The temperatures of the transfer line, the quadrupole and the ion source were set to 300 °C, 150 °C and 250 °C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software. The MS was operating in the total ion current (TIC) mode and in the selected ion monitoring (SIM) mode. The TIC mode (mass range: 40−450 *m/z*) was used for monitoring of non-target compounds and the SIM mode was used for identification and quantification of the target components. In the SIM mode two or three ions were monitored per compound (see Table 1) and all target compounds were identified and quantified running GC/MS programs with two different SIM programs, separately for PAHs and for heterocyclic compounds.

For the quantification measurements, calibration standards were daily prepared in isooctane and external calibration was performed with every sequence of measurements. The limit of detection (LOD) was calculated from the concentration of the lowest standard as the concentration giving a signal-to-noise of three (S/N = 3).

2.6. Statistical analysis

Principle component analysis (PCA) was carried out using Simca software, Version 16.0.1 (Sartorius Stedim, Sweden) to visualize trends and patterns of different compounds within different samples' groups.

3. Results and discussion

3.1. Screening analysis

Suspect screening with GCxGC/ToF-MS was done to investigate wide range of organic chemicals associated with CR, but not routinely analyzed in CR samples. The screening allowed to tentatively identify several compounds present on the suspect's screening list and additionally also several compounds not included in the list (non-target). The presence of 21 heterocyclic aromatic compounds (including polyaromatic nitrogen- sulfur- and oxygen-heterocycles: PANHs, PASHs and PAOHs), 2 aromatic amines and 3 methyl PAHs (1MPYR, 2MPHN, 5MCHY) was confirmed by injection of available standards. Additionally, several methyl-PAHs (structural isomers of 1MPYR, 2MPHN and 5MCHY) and a suite of dimethyl- (and/or ethyl-) dibenzothiophenes (structural isomers of 46DMDBT) were also quantified. Moreover, 16-EPA PAHs together with TPH, BeP, BjF and PER were identified by target analysis.

The GCxGC chromatograms of the raw extracts were rich in many coeluting compounds and showed the presence of an unresolved complex mixture. Several peaks could be tentatively identified based on the NIST library match greater than 90%. We should stress out that non-target analysis was not the main goal of this study, as the tentative identification was based on low-resolution mass spectrometry (LRMS) with its accuracy limitations. We have rather performed suspect target screening and additionally several abundant resolved peaks could be tentatively identified and their identity could be confirmed by the injection of available standards.

The presence of benzo[*b*]naphthofurans, benzo[*b*]naphthothiophenes, alkylated dibenzothiophenes, acridine, N-phenyl-2-naphthylamine and 2-methylthiobenzothiazole could be confirmed in all samples' groups. Their abundance in the analyzed samples emphasize their environmental and sample-specific significance.

Many other resolved peaks could be tentatively identified as alkylated PAHs, aromatic amines and amides, nitro-PAHs, heterocyclic PAHs, alkylated heterocyclic PAHs and halogenated PAHs or halogenated heterocyclic PAHs with NIST MS Search's Substructure Identification program. In addition, different phthalates could be tentatively identified in all extracts but these compounds fell outside the scope of this study. This limited screening approach shows that comprehensive and reproducible non target screening study (Hites and Jobst, 2018; Samanipour et al., 2019) is needed in order to better characterize toxic compounds associated with rubber matrix and ELT.

4. Method optimization

The method described here gave the best results for extraction and analysis of several heterocyclic aromatic compounds (including PANHs, PASHs and PAOHs), with different K_{ow} values, present in CR. The extraction, based on the method developed by of Menichini et al. (2011), was slightly changed by replacing hexane with more polar EtAc. The recoveries of the extractions with DCM/hexane and DCM/EtAc were similar for all studied compounds. Rubber is a very complex chemical matrix and the chromatograms of raw, unprocessed extracts are extremely rich in coeluting compounds. The GC-MS target analysis of the 10-fold diluted raw extracts did not give satisfactory results, because of the coelution of unknown compounds interfering with the target analytes in the SIM mode. Therefore, further clean-up was necessary.

The recoveries for benzothiazole and 2-phenylbenzothiazole obtained on an alumina open column were lower than 50% and many heterocycles and thiazoles did not elute from a Florisil open column. The recoveries of silica open column procedures gave 90−110% for all target compounds. Solid phase extraction, a complementary method, is effective in purifying trace analytes in complex environmental samples (Ionas and Covaci, 2013; Li et al., 2017; Mosekiemang et al., 2019). Similar recoveries were obtained applying fractionation on normal phase silica SPE cartridges, with eluents based on procedure developed by Ionas at al (ulcanization & Ac, 1177) for analysis of flame-retardants. Nonetheless, this SPE procedure did not provide desired results, like e.g. clear-cut fractions. Fractionation on reversed phase SPE cartridges gave better results and allowed to fractionate more target compounds. Most of the heterocyclic PAHs, like PASHs, eluted together with PAHs in the first fraction but this procedure allowed fractionation of PANHs: acridine and carbazole could be separated from the PAHs and from each other. Carbazole eluted in the second fraction and acridine eluted in the fourth fraction. In addition, benzothiazole eluted in the second fraction and could therefore be separated from analyzed BTZ derivatives and other compounds. The recovery of this procedure was also 90−110% for all the compounds. Additional advantages of the SPE method are small amounts of used solvents and the convenience in the use of SPE cartridges. Examples of chromatograms of three fractions of a crumb rubber extract, containing analyzed compounds, are presented in the Supporting Information (see Fig. S1).

4.1. Analytical results

The samples analyzed in this study, 21 in total, were at first divided in three groups; rubber granulate from synthetic turf football fields (F), car tires (C) and rubber tiles (T). The obtained

results revealed that the concentrations found in the samples from the Spanish tiles were ten or even thousand times higher than in the rest of the analyzed samples. The Spanish mats consisted of two types of different CR differing in size and color (green and black). These two types of CR were analyzed separately and coded as separate group S. The two S-samples were 100 times diluted prior clean-up and analysis. The levels of the quantified compounds in the remaining three groups, F, C and T, were mutually comparable. The medians (used to correct for extreme values) and the maximum concentrations for all groups are given in Table 3. The medians were reported when the substance was detected in at least 50% of the samples. All quantification data from the GC-MS analysis are presented in Table S1 in the Supporting Information.

2-Mercaptobenzothiazole (MBZT) could be quantified in only two samples (T3 and C2: see Table S1 in the Supporting Information). The LOD of this compound is rather high (1 mg/kg; see Table 2) and therefore GC analysis seems less suitable for this compound. Quick screening of the samples with Liquid Chromatography - Atmospheric Pressure Chemical Ionization - Mass Spectrometry (LC-APCI-MS) confirmed the presence of MBTZ in almost all samples (data not presented here). Probably other benzothiazoles suffer from the same problem (see Table 2), and were not detected with the GC screening; LC analysis may give better results. The levels of the PAHs found in samples F, T and C are comparable to the levels presented in the RIVM report (Rijksinstituut voor Volks, 2017), but the medians of the sum of 8 EPA PAHs in samples F and T are, with 9.4 and 8.3 μg/g respectively, substantially higher than the median of the pitch concentrations reported in the RIVM report (5.8 μg/g). The PAHs concentrations in the F, T and C samples are below the European safety limits for mixtures but above the safety limits for consumer products (1 μg/g

for the group of 8 carcinogenic PAHs). The mats purchased in Dutch shops exceed the EU limits for articles placed on the market for use by the public. In case of the Spanish mat (S) the levels are exceeded by even a few hundred times, and the concentration of BaP alone (0.3 mg/g) is far above the safety limit for mixtures (0.1 mg/g). The Spanish mat, purchased from a home improvement retailer with stores in Spain, but also in other EU countries, Asia, South America and Africa, is intended for indoor and outdoor use. No other specific characteristics are known.

The extremely high concentrations of PAHs in sample S seems unusual for a ELT-based product. The study of Re Paolinin et al.(Re Depaolini et al., 2017), where large amount of ELTs of different age and origin (European and non-European) was analyzed, reported the values for the ∑8-EPA PAHs ranging from 3.85 to 11.76 μg/g. These values are similar to the values found in this study, where the ∑8-EPA PAHs values for the F, T and C samples are respectively 9.4, 8.3 and 2.5 μg/g, while for the sample S this value reaches 1861 μg/g (see Table 3).

PAHs in tires originate mainly from highly aromatic (HA) extender oils (AatmeeyataSharma, 2010). The oil content in car tires is 6−8% of the whole tire mass (11−16% of the tread only), and the content is much lower in truck tires (4.5% of whole tire, very low in the tread) (European Commission. Opin, 2003). According to the CSTEE opinion from 2003 (European Commission. Opin, 2003) the total PAH content in HA oils is in the range of 300−700 μg/g and the estimated maximum total PAHs content in tires reaches 112 μg/g. Although this document does not specify which PAHs belong to the "total PAHs content", it is plausible to assume that this term refers to the group of 16 EPA PAHs.

The BaP concentration in ELTs ranges between 1 and 16 μg/g (average 5 μg/g) (European Commission. Opin, 2003), while this

Table 3
Median (MED) and maximum concentrations (MAX) of compounds quantified in samples F, T, C; average (AVG) and relative std. Deviation (RSD) of compounds quantified in samples S. MED: the median value is reported if the substances were present in more than 50% of the samples.

Heterocyclic aromatic compounds	F (n = 10) μg/g		T (n = 6) μg/g		C (n = 3) μg/g		S (n = 2) μg/g		PAHs	F (n = 10) μg/g		T (n = 6) μg/g		C (n = 3) μg/g		S (n = 2) μg/g	
	MED	MAX	MED	MAX	MED	MAX	AVG	RSD		MED	MAX	MED	MAX	MED	MAX	AVG	RSD
Dibenzothiophene	0.1	0.5	0.3	0.4	0.1	0.1	123	7	Naphthalene	0.5	0.9	0.4	1.9	0.3	0.3	27	12
Benzothiazole	9.4	40	57	114	14	113	72	13	Acenaphthylene	0.5	0.2	0.7	0.9	0.4	0.6	26	20
2-Methylthiobenzothiazole	2.6	5.9	1.6	2.5	0.5	5.8	34	1	Acenaphthene	−	0.7	0.4	0.4	−	0.3	42	1
2-Phenylbenzothiazole	4.1	8.3	9.8	12	9.0	23	28	7	Fluorene	0.1	0.9	0.5	0.9	−	0.5	283	1
∑Methyl-DBT	0.9	2.0	1.4	2.4	0.7	1.8	37	2	Phenanthrene	0.9	6.3	3.7	4.7	1.4	1.5	1841	20
∑Dimethyl-DBT	8.5	12	8.2	15	5.2	12	71	1	Anthracene	0.6	1.3	0.8	1.2	0.6	0.7	148	13
Benzo[b]naphtho[2,1-d]thiophene	2.7	3.2	1.3	1.4	0.3	1.2	310	22	2-Methylphenanthrene	1.9	3.8	0.9	3.6	0.5	0.6	300	9
Benzo[b]naphtho[1,2-d]thiophene	0.8	1.0	0.4	0.5	−	0.3	68	21	∑Methyl PAHs MW178[2]	2.8	9.8	3.8	6.1	2.7	3.3	624	9
Benzo[b]naphtho[2,3-d]thiophene	0.1	0.6	0.3	0.6	−	0.2	128	25	Fluoranthene	4.9	8.5	6.7	8.7	3.2	4.4	1289	28
∑ PASHs[1]	31	70	79	139	37	148	871	15	Pyrene	11	25	23	27	18.0	20	921	25
Acridine	−	0.2	0.2	0.6	−	−	35	41	1-methylpyrene	0.8	1.1	1.1	1.3	0.2	0.7	56	1
Carbazole	−	0.3	0.3	0.5	0.4	0.4	272	31	∑PAHs MW216[3]	4.2	5.5	5.5	8.6	1.2	2.2	578	9
Dibenzofuran	−	0.1	0.2	0.3	−	−	133	6	Benzo[a]anthracene	1.0	1.4	1.0	2.9	−	0.7	405	22
Benzo[b]naphtho[2,1-d]furan	0.5	0.7	0.3	1.1	−	0.2	220	16	Triphenylene	2.7	3.6	1.5	3.5	0.6	1.0	111	13
Benzo[b]naphtho[1,2-d]furan	0.4	0.4	0.2	0.2	−	0.1	90	13	Chrysene	1.9	2.5	1.5	3.3	0.6	0.8	351	20
Benzo[b]naphtho[2,3-d]furan	−	0.5	−	−	−	−	135	14	∑Methyl PAHs MW228[2]	17	22	10	13	13	13	458	2
Diphenylamine	−	0.9	3.6	4.9	2.6	2.6	21	1	Benzo [b]fluoranthene	1.6	2.6	0.7	2.8	0.1	0.5	289	24
N-phenyl-2-naphthylamine	3.1	7.8	9.7	48	−	−	−	−	Benzo [k]fluoranthene	0.3	0.7	0.5	0.7	−	−	133	22
									Benzo[j]fluoranthene	0.4	0.8	0.7	0.8	0.6	0.8	120	23
									Benzo[e]pyrene	2.9	3.6	2.6	4.0	1.1	2.2	225	23
									Benzo[a]pyrene	1.1	1.5	1.4	2.0	0.8	1.3	284	24
									Perylene	0.3	0.4	0.6	0.9	1.0	1.1	79	24
									Dibenz[a,h]anthracene	−	0.2	−	0.2	−	−	55	17
									Indeno[1,2,3-cd]pyrene	0.8	1.1	0.8	1.2	0.5	0.6	174	26
									Benzo [g,h,i]perylene	5.0	5.7	4.4	−	3.3	6.6	187	28
									∑PAHs[4]	58	82	73	94	47	58	8648	22
									∑16-EPA PAHs	31	52	46	59	30	36	6453	22
									∑8-EPA PAHs	9.4	13	8.3	17	2.5	6.1	1861	19

[1]Sum of all determined PASHs and benzothiazoles without 2-mercaptobenzothiazole; [2]Sum of methylated PAHs (including separately determined isomers); [3]Sum of all PAHs with MW=216: methylated fluoranthenes and pyrenes and/or benzofluorenes [4]Sum of all quantified PAHs: parent and methylated.

concentration in the S sample reaches 284 μg/g. The concentrations of PAHs found in the Spanish paver are thus highly above the concentrations typical for car tires. It is possible, that the paver contains PAHs originating from sources different from car ELTs, like tar coating. The PAHs concentrations in sample S are in the range of the concentrations found in coal-tar-based seal coats (CTBS), where the sum of 16 EPA PAHs ($\sum$16-EPA) reaches 66 mg/g (Mahler et al., 2012) (in this study $\sum$16-EPA = 6.5 mg/g rubber). Coal tar pitch, a distillation product of coal tar, is used as a binder in coatings for roofing and paving (e.g. CTBS) and the $\sum$16-EPA reaches in pure CTP 294 mg/g (Machado et al., 1993). In the previous study of Llompart et al. (2013) different CR samples originating from Spanish playgrounds were analyzed together with purchased CR mats samples. Five of these examined samples contained very high PAH levels, comparable with those found in the present study for sample "S". All these samples were purchased from the same retailer but in different years; namely in 2012 (the 5 tiles analyzed by Llompart et al., in 201,3[46]) and in 2018 (sample S analyzed in this study).

Next to the parent PAHs also methyl PAHs were determined. Several groups of methylated PAHs were tentatively identified by suspect-screening and three methylated PAH groups (MWs=192, 216 and 242) were quantified. It should be noted that the group of PAHs with MW=216 ($C_{17}H_{12}$) might consist of methylated pyrenes and fluoranthenes as well as benzofluorenes; the mass spectra of these isomers are very similar.

Lately, alkylated-PAH, particularly methyl-PAHs, are getting more attention because of their ubiquity and toxicity, the latter sometimes higher than that of the parent PAHs (Lam et al., 2018; Brack and Schirmer, 2003). The concentrations of the three methyl PAHs isomers 5MCHY (possibly carcinogenic to humans; one of the PAHs included by US EPA in the Toxic Release Program (Environmental Protecti, 2018)), 1MPYR and 2MPHN were determined in this study. Several structural isomers were also identified and their concentrations were calculated using the chromatographic responses of these three identified methylated PAHs. The presence of 5MCHY could be detected in several samples but it could not be quantified because of low concentrations and

additionally coelution with other methylated isomers. 5MCHY could be quantified in the S samples resulting in a concentration of ca. 10 μg/g (a chromatogram of partially separated 5MCHY is presented in the Supporting Information; see Fig. S2).

The results presented here show that the three groups of methylated PAHs have a substantial contribution to the sum of all quantified PAHs (see Fig. 1A); the determined methyl PAHs make up for 20−40% of all measured PAHs. In case of the Spanish tile (S) all quantified PAHs make up for almost 1% w/w (ca. 9 mg/g) of the whole tile. This tile shows not only much higher concentrations, but also the pattern of the determined PAHs differs significantly from the F, T and C samples: e.g. phenanthrene makes up for 21% of all quantified PAHs (see Fig. 1A, whereas the group $\sum$MW=178 PAHs consists almost completely of phenanthrene). The percentages of $\sum$MW=178 PAHs are significantly lower in case of the three other sample groups; tiles (T) consist for 6.4% of phenanthrene (mainly) and anthracene and the percentages in C and F are lower. Sample S contains a relatively small fraction of methylated PAHs with MW=242 (5.4%); while these percentages are the highest for samples F and C, exceeding 26% (Fig. 1B). These differences in PAHs' profiles support the assumption concerning the different origin of PAHs present in the Spanish tail.

Principal component analysis (PCA) allowed to distinguish three sample clusters: S, F and a cluster comprising T and C (see Fig. 2). Extremely high concentrations found in samples S shift them far away on PC1. Clusters T + C and F are different based on PC2. This shift on PC2 is due to the patterns of the measured compounds. The loading of the heterocyclic compounds (e.g. benzothiazole) is the strongest on PC2.

In general, the concentrations of all determined compounds are higher in samples T in comparison to F and C (see Table 3 and Table S1 for the detailed results). However, the order of concentrations is different between the compounds, what is depicted in Fig. 1C and D.

The profiles of samples T and C show the best resemblance (see the profiles of analyzed PAHs and PASHs in Fig. 1A and B). These differences between samples F and T + C may be explained by weathering of the football pitch samples. This hypothesis can be
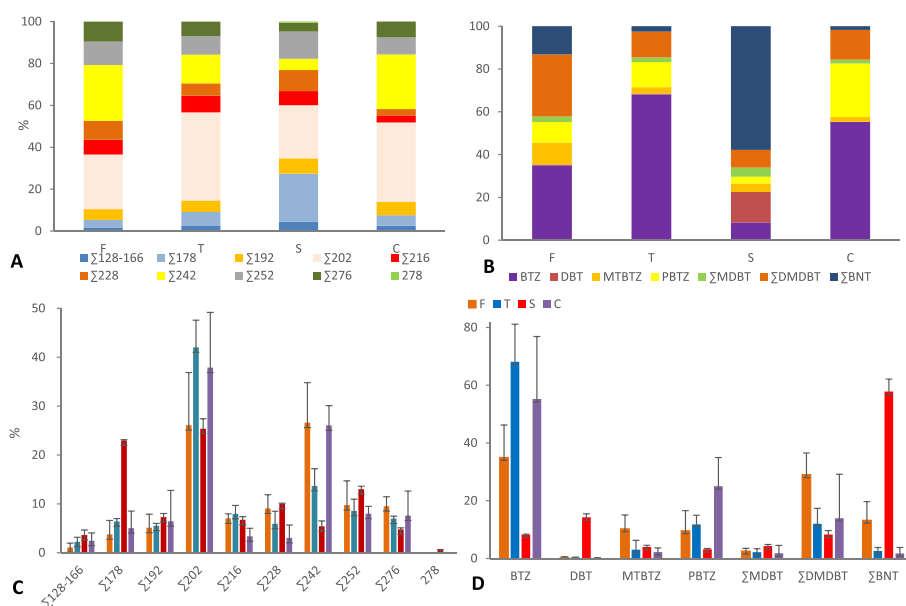
Fig. 1. Profiles of PAHs (A), and PASHs and benzothiazoles (B) observed in turf rubber granulate (F), tiles (T), Spanish tile (S), car tires (C). Profiles of PAHs (C), and PASHs and benzothiazoles (D) depicted per samples' group: $\sum$128−166: NAP, ACY, ACE, FLU; $\sum$178: PHN, ANC; $\sum$192: $\sum$methyl-178; $\sum$202: FLA, PYR; $\sum$216: $\sum$methyl-202; $\sum$228: BaA, TPH, CHY; $\sum$242: $\sum$ methyl-228; $\sum$252: BbF, BkF, BjF, BeP, BaP, PER; $\sum$276: IND, BghiP; 278: DahA.
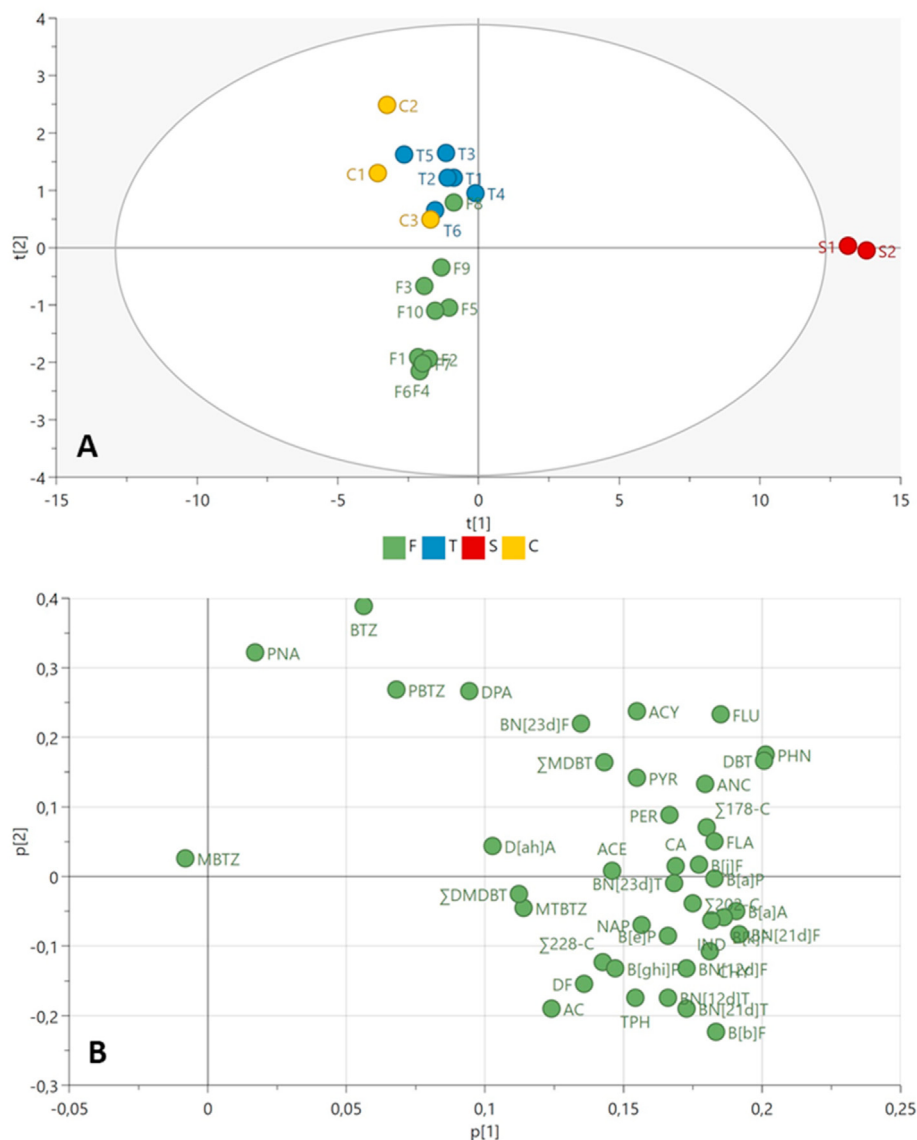
Fig. 2. Principle Component Analysis: (A) Scores and (B) Loadings. 41 variables and 21 observations in four classes: rubber granulate from football fields (F-green), tiles (T-blue), Spanish tile (S-red), car tires (C-yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

supported by setting the percentages ratios of C/F and T/F against the K_{ow} of the measured PAHs (this value is increasing with increasing carbon amount); see Fig. 3. The ratios of C/F and T/F are decreasing, which indicates enrichment of heavier PAHs (with higher K_{ow}) in weathered CR samples (F).

Acridine and carbazole, suspected carcinogens, could be identified in majority of the tile samples (T and S) and in a few weathered football field samples. Azarenes, like acridine, are more water soluble and more mobile than homocyclic PAHs with similar molar masses. Consequently, they can be faster transported to the ground water. The concentration of BZT in rubber turf (F) is lower than in car tires (C) and much lower than in tiles (T). In case of DBT and alkylated DBTs, compounds with K_{ow} values higher than BZT, the differences are not so large. BZT is known to be stable after weathering processes, and was reported as indicator of the contribution of street runoff to sediments in urban areas (Spies et al., 1987). It is also worth noticing that the rubber turf samples (F) contain relatively more benzo [*b*]naphthothiophenes and benzo [*b*]naphthofurans than the mats samples. These compounds have

high K_{ow} values (above 5) and to our knowledge have not been determined in CR samples before.

These findings emphasize the need for longitudinal studies on vaporization, leaking, degradation, sorption and other weathering processes taking place in CR. The RIVM study (Rijksinstituut voor Volks, 2017) reported slightly higher concentration of PAHs measured in new pitches compared to older pitches; this could also indicate leaching, evaporation or degradation.

The applied analytical method allowed identifying and quantifying two aromatic amines: diphenylamine (DPA) and N-phenyl-2-naphthylamine (PNA). Almost all injected samples also showed the presence of a huge peak that was identified by the NIST library as N-1,3-dimethylbutyl-N′-phenyl-p-phenyldiamine (6PPD); an example chromatogram with a distinct peak identified as 6PPD together with the mass spectrum are presented in the Supporting Information in Fig. S3. Due to the absence of a standard we could unfortunately not confirm this one, but this compound was also identified and quantified in the ERASSTRI study published in 2020 (Schneider et al., 2020). A recent study of Tian et al. (2020) showed
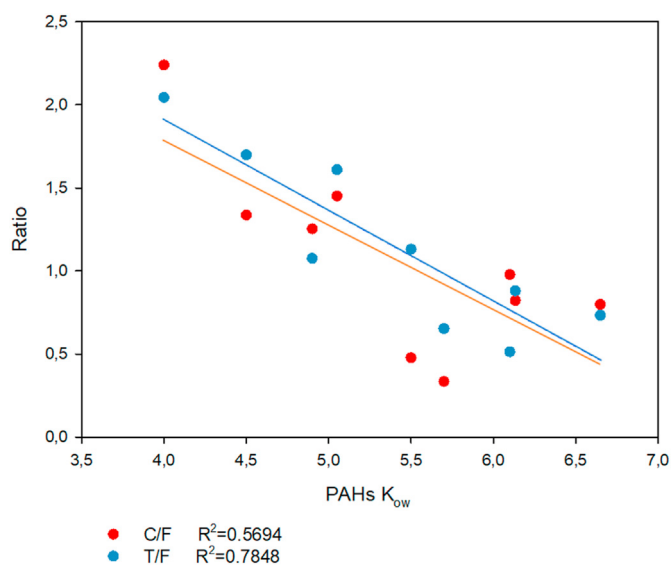
Fig. 3. Ratios of the percentages of PAHs C/F and T/F plotted against the Kow of the measured PAHs.

that 6PPD-quinone present in roadway runoff is highly lethal to Coho salmon. 6PPD-quinone is produced when 6PPD, a primary antioxidant used in rubber tires, reacts with ozone and prevents rubber cracking. PNA, a suspected carcinogen according to ECHA, which can be metabolized to the carcinogenic β-naphthylamine (European Chemical Agency., 2016), is present in the T samples (median ca. 10 μg/g) in and in lower concentrations in rubber turf (F). To our knowledge PNA and DPA, have not been determined in CR before. PNA and 6PPD are common antioxidants used in the rubber production and their presence was also tentatively confirmed in a suspect-screening in the latest study of US EPA (US Environmental Protection Agency (US EPA); Centers for Disease Control and Prevention(CDC), 2019), but they were not quantified in that study.

5. Conclusion

The developed analytical method based on sonication, SPE and GC-MS analysis gave the best results for extraction and analysis of a wide suite of aromatic compounds (including PAHs, heterocyclic PAHs, aromatic amines, etc.) present in recycled rubber. 21 heterocyclic aromatic compounds, two aromatic amines and several methylated PAHs together with the 16 EPA PAHs and TPH, BeP, BjF and PER were identified and quantified in CR samples originating from football pitches, rubber tiles and car tires. Several of these compounds, like benzonapthothiophenes, benzonaphthofuranes, 2-methylthiobenzothiazole, diphenylamine and N-phenyl-2-naphthylamine, have not been determined in CR before.

The results showed that PAHs concentrations in several samples are below the European safety limits for mixtures, but above the safety limits for consumer products. The mats purchased in Dutch shops exceed the EU limits for articles placed on the market for use by the public. In case of one sample, the Spanish mat, these limits are exceeded even a few hundred times. The PAH results presented here show that the methylated PAHs have a substantial contribution to the sum of all quantified PAHs: the determined methyl PAHs make up for 20−40% of all measured PAHs.

The obtained results stress the need for expanding the list of target compounds analyzed in CR and rubber playground tiles. Proper toxicological risk assessment of recycled rubber used in consumer products cannot be based on a limited set of target analytes alone, while ignoring many other hazardous compounds present in CR. The limited screening approach, applied in this study, shows that comprehensive non-target screening study is needed in order to better characterize toxic compounds associated with rubber matrix and ELT.

Credit author statement

Ewa Skoczyńska. Conceptualization, Investigation, Methodology, analysis, data interpretation, writing: Writing − original draft, Prof. dr. Jacob de Boer. Supervision, Conceptualization, Resources, writing: editing and final approval of the version to be published, Prof. dr. Pim E.G. Leonards. Conceptualization, data interpretation, assistance with statistics, writing: editing and final approval of the version to be published, Prof. dr. Maria Llompart. Making Spanish samples available, and information on Spanish situation crumb rubber, writing: interpretation Spanish data, editing and final approval of the version to be published.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130076.

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