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Presence of toxic and hazardous substances in recycled rubber surfaces; environmental distribution and toxicological implications

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TESE DE DOUTORAMENTO

PRESENCE OF TOXIC AND HAZARDOUS SUBSTANCES IN RECYCLED RUBBER SURFACES; ENVIRONMENTAL DISTRIBUTION AND TOXICOLOGICAL IMPLICATIONS

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INDEX


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DE SANTIAGO
DE COMPOSTELA

ABBREVIATIONS


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#

6PPD: N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine

6PPD-quinone: N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine quinone

A

ACN: acetonitrile

ACE: acenapthene

ACY: acenaphthylene

ANC: anthracene

ANOVA: analysis of variance

ATSDR: Agency for Toxic Substances and Disease Registry

B

B[a]A: benzo[a]anthracene

B[a]P: benzo[a]pyrene

B[b]F: benzo[b]fluoranthene

B[e]P: benzo[e]pyrene

B[ghi]P: benzo[ghi]perylene

B[j]F: benzo[j]fluoranthene

B[k]F: benzo[k]fluoranthene

BARGE: bioaccessibility research group of Europe

BBP: benzylbutylphthalate

BHA: butylhydroxyanisole

BHT: butylhydroxytoluene

BPA: bisphenol A

BTZ: benzothiazole

C

CAS: Chemical Abstracts Service

CE: collision energy

CHY: chrysene

D

D[ah]A: dibenzo[a, h]anthracene

DBP: dibutyl phthalate

DCHP: dicyclohexyl phthalate

DEA: diethyl adipate

DEHA: di-2-(ethylhexyl) adipate

DEHP: di-2(ethylhexyl) phthalate

DEP: diethyl phthalate

DIBP: diisobutyl phthalate

DIDP: diisodecyl phthalate

DIHP: diisoheptyl phthalate

DINP: diisononyl phthalate

DIPP: diisophentyl phthalate

DMA: dimethyl adipate

DMEP: dimethoxyethyl phthalate

DMP: dimethylphthalate

DNOP: di-n-octylphthalate

DOE: design of experiments

DPhP: diphenylphthalate

DPP: dipentylphthalate

E

ECHA: European Chemicals Agency

EEA: European Environment Agency

ELTs: End-of-life tires

EFSA: European Food Safety Authority

EI: electron impact

EPA: Environmental Protection Agency

EtAc: ethyl acetate

EU: European Union

F

FLA: fluoranthene

FLU: fluorene

FS: full scan

G

GAC: green analytical chemistry

GC: gas chromatography

GSP: green sample preparation

H

HMMM: hexa(methoxymethyl)melamine

HMW: high molecular weight

I

IDL: instrumental detection limit

IND: indeno[1, 2, 3-cd]pyrene

IQL: instrumental quantification limit

L

LLE: liquid-liquid extraction

LMW: low molecular weight

LOD: limit of detection

LOQ: limit of quantification

M

MBTZ: mercaptobenzothiazole

MeOH: methanol

MOF: metal organic framework

MS: mass spectrometry

MS/MS: tandem mass spectrometry

MW: molecular weight

m/z: mass-to-charge ratio

N

NAP: naphthalene

P

PAH: Polycyclic aromatic hydrocarbon

PHN: phenanthrene

POPs: persistent organic pollutants

PYR: pyrene

Q

Q: quadrupole

QqQ: triple quadrupole mass spectrometer

R

RAC: Committees for Risk Assessment

RSD: relative standard deviation

RTCR: recycled tire crumb rubber

S

SEAC: Socio-Economic Analysis

SIM: selected ion monitoring

SPE: solid-phase extraction

SPME: solid-phase microextraction

SRM: selective reaction monitoring

SVHC: substance of very high concern

T

TBP: 4-tert-butylphenol

TWP: tire wear particles

TRWP: tire and road wear particles

U

UAE: ultrasound assisted extraction

UBM: unified bioaccessibility method

US: ultrasounds

USA: United States of America


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ABSTRACT

Nowadays, the manufacture of new articles from the recycling of end-of-life materials (waste) is an increasingly common practice. Some of these products may come from the reuse of hazardous materials or wastes, or can contain substances that may be harmful, so the composition of these articles needs to be controlled and monitored. One example is the recycling of end-of-life tires (ELTs), which are reused to build various leisure and sports surfaces.

The main purpose of this thesis is the development and application of analytical methodologies for the determination of tire-rubber related organic compounds in recycled tire crumb rubber (RTCR) materials and their occurrence on the environment (air and water), as well as the potential risk of human exposure. For this objective, different sample preparation techniques have been used for the extraction of the compounds of interest prior to analysis by gas chromatography coupled to tandem mass spectrometry.

The present thesis is divided into six sections. Section I JUSTIFICATION AND OBJECTIVES comprises the motivations for the research and the selection of the studied matrices. It also explains the relevance of the selected compounds and the problems associated with them, as well as the need for new analytical methodologies for their evaluation and control.

Section II INTRODUCTION contains the studied substances and matrices, presenting a classification and their physicochemical description, as well as the different applications of RTCR surfaces and their parallelism with the tire wear particles (TWP). In addition, the diffusion of the compounds to the environmental compartments and the toxicological implications are also described. This part is followed by an explanation of the different analytical and extraction techniques employed.

Section III METHODOLOGY describes the analytical methods, including the sample preparation approaches and the analytical tools, employed for the analysis of the different matrices: surfaces made of tire rubber, water, air and synthetic human biological fluids.

Section IV RESULTS AND DISCUSSION displays the experimental work and results obtained during the development of this doctoral thesis, which corresponds to six research articles summarised in three chapters compiled in this section. Each of the articles includes an introduction and specific background, and a bibliographic discussion of the obtained results.

Chapter 1. DETERMINATION OF HAZARDOUS SUBSTANCES IN DIFFERENT RECYCLED RUBBER SURFACES

This chapter includes two publications dealing with the analysis of 42 target compounds, including polycyclic aromatic hydrocarbons (PAHs), plasticizers, antioxidants and vulcanisation additives by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) after ultrasound assisted extraction (UAE) of different recycled rubber samples.

The first article is based on the analysis by UAE-GC-MS/MS of 40 samples of different types of recycled rubber surfaces such as crumb rubber used as infill in artificial turf football pitches, playground flooring, tree root coverings, etc. Commercial samples of recycled rubber tiles and car tire samples were also analyzed. In addition, other alternatives were studied, such as cork infill (football pitches) or sand (playgrounds). In the second article, the issue of the use of recycled rubber granulate as infill in artificial turf pitches worldwide was assessed. A total number of 91 infill samples (78 crumb rubber and 13 alternatives) from 17 countries on 4 continents were analyzed, to determine the compounds mentioned above. Infill alternatives include cork, coconut fibre and thermoplastic elastomers. In both cases, the analytical methodology was validated in terms of analytical quality.

Chapter 2. ENVIRONMENTAL IMPACTS OF RECYCLED TIRE RUBBER: AIR AND WATER

This chapter comprises three articles. The first one is focused on the evaluation of the diffusion of several compounds (PAHs, plasticizers, antioxidants and vulcanisation additives) present in recycled crumb rubber into runoff water. For this purpose, a methodology based on solid-phase microextraction (SPME) followed by GC-MS/MS was

proposed to determine these chemicals in water leachates directly collected from football pitches. In addition, laboratory-scale runoff simulation experiments were carried out to assess whether there could be a persistent input of the target compounds into the runoff water.

The other two publications are based on the development of analytical methods for the sampling and analysis of air samples. These samples were collected in places related to tire rubber, such as locations where recycled tire rubber are placed (children's playgrounds or football pitches) or tires were stored, among others. In the first article a sampling step based on solid-phase extraction (SPE), employing commercial sorbents and a metal-organic framework (MIL-101), followed by UAE extraction was optimized. The second analytical method developed involved the use of SPME (instead of UAE) to isolate the target compounds from the SPE sorbent. In both cases, the extraction techniques were optimized by chemometric tools, and the analysis was performed by GC-MS/MS. After the validation of the analytical methods, the analysis of several real samples was performed.

Chapter 3. EVALUATION OF THE POTENTIAL RECYCLED CRUMB RUBBER BIOACCESSIBILITY

This chapter includes one publication. Currently, there is scarce information available on the risks of accidental oral ingestion of these materials, as well as data on the bioaccessibility of PAHs into human biological fluids. Therefore, a simulation of the digestion of recycled rubber infill was carried out employing a standardised protocol. In order to isolate the compounds, an analytical methodology based on SPE using commercial organic sorbents and an environmentally friendly alternative (pulverized cork) followed by GC-MS/MS analysis was developed. Finally, the bioaccessibility percentage of the PAHs detected in the fluids was calculated and a risk assessment of the ingestion of these materials by a young child was performed.

Section V presents a comprehensive GENERAL DISCUSSION of all the sections discussed above.

And finally, section VI, CONCLUSIONS, features the general conclusions drawn from the research work presented in this doctoral thesis.


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I. JUSTIFICATION AND OBJECTIVES

Over the last decades, waste generation has been an environmental management problem. One example are tires from end-of-life vehicles. This waste is being given a second life by reusing it to manufacture sports and leisure surfaces, such as artificial turf football pitches or playgrounds.

In recent years, social and environmental concerns have been growing regarding these recycled rubber surfaces due to the detection of substances harmful to human health and the environment in their composition. The compounds detected include organic compounds such as polycyclic aromatic hydrocarbons (PAHs), plasticizers, antioxidants, different endocrine disruptors, among others, as well as inorganic compounds and elements such as heavy metals.

In addition, the recycled rubber granules used as infill in football pitches are considered microplastics due to their small particle size, representing the largest intentional source of microplastics in the environment.

The present thesis is focused on the analysis and monitoring of PAHs, different plasticizers (adipates, phthalates and bisphenol A), antioxidants and vulcanisation additives in recycled rubber samples and in different environmental compartments (air and water), as well as the assessment of the oral bioaccessibility of PAHs in humans.

Therefore, different analytical methodologies have been developed, complying as well as possible with sustainability and the green analytical chemistry (GAC) and green sample preparation (GSP) principles, with the aim of determining the above-mentioned chemical compounds in a broad range of matrices of different nature (solid samples, air, water, and biological fluids).

The following objectives have been pursued during this doctoral thesis:

> I. Characterization of recycled rubber surfaces. Comprehensive analysis of different RTCR materials and evaluation of the problem at a global level.

An analytical method based on ultrasound assisted extraction (UAE) followed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) was employed to study 40 different recycled rubber and tire rubber samples, including artificial football pitches infill, children´s playgrounds flooring (indoor and outdoor), tree roots pavements and car tires, among others (**Section IV, Chapter 1, *1.1***).

Furthermore, the previously developed UAE-GC-MS/MS method was used for the global evaluation of recycled rubber granulate used as infill in artificial turf pitches. This research involved 91 samples (78 recycled rubber and 13 recycled rubber alternative materials) from 17 countries of 4 continents. The alternative materials included samples of cork, coconut fibre and thermoplastic polymer fillers (**Section IV, Chapter 1, *1.2***).

Concern about the environmental and human health impact of these surfaces is increasing. Therefore, studies were carried out on the distribution of compounds present in these materials into the runoff water and nearby air.

II. Study of the presence of substances from recycled rubber in runoff water and lab-scale assessment of the continuous diffusion into water.

For the determination of PAHs, plasticizers, antioxidants and vulcanisation additives in runoff water collected directly from football pitches, a method based on solid-phase microextraction (SPME) followed by GC-MS/MS analysis was selected. In addition, lab-scale experiments were performed to assess the continuous leaching of these chemicals into water (**Section IV, Chapter 2, *2.1***).

III. Development of air sampling and analysis methods for the determination of tire related compounds.

For the study of the air above the locations on which these recycled rubber surfaces are placed as well as the surrounding air, a sampling method based on active sampling by solid-phase extraction (SPE) was developed. Commercial sorbents and metal-organic frameworks (MOF) materials were used for the retention of the target compounds. Then, an UAE procedure was optimized by chemometric approach to ensure a high-throughput extraction of the compounds prior to GC-MS/MS analysis (**Section IV, Chapter 2, *2.2***).

To improve the above methodology, an air sampling-analysis method based on SPE-SPME-GC-MS/MS was then developed. This method avoids the consumption of organic solvents and allows an increase in sensitivity, detecting compounds at very low concentration levels (ultra-trace level) (**Section IV, Chapter 2, *2.3***).

Exposure to these substances by users of these surfaces is poorly documented. Therefore, an evaluation of the oral bioaccessibility of PAHs present in recycled rubber used as infill in artificial fields was carried out.

IV. Study of the *in-vitro* bioaccessibility of PAHs from recycled rubber granulate in synthetic human biological fluids.

The unified bioaccessibility method (UBM) developed by the Bioaccessibility Research Group of Europe (BARGE) was used for the simulation of recycled rubber human digestion. After an *in-vitro* digestion, SPE was employed to isolate the target PAHs contained in the bioaccessible fraction before GC-MS/MS analysis. Risk assessment evaluation was carried out to check the potential danger of accidental ingestion of these materials containing harmful and carcinogenic substances, which can occur especially in young children (**Section IV, Chapter 3, *3.1***).

II. INTRODUCTION

1. RECYCLED TIRE CRUMB RUBBER (RTCR)

1.1 INTRODUCTION

For some decades now, tire waste management becomes a problem in most developed societies due to the increasing number of tires discarded each year and the environmental problems associated with them. For example, in 2016 in the town of Seseña (near to Madrid, Spain) the fire at the tire dump that was located there, and illegally accumulated end-of-life tires (ELTs) for 15 years, had a strong environmental and social impact, resulting in the evacuation of thousands of people [1].

In 2019, 3.45 million tonnes of ELTs were generated in Europe (27 European Union countries plus Norway, Serbia, Switzerland, Turkey and United Kingdom) [2]. These waste materials have a high environmental effect as they are not biodegradable. The problems associated with landfill of ELTs have led governments and institutions to look for alternatives for their reuse [3].

Until a few years ago, incineration was one of the most common solutions, employed to obtain energy from ELTs with the consequent release and spread of the harmful substances present in tire rubber into the atmosphere [4]. It is estimated that 94 % of these ELTs are collected and treated to give old tires a second useful life as material recycling (53 %) or energy recovery (40 %), among others like civil engineering [2]. The most common form of recycling is to shred and grind them into rubber granulate, which is then used to manufacture different types of structures such as mulches, tiles, etc., for recreational or sporting purposes [5].

1.2 APPLICATIONS AND ALTERNATIVE MATERIALS

Tires are complex materials with compositions and properties that vary depending on the vehicle for which the tire is manufactured. The main components of a conventional tire are natural rubber, synthetic rubber polymers (styrene and butadiene rubber, SBR), steel wire, fabric cords, filler materials (mainly black carbon and silica) and different antioxidants, antiozonants and curing system (cross linking and vulcanising agents) [6,7]. **Figure 1** shows the different components of a passenger car tire [6,7].

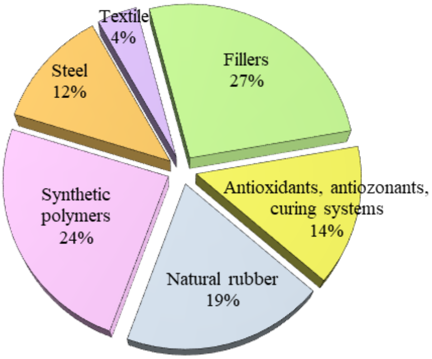

Figure 1. Components of a passenger car tire.

The average life of a tire highly depends on the user's use of the vehicle, but it is estimated to last between 3 and 5 years. Therefore, as commented before, several million tonnes (around 1 billion) of ELTs are generated each year in the world and it is necessary to look for alternatives to give them a second useful life instead of storage them [8].

The need to achieve the reuse of ELTs has motivated different European institutions and organisations to carry out research projects in this area fostering the circular economy and sustainability with the main objective of recycling and constructing new tires employing ELTs [9].

The problem with ELTs is that several chemical components, such as cross-linking agents, are not easily degradable and they reprocessed present difficulties, thus the main way of recycling is shredding and grinding waste tire rubber [3]. The most employed techniques to shredding scrap tires into ground tire rubber are ambient and cryogenic methods, obtaining shredded materials with different characteristics [10]. This recycled product has different applications such as derived rubber granules and powder, a product obtained after shredding and removal the steel and the textile cords from the shredded rubber [11].

1.2.1 Synthetic Turf Football Pitches

The most common application of recycled tire rubber granules is employing this material as infill in synthetic turf pitches. These sport surfaces are comprised of three components: the backing, the infill, and the grass fibres, as is shown in **Figure 2**.

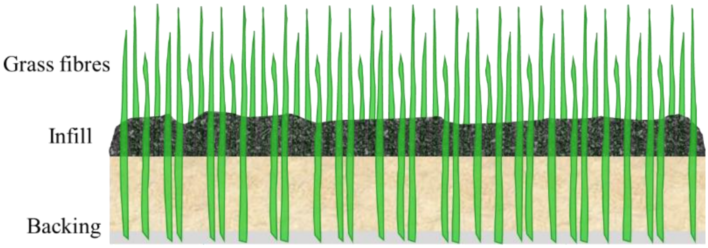

Figure 2. Components a synthetic turf football pitch.

Synthetic turf pitches appeared in the sixties in USA and in the eighties in Europe to be an alternative to natural grass in different places [4]. In 2017, the number of pitches in Europe was 13000 synthetic turf pitches and 47000 mini pitches, both to play football. It is estimated that every year around 1300 new pitches are constructed, some of them for replacing older fields [12]. Also, in USA the number of pitches is similar to Europe [13]. The number of tires employed per football pitch range between 20000 and 40000 to perform the infill of the field [14].

The 95 % of the synthetic turf pitches installed in USA (in Europe a little less) employ crumb rubber as infill, while the remaining 5 % are alternative infill materials to recycled rubber such as cork, coconut fibre or thermoplastic elastomers, among others [4,12].

Rubber granulate infill represents the largest intentionally added source of microplastics in the environment, representing around 38 % (16000 tonnes per year) [15].

1.2.2 Other Applications

Another of the most widespread applications of rubber granulate is the manufacture of tiles or mulches that are used as flooring in playgrounds, gyms, sports fields (athletics), cattle stables and urban pavers (e.g., tree roots protectors), among others. This kind of product is widely used as floor in children´s playgrounds due to the prevention of injuries and the colourful colours they can have, that are more attractive for children than conventional floor [5]. Nevertheless, these surfaces can achieve high temperatures, especially those placed outdoor and cause burn to the users of these surfaces, mainly children [16].

Figure 3. Playgrounds and others crumb rubber applications.

1.3. RTCR CHEMICAL COMPOSITION

The chemical composition of the recycled crumb rubber used in the above-mentioned applications is still under study, although much information has already been reported by several authors. This complex matrix is made up of different inorganic substances including heavy metals and metalloids [17–21]. Several organic compounds conformed this material, including volatile and semivolatile chemicals and persistent organic pollutants (POPs) such as PAHs, different plasticizers (phthalates and adipates), antioxidants and antiozonants, polychlorinated biphenyls (PCBs), chlorinated paraffins, vulcanisation additives, among others [22–26].

In addition, other components of artificial turf fields, such as synthetic grass, contain environmental and health concerning compounds like perfluoroalkyl substances (PFAS) [27].

1.4 LEGISLATION

There is scarce legislation specifically covering these materials constructed with recycled tire rubber, although their composition contains a large number of chemical compounds that can be hazardous to human health and the environment [28,29].

Attending to the legal concentration limits in force in 2018 of 100 mg kg^{-1} for benzo[a]pyrene (B[a]P) and dibenzo[ah]anthracene (D[ah]A), and 1000 mg kg^{-1} for benzo[e]pyrene (B[e]P), benzo[a]anthracene (B[a]A), chrysene (CHY), benzo[b]fluoranthene (B[b]F), benzo[j]fluoranthene (B[j]F) and benzo[k]fluoranthene (B[k]F), the Dutch National Institute for Public Health and the Environment proposed a restriction of 17 mg kg^{-1} for the combined concentration of these eight PAHs based on scientific evidence that the legal limits were high to ensure the health of users of these surfaces [30,31].

The Committees for Risk Assessment (RAC) and Socio-Economic Analysis (SEAC) of the European Chemicals Agency (ECHA) agreed with this lowering of the limit for these compounds with some slight modification (20 mg kg^{-1} instead of 17 mg kg^{-1}), in order to ensure the protection of the health of users and the cancer risk from low levels exposure [28].

After that, the EU member states supported the RAC and SEAC´s decision and adopted it by the European Commission [32]. Since 10 August 2022, in the EU and European Economic Area (EU plus Iceland, Liechtenstein and Norway) countries, it is forbidden the market and the use of granules and mulches if they contain more than 20 mg kg^{-1} of the sum of eight PAHs mentioned above [33].

Regarding the microplastics pollution, in January 2019, the ECHA suggested a restriction proposing two ways to control recycled rubber infill spreading from artificial pitches. One was to require the use of preventive and risk management measures to avoid the release of infill particles after a transition period of three years, and the second was banning the market of this material after a six-year period [34]. The SEAC and RAC supported this proposal with some minor modifications, and it is currently in the hands of the European Commission for a decision with EU member states [35].

Finally, in May 2021, the ECHA published a research in which over 300 chemicals, potentially present in the crumb rubber, were identified [36]. Several of these substances (heavy metals, phthalates, among others) need further evaluations due to their hazardous properties that could affect people and environment.

Therefore, in view of this latest information, in the coming months or years will see updates to the legislation relevant for these materials.


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2. STUDIED COMPOUNDS

As mentioned in the previous section, tires, and therefore materials made from recycled rubber, contain various chemical substances in their composition that can be harmful to both human health and the environment [37].

The present dissemination is focused on the evaluation of four families of tire rubber additives: PAHs, plasticizers, antioxidants and vulcanisation additives; and to a lesser extent in substances of recent environmental concern also present in these materials employed as antiozonants and cross-linking agents.

2.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

2.1.1 Introduction

PAHs are a group of substances catalogued as persistent organic pollutants (POPs) formed during incomplete combustion processes, both naturally and by anthropological activity. Natural sources of PAHs include volcan eruptions and wood fires, among others, while human activity involve the combustion of coal, gas, oil, wood or some organic substances, cigarettes smoke, among other sources [38].

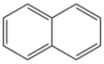
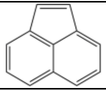
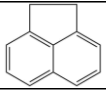
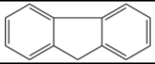
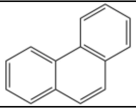
Car tires tread rubber have extended oils (about 15-20 %) in their composition, with highly aromatic oils being the most used, indicating that a high content of PAHs will be present [37]. In addition, carbon black (used as filler in the tire rubber) also contained PAHs [39,40]. Therefore, several PAHs are contained in the recycled rubber materials.

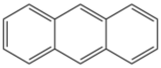
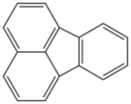
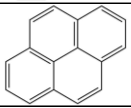
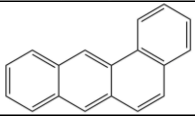
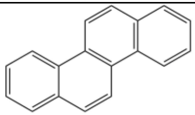
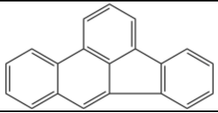
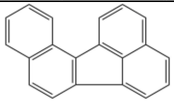
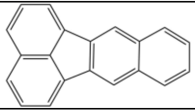
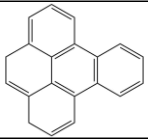
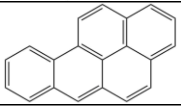
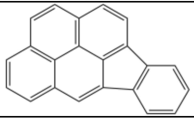
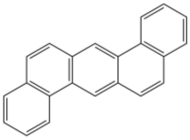
2.1.2. Physico-chemical properties

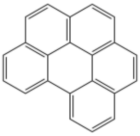
The structure of PAHs consists of at least two fused benzene rings without heteroatoms. The simplest example is naphthalene (NAP). More complex PAHs results for the fusion of more benzene rings or alkyl groups. Rings can be fused together alternately (as in phenanthrene) or linearly (as in anthracene).

In the environment PAHs can be found in soils, water and air. The distribution among these environmental compartments will be influenced by the vapour pressure of the compounds. PAHs with 2-3 rings have a high vapour pressure, so they will evaporate into the gas phase easily. Whereas larger PAHs (4 or more rings) are often found adsorbed on particulate matter (PM2.5 and PM10) and can cause harm to living organisms through inhalation. **Table 1** lists the studied PAHs in the present thesis, their abbreviation, CAS number, molecular weight (MW) and chemical structure. Data was obtained from European Chemicals Agency and chemical structures were constructed employing ChemDraw 20.1.1.

Table 1. Target PAHs. Name, abbreviation, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Compounds	Abbreviation	CAS	MW	Chemical Structure
Naphthalene	NAP	92-20-3	128.2	
Acenaphthylene	ACY	208-96-8	152.2	
Acenaphthene	ACE	83-32-9	154.2	
Fluorene	FLU	86-73-7	166.2	
Phenanthrene	PHN	85-01-8	178.2	

Anthracene	ANC	120-12-7	178.2	
Fluoranthene	FLA	206-44-0	202.3	
Pyrene	PYR	129-00-0	202.3	
Benzo[a]anthracene	B[a]A	56-55-3	228.3	
Chrysene	CHY	218-01-9	228.3	
Benzo[b]fluoranthene	B[b]F	205-99-2	252.3	
Benzo[j]fluoranthene	B[j]F	205-82-3	252.3	
Benzo[k]fluoranthene	B[k]F	207-08-9	252.3	
Benzo[e]pyrene	B[e]P	192-97-2	252.3	
Benzo[a]pyrene	B[a]P	50-32-8	252.3	
Indeno[1, 2, 3-cd]pyrene	IND	193-39-5	276.3	
Dibenzo[a, h]anthracene	D[ah]A	53-70-3	278.3	

Benzo[ghi]perylene	B[ghi]P	191-24-2	276.3	

2.1.3. Toxicity

These compounds can entry into human body by different routes such as oral ingestion, inhalation or dermal contact. Larger PAHs enter the human digestive tract by ingestion of food, and their presence may be due to the type of cooking or absorption at some stage of development of the organism ingested. On the other hand, inhalation is a major route for smaller PAHs [41].

The structure of PAHs is decisive for their biological activity because some of them have a zone known as the bay region. This region is formed by the branching of the benzene rings that make up PAHs with 3 or more rings, e.g., phenanthrene (**Table 1**).

When PAHs enter the human body, they undergo transformations due to the stages of metabolic pathways forming metabolic derivatives which have predicted toxic properties such as neurotoxicity, immunotoxicity, genotoxicity and carcinogenic activity, among others [42].

In summary, PAHs will form epoxides that are highly reactive to nucleophilic attack by DNA. The formation of adducts with DNA (1st stage of chemical carcinogenesis) will lead to altered functioning of the regulation of gene expression. For example, (+)-Benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide is associated with alteration of p53 gene activity, which is responsible for tumour suppression [43]. This compound is related to the cancers provoked by tobacco [44].

2.1.4. Legislation and regulatory considerations

For decades, PAHs have been monitored in the environment and studied for the effects mentioned above. In 1977 the EPA stablished the Priority Pollutants List, a document intended for the practical application of the regulation of individual chemicals, including 16 of the 18 PAHs studied in these thesis (**Table 2**) [45]. In addition, PAHs are listed in the United States governmental Agency for Toxic Substances and Disease Registry (ATSDR) Substance Priority List [46].

In the EU, 8 of the 18 PAHs studied in this thesis are currently considered carcinogenic [47]. In **Table 2** are represent the PAHs considered carcinogenic by ECHA. It is expected that in the future more PAHs will be considered carcinogenic.

As compounds that have been studied for decades, PAHs are regulated in various matrices. Regarding the studied materials, as mentioned in the previous section, only eight PAHs are regulated by the EU. The legal concentration limit is 20 mg kg^{-1} for the sum of B[a]A, CHY, B[b]F, B[j]F, B[k]F, B[a]P, B[e]P and D[ah]A [33].

Table 2. ECHA and EPA classification of the studied PAHs.

Compound	ECHA carcinogen	EPA priority pollutant
NAP		X
ACY		X
ACE		X
FLU		X
PHN		X
ANC		X
FLA		X
PYR		X
B[a]A	X	X
CHY	X	X
B[b]F	X	X
B[j]F	X	

B[k]F	X	X
B[e]P	X	
B[a]P	X	X
IND		X
D[ah]A	X	X
B[ghi]P		X

2.2 PLASTICIZERS AND BISPHENOL A

2.2.1 Introduction

The European Environment Agency (EEA) defines plasticizer as *“a substance which when added to a material, usually a plastic but also paint or an adhesive, makes it flexible, resilient and easier to handle”* [48]. The most common employed plasticizers are phthalates, esters of phthalic acid, and to a lesser extent adipates, esters derived from adipic acid [48]. These chemicals are commonly used to modify the properties of different polymers such as plastic materials and rubber (including tire rubber). Adipates are in different coating, washing and cleaning applications, among others [49].

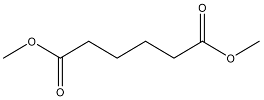
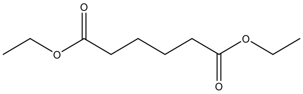
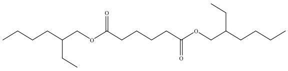
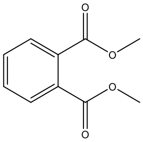
According to the European Food Safety Authority (EFSA), Bisphenol A (BPA) “*is a chemical that is mainly used in combination with other chemicals to manufacture plastics and resins*” [50]. The main use of BPA is to produce polycarbonate plastic which is employed in many consumer goods such as bottles for drinks, CDs and DVDs, among others [51].

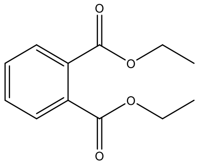
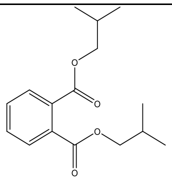
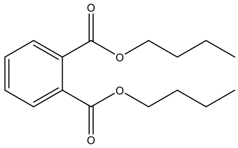
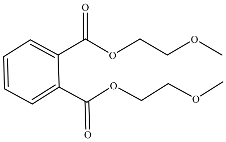
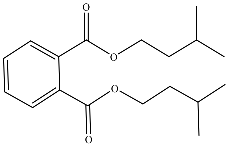
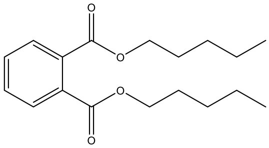
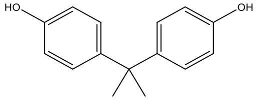
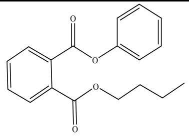
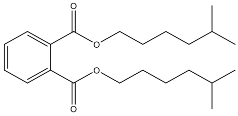
2.2.2 Physico-chemical properties

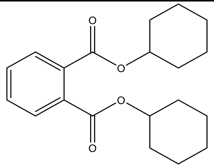
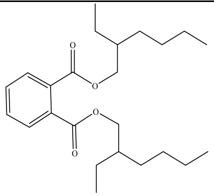
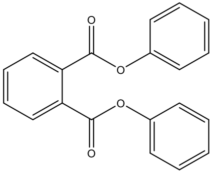
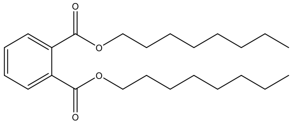
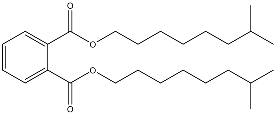
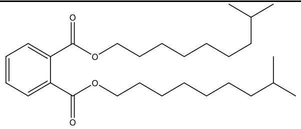
Phthalates are ubiquitous compounds which can be found in environmental compartments, food and personal care products, among other materials. The most known are ortho-phthalates such as di-2(ethylhexyl) phthalate (DEHP) [52]. In Europe, the production of phthalates represents about 80 % of all plasticizers market. Ortho-phthalates are classified into two main groups: low molecular weight (LMW) and high molecular weight (HMW). These subgroups present different characteristics, applications and legal requirements [53].

Phthalates present a low melting and high boiling points make them attractive additives to provide or improve flexibility to different products. These substances are not covalent bond but physically linked to the plastics or rubber materials which may produce the leaching of them or the volatilization of short alkyl chains phthalates [54,55]. In **Table 3** are summarized the studied platicizers in the present thesis, their abbreviations, CAS number, molecular weight (MW) and chemical structure. Data was obtained from European Chemicals Agency and chemical structures were constructed employing ChemDraw 20.1.1.

Table 3. Target plasticizers. Name, abbreviation, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Compounds	Abbreviation	CAS	MW	Chemical Structure
Adipates				
Dimethyl adipate	DMA	627-93-0	174	
Diethyl adipate	DEA	141-28-6	202	
Di-2(ethylhexyl) adipate	DEHA	103-23-1	371	
Orto-phthalates				
Dimethyl phthalate	DMP	131-11-3	194.2	

Diethyl phthalate	DEP	84-66-2	222.2	
Diisobutyl phthalate	DIBP	84-69-5	278.3	
Dibutyl phthalate	DBP	84-74-2	278.3	
Dimethoxyethyl phthalate	DMEP	117-82-8	282.3	
Diisopentyl phthalate	DIPP	605-50-5	306.4	
Dipentyl phthalate	DPP	131-18-0	306.4	
Bisphenol A	BPA	80-05-7	228.3	
Benzylbutyl phthalate	BBP	85-68-7	312.4	
Diisoheptyl phthalate	DIHP	41451-28-9	362.5	

Dicyclohexyl phthalate	DCHP	84-61-7	330.4	
Di-2(ethylhexyl) phthalate	DEHP	117-81-7	390.6	
Diphenyl phthalate	DPhP	84-62-8	318.3	
Di-n-octyl phthalate	DNOP	117-84-0	390.6	
Diisononyl phthalate	DINP	28553-12-0	418.6	
Diisodecyl phthalate	DIDP	26761-40-0	446.7	

2.2.3 Toxicity

In recent years, four LMW phthalates: DIBP, DBP, BBP and DEHP have been classified by the ECHA as substances toxic for reproduction (category 1B) and endocrine disruptors (see **Table 4**). DBP is also under assessment as a persistent, bioaccumulative and toxic compound. They can affect the human hormonal system which can damage fertility or foetus, especially influence the sexual development of male [52,56].

BPA is catalogued as a toxic for reproduction and endocrine disrupting substance as the above-mentioned phthalates, but also as a skin sensitizer by ECHA according the harmonised classification and labelling [57]. Long term exposure to BPA has been also related with cancer and diabetes in adults [58].

In addition, the increased risk for other diseases such as obesity was related to the phthalates and BPA endocrine disruption function [59].

2.2.4 Legislation and regulatory aspects

EPA included six ortho-phthalates in the Priority Pollutants List (see **Table 4**) [45]. Furthermore, 3 phthalates (DBP, BBP and DEHP) are listed in the United States governmental Agency for Toxic Substances and Disease Registry (ATSDR) Substance Priority List [46].

As commented in the previous sub-section, four phthalates are considered as endocrine disrupting and reprotoxic compounds by ECHA. The use of these four mentioned plasticizers is regulated in some materials. The European Commission in December of 2018 approved an amending to the Regulation (EC) No 1907/2006 concerning these four phthalates (DIBP, DBP, BBP and DEHP) limiting the concentration of these four chemicals, individually or in combination, to 1000 µg g^{-1} (0.1 % w/w) in plasticized materials of articles placed on the market after 7 July 2020 [60]. Plasticized material according to this regulation includes rubber material, therefore the limit covers the recycled rubber surfaces and materials.

Recently, ECHA published a report containing an evaluation of ten phthalates according to their toxicity for reproduction properties, concluding that further studies are needed to determine whether the use of these compounds should be restricted because of their effects on human health and the environment [61]. In this document are listed three orto-phthalates studied during this dissemination: diisopentylphthalate, dipentylphthalate and dimethoxyethyl phthalate.

Table 4. ECHA and EPA classification of the studied plasticizers.

Compound	ECHA reprotoxic	EPA priority pollutant
DMA		
DEA		
DEHA		
DMP		X
DEP		X
DIBP	X	
DBP	X	X
DMEP		
DIPP		
DPP		
BPA		
BBP	X	X
DIHP		
DCHP		
DEHP	X	X
DPhP		
DNOP		X
DINP		
DIDP		

2.3 VULCANISATION ADDITIVES AND ANTIOXIDANTS

2.3.1 Introduction

Vulcanisation additives involve the chemical compounds which take part in the vulcanisation process, meaning for vulcanization "*a chemical reaction of sulfur (or other vulcanizing agent) with rubber or plastic to cause cross-linking of the polymer chains*"; with the aim of increas the strength and resiliency of the polymer [62]. Regarding all the vulcanization system (vulcanization agents, activators and accelerators), its components represent around 4 % of the car tire composition [37].

According to the European Commission, antioxidant is "*any substance that prevents or reduces damage caused by free-radicals (highly reactive chemicals containing oxygen) which attack other molecules and modify their chemical structure*" [63]. They are also known as preservatives, with an extensive application in several consumer goods such as personal care products or in food stuff. In tire rubber, several antidegradants, including antioxidants are added to protect the material from oxygen, ozone or wear, among others [64].

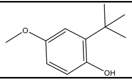
2.3.2 Physico-chemical properties

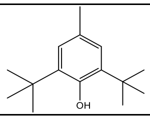
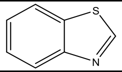
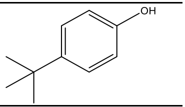
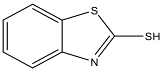
The antioxidants butylhydroxyanisole (BHA) and butylhydroxy toluene (BHT) are added to the tire rubber to prevent the degradation produce by crazing, but no protection effect against ozone has been reported. The principal disadvantage of BHA and BHT is their high volatility [64].

The vulcanization accelerators benzothiazole (BTZ), 4-tertbutylphenol (TBP) and 2-mercaptobenzothiazole (MBTZ) are employed in tire rubber composition to improve mechanical strength and abrasion endurance [64,65]. These substances are volatile compounds that may release easily to the atmosphere [66].

The studied antioxidants and vulcanization additives, their abbreviation, CAS number, molecular weight (MW) and chemical structure are depicted in **Table 5**. Data was obtained from European Chemicals Agency. The chemical structures were constructed employing ChemDraw 20.1.1.

Table 5. Target antioxidant and vulcanization additives. Name, abbreviation, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Compounds	Abbreviation	CAS	MW	Chemical Structure
Butylhydroxyanisole	BHA	121-00-6	180.2	

Butylhydroxytoluene	BHT	128-37-0	220.2	
Benzothiazole	BTZ	95-16-9	135.2	
4-tertbutylphenol	TBP	98-54-4	150.2	
2-mercaptobenzothiazole	MBTZ	149-30-4	167.3	

2.3.3 Toxicity

Due to the volatile nature of the antioxidants and vulcanization agents studied, they may release part of its concentration into the air. Therefore, one main entry route in human organism is through inhalation.

According to the classification provided by companies to ECHA in REACH registrations and CLP notifications, BHA and BHT are very toxic to aquatic life with long lasting effects. BHA is also suspected to be carcinogenic and to damage fertility in foetus, among others [67–69]. BHT is under assessment as endocrine disruptor. It was demonstrated that BHT and some metabolites trend to accumulated in some mice organs like liver and kidney. In addition, it has a potential teratogenic effect in aquatic life [70,71].

Due to the high-water solubility of BTZ and MBTZ, these substances are present in the aquatic environment posing a risk. This information is corroborated for MBTZ according to the harmonised classification and labelling considering this compound as very toxic to aquatic life, very toxic to aquatic life with long lasting effects, and it may produce skin allergy [72]. BTZ present an environmental hazard and it is suspected to cause harmful effects via skin contact [73,74].

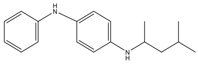
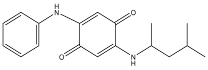
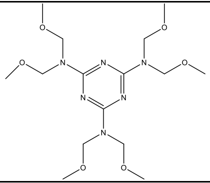
2.3.4 Legislation and regulatory aspects

None of these compounds have any legal limits or recommendations on this type of material.

2.3.5 Other Compounds

Social and environmental concern about the presence of certain substances in tire rubber is increasing due to the recent papers relating the presence of a tire antiozonant product with the premature mortality of coho salmon [75]. For this reason, in the last chapter of this doctoral thesis, a suspect screening was carried out in biological fluids samples to test the possible presence of three controversial compounds present in tire rubber [75,76]. The chemicals names, their abbreviations, CAS numbers, molecular weights (MW) and chemical structures are represented in **Table 6**.

Table 6. Name, abbreviation, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure of the compounds included in the suspect screening.

Compounds	Abbreviation	CAS	MW	Chemical Structure
N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine	6PPD	793-24-8	268	
N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone	6PPDq	-	298	
Hexamethoxymethylmelamine	HMMM	3089-11-0	390	

3. ENVIRONMENTAL AND TOXICOLOGICAL PROBLEMS

3.1 OCCURRENCE IN THE ENVIRONMENT

Recycled rubber surfaces are mainly used outdoors and are exposed to different climatologic conditions, which allows some of their compounds to be released into the environment. Crumb rubber is a material capable of absorbing heat which means that these surfaces can reach high temperatures (70 ºC) on hot days promoting the release of volatile and semivolatile substances present in their composition [14].

On the other hand, the maintenance work of synthetic turf to preserve their properties like periodic watering (specially in hot days) can be a factor that favours crumb rubber leaching and even the diffusion of several compounds to the air [14,20]. Indoor facilities also pose a danger, because although they are more protected against weather conditions and compounds are not released into the environment in the same way, users and spectators are exposed to different hazardous chemicals (specially by inhalation) in an enclosed space.

Moreover, recycled rubber materials have a similar composition to tire and road wear particles (TRWP) which are generating social and environmental concern because are one of the biggest sources of microplastics into the environment with lethal effects to some aquatic organisms [75,77].

3.1.1 Water

The study of the diffusion through water of compounds contained in recycled rubber materials are focused mainly on synthetic turf football pitches infill. Moreover, most studies assessed simulated samples instead of real water collected from the fields [14].

The presence of inorganic and organic hazardous compounds in these matrices makes essential to understand the release of concentrations of these substances through water because of the environmental and health implications for different organisms [78,79]. The leaching of different compounds such as PAHs, antiozonants and heavy metals, from tire rubber and recycled rubber materials has been demonstrated with harmful effects to aquatic environment [20,80–83]. The major contributor to water leaching is zinc, with a minor contribution of organic compounds [84]. The problematic associated with these organic chemicals is that the most hydrophobic compounds will tend to bioaccumulate and may pose a risk to aquatic system.

However, there is no regulation for water leaching surrounding synthetic football fields. Directives 2008/105/EC and 2013/39/EU regarding priority substances in the field of water policy listed different chemical substances and elements, including some compounds present in recycled tire rubber [85,86]. Among the compounds studied in this doctoral thesis, eight PAHs (ANC, FLA, NAP, B[a]P, B[b]F, B[k]F, B[ghi]P and IND) and the phthalate DEHP are on this list of priority pollutants in surface waters. The PAHs ANC, B[a]P, B[b]F, B[k]F, IND and B[ghi]P, and DEHP are considered as priority hazardous chemicals.

Most of the research performs the leaching experiments simulating the conditions and employing standardized methodologies [18,31,40,80,87–92]. Different extraction techniques were employed to isolate and concentrate the target compounds in the water leachates such as solid-phase extraction (SPE) [40,87] and solid-phase microextraction (SPME) [20,89,90]. Research that analyzed real water samples has the limitation of collecting only a few water samples from artificial turf football fields.

3.1.2 Air

Concern about air quality both outdoors and indoors is growing. According to EEA air pollution produces around 1 % of all cancer in Europe and the 2 % of death provoked by cancer [93]. Tire and recycled tire materials contain several hazardous and concerning volatile and semivolatile compounds. The release of these compounds from tire materials to the atmosphere, as well as rubber granulate dust, contributing to air pollution and may pose a risk to human health by inhalation of different harmful substances such as PAHs, phthalates, benzothiazole, etc [20].

In the EU, B[a]P and some heavy metals values in ambient air are limited with the aim to protect human health and environment [94]. This directive stablishes a target value of 1 ng m^{-3} for B[a]P. Other PAHs are monitored by EU under the Atmospheric Pollutants Emission Reductions Directive [95]. However, information about the air composition in environments related to tire rubber materials is scarce.

Air sampling is a difficult step in the air analysis process that often involves the use of a solid sorbent capable to retain the compounds of interest. Different approaches based on active and passive methodologies were found in literature. Passive sampling in most cases involves the use of SPME or sorbent trapping at laboratory scale [5,20,90,96,97]. In these experiments the diffusion of several organic compounds from the recycled rubber material (playgrounds and synthetic pitches infill) to the vapour phase was evaluated, demonstrating the release of different volatile and semivolatile PAHs and plasticizers, among others.

On the other hand, both active and passive air sampling were employed to study real air samples from artificial turf football pitches [98–102]. Passive sampling was performed using silicone and lowdensity polyethylene (LDPE) samplers aiming to determine PAHs and oxygenated PAHs [102]. In these in situ studies, the diffusion of different compounds such as PAHs, phthalates, among other semivolatile and volatile organic compounds into the air was demonstrated.

3.2 TOXICOLOGY AND BIOACCESSIBILITY

The main entry routes of compounds present in crumb rubber into the human body are dermal contact, inhalation and oral ingestion [103]. So far there is little information about the exposure to the compounds present in these recycled materials into the human body. A study from 2019 reported that children cancer risk associated with activities over recycled tire rubber surfaces (playgrounds) is 10 times higher than in conventional (uncovered) [104]. What is becoming increasingly well-known is the ingestion of tire and road wear particles (TRWP) by various non-human living organisms, and diverse noxious effects (including premature death) related to TRWP exposure were reported [105,106].

Due to the proliferation of these types of surfaces in recent years, especially synthetic turf pitches and children’s playgrounds, and their accessibility the general users, it is very important to carry out studies related to exposure to these types of materials through the afore mentioned routes. Oral intake will occur more in young children (3-6 years) because of their hand-to-mouth nature, may accidentally or consciously ingest some of these materials.

Oral bioaccessibility is the portion of a chemical compound or chemical element solubilized from the matrix sample employing test methods that mimic the human gastrointestinal conditions [107]. Until now, there are only a few investigations assessing the human bioaccessibility of recycled rubber granulate, as well as risk assessment, and the toxicological implications it may have [17,108–111]. These research articles were focused on specific organic compounds and some heavy metals. Recently, a study carried out in Japan evaluated the bioaccessibility of heavy metals, PAHs and derivatives, plasticizers, antioxidants, antiozonants and vulcanization additives, among others, demonstrating the presence of many crumb rubber related substances in human biological fluids [21,25,26].

In 2019 the USA National Toxicology Program carried out a report on synthetic turf and recycled tire crumb rubber. In this assessment, an *in-vitro* evaluation of the biological activity of this infill material demonstrated the cytotoxicity of crumb rubber in skin, lung and small intestine employing human cell lines covering the three main entry pathways (dermal contact, inhalation and ingestion) [112]. These results promoted subsequent research investigating the *in-vivo* exposure of female mice fed chow adulterated with recycled rubber [113]. This was the first research of this kind evaluating crumb rubber.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4. SAMPLE PREPARATION

4.1. INTRODUCTION

In analytical chemistry, sampling and sample preparation steps are essential to satisfactory extract the analytes of interest for subsequent analysis. The main purpose of the sample preparation is the isolation (extraction) of some analytes of interest from the matrix sample. In addition, the sample preparation procedure ensures that the concentration levels of the studied substances are suitable for their correct detection during analysis [114].

Until a few years ago, sample preparation processes employed methodologies that involved the use of large volumes of organic solvents, several steps and a high consumption time. But the rise of green analytical chemistry (GAC) and green sample preparation (GSP) principles, in addition to the processes of miniaturization and automation, are encouraging the development of faster, simpler, cheaper and more sustainable analytical methods [115,116]. Even recently, simple software capable of assessing the greenness of the analytical method and sample preparation process have been developed [117,118].

In this doctoral thesis different extraction techniques were employed to isolate the studied compounds from different kind of matrices. Ultrasound assisted extraction (UAE) was selected to extract the target compounds from the recycled tire rubber surfaces. Solid-phase microextraction (SPME) was proposed to perform the runoff water and simulated water experiments. Solid-phase extraction (SPE) was employed to collect air samples in environments related to tire and recycled tire rubber. UAE and SPME were also employed to isolate the substances retained in the sorbent employed in the SPE air sampling after optimized them. Finally, SPE was also employed to isolate the compounds present in human synthetic fluids. In all cases, most critical experimental parameters affecting extraction were optimized by advanced statistical tools to obtain the highest extraction efficiency.

4.2. ULTRASOUND ASSISTED EXTRACTION (UAE)

4.2.1. Introduction

UAE is a technique that is widely used nowadays because of its simplicity and low cost. Its fundament is based on ultrasounds (US), which are sound waves with frequencies higher than the upper limit of the human ear [119]. The procedure consists of using these ultrasound waves to extract the compounds from a liquid or solid to an organic solvent. It is important to note that no direct interaction takes place between the waves and the compounds, the ultrasound energy foster the formation and implosion of bubbles of organic solvent (phenomenon known as cavitation) which facilitate the mass transfer between the matrix and the solvent [120].

4.2.2. Experimental procedure

There are different instruments that can be employed to perform UAE, being the main employed instruments ultrasonic probe and ultrasonic bath. The ultrasound probe consists of an apparatus which can be inserted in the studied sample delivering the energy in a particular zone but achieving more energetic extractions. On the other hand, the ultrasound bath is the most common used due to its availability and lower cost. In these devices, a vessel containing the liquid or solid sample mixed with an extractive phase is immersed into the bath (indirect sonication) and then subjected to a specific and constant ultrasonic frequency (generally between 20 and 100 kHz, frequency range of physical effects) [119,120].

4.2.3. Parameters affecting UAE

UAE efficiency may be affected by different medium parameters [121]:

- Solvent: different types, volume, target compounds solubility, viscosity, surface tension and vapour pressure, among others.

- Temperature: it affects significantly the solvent properties. The cavitation phenomenon is enhanced decreasing the temperature, since the surface tension and viscosity decrease and vapour pressure increases. On the other hand, perform the extraction at lower acoustic intensity may produce that higher temperatures increase the number of cavitation bubbles. The solvent temperature must be controlled within a suitable range, as it is important to take into account the possible degradation of the analytes with temperature.

- Extraction time: the extraction time may influence the efficiency of the extraction, but precautions must be taken because a long extraction time may affect the analytes under study.

4.2.3. Applications

UAE has been successfully applied for the extraction of PAHs, plasticizers, antioxidants and vulcanisation additives from recycled rubber materials [5,20,96].

In this doctoral thesis, UAE was employed in section IV Chapters 1.1 and 1.2 to extract different compounds from recycled tire rubber and alternative materials. In Chapter 2.2, UAE was also selected to isolate the retained compounds in the SPE sorbent to determine the target compounds in the air.

4.3. SOLID-PHASE MICROEXTRACTION (SPME)

4.3.1. Introduction

SPME is a sample preparation technique introduced by Prof. Janusz Pawliszyn and collaborators in 1989 [122]. The SPME device is based on a fused silica fibre, chemically inert and very stable, covered with a stationary ab/adsorbent phase of polymeric nature. The fibre is placed in a stainless-still needle which is hold in a device similar to a syringe.

Figure 4. represents the most common SPME device designed and introduced by Supelco in 1993 [123].

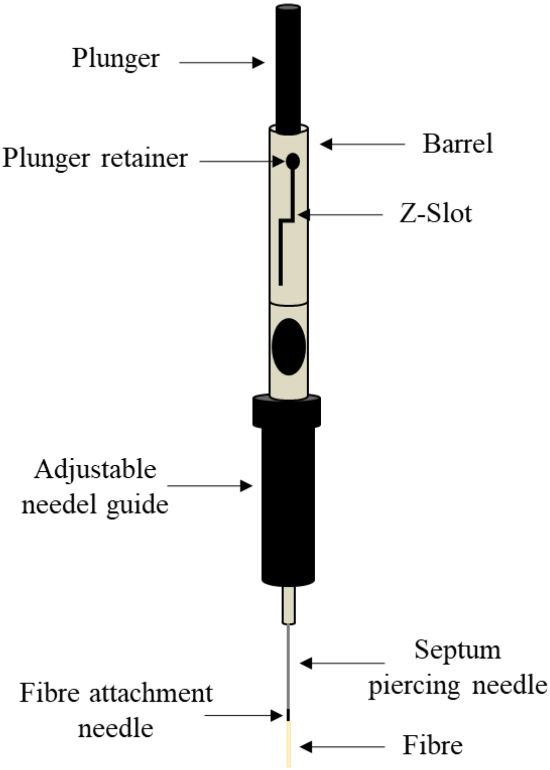

Figure 4. Scheme of the SPME device.

SPME shows a wide variety of advantages. It is a simple extraction technique since the device is easy to handle and to apply in the field. In this context, the sampling, extraction and concentration of the analytes is performed in only one-step. In addition, it can be achieved a high sensitivity as the extracted analytes are concentrated on the fibre and directly desorbed in the gas chromatograph (quantitative transfer). On the other hand, the amount of sample is usually low, the use of organic solvents is minimal, or even absent, and residue generation is negligible, which makes SPME an environmentally friendly approach. Furthermore, SPME is a technique which offers selectivity and easy to automate [123,124].

4.3.2. Experimental procedure

The SPME process basically involves two steps [125,126]:

1. Extraction: the fibre is exposed to the sample for a certain period of time so that the compounds migrate from the sample to the fibre coating (extracting phase) and are retained.

2. Desorption: after the extraction is complete, the fibre containing the analytes is directly transfer to the injection port of a gas (thermally desorbed) or liquid (desorbed in a solvent) chromatograph.

The SPME extraction may be performed by three different modes (see **Figure 5**) [125]:

- Headspace mode (HS-SPME): the fibre is inserted and exposed to the headspace over the sample. The analytes release to the headspace and then they are retained by ab/adsorption mechanisms into the stationary phase. This mode is extensively employed for the determination of volatile and semivolatile compounds.

- Direct immersion mode (DI-SPME): in this extraction mode the fibre coating is directly exposed to the sample (aqueous, liquid or fluid samples) and the substances are transferred from the matrix to the extracting phase. This approach is usually selected for determine non volatile or slightly volatile compounds and for the application in relatively simple matrices.

- Membrane protection mode: the main objective of this mode is the protection of the fibre during the extraction of dirty or complex samples. The compounds are not transfer directly to the fibre but they pass through the membrane. This membrane can be employed to improve the selectivity if it was fabricated with the adequate material.

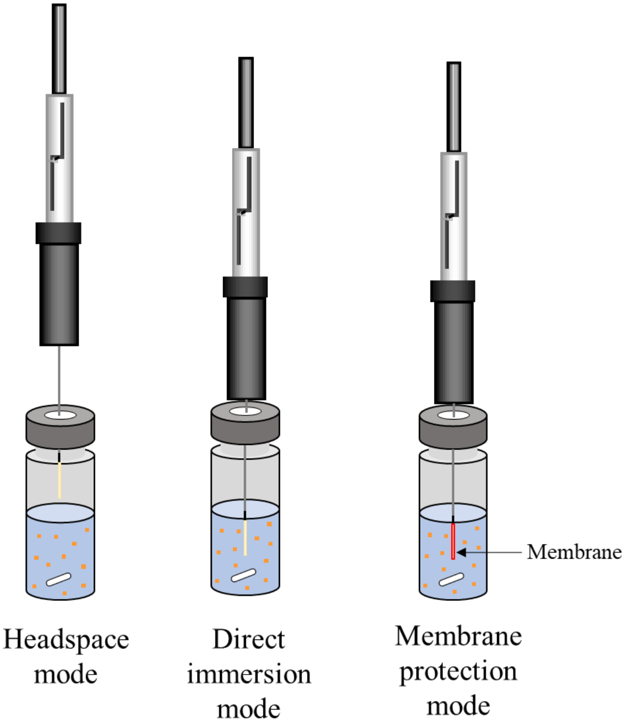

Figure 5. SPME extraction modes.

4.3.3. Parameters affecting SPME

Different factors/steps can influence SPME [123]:

- Fibre coating: there are available different types of polymeric phases (single or mixed), both commercially and laboratory synthetized, with different polarities and thickness. The selection of the fibre depends on the physico-chemical properties (polarity, volatility, etc) of the target analytes.

- Extraction mode: the selection of the extraction method is conditioned by the analytes studied and the matrices used.

- Agitation: stirring affects the transfer of analytes to the fibre and may reduce the extraction time required to reach equilibrium.

- Sample and headspace volume: in HS mode there is a system composed of three phases (sample, fibre and headspace), the number of moles of compounds extracted (n) by the coating at equilibrium are determined by the following equation:

$$n = \frac{K_{fs}V_fV_s}{K_{fs}V_f + K_{hs}V_h + V_s}C_0$$

where K_{fs} is the distribution constant between the fibre (f) and sample (s), and K_{hs} between the headspace (h) and sample; V_f, V_s, and V_h are the volumes of fibre, sample and headspace, respectively; and C_0 is the initial analyte concentration in the sample. It is clear that if the sample volume increases, the amount of analyte extracted increased too but it will increase up to a certain maximum volume after which it will no longer increase. This allows simplifying the above equation, interpreting that the sample volume will not influence the amount of analyte extracted, in the next equation:

$$n = K_{fs}V_fC_0$$

- Sample pH: this parameter is important since SPME coatings are able to extract substances in neutral or undissociated form. Therefore, for acidic species a low pH value will increase the sensitivity, and a high pH value will do the same for basic species.

- Salting-out effect (ionic strength): increasing the ionic strength by adding salts (NaCl, Na_2SO_4, K_2CO_3, etc) to an aqueous sample decreases the solubility of substances in water and promotes their migration into the headspace and/or the SPME fibre.

- Addition of solvent: the addition of organic solvents or water to a solid sample may favour the diffusion of compounds to the fibre.

- Extraction temperature: increasing the sample temperature causes an increase in HS capacity, increasing the mass transfer (diffusion coefficient) to the fibre coating and reducing the extraction time. On the other hand, the decrease in temperature causes the distribution constant between the matrix and the fibre to decrease, resulting in a loss of sensitivity.

- Extraction time: the choice of extraction time in SPME depends to a certain extent on the objectives to be prioritised. SPME is an equilibrium technique, and this implies that the highest amount of analyte will be retained when equilibrium is reached, but sometimes shorter extraction times (pre-equilibrium) can be selected if they ensure correct sensitivity and reproducibility.

4.3.4. Applications

From its development to nowadays, SPME has been used as sample preparation technique in various fields of analysis such as environmental, food science, different “omics” (metabolomics, lipidomics, etc), medical, cosmetics, among others [127–131]. In most cases it is used for the extraction of volatile and semivolatile compounds, but to a lesser extent it is also used to study non volatile analytes.

Regarding the study of tire rubber materials, SPME was selected as extraction technique for the isolation and concentration of PAHs, plasticizers, antioxidants and vulcanisation additives present in water samples [20,90] and in air diffusion experiments at lab-scale [5,20,90,96].

In this doctoral thesis SPME was employed in **Section IV**, Chapter 2.1, for the extraction of different PAHs, plasticizers, vulcanisation additives and antioxidants present in runoff water collected in synthetic turf football pitches and lab-scale water experiments. In addition, a SPME method was optimized for the isolation of compounds from the afore mentioned families retained in SPE sorbents during air sampling, **Section IV**, Chapter 2.3.

4.4. SOLID-PHASE EXTRACTION (SPE)

4.4.1. Introduction

SPE is a widely used sample preparation technique described for the first time by Braus et al. in the 1950s. The employment of this technique aims the isolation, enrichment and/or clean-up of certain analytes of interest contained in liquid, fluid or gas samples. To perform the isolation, the compounds are selectively retained (adsorbed) on the surface of a solid-phase or sorbent material, usually packed in cartridges, prior the elution [126,132].

SPE has several advantages in comparison with conventional sample preparation approaches such as liquid-liquid extraction (LLE); it offers a reduction of organic solvent, more efficient extraction of the analytes and separation from interferences, and the possibility of automating the protocol, among others [132].

4.4.2. Experimental procedure

There are different stages to perform SPE depending on whether the treated sample is gaseous or liquid. The SPE process to aqueous or liquid samples generally involves four steps that are represented in **Figure 6** [132]:

1. Conditioning of the packed sorbent: prior to sample loading, a pre-conditioning step is necessary, in which an organic solvent, usually methanol or acetonitrile or a mixture of both, is passed through the cartridge. This is carried out to eliminate possible impurities and to solvate the sorbent to ensure adequate retention efficiency. A solvent

similar to that containing the target analytes is then used to remove excess organic solvent on the sorbent, e.g. for the analysis of water samples after the addition of methanol, the cartridge is equilibrated with volumes of water before loading the sample.

2. Sample loading: the sample is passed through the sorbent and the compounds are retained on it (adsorbed) based in physico-chemical properties. Sample volumes vary depending on the matrix studied.

3. Washing (rinsing) the packing: an appropriate solvent is used to remove interferences but without eluting part of the analytes with them.

4. Elution and recovery of the analyte: in the final step, an adequate solvent is employed to elute the target analytes retained in the sorbent.

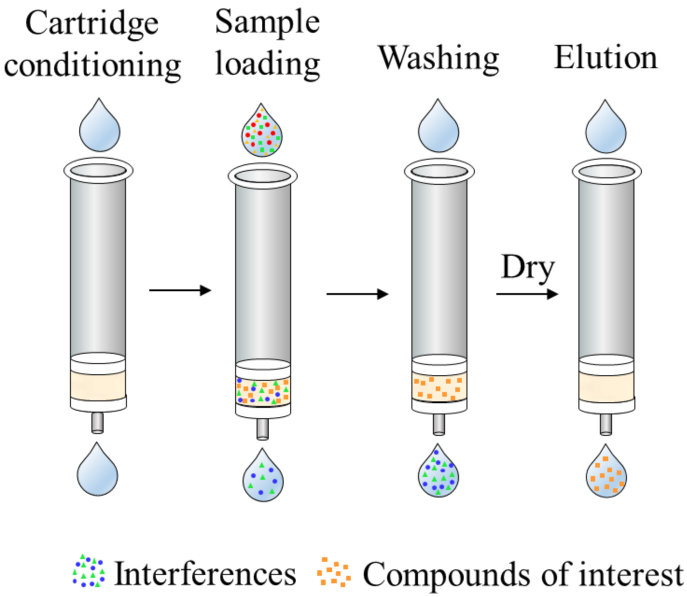

Figure 6. Scheme of the SPE procedure.

Although SPE has been applied to aqueous matrices, it is a suitable technique for air sampling. A schematic representation of the set-up is shown in **Figure 7**. An SPE device similar to the one used for liquid sample preparation is used. The previous stages of conditioning, sample loading, washing and elution are not carried out, but instead, using a vacuum pump, air is passed through the cartridge and the analytes are retained on the sorbent. After that, the compounds of interest retained on the sorbent are extracted by elution with the corresponding organic solvent.

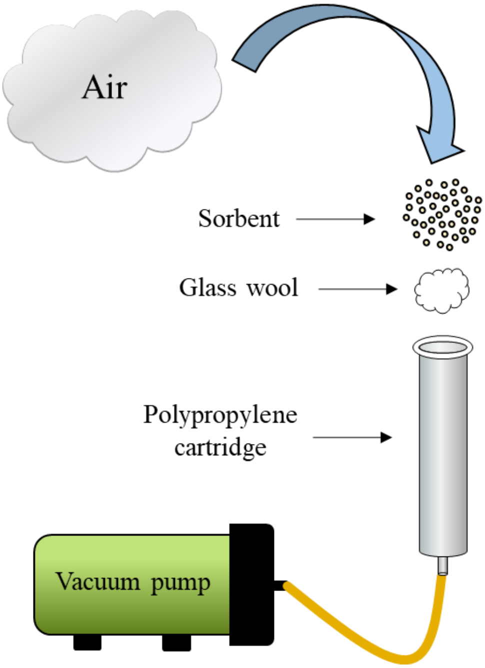

Figure 7. Active air sampling system based on SPE.

4.4.3. Parameters affecting SPE

There are different parameters that can influence the SPE effectiveness such as [126,132]:

- Sorbent: the selection of the adequate sorbent is essential to obtain a high throughput extraction. There are a large number of sorbents both commercially and lab-synthetized available. Sorbents can be classified attending to different physico-chemical properties such as polarity of the solid-phase or the bonding type with the analytes. The most common SPE sorbents are alkyl bonded silicas (C8, C18, among others) and hydrophilic lipophilic balanced polymers (e.g., Oasis HLB), but there are new emerging materials which are employed as SPE sorbents such as molecular imprinted polymers (MIPs), metal organic frameworks (MOFs) or biosorbents like cork [133,134].

- Matrix: the sample used may be liquid, viscous, solid or gaseous, and of varying complexity (e.g. biological fluids). Liquid samples pass directly through the cartridge, viscous samples are usually diluted, and solid samples are dissolved or extracted in a solvent prior to SPE. In the case of air samples, air is passed through the cartridge. Knowledge of the matrix and analytes properties is important for the selection of sorbent, washing and elution solvents.

- Sample volume: select the sample volume to load through the cartridge is important to avoid sorbent clog and ensure that no analyte is lost by exceeding the cartridge capacity.

- Solvent: the choice of the elution solvent, based on physico-chemical characteristics will determine the extraction efficiency. The solvent should be able to extract the analytes retained by the sorbent without eluting interferences with them in a small volume as possible (concentration of the compound).

- Analytes: different parameters such as polarity, functional groups, solubility, stability, pKa, among other chemical properties condition the selection of the adequate sorbent (retention) and solvent (elution).

- Flow rate: this parameter must be balanced as too fast a flow rate causes the analyte-sorbent interaction time to be reduced, which will not allow adequate retention. The same applies to elution, the solvent will not interact enough with the retained analytes to ensure a good extraction.

4.4.4. Applications

SPE is one of the most widely used extraction technique with different applications such as environmental, industrial, pharmaceutical, biological and food, among others, for the determination of different families of compounds like PAHs, pesticides, personal care products, etc [135]. SPE was successfully employed to perform active air sampling to determine synthetic musks, fragrance allergens and pyrethroids in air [136–138]. In addition, this sample preparation procedure was employed to extract PAHs from biological samples [139,140].

In this Thesis, SPE was employed to perform the active air sampling by sorbent trapping, employing commercial sorbents (Tenax TA) and metal organic frameworks (MIL-101), in different locations related to tire rubber materials in **Section IV**, Chapter 2.2 and 2.3. In addition, SPE has been used to isolate PAHs from synthetic human fluids in Chapter 3.1, employing commercial sorbents such as Oasis HLB and biosorbents like cork.


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5. INSTRUMENTAL ANALYSIS

5.1. INTRODUCTION

Gas chromatography (GC) combined with tandem mass spectrometry (MS/MS) was selected for the analysis of the analytes studied in this doctoral thesis.

5.2. GAS CHROMATOGRAPHY (GC)

Gas chromatography (GC) is an analytical separation technique based on the distribution of the analytes present in a sample (solid, liquid or gas) between a gaseous mobile phase, called carrier gas, and the stationary phase, which is usually a high-boiling (non-volatile) liquid immobilized/adsorbed on the surface of a solid (chromatographic column). The mobile phase is an inert gas such as He, N_2 or Ar. The retention of the compounds through the chromatographic column is determined by the interaction between the substances and the column, for example a non-polar chemical has a long retention time on a non-polar column and shorter retention times in polar columns. GC is usually selected for the separation of volatile or semivolatile compounds that are thermally stable [141].

5.3. MASS SPECTROMETRY (MS)

Mass spectrometry is an analytical tool that provides qualitative information such as the elemental composition of samples or chemical structure of substances, and quantitative data, like composition of samples or isotope ratios, from the ions emitted by the molecules studied. One of the most widely used forms of analysis in recent years is the hybridisation of several different analytical techniques [142]. One of the most preferred is the combination of gas chromatography and mass spectrometry (GC-MS). This technique consists of the direct connection of a GC to a mass spectrometer offering quality qualitative and quantitative information, since mass spectrometry offers selectivity, specificity and sensitivity.

The analyzed sample is directly introduced from the chromatograph to the mass spectrometer where the substances present in the sample will be ionized. Three main different parts compose a common mass spectrometer: the ionization source, the mass analyzer and the detector. The GC-MS system is easy to couple because both work with the sample in the gaseous state; to introduce the gas sample into the spectrometer a heated interface is employed. This interface extends from the outlet of the column located inside the gas chromatograph to the ion source of the mass spectrometer.

After the introduction in the ion source, the sample is ionized. One of the most employed ionization sources is the electronic impact (EI). EI is a hard source which electrons bombarded the molecules at a specific energy provoking the emission of an electron and forming positive molecular ions (M^+) and fragment ions.

Then, the mass analyzer receives the ions to consequently go to the detector according to their mass-to-charge ratio (m/z), producing the mass spectrum of the substance [143].

There are different types of analysers such as quadrupoles, time of flight, ion trap, among others. To perform the analysis of this doctoral thesis a triple quadrupole tandem mass spectrometer was employed coupled to gas chromatography. The most employed tandem mass spectrometer is the triple quadrupole mass spectrometer (QqQ). This kind of mass spectrometer consist of the combination of two quadrupoles and a collision cell, with the aim of filtering ions with a specific m/z ratio and their fragmentation. A quadrupole is composed of four cylindrical and parallel bars that serve as electrodes, electrically connected to each other in opposite pairs on which a variable radiofrequency potential is applied. The quadrupole behaves as a mass filter in the following way, only ions with a certain m/z ratio pass through to the detector. All other ions that do not have the m/z ratios recognised by the quadrupole reach the bars (cylinders) and become neutral molecules. This kind of mass analyser instruments are the most compact, lower-priced and most effective [143].

Different ionization working modes can be configured on this instrument:

- Full scan (FS): all masses are registered without filtration.

- Selected ion monitoring (SIM): only ions with a specific mas-to-charge (m/z) are filtered.

- Selective or multiple reaction monitoring (SRM or MRM): the first (Q1) and third (Q3) quadrupoles filter specific m/z of the ions, thus working in SIM mode, while the collision cell (q2) is where the fragmentation of the ions filtered in Q1 takes place. First, Q1 filter some ions (precursor ions) and, after fragmentation by the action of the collision gas (an inert gas such as argon) in the second quadrupole (q2) or collision cell, selected product ions are filtered in Q3. Employing this ionization approach, certain interferences can be minimized or even avoided. Also, this ionization mode offers a sensitive and selective tool that permits obtain chromatograms with very low noise. The configuration of a triple quadrupole mass spectrometer working in SRM mode, as the employed in this PhD thesis, is shown in **Figure 8**.

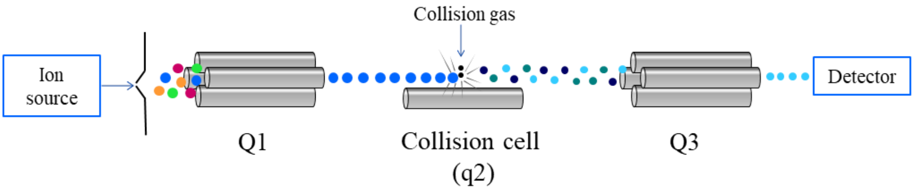

Figure 8. Scheme of a triple quadrupole mass spectrometer working in SRM mode.

GC-MS/MS working in SRM mode was used for the determination of PAHs, plasticizers, antioxidants and vulcanisation additives in different tire and recycled tire rubber surfaces, air, water and biological samples.


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III. METHODOLOGY


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In this doctoral thesis, different sustainable and environmentally friendly sample preparation techniques and methodologies have been developed and applied to rubber crumb materials. To this end, the development of sustainable methodologies based on miniaturization, fast, and low-cost procedures, with little or no waste generation are proposed.

For the simultaneous determination of PAHs, plasticizers, antioxidants and vulcanisation additives in the crumb rubber samples, a miniaturized UAE procedure was proposed. SPME was selected to extract that target compounds from runoff water and lab-scale leachates analysis. On the other hand, a SPE based procedure was optimized to execute air sampling in places related to tire rubber followed by UAE or SPME to extract the target chemicals, retained in a sorbent, prior the analysis. For the isolation of the PAHs from the synthetic biological fluids after the digestion of recycled rubber used in football fields as infill, SPE, employing both commercial and cork by-products as sorbent, was selected as extraction technique.

Chemometric and statistical tools, including design of experiments (DOE) and analysis of variance (ANOVA), were employed for the optimization of the most critical sample preparation parameters affecting extraction, to ensure the most efficient compound extraction, and a proper data interpretation. In all cases, the analytical determination was carried out by GC-MS/MS, optimizing the instrumental conditions to assure the best separation, identification and quantification of the target compounds in the analyzed matrices.

IV. RESULTS AND DISCUSSION

CHAPTER 1. DETERMINATION OF HAZARDOUS SUBSTANCES IN DIFFERENT RECYCLED RUBBER SURFACES


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Rubber from end-of-life tires (ELTs) is a material which is reused to manufacture several sport and leisure surfaces such as infill in synthetic turf football pitches or children´s playgrounds flooring. These materials present in their composition hazardous chemicals like heavy metals, chlorinated paraffins, PAHs, plasticizers, antioxidants, vulcanisation additives, endocrine disruptors, among others. Therefore, these surfaces may pose a risk for the environment and human health and an exhaustive characterization of their content of several harmful substances is essential.

In this chapter, two different studies concerning the identification and quantification of forty-two organic compounds, comprising PAHs, plasticizers, antioxidants and vulcanisation additives, in recycled tire crumb rubber (RTCR) samples from different type of surfaces such as football pitches backfill, playgrounds flooring, tree roots protectors and commercial mulches, and car tires are included. In both studies, UAE was employed to extract the compounds of interest from the samples prior GC-MS/MS analysis.

In the first study, the content of the above-mentioned compounds was evaluated in forty samples from different surfaces including crumb rubber used as infill in artificial fields, playground flooring (indoor and outdoor), commercial mulches, car tires and tree protectors. Furthermore, the content of the target compounds in two green alternative materials, such as cork granules and sand were evaluated.

Following a similar line of research, the global problem of the use of RTCR as infill in synthetic turf football pitches was assessed in a second article. Seventy-eight recycled rubber infills collected from pitches in seventeen countries from four continents were analyzed. In addition, the content of organic compounds in different alternative materials (13 samples), including cork granulate, coconut fibre and thermoplastic elastomers, used as infill in football pitches, was studied.

The two studies have been published in the international journal Science of the Total Environment, and they are presented in next pages.

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1.1.

Hazardous compounds in recreational and urban recycled surfaces made from crumb rubber. Compliance with current regulation and future perspectives

Maria Celeiro, Daniel Armada, Thierry Dagnac, Jacob de Boer, Maria Llompart

Science of the Total Environment, 755 (2021), 142566

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1.2.

Global evaluation of the chemical hazard of recycled tire crumb rubber employed on worldwide synthetic turf football pitches

Daniel Armada, Maria Llompart, Maria Celeiro, Pablo Garcia-Castro, Thierry Dagnac, Jacob de Boer

Science of the Total Environment, 812 (2022), 152542

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CHAPTER 2. ENVIRONMENTAL IMPACTS OF RECYCLED TIRE RUBBER: AIR AND WATER

Recycled crumb rubber is a material employed worldwide for different sport and leisure purposes. The presence of harmful compounds considered as priority pollutants and endocrine disruptors makes essential monitoring the diffusion of these substances into the different environmental compartments such as water and air.

This chapter includes three articles. In the first one, forty compounds, some of them considered as priority substances in the field of water policy in the EU, were determined in runoff water directly collected on artificial turf football pitches. Furthermore, to evaluate the possible continuous transfer of the analytes between the crumb rubber of the football pitches and the water in contact with it, lab-scale runoff water experiments were simulated. The selected methodology for the analysis of the water samples was SPME working in HS mode, followed by GC-MS/MS.

The other two articles assess the diffusion of the compounds present in tire rubber materials to the air adjacent to these surfaces. In the first research, an analytical method based on active air sampling using sorbent trapping (SPE) followed by UAE-GC-MS/MS, was developed to determine forty-one organic compounds (PAHs, plasticizers, antioxidants and vulcanizers) related to tire rubber. The second one explores the improvement in terms of greenness and sensitivity of the first methodology replacing the UAE by SPME. SPME allows performing a more sensitive determination of most volatile compounds, which are the most frequently detected in real environments. Both methodologies were optimized employing chemometrics and validated in terms of quality parameters. Afterwards, they were applied to indoor and outdoor places related to tire rubber materials, including synthetic football fields, children´s playgrounds and tire warehouses, among others.

The three articles were published in Chemosphere, Journal of Separation Science, and Journal of Chromatography A and they are shown below.


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2.1.

Evaluation of chemicals of environmental concern in crumb rubber and water leachates from several types of synthetic turf football pitches

Maria Celeiro, Daniel Armada, Nuno Ratola, Thierry Dagnac, Jacob de Boer, Maria Llompart

Chemosphere, 271 (2021), 128610

ISSN: 0045-6535, Elsevier

DOI: 10.1016/j.chemosphere.2020.128610

2.2.

Miniaturized active air sampling method for the analysis of tire rubber pollutants from indoor and outdoor places

Daniel Armada, Maria Celeiro, Antia Martinez-Fernandez, Piyaluk Nurerk, Thierry Dagnac, Maria Llompart

Journal of Separation Science, 44(8) (2021), 1694-1705

ISSN: 1615-9314, Wiley-VCH GmbH

DOI: 10.1002/jssc.202001249

2.3.

Green methodology based on active air sampling followed by solid phase microextraction and gas chromatography-tandem mass spectrometry analysis to determine hazardous substances in different environments related to tire rubber

Daniel Armada, Maria Celeiro, Thierry Dagnac, Maria Llompart

Journal of Chromatography A, 1668 (2022), 462911

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CHAPTER 3. EVALUATION OF THE POTENTIAL RECYCLED CRUMB RUBBER BIOACCESSIBILITY

Recycled crumb rubber contains different hazardous substances which can entry into the human body through different routes such as inhalation, dermal contact, or ingestion. Surfaces made of recycled tire rubber, such as synthetic fields crumb rubber infill and playground´s flooring, are accessible to the general public, being usually young children the main users. They may unintentionally or voluntary eat some of these materials facilitating the oral ingestion, due to their hand-to-mouth nature.

This chapter includes one article that evaluates the *in-vitro* bioaccessibility of eighteen PAHs from recycled crumb rubber (infill in synthetic fields) into synthetic human biological fluids employing a sustainable analytical methodology. Eight of the target PAHs are considered carcinogenic by ECHA and sixteen as priority pollutants by the US-EPA, among other dangerous properties which can represent a risk to human health. The protocol employed to prepare the synthetic biological fluids and to mimic the human digestion of the crumb rubber was the unified bioaccessibility method (UBM) developed by the Bioaccessibility Research Group of Europe (BARGE).

Firstly, an analytical method based on SPE-GC-MS/MS was optimized to determine the target compounds in the bioaccesible fraction. Two sorbents were proposed for the SPE procedure: a commercial sorbent, such as Oasis HLB, and a biosorbent, crushed cork. The latter sorbent results in a more environmentally friendly methodology and the revalorization of a by-product obtained from the cork industry.

After method optimization and validation, a simulation of the *in-vitro* digestion of crumb rubber samples employed as infill was carried out. In addition, a suspect screening of several hazardous compounds related to tire rubber in the biofluid samples was performed, showing the presence of new emerging pollutants as it is the case of 6PPD-quinone. Finally, a children risk assessment was done to estimate the danger associated to the crumb rubber ingestion.

The article was published in Science of the Total Environment and it is shown below.


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3.1.

Assessment of the bioaccessibility of PAHs and other hazardous compounds present in recycled tire rubber employed in synthetic football fields

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V. GENERAL DISCUSSION

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The present doctoral thesis comprises three distinct parts. The first one deals with the study of the presence of hazardous compounds in different samples of rubber and recycled rubber. The second section studies the diffusion of these substances from recycled rubber surfaces into different environmental compartments such as air and water. Finally, the last part evaluates the oral bioaccessibility of polycyclic aromatic hydrocarbons and other chemicals present in tire rubber materials into human biological fluids, and the risk related to it.

Due to the different matrices analyzed and the wide range of substances determined during the development of this thesis (forty-two in most studies), different analytical approaches were necessary to perform the analysis with the adequate quality. Regarding the samples, different solid materials, including tire and recycled tire rubber materials, and alternative materials (cork granules, coconut fibre, sand and thermoplastic polymers) were analyzed. In addition, environmental matrices (air and water samples), and synthetic human biological fluids were also studied. Attending to the target compounds, four different families of substances were investigated, involving 18 PAHs, 19 plasticizers (three adipates, fifteen phthalates and bisphenol A), 2 antioxidants and 3 vulcanisation additives. Furthermore, during the bioaccessibility research a suspect screening of 5 compounds (vulcanisation, cross linking and antiozonant agents) was carried out.

1.- Determination of hazardous substances in different recycled rubber surfaces

The management of ELTs represents a problem in most developed societies due to the increasing number of tires discarded every year. This material has a high environmental impact since it is not biodegradable. The problematic related to ELTs landfills induced governments and institutions to consider alternatives for their reuse. One of the most useful and attractive applications of ELTs seems to be their conversion into crumb rubber to be employed as infill in synthetic turf football pitches or as children´s playground flooring, among other uses.

Nevertheless, recycled tire rubber utilized to manufacture different kind of sport and leisure surfaces is under scrutiny due to the presence of hazardous substances in its composition. In fact, the presence of heavy metals such as Cd, Pb, Cr, Ni, Cu, Zn, Fe, among others, in crumb rubber materials at concentration up to thousands of µg g^{-1} was demonstrated. Moreover, organic compounds such as polycyclic aromatic hydrocarbons (PAHs), plasticizers, antioxidants, vulcanisation additives, benzothiazoles, chlorinated paraffins, polychlorinated biphenyls (PCBs) or alkylphenols were detected in this kind of surfaces. Thus, this may represent a potential risk to human health and the environment.

The European institutions have taken actions regarding the content of some of these hazardous substances in rubber materials. In this context, since 10 August 2022, eight PAHs considered as carcinogenic (B[a]A, CHY, B[b]F, B[j]F, B[k]F, B[a]P, B[e]P and D[ah]A), have been restricted by the European Union at a maximum concentration of 20 µg g^{-1} in granules or mulches used as infill material in synthetic turf pitches or in loose form, to protect human health (Commission Regulation (EU) 2021/1199 of 20 July 2021).

The United States Environmental Protection Agency (US-EPA) catalogued sixteen PAHs (NAP, ACY, ACE, FLU, PHN, ANC, PYR, FLA, B[a]A, CHY, B[b]F, B[k]F, B[a]P, IND, D[ah]A and B[ghi]P) and six phthalates (DNOP, DEP, DMP, DEHP, BBP and DBP) as priority pollutants.

On the other hand, 4 phthalates (DEHP, BBP, DBP and DIBP) are restricted in plasticized materials, including rubber materials, to 1000 µg g^{-1} for individual concentrations or the sum of them (Commission Regulation (EU) 2018/2005 of 17 December 2018).

In addition, crumb rubber infill is a microplastic material, being the most important source of the microplastics intentionally added to the environment. According to the European Chemicals Agency (ECHA), the emissions are estimated around 38 % of the intentionally added microplastic pollution.

For this reason, an in-depth study was carried out on the presence of forty-two compounds, belonging to the afore mentioned families, in different recycled rubber matrices, including crumb rubber used as infill in synthetic turf football pitches, children´s playgrounds flooring, tree roots protectors, commercial tiles, and car tires. To perform the compounds extraction, a miniaturized ultrasound-assisted extraction (UAE) was selected. This technique allowed employing low amount of solvent (2 mL) and sample (200 mg), being in consonance with the GAC and GSP principles. The UAE step was previously optimized in another research prior to the present thesis. The identification and determination of the target substances in the studied samples was carried out by gas chromatography coupled to tandem-mass spectrometry (GC-MS/MS) employing selected reaction monitoring (SRM) as acquisition mode. This analytical tool offers an unequivocal identification of the compounds, as well as high sensitivity, which is enough for determining trace and ultra-trace concentrations. The analytical method was validated in terms of linearity, limits of detection and quantification, precision and accuracy. Calibration curves covered a concentration range between 0.1 and 1000 μg L^{-1} for most compounds, excluding two phthalates (diisononyl phthalate, DINP, and diisodecyl phthalate, DIDP), which are mixtures of branched chain isomers, and one vulcanisation additive (2-mercaptobenzothiazole, MBTZ). Linearity results showed coefficients of determination (R^2) higher than 0.9906. Instrumental quantification limits (IQLs) and limits of quantification (LOQs) were also calculated, and they were at the sub μg L^{-1} and low ng g^{-1} level, respectively. Additionally, the instrumental method precision was assessed within a day (n = 3) and among several days (n = 6) obtaining a relative standard deviation (RSD) lower than 12 % and 15 %, respectively. Finally, the accuracy of the whole UAE-GC-MS/MS process was evaluated by recovery studies at two concentration levels (1 and 10 μg g^{-1}), for most compounds obtaining values between 72 and 116 % with RSD lower than 15 %. It is important to mention that to the best of our knowledge, recovery studies were carried out for the first time in this type of recycled material.

Once the method was validated, a total of forty tire rubber materials from different sport, leisure and urban surfaces and two alternative materials were analyzed. Thirty of the forty-two compounds were found in the recycled rubber samples collected in football fields and children´s playgrounds. All crumb rubber infill from synthetic pitches contained PAHs, reaching total concentrations between 8 and 95 μg g^{-1}. Also, highlight that PYR, a persistent, bioaccumulative and toxic compound, was detected in all samples with concentrations up to 40 μg g^{-1}, and B[a]P, which is considered the most carcinogenic PAH, was detected in all crumb rubber samples. However, only PHN was detected in the analyzed football pitch material alternative, based on cork. This could be explained by the fact that the field where it is found had previously been filled with recycled rubber. On the other hand, several dangerous (both to environment and human health) plasticizers and other materials were detected in these samples. For example, DEHP (toxic for reproduction phthalate and substances of very high concern, SVHC) was detected in all analyzed samples at concentrations up to 28 μg g^{-1}.

Regarding playgrounds higher concentrations were found outdoors (between 1.0 and 6.0 μg g^{-1}) than indoors (between 9 and 25 μg g^{-1}) for the mean total PAH concentration. The profile of the playgrounds is slightly different from that of crumb rubber samples collected from synthetic turf football pitches. In the latter case, PYR and FLA were the most important contributors to the total PAH content, with a difference of up to two orders of magnitude compared to the other PAH detected. A similar behaviour was observed in the indoor playground samples. On the other hand, for the outdoor playgrounds the PAH with the highest contribution to the total concentration was PYR, followed by the carcinogenic compound CHY. Regarding the PAH profiles, the concentrations of PYR, FLA and PHN were up to two orders of magnitude higher in the indoor playgrounds than in the outdoor playgrounds. This could be explained since the outdoor surfaces are exposed to meteorological conditions (rain, temperature changes, etc) and may release some of the concentration of their compounds into the environment over time. Nevertheless, the average PAH concentrations in both cases (indoor and outdoor playgrounds) achieved lower concentrations for PAHs than in the fields. In most playgrounds flooring samples, toxic for reproduction plasticizers, such as DBP, DIBP and DEHP, and other concern compounds like BTZ, TBP and

BHT were detected. The concentration of DBP, DIBP and DEHP were up to two orders of magnitude higher in the indoor facilities and commercial pavers than in the outdoor ones, which refutes the idea that outdoor surfaces could release some of their components into the environment. BPA, a controversial compound with reprotoxic and endocrine disrupting properties, was detected in the 50 % of the outdoor samples and in all indoor ones. It is important to underline that a sand sample collected in a playground did not show any of the compounds studied, being an interesting alternative to rubber surfaces.

In the tree roots protectors, thirteen PAHs, nineteen plasticizers, BHT, BTZ and TBP were detected which can represent a risk to the environment due to their direct contact with roots and soil. Concerning the car tires, fifteen of the eighteen target PAHs were found, reaching concentrations up to 40 µg g^{-1}. Regarding the commercial recycled rubber granules, the PAH profiles were quite similar to the crumb rubber directly collected in the artificial pitches, although the mean concentrations were higher. Also, several compounds from the other studied families were detected. The PAHs concentration were similar between the commercial crumb rubber and the car tires. In the tires, eight plasticizers were found, reaching concentrations up to 91 µg g^{-1} for DEHP. The antioxidant BHT and the vulcanisation additives BTZ and TBP were detected in all tire samples.

Finally, commercial rubber tiles were analyzed, achieving concentrations of several orders of magnitude higher than the other surfaces studied. The results for PAHs were extremely high in comparison with the other studied samples. These tiles achieved a mean PAH concentration around 6000 µg g^{-1}. Toxic plasticizers were present in these samples as well as the multiple phthalates DINP and DIDP, BTZ, TBP and BHT, with concentrations similar to the registered in the indoor playgrounds.

In summary, hazardous compounds were detected in all types of recycled rubber samples although they were, in most cases, compliant with European regulations. In addition, alternative materials such as cork and sand appear to be sustainable alternatives in terms of health and environmental implications.

To find out whether this problem was worldwide, it was decided to study the recycled rubber granulate used as infill in synthetic turf football pitches in various countries to look if the levels of hazardous and harmful compounds were similar.

A total of seventy-eight crumb rubber infill samples from Europe (Albania, Croatia, Finland, France, Germany, Greece, Italy, Netherlands, Poland, Portugal, Spain, Sweden, Turkey and United Kingdom), America (Chile and United States), Asia (Thailand and Turkey) and Canary Islands, considered from Africa due to the geographical situation, were analyzed. In addition, thirteen samples of alternative materials such as cork granules, coconut fibre and thermoplastic elastomers were included and their content in the target compounds was compared with the values found in crumb rubber. This was the largest study attending the number of samples as well as the number of sampling countries involved. Statistical tools aiming to look for a relation between the geographical origin and the chemical profiles of the crumb rubber were employed.

For the determination of the target compounds the same methodology based on UAE-GC-MS/MS was employed. After the verification of the methodology in terms of linearity, sensitivity and precision (intra and inter-day), it was applied to ninety-one real infill samples collected in synthetic turf football pitches.

Attending to the crumb rubber PAHs content, the results show similar trends in most countries. The most abundant compound was PYR in all countries followed by FLA in most of them, both considered as persistent, bioaccumulative and toxic. Other harmful compounds such as PHN, which are considered toxic to aquatic life, and carcinogenic substances like CHY and B[e]P were found at concentrations up to 11 $\mu g\ g^{-1}$. Although it is very difficult to relate the age of a field to the concentrations found in the granulate analyzed, as more new rubber infill is added periodically, a higher concentration for PAHs in rubber granules collected from newly constructed football pitches (0.5 to 2 years) can be observed. This is probably because this recycled rubber infill was not used and subjected to much weathering.

Regarding the European regulation for the 8 PAHs considered carcinogenic among other properties by ECHA, one sample collected in an EU country surpassed the limit of 20 μg g^{-1}. In addition, although the non-EU countries are not covered by this legislation, two samples exceeded this limit. This regulation did not include several compounds detected in the crumb rubber samples such as NAP, PHN, ANC, FLA, PYR and B[ghi]P which are classified as persistent, bioaccumulative, toxic and/or SVHC by ECHA, and priority pollutants by EPA.

On the other hand, considering the plasticizers studied on this research that are regulated by EU (DIBP, DBP, BBP and DEHP) in this kind of samples, three samples, one from an EU country and other two from non-EU countries surpassed the legal limit of 1000 μg g^{-1}. Furthermore, six of the studied phthalates (DNOP, DEP, DMP, BBP, DBP and DEHP) considered as priority pollutants by EPA were detected in the crumb rubber samples.

Vulcanisation additives and antioxidants were also detected in the crumb rubber samples. Some of these compounds are toxic for aquatic life with long lasting effects, skin sensitizers and endocrine disruptors like BTZ, TBP and MBTZ. The two antioxidants studied BHA and BHT, are currently under assessment as endocrine disruptors.

Nowadays, new infill alternative materials are employed in synthetic turf football pitches to substitute recycled crumb rubber, including in this research three types of alternative backfills: cork, thermoplastic elastomers and coconut fibre.

Some PAHs were found in the cork and thermoplastic elastomer samples, although at much lower levels than in the crumb rubber samples. This could be explained due to the previous presence in those football fields of crumb rubber infill. Furthermore, high concentrations of some plasticizers were detected in some thermoplastic elastomer samples, which was to be expected given their plastic-based composition. On the other hand, rubber granulate and thermoplastic polymers are considered microplastics and can be released into the environment increasing microplastic pollution. Taking all this into account, cork filler seems to be a good material to replace recycled rubber as a filler due to the lower or no content of hazardous chemicals. It can therefore be proposed as a safer and more sustainable alternative.

Finally, a statistical analysis employing one-way analysis of variance (ANOVA) was carried out trying to find significant differences between the crumb rubber infill considering different geographical origin of the pitch, age of the field and indoor/outdoor place. For some compounds such as PYR, DMP, BTZ and BBP higher concentrations were detected in the indoor samples than in the outdoor ones. However, it was not possible to find a relation between the chemical composition of crumb rubber and the geographical origin of the infill or the age of the field. This could be due to the globalization which makes that one crumb rubber manufacturer can provide several countries all around the world. In addition, the fields are refilled periodically with fresh recycled rubber backfill which could make difficult stablish a relation between composition and age.

Therefore, a significant number of hazardous substances in the recycled crumb rubber surfaces even at concentrations above the legal limit were detected, demonstrating that this is a global problem. The use of new alternative materials to replace the crumb rubber is increasing due to the absence or presence at lower concentrations of hazardous compounds. Despite the recent approved legal limits, which only cover a reduced number of compounds, different stakeholders (legislators, environmental and health agencies, academia, among others) must collaborate and proposed strategies together to protect not only human health but also the environment.

2.- Environmental impacts of recycled tire rubber: air and water

Most surfaces made from recycled tire rubber are located outdoor, particularly synthetic turf football pitches. Therefore, they are exposed to different weather and atmospheric conditions. The diffusion of the hazardous compounds present in these surfaces into the environment can represent an environmental problem and have an impact on the health of humans and other organisms. For this reason, an evaluation of the possible substances release present in the recycled rubber into different environmental compartments including water, through water leachates, and air, was carried out. Several of the detected compounds in the recycled crumb rubber samples are considered by the European Union (additionally to the concerning properties mentioned above) as priority pollutants in surface waters (Directive 2008/105/EC) like the PAHs ANC, B[a]P, B[b]F, B[ghi]P, B[k]F, FLA, IND and NAP, and the plasticizer DEHP.

For this reason, it is very important monitoring and control the presence of these chemicals in water which is in contact with this type of rubber infill and others, such as playgrounds flooring or others tire rubber materials. To evaluate the transfer of the compounds through the water, runoff water samples were directly collected in synthetic turf football pitches in rainy days. A total of forty compounds, belonging to the above-mentioned families, were evaluated in these matrices. Solid-phase microextraction (SPME) was the selected technique to carry out the experiments followed by GC-MS/MS analysis. SPME allows performing the extraction and preconcentration of the analytes in a single step, avoiding the use of organic solvents and making the sample preparation step as more sustainable and safer. In addition to real runoff water samples, runoff water experiments were simulated in lab-scale. The experiments were performed employing irrigation water (these synthetic fields are periodically watering to keep their characteristics, especially during warm stations) and rainwater. For this study, different crumb rubber infill samples were placed in contact with irrigation water and rainwater for twenty-four hours. Afterwards, the water was analyzed by SPME-GC-MS/MS. In addition, to investigate the continuous inflow of the substances from the crumb rubber into the water, the same crumb rubber infill sample was put in contact with a fresh rainwater aliquot each twenty-four hours for one week, and the collected extract was analyzed each twenty-four hours.

The SPME-GC-MS/MS methodology was validated in terms of analytical quality. Linearity was assessed in a wide concentration range employing ultrapure water spiked with the studied compounds from 0.001 to 0.5 µg L^{-1} for all target compounds (nine concentration levels), obtaining coefficients of determination (R^2) higher than 0.9900 in all cases. LODs were between 0.03 and 15 ng L^{-1}, demonstrating that the validated methodology allows the determination of trace levels of the target compounds in water samples. Precision was tested within a day (n = 3) and amongst several days (n = 5) for different concentration levels, achieving RSD values lower than 10 % in most cases. To evaluate the accuracy of the whole analytical process (SPME-GC-MS/MS), recovery studies were accomplished employing rainwater spiked with 0.1 µg L^{-1} of the studied compounds, obtaining recovery values between 74 and 120 % with RSD values lower than 10 % in most cases. Finally, the analysis of 8 real water samples and 4 simulated runoff samples was carried out.

Several PAHs were detected in the real runoff water samples collected on rainy days in synthetic turf football fields, such as the priority pollutants in surface waters NAP, ANC and FLA, which were present in seven, four and eight samples, respectively. PHN and PYR, both considered as substances of very high concern, appeared in all samples, reaching concentrations up to 0.13 and 0.90 µg L^{-1}, respectively. CHY, which is considered carcinogenic, persistent, bioaccumulative and very toxic to aquatic life (with long lasting effects) according to EU, was found in six samples.

Regarding plasticizers, DEHP, catalogued as priority pollutant in surface water, was found in five samples. Moreover, the plasticizers DMP, DEP, DBP and BBP, considered as priority pollutants by EPA, were detected in 6, 7, 8 and 7 of the analyzed samples, respectively. DBP, BBP and DEHP are also considered toxic for reproduction, as well as DIBP, which was found in all samples. On the other hand, the vulcanisation additives BTZ and TBP, and the antioxidant BHT, were detected in all samples. It is important to note the presence of TBP at concentrations up to 4 µg L^{-1}, since this compound is very toxic to aquatic life with long lasting effects and is an endocrine disruptor.

The results of the simulated runoff water experiments showed that crumb rubber is a continuous source of PAHs, phthalates, antioxidants and vulcanising additives. Regarding PAHs, 13 out of the 16 studied were detected, and most of them were found in the water samples collected in different days for all the samples, revealing a continuous transfer of these compounds over time. Most volatile PAHs achieved similar concentrations to those detected in the real waters and the comparison of the real and simulated water profiles showed similarities demonstrating that the source of the aquatic chemical pollution comes from the crumb rubber infill. As regards plasticizers, seven phthalates and BPA were detected in the water from the lab-scale experiments at concentrations up to 2.4 µg L^{-1}, which was in consonance with the results obtained in the real runoff waters. The antioxidant BHT and the vulcanisation additives BTZ and TBP were also detected in the samples. BTZ was detected at concentrations higher than 10 µg L^{-1}. This may pose an environmental problem as several of these chemicals pose a potential risk to the aquatic environment. Also, these families of compounds appeared a similar concentration between the simulated water and water samples collected directly above the synthetic football fields.

Air diffusion is another way of entry of the tire related compounds into the environment which can pose a risk to human health since they can be taken up through inhalation. These surfaces in warm days can achieve temperatures between 60 and 80 ºC, giving rise to the diffusion into the air of several of the compounds present in crumb rubber. For this reason, in the present doctoral thesis, two analytical approaches based on active air sampling, employing sorbent trapping, to measure and demonstrate the air pollution produced by this type of surfaces were developed. In both analytical methodologies, an active air sampling based on solid-phase extraction (SPE) was performed to catch the air samples and retain the target compounds, using a vacuum pump at a controlled flow rate (60 L min^{-1}). After the SPE step, different extraction techniques such as UAE and SPME were employed to isolate the retained substances in the SPE sorbent prior GC-MS/MS analysis.

In the first research, the SPE-UAE-GC-MS/MS methodology was optimized for the analysis of 41 compounds belonging to the families mentioned above. In preliminary studies, the type (Florisil, Tenax TA and MIL-101) and amount (25 mg) of sorbent were studied. Afterwards, a full factorial experimental design (2^4, 16 experiments) was carried out to evaluate the most critical parameters affecting the extraction procedure, including extraction time (2 and 10 min), extraction technique (vortex or UAE), type of solvent (ethyl acetate and hexane) and type of sorbent (MIL-101 and Tenax TA). The sorbent and the solvent were statistically significant, as well as the interactions between them, and sorbent and extraction technique interaction. Based on these results, the most favourable conditions for the simultaneous sampling and extraction of the compounds imply the use of Tenax TA and desorption of the analytes with ethyl acetate (1 mL) using UAE for 2 minutes. After the application of chemometric tools to optimize the method, the air breakthrough volume was tested, obtaining as the most practical air volume 1 m^3, offering a short sampling time (16 min) and high throughput. Then, the SPE-UAE-GC-MS/MS methodology was validated in terms of linearity, sensitivity, precision (intraday and interday) and accuracy (recovery studies of the whole method). Linearity was assessed from 0.1 to 1000 µg L^{-1}, obtaining R^2 values ≥ 0.991. The method precision was evaluated intra and interday, showing a RSD values lower than 8 and 12 %, respectively. The whole method accuracy registered recovery values between 71 and 110 % with RSD between 2 and 17 %. IDLs and LODs were at the sub ng mL^{-1} and sub ng m^{-3} for most compounds.

Finally, the optimized and validated method was applied to real outdoor and indoor air samples collected in the city of Santiago de Compostela including parkings, a motor garage, a warehouse containing some recycled rubber tiles, a bus station and a synthetic turf football pitch. In the indoor sites between nine and fifteen PAHs were found, and in the outdoor ones between nine and eleven. Attending to the sum of PAHs, it ranged between 8 and 41 ng m^{-3}, excluding the warehouse air sample. In this warehouse, where several recycled rubber tiles were stored and has no ventilation, the highest concentrations for PAHs were found (1415 ng m^{-3}). In the football field, air was sampled in three different days, being one a warm day with temperatures around 30 ºC and the other two days between 10 and 15 ºC. It is important to emphasize that the highest concentrations were detected the hottest day. Several plasticizers were found in the analyzed air samples, including the priority pollutants and toxic for reproduction DMP, DEP, DIBP, DBP and DEHP. It is important to underline the detection of DIBP in concentrations close to or above 100 ng m^{-3} in the warehouse and in the football pitch samples. In this last sample, DEHP reached concentrations up to 218 ng m^{-3}. Hence, the sample with the highest level of plasticizers (phthalates) was the air collected in the football pitch during summer, in spite of being an outdoor sample. The vulcanisation additives BTZ and TBP were detected in most air samples with concentrations up to 100 ng m^{-3}. Regarding antioxidants, BHT was detected in all samples with concentration levels higher than 150 ng m^{-3}.

In view of the obtained results, further research was carried out for the analysis the same substances in air samples collected in tire related places. In this case, SPME was selected as extraction technique trying to avoid or reduce the volume of organic solvent making greener and more sustainable the sampling-analysis process. Additionally, SPME is a good choice for the analysis of volatile and semivolatile compounds, which were detected at higher concentrations in the previous article. To optimize the SPME step, two fibre coatings (PA and PDMS/DVB) were selected according to preliminary results. After that, a mixed level fraction experimental design ($3 \cdot 2^{3-1}$ + 3 central points) was carried out evaluating four factors including the type of fibre coating (PA and PDMS/DVB), solvent addition (0 and 100 µL of acetone), headspace volume (1.8 and 10 mL vial) and extraction stirring. The optimal experimental conditions were obtained by adding 100 µL of acetone to the sorbent (Tenax TA) using a PDMS/DVB fibre in a 10 mL vial without stirring. The SPME time profile showed an optimum extraction time of 45 minutes to obtain a sensitive and high throughput compound isolation. Before the analysis of real air samples, the developed methodology was validated in terms of analytical quality. Linearity was performed covering a wide concentration range, obtaining values for R^2 higher than 0.9900. LODs and LOQs were at the sub ng m^{-3} for most compounds. Method precision was also evaluated, obtaining results for RSD lower than 14 % and 16 % for intra-day and inter-day precision, respectively. Finally, recovery experiments demonstrated a good accuracy.

On the other hand, the whole SPE-SPME-GC-MS/MS protocol was compared to SPE-UAE-GC-MS/MS method, previously developed, revealing that the first methodology has higher chromatographic responses for the most volatile compounds (the most frequently found in air). This, together with the avoidance of organic extraction solvents and waste generation, makes this second method a more sustainable alternative and achieves better responses for the compounds expected to be detected in real samples. In addition, detection levels are equal or lower, with most compounds reaching ultra-trace levels and the rest of them below 4.6 ng m^{-3}.

In this study, air samples collected in three different types of places were analyzed, including 2 samples from synthetic turf football pitches filled with recycled rubber granules, 3 from air adjacent to playgrounds with recycled rubber flooring and 2 tire warehouses. Several toxic and carcinogenic PAHs (comprising some of the 8 ECHA and 16 EPA PAHs) were detected in all samples, as well as different toxic and priority pollutants plasticizers, the vulcanisation additives BTZ and TBP, and the antioxidant BHT. Some of these compounds reached dozens of ng m^{-3}, even in specific cases hundreds of ng m^{-3}.

The presence of these compounds in the air adjacent to the playgrounds was shown to be due to the recycled rubber, since in the surrounding area (around 25 metres away from the playground) no or lower concentrations of these compounds were detected. Furthermore, concentrations of hazardous substances were detected in the stands of the football pitches, so spectators may be affected by the diffusion of these compounds in the air, and not only the users of the surfaces. For car tire warehouses, and specially for the ELTs store, higher concentrations than in playgrounds and fields were detected. In particular, much higher sum PAH concentrations were found in the warehouses, up to 160 ng m^{-3}, and the highest concentrations for BTZ (around 1000 ng m^{-3}) and TBP (around 12 ng m^{-3}) were also found in these samples. On the other hand, the concentrations for the sum of PAHs were similar in the playgrounds and on the football pitches, between 7.3 and 27 ng m^{-3}, while the detected concentrations were slightly or significantly higher (depending on the substance) in the air sampled in the children´s playgrounds than in the air collected over the football pitches.

Hence, the transfer of concern chemicals from recycled tire rubber to different environmental compartments including runoff water and air was demonstrated. Several hazardous substances were detected in water leachates which can pose a risk to the environment, especially aquatic ecosystem. It has been shown in lab-scale studies that there is continuous leaching of these compounds from the recycled rubber into the runoff water. On the other hand, the presence of different harmful substances in the ambient air in spaces with rubber materials and recycled rubber may represent a hazard for human and environmental health. It is worth noting the higher concentration detected in the air on hot days.

3.- Evaluation of the potential recycled crumb rubber bioaccessibility

Oral ingestion is one of the main entry routes of crumb rubber compounds into human body, especially in the case of children due to their hand-to-mouth nature. It was estimated that approximately 200 mg of soil and dust per day are ingested by a four-year-old child according to US-EPA. *In-vitro* studies are needed to assess the potential risk to humans caused by organic compounds or metals and metalloids present in recycled rubber. To simulate the human digestion fluids (saliva, gastric juice, bile and duodenal) and mimic the crumb rubber digestion, the unified bioaccessibility method (UBM), developed by the Bioaccessibility Research Group of Europe (BARGE), was applied. An appropriate sample preparation treatment is essential to isolate the target analytes, in this case eighteen PAHs, including the eight ECHA and the sixteen EPA, present in the synthetic biological fluids. The selected technique to extract these compounds was SPE. To perform the extractions commercial sorbents, such as Oasis HLB, and a cork based biosorbent were employed. The analyses were carried out by GC-MS/MS. Before SPE-GC-MS/MS validation, some preliminary experiments were performed to optimize the type of sorbent, type of elution solvent, elution volume and sample volume loaded. Under the optimal conditions (Oasis HLB or cork as sorbent, 1 mL of ethyl acetate as solvent elution and 5 mL of synthetic fluids sample), the whole UBM-SPE-GC-MS/MS method was validated in terms of linearity, sensitivity, precision and accuracy. The method achieved good linearity in a wide concentration range with R^2 higher than 0.9974. IQLs and LOQs were at low ng L^{-1}. Additionally, satisfactory precision values with RSD lower than 12 % were achieved. The whole method accuracy was tested employing synthetic biological fluids spiked with the eighteen PAHs at three different concentration levels (three replicates per level), obtaining mean recovery values around 93 %. Furthermore, the feasibility of the cork biosorbent for the extraction of the compounds was demonstrated, as well as the suitability of the UBM method to perform the digestion with good accuracy.

Once the methodology was completely optimized and validated, it was applied to real crumb rubber samples. The samples include six recycled crumb rubber employed as infill directly collected from the fields, and two crumb rubber samples purchased from local providers. The results demonstrated the presence of seventeen of the eighteen target PAHs (all EPA PAHs were detected) in the analyzed fluids. In general, the most volatile like NAP, ACY, ACE, FLU, PHN and ANC, reached higher bioaccessibility percentages (between 0.l and 6.6 %, except for NAP, which reached percentages between 19 and 49 %) in digestive fluids. Regarding the heaviest PAHs, between 4 and 6 rings, they achieved the highest concentrations in the fluids. It is important to note, that the carcinogenic substance B[a]P was detected in the 75 % of the bioaccessible fractions, at concentrations up to 2.5 ng g^{-1}. Finally, an exposure assessment of children was carried out, concluding that the values obtained in the oral bioaccessibility tests were lower than the EPA's proposed oral reference dose (RfD) of 300 ng kg^{-1} day^{-1} for B[a]P. However, dozens of hazardous and problematic compounds are present in this type of material and must be taken into account in order not to underestimate the risk.

In addition to the target analysis, a suspect screening was performed to the same extracts to look for other tire-related compounds with harmful properties. Some of these compounds were detected in the fluids such as N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPDq), recently related with the death of coho salmon, and hexa(methoxymethyl)melamine (HMMM), among others.

To summarize, hazardous compounds from different chemical families such as PAHs, plasticizers, antioxidants and vulcanising additives were detected on different surfaces made from recycled rubber, confirming that the problem is global and does not only affect specific locations. Although carcinogenic substances, endocrine disruptors and reprotoxic compounds, that can cause problems for human health and the environment, have been identified in the crumb rubber samples studied, most of the analyzed samples comply with the current European legislation for some PAHs and plasticizers. Furthermore, recycled crumb rubber is an important source of microplastic pollution, specifically the main source of microplastic intentionally added in the environment. The ECHA estimates that around 16000 tonnes of recycled rubber granules, which represents around a 38 % of the total intentionally added microplastics in the environment. The environmental release of some of these chemicals through water and air has also been assessed. Several controversial compounds were detected in runoff water leachates and the continuous leaching of these substances was demonstrated in lab-scale studies. Regarding the air analysis, an active air sampling methodology followed by sustainable sample preparation techniques was developed. These surfaces release PAHs, plasticizers and other compounds into the air at levels of ng m^{-3}. It has been shown that on hot days these concentrations increase. Finally, *in-vitro* bioaccessibility studies were performed to evaluate the potential oral bioaccessibility of PAHs present in recycled crumb rubber, proving that most PAHs studied and other hazardous compounds were present in the synthetic biological fluids. On the other hand, in this thesis it was revealed that alternatives to crumb rubber contained no or lower concentrations of the target compounds. In addition, to perform the analysis, sustainable methodologies which complies with green analytical principles were employed, such as the implementation of UAE with low solvent extraction volume, or the SPME trying to avoid the organic solvent and gaining sensitivity. To conclude, although this thesis has allowed a deeper understanding of these materials and some of their environmental and toxicological implications, further studies are needed to identify and characterize more potentially hazardous substances in their composition, as well as compounds derived from the degradation or interaction of these materials in the environment.


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VI. CONCLUSIONS


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Throughout the development of this doctoral thesis, knowledge about the presence of toxic substances, including PAHs, plasticizers, antioxidants and vulcanisation additives, in materials made from recycled tire rubber has been deepened. Moreover, analytical methodologies to assess the possible diffusion of these compounds in the environment (adjacent air and runoff water) and the oral bioaccessibility of these compounds in human body have been developed.

Due to the different nature of the studied matrices (solid, liquid and gas), different sample preparation techniques have been used. All of them have tried to comply with the principles of green analytical chemistry and to be as simple as possible favouring implementation in any analytical laboratory. For the analysis of recycled crumb rubber materials ultrasound assisted extraction (UAE) was employed. Solid-phase extraction (SPE) was used to perform the active air sampling, based on sorbent trapping, followed by UAE and solid-phase microextraction (SPME) for the extraction of the target compounds. For the runoff water analysis SPME was also used. To isolate the bioaccessible fraction of the compounds, present in crumb rubber, into human biological fluids, SPE was the selected technique.

Analysis was performed employing gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) in all cases. This analytical tool provides an excellent selectivity and sensitivity to identify the compounds unequivocally.

To optimize the sample preparation step chemometric tools such as analysis of variance (ANOVA) and design of experiments (DOE) were employed. In some cases, ANOVA was also used to interpret the results data.

All the analytical methodologies applied and developed in this PhD thesis were validated in terms of analytical quality parameters: linearity, limits of detection and quantification, precision (intra-day and inter-day) and accuracy. Once the methods were successfully validated, they were applied to a large number of real samples.

The general conclusions of each chapter are included below:

Chapter 1. Determination of hazardous substances in different recycled rubber surfaces

- A simple methodology, employing low sample amount and solvent volume, based on UAE-GC-MS/MS has been validated and used for the analysis of 42 organic compounds in recycled tire rubber samples and alternative materials.

- The results showed the presence of several PAHs considered carcinogenic or priority pollutants, as well as any reproductive toxic or endocrine disrupting plasticizers in all recycled crumb samples rubber studied.

- Almost all recycled rubber samples fulfilled the ECHA limit of 20 µg g^{-1} established by the EU for the sum of the eight ECHA PAHs and the limit of 1000 µg g^{-1} for the individual concentration or the sum of the toxic plasticizers DIBP, DBP, BBP and DEHP.

- Cork granules (pitches infill) and sand (playground flooring) appear to be a good substitutes of recycled rubber materials in terms of hazardous chemical content. These materials were low-cost and biodegradable.

- Moreover, crumb rubber infill and thermoplastic elastomers (alternative materials) are considered as microplastics contributing to the environmental microplastic pollution, which is nowadays an emerging concern.

- The problematic associated to the presence of toxic substances in several type of recycled rubber materials (granules, mulches, tires, trees protectors, etc) and the global dimension of this problem was demonstrated.

Chapter 2. Environmental impacts of recycled tire rubber: air and water

- A methodology based on miniaturized SPME-GC-MS/MS has been validated for the analysis of 40 compounds in runoff water directly collected over synthetic turf football pitches.

- Compounds present in the rubber granulate used as infill in the same fields were detected in the runoff water, and laboratory-scale experiments were also carried out demonstrating the continuous leaching of toxic compounds from the rubber granulate into runoff and irrigation water.

- Two methodologies based on active air sampling (SPE) followed by UAE and SPME, respectively, were optimized employing DOE and validated in terms of analytical quality to study the presence of organic compounds related to tire and recycled tire rubber materials in air adjacent to these surfaces.

- Compounds from all the studied families were detected in air samples collected at different locations related to tires or recycled rubber (football fields, parks, tire warehouses, car parks, etc).

- Furthermore, it was shown that the composition of the air and the recycled rubber used in fields and parks had a similar distribution, so there is clearly a relationship between air pollution and the presence of these surfaces in the environment.

Chapter 3. Evaluation of the potential recycled crumb rubber bioaccessibility

- The potential oral bioaccessibility of 18 PAHs present in recycled rubber granulates was studied. The UBM method was used to simulate the digestion of recycled rubber granulate used as infill in artificial football pitches.

- An analytical method based on SPE employing commercial sorbents such as Oasis HLB and a new biosorbent based on crushed cork followed by GC-MS/MS was developed and validated to perform the isolation of 18 PAHs from the human synthetic biological fluids.

- For the first time the oral bioaccessibility of 18 PAHs present in real crumb rubber samples used as infill in synthetic turf football pitches into synthetic human biological fluids was demonstrated.

- A method based on UBM-SPE-GC-MS/MS was optimized and validated to digest the samples and analyse the synthetic gastrointestinal fluids. As SPE sorbents, commercial sorbents such as Oasis HLB and a new biosorbent based on cork were employed.

- Most of the 16 EPA PAHs and 8 ECHA PAHs were detected in the studied biological fluid samples at levels of ng g^{-1}.

- In addition, a target screening to determine the possible bioaccessibility of other hazardous compounds to the environment and the health of aquatic organisms such as 6PPD, 6PPD-quinone or HMMM was performed. The presence of them in all samples was revealed.

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ANNEX I: LIST OF PUBLICATIONS


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- Maria Celeiro, Daniel Armada, Nuno Ratola, Thierry Dagnac, Jacob de Boer, Maria Llompart. Evaluation of chemicals of environmental concern in crumb rubber and water leachates from several types of synthetic turf football pitches, *Chemosphere*, 270 (2021), 128610

  DOI: 10.1016/j.chemosphere.2020.128610
  Impact factor: **JCR**: 8.943. **CiteScore**: 11.7 (2021). **SJR**: 1.505 (2021). **JCR-Q1**: Environmental Sciences **SJR-Q1**: Chemistry (miscellaneous); Environmental Chemistry; Environmental Engineering; Health, Toxicology and Mutagenesis; Medicine (miscellaneous); Pollution; Public Health, Environmental and Occupational Health (2021).
  Contribution: Formal analysis, Investigation, Data curation, Writing-review.

- Maria Celeiro, Daniel Armada, Thierry Dagnac, Jacob de Boer, Maria Llompart. Hazardous compounds in recreational and urban recycled surfaces made from crumb rubber. Compliance with current regulation and future perspectives, *Science of The Total Environment*, 755 (2021), 142566

  DOI: 10.1016/j.scitotenv.2020.142566
  Impact factor: **JCR**: 10.753. **CiteScore**: 14.1. **SJR**: 1.806 (2021). **JCR-Q1**: Environmental Sciences. **SJR-Q1**: Environmental Chemistry; Environmental Engineering; Pollution; Waste Management and Disposal (2021).
  Contribution: Formal analysis, Investigation, Data curation.

- Daniel Armada, Maria Celeiro, Antia Martinez-Fernandez, Piyaluk Nurerk, Thierry Dagnac, Maria Llompart. Miniaturized active air sampling method for the analysis of tire rubber pollutants from indoor and outdoor places, *Journal of Separation Science*, 44 (2021), 1694-1705

DOI: 10.1002/jssc.202001249
Impact factor: **JCR**: 3.614. **CiteScore**: 5.5. **SJR**: 0.58 (2021)
JCR-Q2: Analytical Chemistry. **SJR-Q2**: Analytical Chemistry; Filtration and Separation (2021).

- Daniel Armada, Maria Llompart, Maria Celeiro, Pablo Garcia-Castro, Nuno Ratola, Thierry Dagnac, Jacob de Boer. Global evaluation of the chemical hazard of recycled tire crumb rubber employed on worldwide synthetic turf football pitches, *Science of The Total Environment*, 812 (2022), 152542

  DOI: 10.1016/j.scitotenv.2021.152542
  Impact factor: **JCR:** 10.753. **CiteScore:** 14.1. **SJR:** 1.806 (2021). **JCR-Q1:** Environmental Sciences. **SJR-Q1:** Environmental Chemistry; Environmental Engineering; Pollution; Waste Management and Disposal (2021).
  Contribution: Validation, Formal analysis, Investigation, Data curation, Writing-original draft, Writing-review & editing, Visualization.

- Daniel Armada, Maria Celeiro, Thierry Dagnac, Maria Llompart. Green methodology based on active air sampling followed by solid phase microextraction and gas chromatography-tandem mass spectrometry analysis to determine hazardous substances in different environments related to tire rubber, *Journal of Chromatography A*, 1668 (2022), 462911

  DOI: 10.1016/j.chroma.2022.462911
  Impact factor: **JCR**: 4.601. **CiteScore**: 7.3. **SJR**: 0.813 (2021)
  JCR-Q1: Analytical Chemistry. **JCR-Q2:** Biochemical Research Methods. **SJR-Q1**: Analytical Chemistry, and Organic Chemistry. **SJR-Q2:** Biochemistry, and Medicine (miscellaneous) (2021).

Contribution: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-original draft, Writing-review & editing, Visualization.

- Daniel Armada, Antia Martinez-Fernandez, Maria Celeiro, Thierry Dagnac, Maria Llompart. Assessment of the bioaccessibility of PAHs and other hazardous compounds present in recycled tire rubber employed in synthetic football fields, *Science of The Total Environment*, 857 (2023), 159485

  DOI: 10.1016/j.scitotenv.2022.159485
  Impact factor: **JCR:** 10.753. **CiteScore:** 14.1. **SJR:** 1.806 (2021). **JCR-Q1:** Environmental Sciences. **SJR-Q1:** Environmental Chemistry; Environmental Engineering; Pollution; Waste Management and Disposal (2021).
  Contribution: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-original draft, review & editing, Visualization.


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ANNEX II: JOURNAL PERMISSIONS


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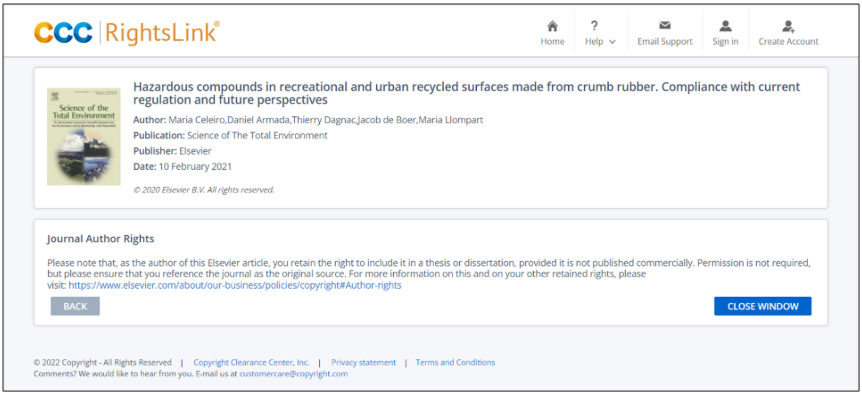
- M. Celeiro, D. Armada, T. Dagnac, J. de Boer, M. Llompart. Hazardous compounds in recreational and urban recycled surfaces made from crumb rubber. Compliance with current regulation and future perspectives, *Sci. Total Environ.*, 755 (2021), 142566



Hazardous compounds in recreational and urban recycled surfaces made from crumb rubber. Compliance with current regulation and future perspectives

Author: Maria Celeiro,Daniel Armada,Thierry Dagnac,Jacob de Boer,Maria Llompart
Publication: Science of The Total Environment
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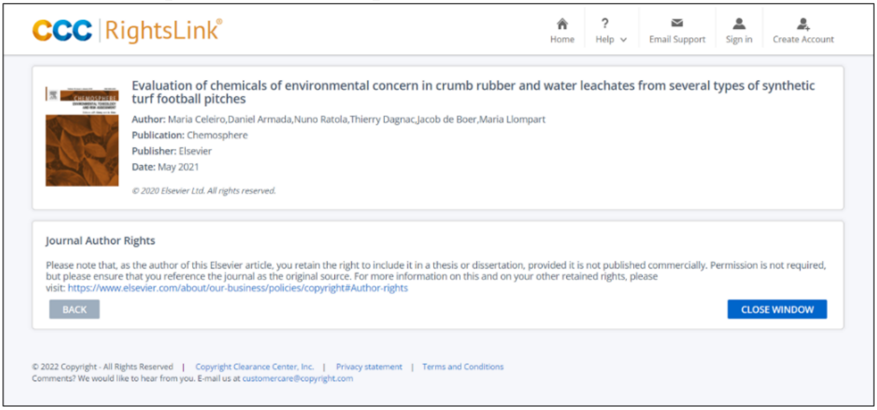
- M. Celeiro, D. Armada, N. Ratola, T. Dagnac, J. de Boer, M. Llompart. Evaluation of chemicals of environmental concern in crumb rubber and water leachates from several types of synthetic turf football pitches, *Chemosphere*, 270 (2021), 128610



Evaluation of chemicals of environmental concern in crumb rubber and water leachates from several types of synthetic turf football pitches

Author: Maria Celeiro,Daniel Armada,Nuno Ratola,Thierry Dagnac,Jacob de Boer,Maria Llompart
Publication: Chemosphere
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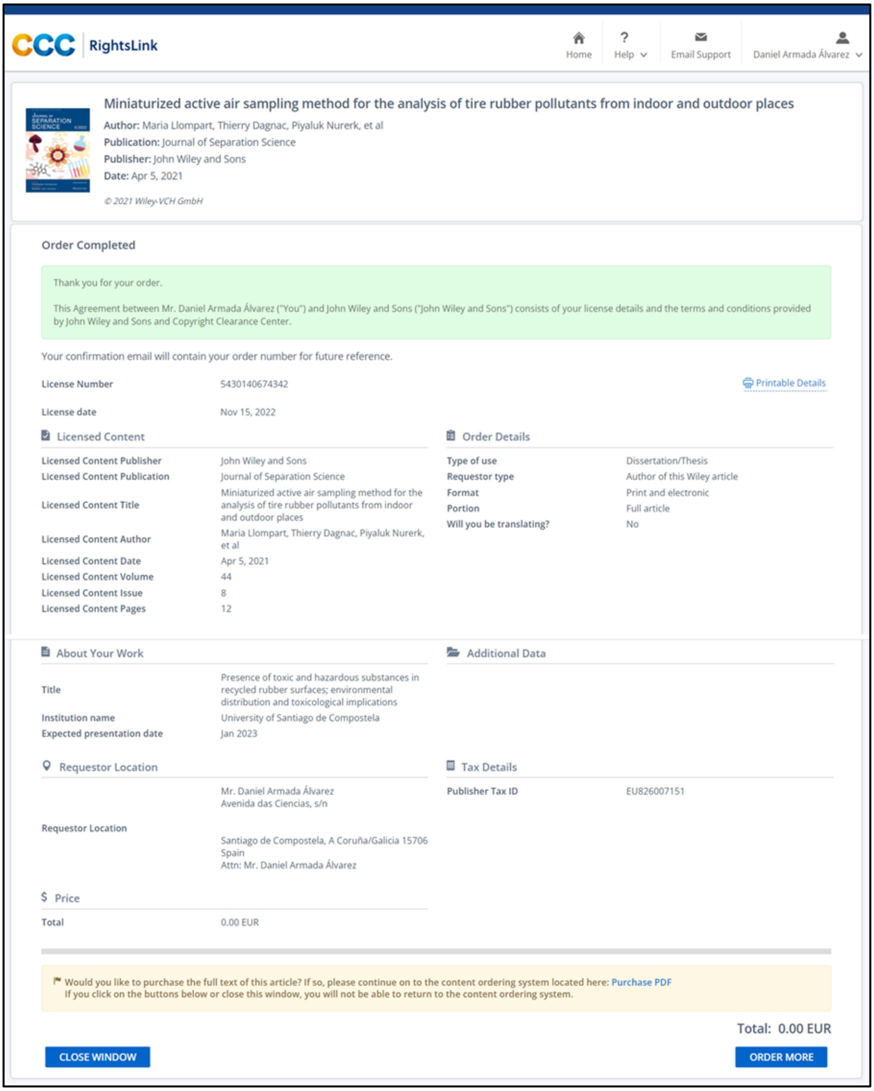
- D. Armada, M. Celeiro, A. Martinez-Fernandez, P. Nurerk, T. Dagnac, M. Llompart. Miniaturized active air sampling method for the analysis of tire rubber pollutants from indoor and outdoor places, *J. Sep. Sci.*, 44(8) (2021), 1694-1705



Miniaturized active air sampling method for the analysis of tire rubber pollutants from indoor and outdoor places

Author: Maria Llompart, Thierry Dagnac, Piyaluk Nurerk, et al
Publication: Journal of Separation Science
Publisher: John Wiley and Sons
Date: Apr 5, 2021

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Global evaluation of the chemical hazard of recycled tire crumb rubber employed on worldwide synthetic turf football pitches

Author: Daniel Armada,Maria Llompart,Maria Celeiro,Pablo Garcia-Castro,Nuno Ratola,Thierry Dagnac,Jacob de Boer

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- D. Armada, M. Celeiro, T. Dagnac, M. Llompart. Green methodology based on active air sampling followed by solid phase microextraction and gas chromatography-tandem mass spectrometry analysis to determine hazardous substances in different environments related to tire rubber, *J. Chromatogr. A*, 1668 (2022), 462911 (*Open Access*)

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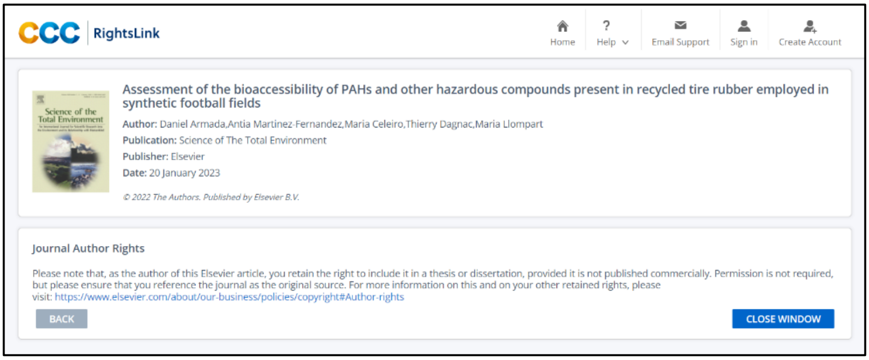
- D. Armada, A. Martinez-Fernandez, M. Celeiro, T. Dagnac, M. Llompart. Assessment of the bioaccessibility of PAHs and other hazardous compounds present in recycled tire rubber employed in synthetic football fields, *Sci. Total Environ.*, 857 (2023), 159485 (Open access)



Assessment of the bioaccessibility of PAHs and other hazardous compounds present in recycled tire rubber employed in synthetic football fields

Author: Daniel Armada,Antia Martinez-Fernandez,Maria Celeiro,Thierry Dagnac,Maria Llompart
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ANNEX III: INDEX OF FIGURES AND TABLES

FIGURES

TABLES


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ANNEX IV: RESUMO


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A presente tese doutoral aborda o estudo da presenza de diversas familias de compostos perigosos en mostras de caucho e granulado de caucho reciclado así como a difusión de ditas substancias dende os materiais de caucho aos compartimentos ambientais, como a auga e o aire. Por outra banda, tamén se avalía a bioaccesibilidade oral de hidrocarburos policíclicos aromáticos e outros compostos presentes no caucho en fluídos biolóxicos humanos.

Debido á variedade que presentan as matrices de estudo (sólidas, líquidas e gaseosas) e a ampla gama de substancias determinadas (corenta e dous na maioría dos casos), foron necesarios distintos enfoques analíticos empregando diversas técnicas de mostraxe e extracción e realizando a análise en todos os casos mediante cromatografía de gases seguida de espectrometría de masas en tándem (GC-MS/MS).

En canto ao tipo de mostras, analizáronse diferentes materiais sólidos, incluíndo materiais de caucho reciclado procedente de pneumáticos e outros materiais alternativos como gránulos de cortiza, fibra de coco, area ou polímeros termoplásticos. Ademais, tamén se estudaron mostras de auga, aire e fluídos biolóxicos.

Atendendo aos compostos de estudo, investigáronse catro familias de substancias, entre as cales se atopan dezaoito hidrocarburos policíclicos aromáticos (PAHs), dezanove plastificantes (tres adipatos, quince ftalatos e bisfenol A), dous antioxidantes e tres aditivos de vulcanización. Adicionalmente, nos fluidos biolóxicos humanos realizouse un cribado de sospeita de cinco compostos.

Polo tanto, as distintas investigacións levadas a cabo dividíronse en tres capítulos que se describen a continuación:

1.- Determinación de substancias perigosas en diferentes superficies de caucho reciclado

A xestión dos pneumáticos fóra de uso representa un problema debido ao crecente número de pneumáticos desbotados cada ano. Este material ten un alto impacto ambiental xa que non é biodegradable. A problemática relacionada cos vertedoiros dos pneumáticos fóra de uso provocou que os gobernos e institucións considerasen alternativas para a súa reciclaxe e reutilización. Deste xeito, unha das aplicacións máis útiles e atractivas dos pneumáticos fóra de uso, é a súa conversión en gránulos de caucho para ser empregado como recheo en campos de fútbol de céspede artificial ou como pavimento de parques infantís, entre outros usos.

Non obstante, o caucho reciclado empregado para ditas superficies deportivas e de ocio está no punto de mira debido á presenza de substancias perigosas na súa composición. De feito, xa se atoparon metais pesados como Cd, Pb, Cr, Ni, Cu, Zn ou Fe, entre outros, no granulado de caucho en concentracións de ata miles de µg g^{-1}. Neste tipo de superficies tamén se detectaron PAHs, plastificantes, antioxidantes, aditivos de vulcanización, benzotiazoles, parafinas cloradas, bifenilos clorados ou alquilfenois. Polo tanto, isto pode presentar un risco non só para o medio ambiente, senón tamén para a saúde humana.

As institucións europeas tomaron medidas en relación co contido dalgunhas destas substancias perigosas en materiais de caucho. Neste contexto, o 10 de agosto de 2022 a Unión Europea restrinxiu oito PAHs considerados canceríxenos (benzo[a]antraceno (B[a]A), criseno (CHY), benzo[b]fluoranteno (B[b]F), benzo[j]fluoranteno (B[j]F), benzo[k]fluoranteno (B[k]F), benzo[a]pireno (B[a]P), benzo[e]pireno (B[e]P) e dibenzo[ah]antraceno (D[ah]A)) a unha concentración máxima de 20 µg g^{-1} en gránulos, losetas ou mantas empregadas como material de recheo en campos de céspede sintético ou en forma solta, para protexer a saúde humana. (Regulamento (UE) 2021/1199 da Comisión, de 20 de xullo de 2021).

Ademais, a Axencia de Protección Ambiental dos Estados Unidos (US-EPA) catalogou dezaseis PAHs (naftaleno (NAP), acenaftileno (ACY), acenafteno (ACE), fluoreno (FLU), fenantreno (PHN), antraceno (ANC), pireno (PYR), fluoranteno (FLA), B[a]A, CHY, B[b]F, B[k]F, B[a]P, indeno(1,2, 3-cd)-pireno (IND), D[ah]A e benzo[ghi]perileno (B[ghi]P) e seis ftalatos (di-n-octil ftalato (DNOP), dietil ftalato (DEP), dimetil ftalato (DMP), di-2(ethylhexil) ftalato (DEHP), butil bencil ftalato (BBP) e di-N-butil ftalato (DBP)) como contaminantes prioritarios.

Por outro lado, catro ftalatos (DEHP, BBP, DBP e diisobutilftalato, DIBP) están restrinxidos nos materiais plastificados, incluídos os materiais de caucho, a 1000 μg g^{-1} para as concentracións individuais ou a suma delas (Regulamento (UE) 2018/2005 da Comisión, de 17 de decembro de 2018).

Tamén cabe destacar que o granulado de caucho é considerado un microplástico, sendo a fonte máis importante dos microplásticos engadidos intencionadamente no medio ambiente, representando en torno ao 38 %.

Por este motivo, na presente tese levouse a cabo un estudo en profundidade da presenza de corenta e dous compostos orgánicos (PAHs, plastificantes, antioxidantes e aditivos de vulcanización) en diferentes matrices de caucho reciclado. Entre os diferentes tipos de mostras atopáronse granulado de caucho empregado en campos de fútbol sintéticos, superficies de parques infantís, protectores de raíces de árbores, baldosas comerciais e pneumáticos de automóbiles.

A técnica de preparación da mostra empregada para a extracción dos compostos foi a extracción asistida por ultrasóns (UAE) e a identificación e determinación das substancias realizouse mediante GC-MS/MS. O método analítico, optimizado noutra investigación previa á presente tese, validouse en termos de linearidade, límites de detección e cuantificación, precisión e exactitude. Cabe destacar que foi a primeira vez que se realizaron estudos de recuperación neste tipo de material reciclado e que se obtiveron resultados satisfactorios, entre o 72 e 116 % con desviacións estándar relativas inferiores ao 15 %.

Posteriormente, analizáronse un total de corenta materiais de caucho de pneumáticos empregados en superficies deportivas, urbanas e de ocio e dous materiais alternativos.

No caucho procedente de parques infantís e campos de fútbol de céspede artificial, atopáronse trinta dos corenta e dous compostos analizados. Dos PAHs, o composto que apareceu a maior concentración foi o PYR, sendo unha substancia persistente, bioacumulable e tóxica. Ademais, é de gran interese mencionar que os parques infantís interiores presentaron concentracións máis altas que os exteriores. Isto podería ser debido a que as superficies exteriores están expostas ás condicións meteorolóxicas (choiva, cambios de temperatura, etc) e, co paso do tempo, poden liberar parte da concentración dos seus compostos ao medio ambiente. Non obstante, as concentracións medias de PAHs en ambos casos (parques interiores ou exteriores) alcanzaron concentracións máis baixas que nos campos de fútbol de céspede artificial.

Pos outra banda, en ambas superficies puideron determinarse plastificantes e outras substancias perigosas, como o DEHP que é un ftalato tóxico para a reprodución e unha substancia altamente preocupante.

En canto aos materiais alternativos analizados, na cortiza procedente dun campo de fútbol, só se detectaron catro dos compostos obxectivo (PHN, DEP, BPA e BHT). A presenza destes compostos poderíase explicar polo feito de que o campo no cal se atopa, fora previamente recheo con granulado de caucho reciclado. Non obstante, a area recollida nun parque infantil estaba totalmente libre de contaminantes. Polo tanto, ambos materiais demostraron ser alternativas interesantes ao caucho reciclado.

Nos protectores das raíces das árbores, detectáronse trece PAHs, dezanove plastificantes, BHT, BTZ e TBP, podendo representar un risco para o medio ambiente debido ao seu contacto directo coas raíces e co solo.

A maiores, tamén se analizaron pneumáticos de coche, encontrado quince dos dezaoito PAHs de estudo, oito plastificantes, BHT, BTZ e TBP. Ademais, comparáronse os gránulos de caucho reciclado comercial con aqueles recollidos directamente nos terreos de campos sintéticos. En ambos casos, os perfís de PAHs foron bastante similares, aínda que no granulado comercial as concentracións foron maiores.

Por último, analizáronse as baldosas de caucho comerciais, alcanzando concentracións de varios ordes de magnitude superiores ás das demáis superficies estudadas. Os resultados para os PAHs foron extremadamente altos en comparación coas demais mostras, obtendo unha concentración media de PAHs ao redor dos 6000 µg g^{-1}. Non obstante, algúns plastificantes, BTZ, TBP e BHT, amosaron concentracións similares ás rexistradas nos parques infantís interiores.

Para resumir, en todos os tipos de mostras de caucho reciclado, detectáronse compostos perigosos, aínda que na maioría dos casos cumpriron a normativa europea. Ademais, os materiais alternativos, como a coriza e a area, parecen ser alternativas sostibles en canto ás implicacións para a saúde e o medio ambiente.

Co obxectivo de coñecer se este problema era global, levouse a cabo un estudo do granulado de caucho empregado como recheo nos campos de fútbol de céspede sintético de varios países. Deste xeito, analizáronse un total de setenta e oito mostras de caucho reciclado procedentes de Europa (Albania, Croacia, Finlandia, Francia, Alemaña, Grecia, Italia, Países Baixos, Polonia, Portugal, España, Suecia, Turquía e Reino Unido), América (Chile e Estados Unidos) e Asia (Tailandia e Turquía). Ademais, incluíronse trece mostras de materiais alternativos como gránulos de cortiza, fibra de coco e elastómeros termoplásticos.

Neste caso empregouse a mesma metodoloxía que a investigación anterior, utilizando UAE-GC-MS/MS. Tras a verificación do método en termos de linearidade, sensibilidade e precisión, aplicouse ás noventa e unha mostras e tentouse buscar unha relación entre a orixe xeográfica e os perfiles químicos do caucho facendo uso de ferramentas estatísticas.

A tendencia no contido de PAHs foi similar en todos os países, sendo PYR o composto máis abundante, seguido na maioría dos casos por FLA, ambos considerados persistentes, bioacumulables e tóxicos. Aínda que é moi difícil relacionar a idade dun campo coas concentracións encontradas no granulado analizado, xa que se engade periodicamente máis recheo de caucho novo, obsérvase unha maior concentración para os PAHs nas mostras recollidas en campos de fútbol de recente construción (de 0,5 a 2 anos). Isto probablemente se debe a que dito caucho, practicamente, non se utilizou e non estivo tanto tempo á intemperie.

Tendo en conta a normativa europea para os oito PAHs considerados canceríxenos entre outras propiedades pola Axencia Europea dos Produtos Químicos (ECHA), unha das mostras recollidas nun país da UE superou o límite de 20 µg g^{-1}. Da mesma maneira, aínda que os países non pertencentes á UE non están cubertos por esta normativa, dúas das mostras tamén superaron este límite. Por outro lado, considerando os plastificantes estudados nesta investigación que están regulados pola UE (DIBP, DBP, BBP e DEHP) neste tipo de material, tres mostras, unha dun país da UE e outras dúas de países non comunitarios, superaron o límite legal de 1000 µg g^{-1}. Ademais, das substancias mencionadas, tamén se detectaron outros dos PAHs, plastificantes, antioxidantes e aditivos de vulcanización que foron obxecto de estudo.

Non obstante, hoxe en día empréganse outros materiais alternativos para substituír o caucho dos campos de fútbol sintéticos. Nos tres materiais alternativos analizados (corcho, fibra de coco e elastómeros termoplásticos) detectáronse PAHs, mais os niveis de concentracións foron máis baixos que no granulado de caucho. A presenza destes compostos, podería deberse a que anteriormente eses campos estiveron recheos con caucho reciclado. En canto aos plastificantes, determináronse unhas altas concentracións na alternativa de elastómeros termoplásticos, o que era de esperar dada a súa composición a base de plástico. Ademais, tanto os gránulos de caucho como os polímeros termoplásticos considéranse microplásticos que se poden liberar no medio ambiente. Tendo en conta todos os resultados obtidos, a cortiza pareceu ser a mellor alternativa para substituír o caucho reciclado nos campos de fútbol artificial debido ao seu baixo ou nulo contido de substancias químicas perigosas e a súa biodegradabilidade.

Por último, fíxose unha análise de varianza (ANOVA) tratando de encontrar diferencias significativas entre o recheo de caucho considerando a súa orixe xeográfica, idade do campo e lugar interior/exterior. Como resultado, observouse que para algúns dos compostos como PYR, DMP, BTZ e BBP detectáronse maiores concentracións nas mostras de interior que nas de exterior. Non obstante, non foi posible atopar unha relación entre a composición química do caucho e a súa orixe, posiblemente debido á globalización, que fai que un fabricante deste material poida subministrar a varios países de todo o mundo. Do mesmo xeito, tampouco se obtido unha relación clara coa idade do campo, xa que os campos son recheos periodicamente con granulado de caucho reciclado fresco.

2.- Impactos ambientais do caucho de pneumático reciclado: aire e auga

A maioría das superficies fabricadas con caucho de pneumático reciclado atópanse en espazos exteriores e, polo tanto, están expostas a diferentes condicións meteorolóxicas e atmosféricas. A difusión dos compostos perigosos presentes nestas superficies no medio ambiente pode representar un problema ambiental e ter un impacto na saúde dos seres humanos e outros organismos. Por esta razón, levouse a cabo unha avaliación da posible liberación de substancias presentes no caucho reciclado en diferentes compartimentos ambientais, incluíndo a auga, a través de lixiviados, e o aire. Varios dos compostos detectados nas mostras de caucho reciclado son considerados pola Unión Europea contaminantes prioritarios en augas superficies (Directiva 2008/105/CE) como os PAHs ANC, B[a]P, B[b]F, B[ghi]P, B[k]F, FLA, IND e NAP, e o plastificante DEHP.

Por esta razón, a vixilancia e o control da presenza destas substancias na auga que está en contacto con este tipo de recheo de caucho ou cos pavimentos dos parques infantís presenta gran importancia. Neste estudo, recolléronse mostras de auga de lixiviación de campos sintéticos en días de choiva para avaliar a transferencia de corenta compostos dende o caucho á auga. Ademais, simuláronse experimentos de auga de lixiviación a escala de laboratorio. Para iso empregouse auga de regadío e auga de choiva, poñendo as mostras de caucho reciclado en contacto coa auga durante 24 horas. Tamén, para investigar a difusión continua das substancias do granulado de caucho cara á auga, a mesma mostra de caucho púxose en contacto cunha nova alícuota de auga cada 24 horas durante unha semana, analizando todos os extractos recollidos cada 24 horas.

A técnica de extracción seleccionada foi a microextracción en fase sólida (SPME) seguida dunha análise por GC-MS/MS. Previamente á análise de oito mostras de augas reais e catro augas simuladas, a metodoloxía SPME-GC-MS/MS validouse en termos de calidade analítica, avaliando a linearidade, límites de detección, precisión e recuperacións.

Nas mostras de auga de lixiviación reais recollidas en días de choiva, detectáronse varios dos PAHs e o plastificante DEHP, considerados como contaminantes nas augas superficiais. Do mesmo xeito, tamén se encontraron outros dos compostos de estudo, destacando a presenza de CHY que é canceríxeno, persistente, bioacumulable e moi tóxico para a vida acuática segundo a UE e do TBP que ademais de ser un disruptor endócrino, tamén é moi tóxico para a vida acuática con efectos duradeiros.

En canto aos resultados dos experimentos con augas simuladas, amosouse que o granulado de caucho é unha fonte continua de PAHs, ftalatos, antioxidantes e aditivos de vulcanización. Por exemplo, os PAHs que foron encontrados nestas augas, detectáronse nas mostras recollidas nos diferentes días, polo que revela unha transferencia continua destes compostos ao longo do tempo. Polo tanto, a similitude dos resultados entre as augas reais e as simuladas demostra que a contaminación química destas augas provén do recheo de caucho.

A difusión no aire é outra vía de entrada das substancias relacionados cos pneumáticos no medio ambiente, e isto pode supoñer un risco para a saúde humana xa que poden ser inhalados. Estas superficies en días de calor poden chegar a alcanzar temperaturas entre 60 e 80 ºC o que facilita a difusión no aire de varios dos compostos presentes no granulado de caucho. Por este motivo, na presente tese de doutoramento desenvolvéronse dúas metodoloxías analíticas baseadas na mostraxe activa de aire, para medir e demostrar a contaminación do aire producida por este tipo de superficies. En ambos métodos, a mostraxe activa de aire levouse a cabo mediante extracción en fase sólida (SPE) para capturar o aire e reter os compostos obxectivo, utilizando una bomba a un caudal controlado (60 L min^{-1}). Despois da etapa de SPE, utilizáronse diferentes técnicas de extracción, como UAE e SPME, para illar as substancias retidas no adsorbente na etapa de SPE antes da análise mediante GC-MS/MS.

Na primeira investigación optimizouse a metodoloxía SPE-UAE-GC-MS/MS, para analizar 41 compostos pertencentes ás familias mencionadas anteriormente, mediante estudios preliminares, nos que se seleccionou o tipo (Florisil, Tenax TA e MIL-101) e a cantidade (25 mg) de adsorbente. Posteriormente, levouse a cabo un deseño experimental factorial completo (2^4, 16 experimentos) para avaliar o tempo de extracción (2 e 10 min), a técnica de extracción (vórtex e UAE), o tipo de disolvente (acetato de etilo e hexano) e o tipo de adsorbente (MIL-101 e Tenax TA). En base aos resultados obtidos, as condicións máis favorables para a mostraxe e extracción simultánea dos compostos implican o uso de Tenax TA e a desorción dos analitos con acetato de etilo (1 mL) utilizando UAE durante 2 minutos. Logo estudouse o volume de ruptura do aire bombeado, obténdose como volume de aire máis práctico 1 m^3 xa que ofrece un tempo de mostraxe corto (16 min) e un alto rendemento. A continuación, o método SPE-UAE-GC-MS/MS validouse en canto a linearidade, sensibilidade, precisión e exactitude.

Finalmente, o método optimizado e validado aplicouse a mostras reais de aire exterior e interior recollidas en aparcamentos de automóbiles, un almacén que contiña baldosas de caucho recicladas, unha estación de autobuses e un campo de fútbol de céspede sintético. Nos espazos interiores atopáronse entre nove e quince PAHs, e nos exteriores entre nove e once. As maiores concentracións determináronse no almacén, xa que ademais de que se almacenaban varias baldosas de caucho reciclado, non presentaba ningún tipo de ventilación. Por outro lado, no campo de fútbol a mostraxe realizouse en tres días distintos, sendo un deles un día cálido con temperaturas en torno aos 30 ºC e os outros dous días entre 10 e 15 ºC, destacando que as concentracións máis altas se detectaron o día más caloroso. Ademais, esta mostra foi na que se encontraron os maiores niveis de plastificantes, a pesar de ser unha mostra dun espazo exterior.

Á vista dos resultados, levouse a cabo outro estudo para a análise de mostras de aire recollidas en lugares relacionados con pneumáticos, pero neste caso empregando SPME como técnica de extracción, tratando de evitar ou reducir o volume de disolvente orgánico e facer un proceso de mostraxe-análise máis sostible. Para optimizar a etapa de SPME, seleccionáronse dous recubrimentos de fibra (PA e PDMS/DVB) segundo os resultados preliminares. A continuación, fíxose un deseño experimental de fracción de nivel mixto ($3 \cdot 2^{3-1} + 3$ puntos centrais) no que se estudaron catro factores que incluían o tipo de recubrimento de fibra (PA e PDMS/DVB), a adición de disolvente (0 e 100 µL de acetona), o volume do espazo de cabeza (1,8 e 10 mL de vial) e a axitación do adsorbente. As condicións experimentais óptimas obtivéronse engadindo 100 µL de acetona ao adsorbente (Tenax TA) utilizando a fibra PDMS/DVB nun vial de 10 mL sen axitación. O tempo de extracción óptimo de SPME resultou ser 45 minutos para obter un illamento dos compostos sensible e de alto rendemento. Posteriormente, o método validouse en termos de calidade analítica, estudando a linearidade, a precisión, límites de detección e cuantificación e estudos de recuperación.

Por outro lado, o protocolo SPE-SPME-GC-MS/MS completo comparouse co método SPE-UAE-GC-MS/MS, desenvolvido previamente, revelando que coa primeira metodoloxía se obteñen maiores respostas cromatográficas para as substancias máis volátiles (as máis presentes no aire), sendo ademais unha alternativa máis sostible debido á redución do uso de disolventes orgánicos e á xeración de residuos.

Neste estudo, analizáronse mostras de aire recollidas en tres tipos de lugares diferentes, incluíndo dúas mostras de campos de fútbol de céspede sintético recheo con gránulos de caucho reciclado, tres de aire adxacente a parques infantís con pavimento de caucho reciclado e dous almacenes de pneumáticos. Os resultados demostraron que en todas as mostras se detectaron varios PAHs tóxicos e canceríxenos, plastificantes, os aditivos de vulcanización BTZ e TBP, e o antioxidante BHT.

Ademais, demostrouse que a presenza destas substancias no aire adxacente aos parques infantís debíase ao caucho reciclado, xa que na zona circundante (a uns 25 metros de distancia do parque infantil) non se detectaron concentracións destes compostos ou estas foron menores. Por outra banda, nas gradas dos campos de fútbol si que se atoparon algunhas destas substancias perigosas, polo que ademais dos usuarios destas superficies, os espectadores tamén se poden ver afectados pola difusión destes compostos no aire. En canto aos almacéns de pneumáticos de automóbiles, e especialmente nos almacenes dos pneumáticos fóra de uso, detectáronse concentracións máis elevadas. que nos parques infantís e nos campos artificiais.

3.- Avaliación da potencial bioaccesibilidade do granulado de caucho reciclado

A inxestión oral é unha das principais vías de entrada dos compostos presentes no granulado de caucho no corpo humano, especialmente en nenos. Para iso, necesítanse estudos *in-vitro* para avaliar o potencial risco para os seres humanos causado polas substancias orgánicas ou os metais e metaloides presentes no caucho reciclado. Para simular os fluídos da dixestión humana (saliva, xugo gástrico, bile e duodenal) e imitar a dixestión do caucho, aplicouse o *unified bioaccessibility method* (UBM). Un tratamento adecuado de preparación da mostra é esencial para illar os compostos obxectivo, neste caso dezaoito PAHs, presentes nos fluídos biolóxicos sintéticos. Deste xeito, a técnica seleccionada para extraer ditas substancias foi a SPE, empregando adsorbentes comerciais, como o Oasis HLB, e un bioadsorbente a base de cortiza. A análise realizouse por GC-MS/MS. Primeiro fixéronse experimentos preliminares para optimizar a etapa de SPE, como o tipo de adsorbente, o disolvente e volume de elución e o volume de carga de mostra. Baixo condicións óptimas (Oasis HLB ou cortiza como adsorbente, 1 mL de acetato de etilo como disolvente e 5 mL de mostra de fluídos sintéticos) validouse o método UBM-SPE-GC-MS/MS completo en termos de linearidade, sensibilidade, precisión e exactitude.

Posteriormente, a metodoloxía aplicouse a mostras reais de granulado de caucho, incluíndo seis recollidas en campos fútbol de céspede sintético e dúas compradas a provedores locais. Os resultados demostraron a presenza de dezasete dos PAHs de estudo nos fluídos analizados. En xeral, os PAHs máis volátiles (NAP, ACY, ACE, FLU, PHN e ANC), alcanzaron maiores porcentaxes de bioaccesibilidade (entre 0,l e 6,6 % na maioría dos casos) nos fluídos dixestivos. Cabe destacar que a substancia canceríxena B[a]P detectouse no 75 % das fraccións bioaccesibles en concentracións de ata 2,5 ng g^{-1}. Por último, levouse a cabo un estudo da avaliación do risco de exposición dos nenos, concluíndo que os valores obtidos nas probas de bioaccesibilidade oral eran inferiores á dose oral de referencia (RfD) proposta pola EPA de 300 ng kg^{-1} $día^{-1}$ para o B[a]P. Non obstante, neste tipo de material hai decenas de compostos perigosos e problemáticos que deben terse en conta para non subestimar o risco.

A maiores, realizouse un cribado de sospeita para buscar outros compostos relacionados cos pneumáticos, podendo detectar nos fluídos a N-(1,3-Dimetilbutil)-N′-fenil-p-fenilendiamina-quinona (6PPDq), recentemente relacionada coa morte do salmón coho, e a hexa(metoximetil)melamina (HMMM), entre outros.


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This thesis focuses on the study of recycled tire rubber employed as infill in artificial turf football pitches and as flooring in children's playgrounds, among other uses. Different compounds such as polycyclic aromatic hydrocarbons, plasticizers, antioxidants and vulcanisation additives were determined in a large number of samples of different recycled tire rubber matrices. In addition, the diffusion of these substances into the environment (air and water) and the potential bioaccessibility via oral ingestion were studied.

For this purpose, different analytical methodologies were developed and validated. Due to the wide variety of the studied samples, different simple and sustainable sample preparation techniques were employed prior to analysis by gas chromatography coupled to tandem mass spectrometry.