BISPHENOL RESIDUES IN THE AQUEOUS ENVIRONMENT: OCCURRENCE AND FATE

Ana Kovačič

Doctoral Dissertation
Jožef Stefan International Postgraduate School
Ljubljana, Slovenia

Supervisor: Prof Dr Ester Heath, Jožef Stefan Institute, Ljubljana, Slovenia
Co-Supervisor: Assist Prof Dr Tina Kosjek, Jožef Stefan Institute, Ljubljana, Slovenia

Evaluation Board:

Prof Dr Milena Horvat, Chair, Department of Environmental Sciences, Jožef Stefan Institute, 1000 Ljubljana, Slovenia
Prof Dr Helena Prosen, Member, Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, 1000 Ljubljana, Slovenia
Prof Dr Adrian Covaci, Member, Toxicological Center, University of Antwerp, 2610 Wilrijk, Belgium

MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL

Ana Kovačič

BISPHENOL RESIDUES IN THE AQUEOUS ENVIRONMENT: OCCURRENCE AND FATE

Doctoral Dissertation

OSTANKI BISFENOLOV V VODNEM OKOLJU: POJAVNOST IN KROŽENJE

Doktorska disertacija

Supervisor: Prof. Dr. Ester Heath

Co-Supervisor: Assist. Prof. Dr. Tina Kosjek

Ljubljana, Slovenia, November 2020

“Exploration is curiosity put into action.”
Don Walsh

Acknowledgements

First of all, I would like to thank my supervisor *Prof Dr Ester Heath* for her consistent support and guidance during my PhD at the Department of Environmental Sciences, Jožef Stefan Institute and Jožef Stefan International Postgraduate School, Ljubljana.
I would also like to thank my co-supervisor *Assist Prof Dr Tina Kosjek* for her shared advice and time.

I would like to thank *Dr David Heath*, especially for his thoughtful comments and recommendations on this thesis as well as all scientific papers.

An important part of this work was realized thanks to *Prof Dr Adrian Covaci* and *Celine Gys* at Toxicological Center, University of Antwerp. Special thanks to Adrian for hosting me two times during my two-month visit where I enriched my scientific knowledge. I also thank my colleague and friend *Celine* for her selfless knowledge sharing.

Next, I would like to thank my co-workers at the Jožef Stefan Institute, *Dr Marjeta Česen, Tjaša Gornik, Martin Rafael Gulin, Milka Ljoncheva, Taja Verovšek, David Škufca, Žiga Tkalec*, *Tamara Gajšt* and *Maria Laimou-Geraniou* for sharing ideas and experience, and especially for making my work and life as enjoyable as possible. *Marjeta*, thank you for your selfless support which was crucial for my progress at the beginning.

I would like to acknowledge the Slovenian Research Agency (ARRS) for financially supporting my work through the Young Researcher Grant, Program Group P1-0143 and Projects N1-0047 (FWO), J2-8162 and L7-1848. This work was also supported by the EU-funded project MASSTWIN (European Union's Horizon 2020 Research and Innovation Program under grant agreement no. 692241).

Finally, I want to thank the members of the evaluation board*: Prof Dr Milena Horvat, Prof Dr Adrian Covaci* and *Prof Dr Helena Prosen* for reviewing and evaluating the work of my thesis.

Dragi moji najbližji, prijatelji!
Tjaša, hvala, ker že od najinega prvega univerzitetnega dne hodiš z menoj.
Starša, neizmerno sem hvaležna za vajino neskončno podporo, motivacijo in razumevanje, tudi ko padam. Mitja, hvala za strokovno in moralno pomoč, predvsem pa hvala, ker mi skupaj s svojo družino vedno stojiš ob strani. David, hvala ker me razumeš, poslušaš, podpiraš in si vedno ob meni.
Hvala, ker ste vedno bili in ste ob meni in ker verjamete vame!

Abstract

Bisphenols are a group of industrial chemicals increasingly recognized as contaminants of emerging concern because of their presence in the environment and endocrine disrupting effects. They are used in the production of polycarbonate plastic, epoxy resins and thermal paper, in most cases without restriction. The global exception is bisphenol A, which is banned in infant bottles and is, together with bisphenol S, authorized under European regulation with a specific migration limit for plastic food contact materials. Bisphenol A is the most abundant and well-studied of the group, whereas for other bisphenols available studies do not adequately reflect their occurrence, fate and potential toxicity. This absence of data is a combination of the lack of awareness of bisphenol A analogues and related toxicity and validated analytical methods.

In this work, reliable, sensitive and accurate gas and liquid chromatography MS-based analytical methods for target analysis of 18 bisphenols and suspect and non-target based analysis of their metabolites/transformation products were developed. These methods were applied to determine trace amounts of bisphenols in the aqueous environment, which, in this thesis, includes surface and drinking water, wastewater and aqueous-based food simulants. With developed methods, their stability, removal efficiency, reaction kinetics, and the identification of metabolites/transformation products during biological and photochemical processes were addressed. The migration of bisphenols from food contact materials, a source of bisphenols to humans and environment, was also investigated, and their toxicity towards algae and bacteria studied.

The results showed that bisphenols are stable for up to four weeks under experimental conditions allowing the development of sampling and analytical protocols with assured stability of tested compounds. All tested bisphenols were present in wastewaters at ng L^{-1} levels, their removal efficiency and kinetic depended on the type of treatment, and mostly followed pseudo-first-order kinetics. Biological, UV photolysis and advance oxidation processes yielded high removal efficiencies. However, a single treatment did not lead to complete mineralization, and transformation products were confirmed: three new bisphenol S metabolites, 11 novel phototransformation products of bisphenol F, S and Z and 11 newly identified bisphenol F and S biotransformation products. Bisphenols were also shown to leach from food contact materials into food simulants for different beverages (ng L^{-1}–µg L^{-1}), and it was also shown that the degree of leaching was dependent on migration conditions. Linings of beverage cans and reusable steel bottles were identified as the source of bisphenol A and bisphenol F isomers. Moreover, toxicity models of bisphenol A and F towards algae and bacteria revealed an underestimation of mixture toxicity towards experimental data.

In summary, the confirmed presence of bisphenols in the aqueous environment emphasizes the need for new treatment technologies or combinations of the existing ones. The thesis delivers new insights into the stability of bisphenols during analysis and their fate during water treatment, addressing not only the parent compounds but also their metabolites/transformation products. It also provides new knowledge about the migration

of bisphenols from food contact materials, which represent a significant source of human exposure.

Povzetek

Bisfenoli so skupina industrijskih spojin, ki postajajo zaradi svoje prisotnosti v okolju in endokrinih učinkov prepoznani kot prioritetna organska onesnaževala. Uporabljajo se predvsem pri izdelavi polikarbonatne plastike, epoksi smol in termičnega papirja. Večinoma je njihova uporaba neregulirana. Izjema je bisfenol A, ki je prepovedan v stekleničkah za dojenčke na globalnem nivoju in ima skupaj z bisfenolom S predpisano mejo migracije iz plastičnih materialov v stiku z živili. Bisfenol A je med bisfenoli najbolj razširjen in preučen, medtem ko za ostale bisfenole razpoložljive študije pomanjkljivo opisujejo pojavnost, kroženje in strupenost. Pomanjkanje podatkov je predvsem posledica pomanjkljivega osveščanja o nadomestkih bisfenola A ter podatkov o njihovi strupenosti in pomanjkanja validiranih analiznih metod.

V okviru raziskave smo razvili zanesljive, občutljive in natančne analizne metode na osnovi plinske in tekočinske kromatografije sklopljene z masno spektrometrijo za izvajanje tarčnih analiz izbranih bisfenolov in analiz na principu netarčnih postopkov za njihove presnovne/transformacijske produkte. Metode smo uporabili za določanje sledov bisfenolov v vodnem okolju, ki v okviru predstavljenega doktorskega dela vključuje površinske, pitne in odpadne vode ter simulante živil. Z uporabo razvitih metod smo obravnavali njihovo stabilnost in učinkovitost odstranjevanja med čiščenjem vod, kinetiko reakcij ter identifikacijo presnovnih/transformacijskih produktov med biološkimi in fotokemijskimi procesi. Raziskovali smo tudi sproščanje bisfenolov iz materialov v stiku z živili, ki predstavljajo pomemben vir človeške in okoljske izpostavljenosti, in ocenili strupenost izbranih bisfenolov na alge in bakterije.

Rezultati kažejo, da so bisfenoli stabilni do štiri tedne pod izbranimi pogoji, kar je omogočilo razvoj postopka vzorčenja in analize z zagotovljeno stabilnostjo preučevanih spojin. Vsi analizirani bisfenoli so v odpadni vodi prisotni v ng L^{-1}. Učinkovitost njihovega odstranjevanja in vrsta kinetike sta odvisna od tehnologije čiščenja, a v večini primerov nakazuje reakcijo psevdo-prvega reda. Z biološkimi postopki, UV fotolizo in naprednimi oksidacijskimi postopki čiščenja vod smo dosegli visoko učinkovitost odstranjevanja preučevanih bisfenolov. Uporaba samostojnega postopka čiščenja običajno ne vodi do mineralizacije teh spojin, saj smo potrdili nastanek transformacijskih produktov: tri nove produkte presnove bisfenola S, enajst novih fototransformacijskih produktov bisfenolov F, S in Z ter enajst novih biotransformacijskih produktov bisfenolov F in S. Bisfenoli se prav tako sproščajo iz materialov, ki pridejo v stik z živili v simulante živil različnih pijač (ng L^{-1} − µg L^{-1}), pri čemer je kinetika sproščanja odvisna od migracijskih pogojev. Premazi pločevink za pijače in steklenic iz jekla za večkratno uporabo so glavni vir bisfenola A in izomerov bisfenola F. Modeli strupenosti bisfenola A in F na alge in bakterije so razkrili podcenjevanje strupenosti mešanic teh spojin glede na eksperimentalne podatke.

Predstavljeno doktorsko delo potrjuje prisotnost bisfenola A in njegovih nadomestkov v vodnem okolju in poudarja potrebo po novih tehnologijah čiščenja ali kombinacijah že poznanih. Ponuja nov vpogled v stabilnost bisfenolov med analizo in njihovo kroženje med čiščenjem vode, pri čemer ne obravnava le izhodne spojine, temveč tudi

presnovne/transformacijske produkte. Poleg tega ponuja nova znanja o sproščanju snovi iz materialov v stiku z živili, ki predstavljajo pomemben vir človeške izpostavljenosti.

Contents

List of Figures

List of Tables

Abbreviations

$^{\cdot}OH$	...	hydroxyl radicals
AOP	...	advanced oxidation process
API	...	atmospheric pressure ionization
APCI	...	atmospheric pressure chemical ionization
AF	...	assessment factor
BAF	...	bioaccumulation factor
BCF	...	bioconcentration factor
BOD	...	biological oxygen demand
Bio-TP	...	biotransformation product
c_{eff}	...	effluent concentration
c_{inf}	...	influent concentration
CD	...	cyclodextrin
CECs	...	contaminants of emerging concern
CI	...	chemical ionization
CID	...	collision-induced dissociation
COD	...	chemical oxygen demand
e^-	...	photoelectron
h^+	...	photohole
EC	...	effective concentration
EC_{50}	...	concentration that provokes 50 % of the maximum response
EDC	...	endocrine disrupting chemical
EI	...	electron ionization
ESI	...	electrospray ionization
EU	...	European Union
FDA	...	Food and Drug Administration
$Fe^{2+/3}$	...	ferric ions
FWHM	...	full peak width at one-half maximum
EFSA	...	European Food Safety Authority
EQS	...	environmental quality standards
EPA	...	Environmental Protection Agency
EPI Suite	...	Estimation Program Interface Suite
FCM	...	food contact material
GC	...	gas chromatography
HC	...	hydrodynamic cavitation
HCD	...	high-energy collision-induced
HPLC	...	high performance liquid chromatography
HRMS	...	high resolution mass spectrometry
HRT	...	hydraulic retention time
K_{aw}	...	air-water partition coefficient
K_d	...	sorption coefficient
K_H	...	Henry's coefficient

K_{oc}	organic carbon/water partition coefficient
K_{ow}	octanol/water partition coefficient
LC	liquid chromatography
LC_{50}	concentration that causes death of 50 % of the population
LIT	linear ion trap
LOD	limit of detection
LOQ	limit of quantification
MEC	measured environmental concentrations
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MS^n	multiple stage mass spectrometry
m/z	mass-to-charge
NCI	negative chemical ionization
NIST	National Institute of Standards and Technology
NOEC	no observed effect concentration
Q	quadrupole mass analyzer
QqLIT	quadrupole – linear ion trap
QqQ	triple quadrupole mass analyzer
QqTOF	quadrupole – time-of-flight mass analyzer
PEC	predicted environmental concentrations
pKa	dissociation constant
PNEC	predicted no effect concentration
REACH	European Registration, Evaluation and Authorization of Chemicals
ROSs	reactive radical species
SML	specific migration limit
SPE	solid phase extraction
SPME	solid phase microextraction
SRM	selected reaction monitoring
SRT	sludge retention time
TDI	tolerable daily intake
t-TDI	temporary tolerable daily intake
TIC	total ion chromatogram
TOF	time-of-flight mass analyzer
TP	transformation product
UHPLC	ultra performance liquid chromatography
U.S.	United States
UV	ultraviolet
WFD	Water Framework Directive
WHO	World Health Organization
WWT	wastewater treatment
WWTP	wastewater treatment plant
λ_{ex}	excitation wavelength
λ_{max}	maximum wavelength

Chapter 1

Introduction

1.1 Background and Application

The rapid growth in the variety and quantity of contaminants of emerging concern (CECs), such as bisphenols, reported in the environment and wastewaters (WWs), indicates the necessity for investigating their occurrence and fate. Bisphenols are a class of anthropogenic chemicals with two hydroxyphenyl groups linked by a hydrocarbon bridge, produced by the electrophilic addition of aldehydes or ketones, e.g. acetone to aromatic hydroxy compounds, e.g. phenol in the presence of an acid as catalyst (Figure 1) [1].

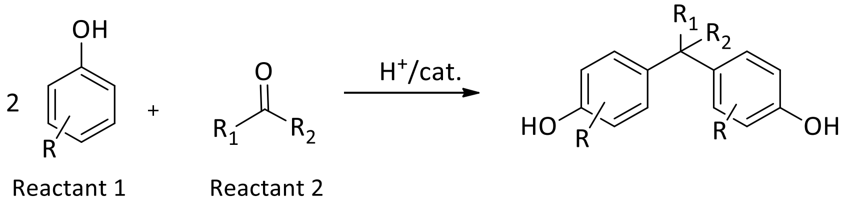


Figure 1: Synthesis of bisphenols [2].

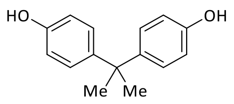
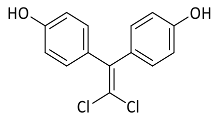
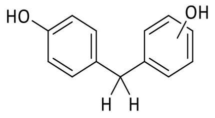
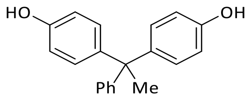
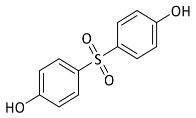
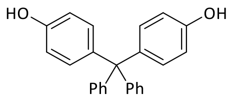
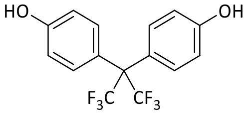
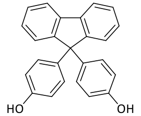
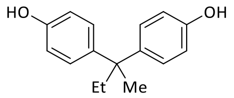
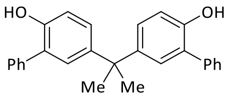
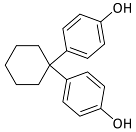
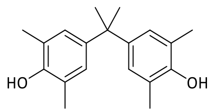
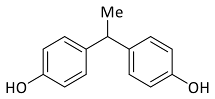
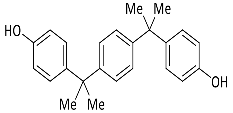
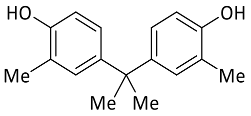
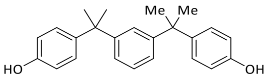
This synthesis is a well-known electrophilic aromatic substitution. It is selective due to the high electronic density at the para position on the phenyl ring, which enables large conversion levels to *para-para (p/p)* bisphenol products resulting in efficient polymerizations.

Bisphenol A (BPA) was synthesized in 1891 by chemist Aleksandr Dianin [3] and reported by Theodor Zincke in Germany in 1905 [4]. However, it was not until 1953 when Hermann Schnell and Dan Fox discovered its practical application as a monomer in the production of various polymers, e.g. polycarbonates, epoxy resins, unsaturated polyesters, and polysulfone resins. The synthesized materials exhibited good thermal stability, chemical resistance, and excellent mechanical properties, such as optical clarity, shatter-resistance and high heat-resistance [1], [2]. Commercial and large-scale production of BPA began in the United States (U.S.) in 1957 and then in Europe a year later [5] leading to its wide use in various industrial and commercial applications, with BPA being the prototype compound [2], [6]. Bisphenol A is one of the highly produced chemicals worldwide with an annual production exceeding 5 million tons [7][8].

The first evidence of BPA toxicity came from experiments on rats carried out by Edward Charles Dodds in the late 1930s, who discovered its hormonal action [9], [10]. Since then, its presence in different environmental matrices and food products has aroused the interest of many scientists [11], but it was not until the early 1990s that the adverse effects of low-dose exposure on laboratory animals were first reported [12]. In 1996, the European Commission classified BPA as “xenobiotic” and harmful to human health due to its potential action as an endocrine disrupting chemical (EDC), i.e., mimicking, blocking, or interfering with hormones of the endocrine system [13].

Grave concern over the adverse effects of BPA has had two main consequences: (1) in science, much effort has gone into researching the environmental occurrence, human exposure, and the toxicity of BPA [2], and (2) industry has begun the search for BPA alternatives having the same or even better properties [3]. In contrast, the background histories of BPA alternatives (Table 1) are less well known. However, recent studies report their presence, especially bis(4-hydroxyphenyl)methane (bisphenol F, 44BPF), bis(4-hydroxyphenyl)sulfone (bisphenol S, BPS), 1,1,1,3,3,3-hexafluoro-2,2-bis(4-hydroxyphenyl)propane (bisphenol AF; BPAF), 2,2-bis(4-hydroxyphenyl)butane (bisphenol B, BPB) in the environment, foodstuffs, personal care products, and human biological samples [14].

Table 1: Chemical classification and structures of the studied compounds.

Name	**CAS**	**Structure**	**IUPAC Name**	**Name**	**CAS**	**Structure**	**IUPAC Name**
Bisphenol A BPA	24-26-37		4,4'-propane-2,2-diyldiphenol	Bisphenol C2 BPC2	14868-03-2		4,4'-(2,2-dichloroethene-1,1-diyl)diphenol
Bisphenol F (BPF) 44BPF 24BPF 22BPF	620-92-8 2467-03-0 2467-02-9		4,4'-methylenediphenol 2,4'-methylenediphenol 2,2'-methylenediphenol	Bisphenol AP BPAP	1571-75-1		4,4'-(1-phenylethane-1,1-diyl)diphenol
Bisphenol S BPS	843-55-0		4,4'-sulfonyldiphenol	Bisphenol BP BPBP	1844-01-5		4,4'-(diphenylmethanediyl)diphenol
Bisphenol AF BPAF	1478-61-1		4,4'-(1,1,1,3,3,3-hexafluoropropane-2,2-diyl)diphenol	Bisphenol FL BPFL	3236-71-3		4,4'-(9*H*-fluorene-9,9-diyl)diphenol
Bisphenol B BPB	77-40-7		4,4'-butane-2,2-diyldiphenol	Bisphenol PH BPPH	24038-68-4		4,4'-propane-2,2-diylbis(2-phenylphenol)
Bisphenol Z BPZ	843-55-0		4,4'-cyclohexane-1,1-diyldiphenol	Bisphenol DM BPDM	5613-46-7		2,2-bis(3,5-dimethyl-4-hydroxyphenyl)propane
Bisphenol E BPE	620-92-8		4,4'-ethane-1,1-diyldiphenol	Bisphenol P BPP	2167-51-3		4-bis(2-(4-hydroxyphenyl)-2-propyl)benzene
Bisphenol C BPC	79-97-0		4,4'-propane-2,2-diylbis(2-methylphenol)	Bisphenol M BPM	13595-25-0		1,3-bis[2-(4-hydroxyphenyl)-2-propyl]benzene

Some BPA analogues, namely 44BPF, BPS, BPAF and BPB are found in similar materials and used in similar applications to BPA, in plastic materials and metal coatings for (food) containers (e.g. polycarbonate plastic and epoxy resins), as a developer for thermal paper and as a cross-linking reagent in the production of fluoropolymers and fluoroelastomers used in food contact materials (FCMs) (Table 2). The European Chemicals Agency reports the annual production or imports of BPF and BPS up to 10,000 and 100,000 tons in the European Economic Area, respectively, while the annual production of BPAF is reported to be in the U.S. between 5 to 250 tons from 1986 to 2002 [3]. The production and usage data of BPA analogues is missing, but the production and the number of materials that contain BPA analogues are expected to increase globally [15]–[18].

Table 2: Bisphenols and their application.

Bisphenol	**Material**	**Application**	**Reference**
BPA	polycarbonates epoxy resins polysulfones polyester resins	beverage and food containers, resin coatings, infant feeding bottles (banned), digital and electronic equipment, construction glazing, sports safety equipment, thermal paper, medical devices and dental sealants	[19]
44BPF	polycarbonates epoxy resins natural sources	manufacture lacquers and varnishes, resin coatings and adhesive plastic, dental material, beverage and food containers, mustard	[20]
BPS	polycarbonates epoxy resins polyethersulfone polyester resins	beverage and food containers, additives in pesticides, dye agents, leather tanning agents, fiber improvers, thermal paper	[21]
BPAF	polycarbonates epoxy resins fluoroelastomer fluoropolymer	beverage and food containers, dental material	[22]
BPB	polycarbonates epoxy resins	beverage and food containers, resin coatings	[23]

At the same time, given their structural similarities to BPA, its analogues are likely to exert similar endocrine disrupting activity and adverse effects on the reproductive system of organisms [14], [17]. As for BPA, the endocrine disrupting properties of BPB and 44BPF were first reported in 1930 [9], but more recently other bisphenols: BPE, BPS, and BPAF, have been found to also exhibit endocrine disrupting effects (e.g. estrogenic and antiandrogenic activities) [17], [24]–[26].

The available literature suggests that 44BPF and BPS are the most commonly used BPA alternatives, but over twenty other bisphenols are known and applied commercially [16], [27]. However, except for BPA, available data regarding their occurrence and fate in the environment and humans is limited at best, but studies have shown their presence in different environmental and biological samples at ng L^{-1} levels [16], [28]–[30]. Therefore, an investigation on the occurrence, fate, and transformation of such highly potent species (Table 1) in the environment is warranted.

1.1.1 Legislation

The potential concern over BPA began in the early 1980s in the U.S. [10] and accordingly, research interest in the study of synthetic and environmental estrogens started to increase [10]. The recognition of BPA in 1988 by the U.S. Environmental Protection Agency (U.S. EPA) [31] as a weak estrogenic chemical led to restrictions on its use in the early 1990s [3]. Knowing that a primary route for human exposure to bisphenols is through ingestion of food and drinks [16], the first Tolerable Daily Intake (TDI) of 0.05 mg/kg bw/day for BPA was established in 1993 [32]. Thus, the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) continued to issue a BPA safety evaluation for FCMs [33]. In 2006, EFSA adopted the same TDI, and the FDA followed by releasing a document entitled "*Draft Assessment of Bisphenol A for Use in Food Contact Applications*" in 2008. While EFSA and the FDA continued to review additional studies, Canada was the first country to regulate BPA by banning its use in baby bottles in 2008 [34]. Later, in 2011, the European Commission assembled a list of substances used in the production of plastic FCMs requiring regulation (EU Regulation 10/2011) including BPA and BPS [35].

In the European Union (EU), the use of BPA was prohibited in the production of baby bottles in 2011, and set specific migration limits (SML) of 0.6, and 0.05 mg kg^{-1} from a material or article into food or food simulants for BPA and BPS, respectively [35]. Individual EU Member States have introduced further national bans on the use of BPA in FCMs and coatings [36]. An example is France, where, since 2015, the use of BPA has been prohibited in all packaging intended to come into direct contact with food [37]. In the same year, EFSA decided to perform a comprehensive review of BPA exposure and toxicity taking into consideration non-dietary sources and after a full re-evaluation reduced the TDI to 4 mg/kg bw/day [38]. This TDI is, however, only temporary (t-TDI), and in 2018, EFSA set up a working group to evaluate recent toxicology data in order to publish an updated assessment. The report is scheduled for 2020 [39], but the task is yet to be finalized. Also in 2018, the European Commission amended Regulation (EU) No 10/2011 lowering the SML for BPA from plastic FCMs to 0.05 mg/kg and additionally the same SML was applied to varnishes and coatings [40]. Although the World Health Organization (WHO) indicated that currently there is no evidence of risks to health concerning BPA in drinking water, which is a minor source of exposure, the benchmark value for BPA (0.01 µg L^{-1}) was included in the Drinking Water Directive based on the precautionary principle [41].

Migration from FCMs also contributes to the release of bisphenols in the environment, and WW effluents are recognized as the main point source [42]. Bisphenol A is not regulated in WWs. However, besides legislation covering food and FCMs, each member state adopts active EU regulations for its monitoring. For example, for Slovenia, the umbrella "*Decree on the emission of substances and heat when discharging WW into surface waters and the public sewage system*" [43] (see Attachment 2) defines discharge limit values for BPA when it is directly or indirectly discharged to surface water (0.16 mg L^{-1}) or municipal wastewater (0.16 mg L^{-1}) [43]. This decree also has a subset of decrees regulating different (e.g., municipal and industrial) WWs and as reported above, mainly concerns industrial WWs. Since WW effluents are the main inflow of pollutants including bisphenols into inland surface waters, the Water Framework Directive (WFD) introduced a holistic approach to the management of water quality, which requires the protection and improvement of all aspects of the water environment including rivers, lakes, estuaries, and coastal waters. The WFD in 2000 [44] established a list of 45 priority compounds for which environmental quality standards (EQS) in surface waters have been set. In 2008 [45], the WFD added a further 17 compounds to the Watch list, including BPA. The latter has not yet assigned a guideline value, but as a possible future environmental quality standard (EQS), a benchmark value of 0.01 µg L^{-1} is proposed. In Slovenia, the "*Decree on surface water status*" defines the annual average-EQS of specific pollutants for inland surface waters [46]. The decree includes BPA and assumes a risk for aquatic organisms if the environmental concentration of BPA exceeds its EQS of 1.6 ug L^{-1}. It is possible that in the future, also the inclusion of selected BPA alternatives will follow if sufficient evidence of their occurrence and effects will be shown.

1.2 Bisphenol Residues in Surface Water, Drinking Water and Wastewater

Concern over BPA and its replacement by BPA analogues and their potential toxicity has led to increasing interest in studying their occurrence and cycling in the aqueous environment, which in this study includes, apart from the surface and drinking water, also WW.

1.2.1 Sources and exposure pathways

Human exposure to bisphenols occurs primarily through oral ingestion and dietary sources, e.g., dental composite, canned and plastic FCMs, tap water and fresh foods, as well as through inhalation (dust, indoor/outdoor air) and dermal routes (cosmetics, thermal paper) [16], [47]. Upon oral ingestion, bisphenols are almost completely excreted *via* urine in conjugated (glucuronide and sulfate) forms within 24 h, while for example, the excretion upon dermal absorption is slower, resulting in incomplete conjugation [47], [48]. Figure 2 shows the potential pathways of bisphenols, their metabolites, and TPs into the environment. Once excreted, they end up in household WW that enters the sewerage system, connected to a municipal wastewater treatment plant (WWTP). If they are not entirely removed during WW treatment, residues will enter surface waters and potentially reach groundwater and drinking water. Thus, WW effluents from municipal or industrial WWTPs are major sources of bisphenols in the environment. Other potential sources are also industrial discharges, runoff and landfill leachate.

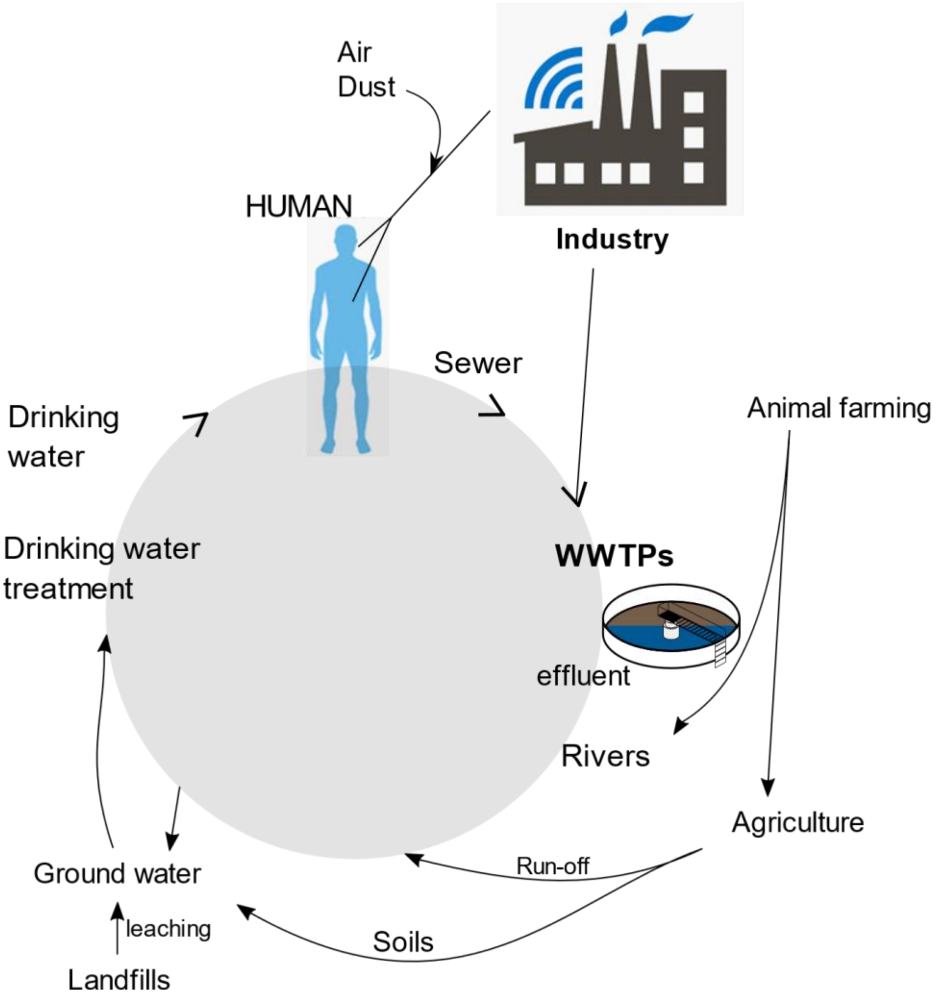


Figure 2: The exposure pathways of bisphenols in the environment modified from Petrovic et al., 2003 [49].

1.2.2 Fate and behavior

The fate and behavior (e.g., transport, bioavailability, distribution, and transformation) into various environmental compartments (e.g., water, air, soil, and biota) of a compound are governed by its chemical structure and physico-chemical properties. These include water solubility, dissociation constant (pK_a), *n*-octanol-water partition coefficient (K_{ow}), organic carbon partition coefficient (K_{oc}), air-water partition coefficient (K_{aw}), Henry's coefficient (K_H), bioconcentration factor (BCF), and bioaccumulation factor (BAF). The experimentally determined data on these parameters for bisphenol analogues are still limited, but can be to some extent predicted from their chemical structures and calculations using different approaches, such as the U.S. EPA Estimation Program Interface (EPI) Suite (Table 3) [15].

The solubility of bisphenols (120–1100 mg L^{-1}) in water is higher than their typical environmental concentrations [50]; hence, it does not limit their occurrence in aquatic compartments. Based on their estimated pK_a values (between 7.6−10.3), most bisphenols in environmental waters (pH = 5–9) are in their neutral and partially dissociated forms [16]. The low/moderate water solubility, high melting points and low K_H ($K_H = 1.0 \times 10^{-15}$–$1.0 \times 10^{-10}$) and K_{aw} ($\log K_{aw} < -5$) values are also typical for bisphenols; thus, the proportion of bisphenols in the gas phase is expected to be negligible [51]. Sorption of a compound to organic matter is defined by the K_{ow} and the K_{oc} (Table 3) [48]. Bisphenols with $\log K_{ow} < 4$, (BPA, BPB, BPF isomers, BPE, BPC2, and BPS), will be mostly present in the water phase, while bisphenols with $\log K_{ow} = 4–7$ (BPBP, BPP, BPZ, BPM, BPC, and BPPH) are more likely to be in soils and sediments and have the potential to accumulate in fat tissues of living organisms [15], [16]. The experimental or calculated data on bisphenol K_{oc} are, to the best of our knowledge, not available for all bisphenols. The only $\log K_{oc}$ of 4.7, 4.6, 3.8, 3.7 and 3.5 mL g^{-1} for 44BPF, BPAP, BPA, BPAF, and BPS, respectively, were experimentally established by Jin et al. [52] and imply that sediment and soil are important sinks for bisphenols. In addition, the potential accumulation of bisphenols in aquatic organisms can be predicted from their calculated BCF and BAF values [53]. These factors are similar for most bisphenols and range from 3.5–640 L kg^{-1}, which suggests a low potential for bioconcentration in aquatic organisms. Indeed, according to the European Union Registration, Evaluation and Authorization of Chemicals (REACH) regulation, only BPM and BPP are considered bioaccumulative based on their calculated BCF and BAF values, i.e., 2000 and 1000 L kg^{-1}, respectively [15]. Bisphenols, based on their predicted half-lives, are more persistent in sediments (135–1621 days) than in soil (30–360 days), and water (15–180 days) [15]. Overall, the physico-chemical properties of the bisphenols (Table 3) imply there is a higher tendency for them to partition in sediments and soil than in the aqueous phase, but their trace concentrations show they are also present in aqueous matrices as well. The exception is BPS that is more likely to be in the aqueous phase on account of its unique structure, which is more rigid and contains polar SO_2 groups. This fact also explains its higher temperature and light stability [19].

Table 3: Physical and chemical properties.

Physico-chemical properties	MW [g mol^{-1}]	pK$_a$	Solubility in water [mg L^{-1}]	logK$_{ow}$*	logK$_{aw}$*	logK$_{oc}$	K$_{H^*}$ [(atm m^3) mol^{-1}]	Half-live* [day]			BCF* [L kg^{-1}]	BAF* [L kg^{-1}]
Bisphenol								Water	Soil	Sediment		
BPA	228.3	10.29	120– 300	3.43	-9.43	3.8	5.34×10^{-7}	38	75	338	172.7	172.8
44BPF**	200.2	9.91	190– 360	3.06	-9.55	-	1.22×10^{-6}	15	30	135	28.02	28.02
BPS	250.3	7.64	1000– 1100	1.65	-12.96	3.5	7.8×10^{-11}	15	30	135	3.535	3.353
BPAF	336.2	8.74	22	4.47	-7.63	3.7	3.38×10^{-5}	180	360	1621	639.3	643
BPB	242.3	10.27	44	4.13	-10.64	-	2.2×10^{-7}	38	75	338	170.2	170.3
BPZ	268.4	9.97	14	5	-9.41	-	2.2×10^{-8}	38	75	338	271.4	272.1
BPE	214.3	10.1	99	3.19	-10.55	4.7	3.53×10^{-6}	15	30	135	45.61	45.61
BPC	256.3	-	26	4.74	-11.86	-	1.24×10^{-6}	38	75	338	112.6	112.6
BPCII	281.1	-	-	3.75	-9.34	-		38	75	338	227.5	227.6
BPAP	290.4	10.22	13	4.86	-10.64	4.6	1.33×10^{-9}	38	75	338	249.7	250.1
BPBP	352.4	-	-	6.08	-9.3	-		38	75	338	385.1	416.4
BPFL	350.1	-										
BPPH	380.48	-	-	7.17	-11.66	-		38	75	338	192.8	585.9
BPDM	284.2	-										
BPP	346.5	10.31	0.59	6.25	-10.23	-	3.21×10^{-11}	60	120	542	1970	9097
BPM	346.5	-	-	6.55	-10.23	-		60	120	542	1970	9097
Reference		[15], [54]	[26], [50], [51]	[15], [16], [54]	[16]	[52]	[55]	[15]				

*Calculated physico-chemical properties of bisphenol analogues based on EPI Suite 4.1.

**The structural similarity between isomers of BPF is also suggesting to share similar physico-chemical properties [54].

1.2.3 Occurrence

The presence of bisphenols in the environment is governed by their physico-chemical parameters, the quantities produced, the metabolism and elimination from the human body and their removal during WWT, which represents the primary point source to the environment. Their physico-chemical properties (Table 3) vary and imply their occurrence in sediments and soils as well as in aquatic compartments, e.g. surface water, groundwater, and drinking water. Compared to BPA, which can reach hundreds of ng to µg per liter in the treated effluent [56], there is only limited data on BPA analogues. A particular gap exists concerning the occurrence of bisphenols in European countries [3]. So far, only a few studies report the occurrence of BPA analogues in different environments, e.g. samples of sediments, soil, surface water, dust, WWs, aquatic organisms, drinking water, different kind of foods and beverages, food simulants, paper, and human urine and blood [15], [42], [57]–[65]. Although their reported concentrations are generally several times lower (ng L^{-1}) than those of BPA, the confirmed presence of 44BPF, BPS, BPAF, BPE, BPP, and BPB in different environmental compartments [52], [66], [67] makes their monitoring essential.

1.2.3.1 Surface and drinking water

The occurrence data of bisphenols in surface waters (rivers, freshwater lakes, urban rainfall-runoff) and drinking water (source and drinking water) are given in Table 4. This thesis is limited to those studies where bisphenols are the focus and excludes those studies that investigate only BPA. The literature shows that BPA is the dominant bisphenol in the aquatic environment with a maximum reported concentration of 14,800 ng L^{-1} [68] and has the highest frequency of detection. The data show (Table 4) that levels of 44BPF, BPS, and BPAF in surface water are similar to, or even exceed, those of BPA (<0.01–65,600, <0.002-246, and <0.01–28 ng L^{-1}, respectively). Repeated sampling of Lake Taihu in China [52], [69] showed an increase in the concentrations of bisphenols, including BPA of one order of magnitude and 44BPF and BPS of two orders of magnitude over a three-year period (2013-2016), with 44BPF becoming the predominant bisphenol. The bulk of the studies come from Asia (e.g. China, Japan, South Korea, and India), most notably from China and from solely two European countries (Slovenia and Croatia) and none from the U.S. (Table 4). Given this evident lack of data regarding the concentration of the bisphenols in seawater, groundwater, and tap water, as well as being aware of how sampling technique and sampling time and rate together with the analytical methodology affect the results, reliable data for bisphenols in the aqueous environment have yet to be gathered.

Table 4: The occurrence of bisphenols in surface and drinking water.

Location	Sample	Concentration range [ng L^{-1}]															Reference
		BPA	44BPF	BPS	BPAF	BPB	BPZ	BPE	BPAP	BPP	BPC	BPFL	22BPF	BPC2	BPBP	BPPH	
China	surface water	2.84-3.47	0.28-67	0.90-245.69	<0.11	-	-	-	-	-	-	-	-	-	-	2.84-3.47	[23]
China South Korea Japan India		<1.9-2850	<5.0-7200	n.d.	n.d.	n.d.	-	n.d.	n.d.	-	-	-	-	-	-	<1.9-2850	[70]
China		14.7-24.8	-	-	<0.11	-	-	-	-	-	-	-	-	-	-	14.7-24.8	[71]
China		0.5-5.6	0.28-67	0.31-11	< 0.078	<0.026-0.70	-	0.01-0.08	-	<0.062	<0.05-0.069	-	-	-	-	0.5-5.6	[52]
China		<(0.5-10)-1600	<(0.5-10)-1600	0.7-84	<(0.5-10)-28	<(0.5-10)-45	-	1.0-56	-	-	-	-	-	-	-	<(0.5-10)-1600	[69]
China		26-720	4.1-160	110-140	18-46	17	-	1.6-2.9	-	-	-	-	-	-	-	26-720	[72]
China		<(0.30-12)-474	9.9-65,600	-	-	-	-	-	-	-	-	-	-	-	-	<0.30-12-474	[73]
Slovenia		63.7	0.52-36.2	<0.58	<0.08	<0.13	<0.01	<0.23	<0.09	<0.02	<0.29	1.76-26.9	0.10	<0.23	<0.09.	63.7	[74]
China		-	0.18-14.9	-	-	-	-	-	-	-	-	-	-	-	-	-	[75]
China		0.05-36.7	0.08-293	0.05-9.49	-	-	-	-	-	-	-	-	-	-	-	0.05-36.7	[76]
Slovenia, Croatia		<1.04	1.68-35.2	6.45-6.45	<0.08	0.25-9.11	<0.01	0.54-0.90	<0.09	<0.02	<0.29	2-17.1	0.37-2.10	<0.23	<0.09.	<0.09	[77]
India		38.3-14,800	<16.7-209	<16.7-341	n.d.	n.d.	-	n.d.	n.d.	-	-	-	-	-	-	n.d.	[68]
China		0.24-255	0.07-131	<0.06-6.59	-	<0.04-1.09	<0.13-2.69	-	<0.13-1.93	-	-	-	-	<0.61-0.43	<0.04-1.03	0.24-255	[78]
China	drinking water	<0.5-12.6	<0.01-5.2	<0.002-10.8	<0.01-14.3	<0.01	<0.02-6.2	<0.02	<0.02	<0.005	-	-	-	<0.06	<0.004	<0.02-12.6	[79]
China		<0.5-0.9	<0.01-1.6	<0.002-4.7	<0.01-3.2	<0.01	<0.02-0.6	<0.02	<0.02	<0.005	-	-	-	<0.06	<0.004	<0.02-0.9	

n.d.: not detected (no LOD available)

1.2.3.2 Wastewater

Bisphenols can reach WWTPs through the sewer system, and like most organic micropollutants, they enter the environment mainly *via* WW discharge [64]. A review by Hu et al. [3] reports numerous studies, which show the presence of BPA in WWs and sludge at concentrations ranging from ng L^{-1}–mg L^{-1} and μg kg^{-1}–mg kg^{-1}, whereas, again, there is only limited information available on the global occurrence and fate of BPA analogues in WWs [57]. In Table 5, the available studies addressing the presence of BPA analogues in WW influents and effluents are presented. The data show that the levels of BPA in WWs are generally one or two orders of magnitude higher than other BPA analogues and that 44BPF and BPS are the most frequently detected BPA analogues at concentrations between 0.6–1170 and <0.32–427 ng L^{-1} in influent and effluent, respectively, followed by BPAF, BPB, and BPE (Table 5). Furthermore, deviation regarding the quality of sampling and analysis, source and WWTP type and provided results, either as a concentration range (e.g., 8.65–292 ng L^{-1}), median (e.g., 58.9 ng L^{-1}) or average concentration (e.g., 3.39 ng L^{-1}), hinders the comparability of such data, not only on the global scale but already at the regional level.

Few studies show noticeable seasonal, spatial, temporal and source variations responsible for the discrepancies in bisphenol concentrations in WWTPs [3], [57], [74]. These fluctuations in influent are due to different WW sources as confirmed by Sun et al. [57], while smaller fluctuations observed in WW effluents likely result from different WWT technologies [3].

Table 5: The occurrence of bisphenols in WWTP influent or effluent.

Location	Sample	Mean/median concentration or range [ng L^{-1}]											Reference
		BPA	44BPF	22BPF	BPS	BPAF	BPB	BPZ	BPE	BPAP	BPP	BPC	
China	wastewater influent	1318; 189-20,400	50; <1.16-166	-	48; <0.43-746	0.282; <0.04-1.02	<0.55	<1.73	3.7; <1.32-97.6	-	-	-	[57]
	wastewater effluent	117; 16.2-1,100	<1.16; <1.16-35.4	-	<0.43; <0.43-3.70	0.714; <0.04-16.6	-	-	3.64; <1.32-75.2	-	-	-	
South Korea*	wastewater effluent	93.0-1100	8.65-292	-	8.93-95.3	0.003-0.02	-	0.07	-	0.01	0.001-0.005	-	[80]
India	wastewater influent	60.5; 184.5-20.1	10.4; 2.9-27.6	-	14.7; 6.7-32.3	1.1; n.d.-2.8	2.5; 0.6-5.5	0.6: n.d.-3.2	-	0.3; n.d.-1.7	7.8; n.d.-26.6	-	[81]
	wastewater effluent	5.2; 1.1-14.2	0.6; n.d.-2.1	-	2.4; n.d.-4.3	n.d.	0.6; n.d.-0.6	n.d.	-	n.d.	0.8; n.d.-2.8	-	
	wastewater influent	654; 36.8-8,990	81.5; <16.7-333	-	78.3; <16.7-438	n.d.	n.d.	n.d.	-	n.d.	n.d.	-	[68]
New York State, USA	wastewater influent	71.6; <30-8500	90.2; <100-1170	-	29.4; <20-707	-	-	-	-	-	-	-	[64]
	wastewater effluent	39.1; <30-3380	64.9; <100-325	-	25.5; <20-427	-	-	-	-	-	-	-	
Slovenia	wastewater effluent	58.7	3.39	5.04	28	2.24	<0.23	-	<0.21	-	-	-	[74]
		540/177	3.77/3.77	27.1/35.6	<0.32	0.037/0.042	<0.23		<0.21				[77]
Croatia		1690/2340	71.2/58.9	15.4/15.4	316/404	2.43/58.9	27.1/27.1		476/476				

n.d. not detected (no LOD available). * g day^{-1} WWTP^{-1}.

According to Table 5, the performance of the WWTP plays an important role in reducing the potential risk of bisphenols in the environment. In this regard, the capacity of different WWTP to remove bisphenols, or any other WW contaminants, is often based on the aqueous phase removal percentage, calculated as follows:

$$Removal\ efficiency\ [\%] = \left(c_{inf} - c_{eff}\right) \times 100/c_{inf} \qquad \text{Equation 1}$$

where c_{eff} and c_{inf} are WW effluent concentration and WW influent concentration, respectively.

Biological treatment is among other treatment technologies considered to play a key role in reducing the levels of CECs in WWs [57] including BPA by obtaining > 90% removal efficiencies [82]. Nevertheless, BPA is commonly reported in treated effluents which are discharged into the environment. The pathway by which BPA analogues enter the environment will be similar because of their structural similarities to BPA [42]. However, variations regarding their occurrence in the effluent may result from them being either less susceptible to degradation compared to BPA, e.g. BPAF and BPE, or more biodegradable, e.g. BPAP, BPP and 44BPF [83]. Importantly, even though the removal efficiencies of CECs are high, they can still be released in sufficient amounts into the environment, especially when present in high influent concentrations. Equally, their poor removal efficiencies, although with lower influent concentrations, mean that BPA analogues could still be released into the environment. Accordingly, the occurrence and fate of bisphenols, especially BPA analogues, in WWTPs have to be explored to understand their potential environmental risks.

1.2.4 Adverse effects

One of the parameters used to describe the toxicity of compounds are the median effective concentration (EC_{50}) that produces a specific effect in 50% of test organisms and the lethal concentration (LC_{50}) that causes 50% death of the tested population [84]. Studies have shown that BPA analogues can have toxic effects, namely endocrine disruption, cytotoxicity, genotoxicity, reproductive toxicity, dioxin-like effects, and neurotoxicity [14], [15].

One of the most researched toxic effects, e.g. endocrine disruption, has only recently been addressed regarding bisphenol alternatives including BPS, 44BPF, and BPAF [14]. In general, available data is from *in vitro* studies [85], which suggest that they interact with physiological receptors [17], such as estrogen α and β, androgenic, glucocorticoid, aryl hydrocarbon receptors and thyroid hormone receptors [14]. Recent literature reviews by Chen et al. [15] and Wang et al. [83] showed that 44BPF, BPS, BPAF, BPB, BPZ, BPC, BPP, BPAP, and BPE exhibit estrogenic and antiandrogenic effects similar or even greater than that of BPA. Based on the maximum estrogenic potencies reported ($7.8 \times 10^{-11} - 7.74 \times 10^{-3}$), the most estrogenic bisphenol was BPAF followed by BPB > BPZ > BPA > BPC > BPP > BPAP > 44BPF > BPE > BPS. Moreman and co-workers [85] demonstrated an estrogen receptor-dependent mechanism in zebrafish embryo-larvae. Their ranking orders for toxicity and teratogenic effect were BPAF (1.6 mg L^{-1}) > BPA (12 mg L^{-1}) > 44BPF (32 mg L^{-1}) > BPS (199 mg L^{-1}) and estrogenicity BPAF (0.01–0.1 mg L^{-1}) > BPA = 44BPF (0.1–1 mg L^{-1}) > BPS (5–50 mg L^{-1}), respectively. Similar estrogenic and antiandrogenic effects of 44BPF and BPS as BPA at higher concentrations than BPA were also observed by Park et al. [86]. Moreover, BPA, 44BPF and BPS mixture had increased endocrine-disrupting activity at lower concentrations compared to that of a single bisphenol. This increase demonstrates a synergistic effect, namely that inactive concentrations of a single bisphenol can be harmful when mixed. Thus, it is necessary to consider the effects of mixtures when assessing endocrine disruption since organisms are exposed to mixtures of chemicals, rather than single ones. Besides BPAF, BPB also exerts a higher estrogenic activity than BPA via a non-genomic G-protein [87].

Generally, BPS seems to be less estrogenic and antiandrogenic than BPA but has a larger effect on 17α-hydroxyprogesterone [88]. Additionally, Stossi et al. [89] also report antiestrogenic and androgenic activities for BPAF, BPAP, BPB, BPC, and BPZ, which exhibited higher affinity for estrogen β than for α and acted as estrogen β antagonists with one order of magnitude lower EC_{50} compared to BPA. Limited studies also revealed greater or similar genotoxicity of some analogues. However, since the information of effects other than estrogenic is even more scarce, the data comparison between *in vitro* studies is difficult to interpret as the metabolic capabilities for most cell-based assay systems can vary according to tissue or species type [15], [85]. However, it was shown that 44BPF, BPS and BPAF enhanced the formation of reactive oxygen species damaging lipids and proteins [8], [62], 44BPF and BPS induce significant DNA damage in HepG2 cells, and BPS was observed to affect gene expression related to fetal development [84], [90]. However, only BPA affected genes related to the immune system [91]. Tišler et al. [26] demonstrated the higher toxicity of BPAF to *Daphnia magna*, *Danio rerio*, *Desmodesmus subspicatus* compared to BPA and 44BPF. In most cases, BPA was more toxic to these aquatic organisms compared to 44BPF, but the pigmentation of zebrafish embryos and reproduction of water fleas *Daphnia magna* after test exposure to 44BPF were much more impaired.

Bisphenols can bind to multiple nuclear receptors (androgen, glucocorticoid, and thyroid hormone receptors) also in the human body. Recent animal studies have shown the potential of some BPA analogues, e.g., BPS, BPF and BPAF to induce a similar neurobehavioral disruption as BPA [14], [92]. The human metabolism of bisphenols can also provide an insight into the fate and effects of possibly formed metabolites in the environment [93]. Despite human metabolism of BPA [22], [94], only a few studies have used *in vitro* and *in silico* experiments to provide relevant information regarding other bisphenols [22], [95]. Conjugation with glucuronic acid or sulfate is generally regarded as the main detoxification pathway for most bisphenols. However, based only on their structural similarity, it cannot be assumed that their metabolism is interchangeable [22]. Limited studies evaluate the formation of phase I and phase II metabolites of BPF, BPS and BPAF and apart from glucuronide, and sulfate conjugates oxidative metabolites, dimers and glutathione adducts, were elucidated [22], [96], [97]. In contrast to conjugated metabolites, enhanced endocrine activities were presented for several oxidative metabolites [22]. Recently, it was shown that co-exposures exhibit significant inhibitory effects on BPA metabolism, demonstrating the underestimated risks of simultaneous exposure to environmental toxicants [97]. Thus, there is a vast knowledge gap in the elucidation of metabolites to investigate, not only the effects of co-exposure to the bisphenol mixture, but also the effects of their interaction with their metabolites.

A common approach for the assessment of the impact on the ecosystem, when being affected by one or more environmental stressors, is the calculation of risk quotient (RQ), accepted and adopted in the development of environmental risk assessment guidelines [98]. Herein, the RQ is calculated as the ratio of the measured or predicted environmental concentrations (MEC or PEC) to the appropriate threshold value or predicted no-effect concentration (PNEC), which can be delivered as the ratio of toxicological endpoint data ($L(E)C_{50}$ or no observed EC (NOEC)) to the assessment factor (AF) [99], [100]. In general, the $RQ > 1$, corresponds to high risk, $0.1 < RQ > 1$ to medium risk and to low, when RQ is < 0.1 [100]. Thus, a recent review by Lie et al. [18] evaluates the ecological risk based on the calculated RQ using the available data from Table 4. The ecological risk of BPA and BPS from surface waters in China, Japan, South Korea and India is reported to be low or medium, while the risk associated with 44BPF from China, Japan and South Korea is reported to be relatively high.

Overall, little is known about the toxicity of other BPA analogues and the toxicity of bisphenol metabolites and TPs. Accordingly, studies are needed to elucidate their environmental occurrence, sources and pathways of environmental and human exposure [86]. There is also a need for epidemiological and *in vitro* studies addressing the toxicity of bisphenol mixtures (co-exposure) and their TPs formed during environmental or metabolic processes instead of considering only the single parent compounds [14]. Exposure to mixtures of bisphenol residues and other environmental contaminants is needed. These missing pieces of information regarding the toxic effects of bisphenols could prevent the underestimation of the actual risk posed by bisphenols [16].

1.3 Bisphenols in Water Treatment Systems

1.3.1 Treatment technologies for the removal of bisphenols

Research dealing with the development of effective water technologies to remove a specific chemical, pathogens, or a group of chemicals has increased in the last decades due to stringent water quality control and regulations [101]. Various WWT processes are capable of removing such contaminants while producing environmentally safe, treated WW effluent [6]. A traditional WWTP is usually comprised of physical and biological processes, including preliminary, primary, secondary, and occasionally a tertiary step. Wastewater can be treated using different processes and operations within a single step according to its source, type, flow, characteristics, and intended usage [102]. Preliminary WW treatment mechanically eliminates the coarse solids by rakes and sieves. The WW enters the primary treatment, where smaller particles are physically removed by sedimentation [102]. Afterwards, WW reaches the secondary level, which typically involves biological treatment using suspended (conventional) activated sludge [82]. This process is designed to degrade/transform or preferably mineralize organic substances and nutrients (organic matter, nitrogen, and phosphorus) by the involvement of two main mechanisms: biological degradation and ad(b)sorption [83]. The prevalence of a particular mechanism depends on the WWTP performance and characteristic (e.g., size, configuration), and especially on the compound's physico-chemical properties, namely for compounds with $\log K_{ow} < 4.0$ biodegradation is expected to be the primary elimination mechanism, while a greater tendency for ad(b)sorption is expressed in the case of compounds with $\log K_{ow} > 4.0$ [6], [80].

Although biological methods can efficiently remove numerous pollutants and are by far the most widely used WWT technology, they cannot mineralize all organic compounds including BPA analogues, e.g. BPAF and BPS [19], [103], [104]. Thus, the possible release of such compounds into the environment is implying the desire for the technical and operational solutions that could improve purification [102], [105] by upgrading existing conventional biological treatment [106] as well as by developing new treatment technologies. Recently, advanced oxidation processes (AOPs) have shown promise for removing persistent compounds, including bisphenols [107], albeit data on their fate and removal during different WW and water treatment technologies and the possibility for such technologies to be scaled-up are scarce. Also, besides parent compounds, their TPs formed during water treatment should be addressed since the possibility to be more toxic compared to parent compounds cannot be excluded [15].

1.3.1.1 Biological treatment

The most commonly applied conventional activated sludge process is a continuous or semi-continuous aerobic treatment, where WW is subject to microbial degradation and undergoes nitrification. Its performance and characteristics are evaluated by monitoring standard WW parameters including pH, temperature, RedOx potential, hydraulic retention time (HRT), suspended retention time (SRT), total nitrogen (nitrate (NO_3-N), nitrite (NO_2-N) and ammonia (NH_4-N), total phosphorus, chemical oxygen demand (COD), biological oxygen demand (BOD), and suspended solids (the type of microorganism, food-to-microorganisms ratio) [102]. Alternative biological treatment technologies include, e.g., trickling filter or biological aerated filter processes, where biomass is attached to media in a packed tower or on special carriers, and membrane bioreactors or hybrid mechanisms [108], [109]. Despite their simplicity (e.g., smaller space demand) and low energy input (e.g., lower hydraulic retention time), these treatment technologies offer high removal efficiencies for specific compounds, including BPA when compared to suspended biomass due to their higher biomass retaining period, higher active biomass concentration, as well as low sludge production [110][78], [79].

The literature describing the removal of BPA analogues (%) from the aqueous phase (Eq. 1) during biological treatment is presented in Table 6 and demonstrates a lack of data, with temporal and spatial variations, as well as different sampling methods, and treatment type. These differences make it challenging to compare removal efficiencies between different treatment technologies and between individual bisphenols. The calculated average removals based on activated sludge processes vary greatly and demonstrate a discrepancy between different full-scale studies at the real municipal or industrial WWTPs as well as at different scales (full- or lab-scale) studies (Table 6). However, the highest removal during biological treatment at WWTPs was observed for BPAP, BPP, and BPZ, followed by BPA, 44BPF, BPS, and BPB, and the lowest for BPAF and BPE. The negative values of calculated removals for BPAF and BPE suggested that these bisphenols are the two most recalcitrant BPA analogues [111]. The possible explanation for significantly lower removal efficiencies of BPA, 44BPF and BPS observed by Xue et al. [64], when compared to other studies, could be in the poor performance of the WWTP rather than in the treatment type. Generally, higher removal efficiencies were obtained in lab-scale experiments, while showing the characteristic of the bacteria to degrade specific bisphenols preferably, i.e. *Bacillus amyloliquefaciens* prefer to degrade 44BPF rather than BPA. At the same time, the contrary was observed for *Sphingomonas species*. According to Huang et al. [112], removal efficiency is enhanced when exposing BPS to the microbial community instead of isolated bacteria. In addition, lab-scale experiments suggest that the removal efficiency of bisphenols decreases with increasing initial concentrations [104], [113].

Investigations of the BPA analogue biodegradability during WW treatment conducted at WWTP or on the lab-scale, and especially pilot-scale experiments, are limited. Additionally, these investigations address only a single compound at the non-environmental concentrations or isolated organisms. Thus, there is an urgent need for studies that would more realistically reflect actual treatment conditions. There has also been no study concerning the removal of BPA analogues using an advanced biological treatment, such as attached growth biomass.

Table 6: Removal efficiency of bisphenols.

	Sampling type	**Type of WWTP (number)**	**Removal efficiency**										**Reference**
			BPA	**44BPF**	**BPS**	**BPAF**	**BPB**	**BPZ**	**BPE**	**BPAP**	**BPP**	**BPC**	
Full-scale at the real WWTP	composite (0-24 h, 7 days)	industrial municipal (n=7)	78	94	99	-153	-	-	-86	-	-	-	[57]
	grab	municipal (n=5)	72	95	83	100	69	100	-	100	95	-	[81]
	composite (0-24 h, 7 days)	municipal (n=2)	23	28	13	-	-	-	-	-	-	-	[64]
	Organism/Type of matrix	**Testing time / C_i [mg L^{-1}]**	**Removal efficiency**										**Reference**
			BPA	**44BPF**	**BPS**	**BPAF**	**BPB**	**BPZ**	**BPE**	**BPAP**	**BPP**	**BPC**	
Lab-scale	*Bacillus amyloliquefaciens*/activated sludge	24 h / 10−250	77	69	-	-	-	n.r.	77	-	-	95	[113]
	Sphingomonas sp. NP5/activated sludge	15 min / 0.278−0.680	54	100	77	-	-	59	67	-	-	45	[114]
	microbial community/activated sludge	8 days / 50	-	-	100	-	-	-	-	-	-	-	[112]
	laccase/fungi-immobilized on polyacrylonitrile beads	90 min / 200−250	100	100	-	-	100	-	-	-	-	-	[115]
	tyrosinase/fungi-immobilized on polyacrylonitrile beads		92	94	-	-	93	-	-	-	-	-	
	laccase/fungi-immobilized on spongin	24h / 2−50	98	96	47	-	-	-	-	-	-	-	[104]

n.r.: not reported

1.3.1.2 Advanced oxidation processes

Advanced oxidation processes have mostly been used to disinfect drinking water rather than treat complex matrices like WW and landfill leachates due to cost [116]. They are effective treatment technologies due to their *in-situ* generation of highly reactive oxygen species (ROS) [117] [107]. However, the same studies also highlight their potential as pre- or tertiary WW treatments [56]. Although AOPs comprise a range of different strategies, they are characterized by the production of hydroxyl radicals (˙OH). The most researched AOPs include homogeneous and heterogeneous photochemical reactions, heterogeneous photocatalysis and other reactions generating ˙OH, based on ozone (O_3), hydrogen peroxide (H_2O_2), titanium dioxide (TiO_2), Fenton reagent, UV irradiation or cavitation [118], [119]. Among the other oxidants like O_3, H_2O_2 and $KMnO_4$, these types of radicals are the most oxidizing species (oxidation potential=2.8 V) that can readily and non-selectively attack organic compounds, preferably resulting in their complete mineralization [56].

UV photolysis is a very effective and widely applied process used in advanced water treatment technologies for disinfection of groundwater and drinking water [116]. It involves the source of artificial light and is classified into direct and indirect photolysis [120]. In both cases, low-pressure mercury lamps emitting light at 254 nm are the most commonly used UV sources [116]. In direct photolysis, the target compound itself absorbs photons resulting in structural changes, which preferentially leads to its complete mineralization. Thus, the removal of contaminants in such treatment highly depends on the structural characteristics of the compound, requiring the presence of functional groups responsible for photoactivity (e.g. aromatic rings, heteroatoms, and double bonds). In indirect photolysis, the structural degradation of contaminants is facilitated by other sensitizing species, which absorb radiation to reach an excited state and generate ROS [120]. UV photolysis alone is not an AOP; however, since many AOPs involve UV irradiation as an important step in the synthesis of ˙OH that leads to more efficient treatment technology compared to the same operation in the absence of irradiation, we included this process in this paragraph [121]. This phenomenon was already confirmed in studies of BPA elimination from aqueous solutions and only recently in the case of the elimination of 44BPF, BPS, BPAF, BPB, BPE, and BPP (Table 7). In addition, the photolysis of BPA analogues was studied by Cao et al. [21] who reported increased and more rapid degradation when exposing BPS to UV radiation lamp (λ_{ex} = 254 nm) compared to a metal halide lamp (λ_{ex} > 314 nm). The reason is that BPS absorbs almost no light at wavelengths above 320 nm [122].

The combined action of UV irradiation and an oxidant (ozone, hydrogen peroxide, and halogens) or catalyst (Fenton, cyclodextrin, and humic acid), is carried out in homogenous phases. Among various combinations of these methods (UV/H_2O_2, UV/O_3, $UV/H_2O_2/O_3$, H_2O_2/O_3, photo-assisted Fenton reaction), UV/H_2O_2 is the most commonly applied for the degradation of EDCs including BPA [121]. These combinations, as well as the majority of AOPs discussed, have not been investigated regarding BPA analogues.

The photolysis of O_3, H_2O_2 or Fenton reagent enhances the formation of ˙OH according to the following reactions [123], [124]:

$$O_3 + H_2O + h\nu \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + h\nu \rightarrow 2OH^{\cdot}$$

$$Fe^{2+} + H_2O_2 + h\nu \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH^{\cdot} + H^{+}$$

These mechanisms reduce the need to recover the dissolved catalyst, a characteristic of the homogeneous catalytic process, and make the Fenton reaction one of the most efficient methods for $^{\cdot}OH$ formation. The degradation rate using Fenton with this reaction has shown to be dependent on the pH and the initial concentration of H_2O_2 and Fe^{2+} [125]. The complete removal of BPA within less than 10 min shows the potential of this technology for the treatment of (waste) water containing bisphenols [126]. So far, only one study of BPAF [127] and two of BPS [21], [122] have applied the Fenton-like reaction for their elimination from the water. The results showed that both compounds exhibited enhanced degradation in the presence of Fe^{2+} ions.

Cyclodextrin (CD) enhanced photolysis has also emerged as a prospective photocatalytic method to remove contaminants from the water. The addition of β-cyclodextrin (β-CD) to the reaction medium also gives the ability to catalyze the degradation of BPA, 44BPF, BPZ, BPE, and BPP. Their enhancement of photodegradation (up to 9-fold increase) with removal in less than 2 h is thought to result from moderate inclusion of bisphenol molecules in the β-CD cavity which in turn increases the production of $^{\cdot}OH$ radicals [128]–[131] (Table 7).

The complete mineralization of BPA was also achieved by titanium dioxide (TiO_2) photocatalysis [132], [133] as one of the AOPs and a representative of heterogeneous photocatalysis, where the generated photoelectron (e^-)/photohole (h^+) pairs by light activation of semiconductor material are responsible for $^{\cdot}OH$ formation [132]. Due to the excellent properties (photoactivity, physical and chemical stability, low cost, and easy access) of this semiconductor, the TiO_2 based photocatalysis may find application as a part of sequential treatment technology [126]. Erjavec et al. [107] compared photolysis and TiO_2 supported photocatalytic degradation of BPA, 44BPF, and BPAF. Under the conditions described, all three bisphenols were removed entirely from the water within 4 h, while more than 80% remained after being exposed to UV irradiation only. Bisphenol A and 44BPF were entirely mineralized. The presence of two CF_3 groups and restricted cleavage of strong C – F bonds in the structure of BPAF meant that it was the most photolytically/catalytically stable compound among the examined bisphenols. Similarly, Vela et al. [134] found that photolysis was less efficient at removing BPA and BPB compared to photocatalysis with TiO_2.

Cavitation is another promising AOP that results in a significant enhancement in compound removal from aqueous media, especially when combined with some other process [101], [135], [136]. During cavitation, gas-filled bubbles are formed and subsequently collapse. This implosion occurs rapidly resulting in the release of high energy resulting in the homolytic cleavage of water molecules into $^{\cdot}OH$ [137]. Hydrodynamic and acoustic cavitation [119] have both been shown to efficiently degrade BPA (> 90%) in water [138], [139]. In hydrodynamic cavitation, cavities are formed because of a sudden pressure drop due to increasing fluid velocity (Bernoulli principle) or boundary layer separation. In acoustic cavitation, cavities under the influence of an ultrasonic field are generated using an ultrasonic transducer [137]. The current improvements of hydrodynamic cavitation, namely higher bubble densities and better scale-up and economic possibilities, make this technology a promising solution for treating (waste) water. Encouraging results suggest the need to investigate further the removal efficiency of this type of treatment technology for mixtures of bisphenol, especially from WW at pilot- and full-scale.

As in the case of biological treatment, BPA has been by far the most widely studied bisphenol by AOPs [126], [133], [140]–[142]. The data suggest that these processes are more effective than biological treatment [143], while the economic perspective needs to be further investigated. Table 7 shows how only a few studies have reported the fate of BPA analogues during photolysis or AOPs in aqueous solutions and demonstrate that the removal efficiency of AOPs depends on the compound and on additive concentration as well as process conditions (e.g., pH, temperature, and time). Unlike in biological treatment, degradation rate increases with increasing initial concentration [21], [130], [144].

Table 7: AOP removal efficiency of BPA analogues.

Bisphenol	**AOP**	**Matrix type**	**Condition**	**c_i [mg L^{-1}]**	**Degradation [%] (time)**	**Mineralization [%] (time)**	**Reference**
44BPF	UV	ultrapure water, lab-scale	150 W, $\lambda_{max} = 365$ nm	8.8	20 (4 h)	-	[26]
	TiO_2/UV		150 W, $\lambda_{max} = 365$ nm, TiO_2, pH>6		100 (4 h)	100 (6 h)	
	UV		30 W, $\lambda_{max} = 254$ nm	2.5−15 (10)	25 (60 min)	-	[130]
	UV/β-CD		30 W, $\lambda_{max} = 254$ nm, β-CD: 0.57 g L^{-1}, pH = 7		65 (60 min)	enhanced	
BPAF	UV		150 W, $\lambda_{max} = 365$ nm	14.7	10 (4 h)	-	[26]
	TiO_2/UV		150 W, $\lambda_{max} = 365$ nm, TiO_2, pH>6		100 (4 h)	70 (6 h)	
	UV		250 W, $\lambda_{max} = 365$ nm	13.5	<5 (120 min)	-	[127]
	UV/montmorillonite KSF dispersion (Fe^{2+})		250 W, $\lambda_{max} = 365$ nm, KSF: 1.0 g L^{-1}, pH=3.7		98 (120 min)	78 (120 min)	
BPS	Photolysis		40 W metal halid lamp, $\lambda_{ex} >$ 313 nm, pH = 11	5−50	2.7 (120 min)	-	[21]
			20 W UV lamp, $\lambda_{max} = 254$ nm, pH = 11		33.3 (120 min)		
			40 W UV lamp, $\lambda_{max} = 254$ nm, pH = 11		83.3 (120 min)		
	UV/NaCl		20 W $\lambda_{max} = 254$ nm, NaCl: 0.05 mol L^{-1}	15	100 (120 min)		
	UV/Fe^{2+}		40 W $\lambda_{max} = 254$ nm, Fe^{2+}: 0.04 mol L^{-1}, pH = 3		73.4 (180 min)		

BPS	photolysis	ultrapure water, lab-scale	350 W xenon lamp, λ_{ex} = solar spectrum	10	72 (5 h)	-	[122]
	photolysis/humic acid				80 (5 h)		
	photolysis/Fe^{3+}				91 (5 h)		
BPE	UV		xenon flash laser lamp, λ_{max} = 266 nm	75−210	φ = 0.004 (5-6 ns)	-	[129]
	UV/β-CD		xenon flash laser lamp, λ_{max} = 266 nm, β-CD: 8.8 g L^{-1}		φ = 0.019 (5-6 ns)		
BPZ	UV		35 W λ_{max} = 254 nm	2−30 (15)	20 (16 min)	30 (80 min)	[131]
	UV/β-CD		35 W λ_{max} = 254 nm, β-CD: 136 mg L^{-1}, pH = 7		100 (16 min)	70 (80 min)	
BPP	UV/TiO_2		250 W, λ_{max} = 365 nm, TiO_2: 1 g L^{-1}, pH=12	0−10 (10)	70 (60 min)	40.6 (100 min)	[128]
	UV/TiO_2/β-CD		250 W, λ_{max}= 365 nm, β-CD: 6.8 mg L^{-1}, pH = 6		100 (60 min)	94.8 (100 min)	
BPB	photolysis	WW effluent, pilot-scale	natural sunlight irradiation, λ_{ex} = 200−1100 nm	0.3	50 (240 min)	40 (240 min)	[134]
	UV/TiO_2/$Na_2S_2O_8$		natural sunlight irradiation, λ_{ex} = 200−1100 nm, TiO_2: 200 mg L^{-1}, $Na_2S_2O_8$: 250 mg L^{-1}, pH = 7		100 (240 min)	80 (240 min)	

1.3.2 Transformation of bisphenols

During water treatment compounds undergo similar transformation reactions as in the environment, i.e. organisms in biological processes and chemical/photochemical processes can mineralize or transform the chemical structure of organic compounds [42], [113]. The complete mineralization of aqueous pollutants to non-toxic products like carbon dioxide, water, and mineral acids or transformation into harmless organic species is the main objective of any water treatment technology. However, TPs can form [145] and may behave differently in terms of environmental persistence and toxicity compared to their parent compounds [70].

Different degradation pathways and TPs have been identified for BPA during biological treatment and AOPs. Various bacteria, fungi, and algae included in the biological processes have been shown to degrade and transform BPA following two primary mechanisms: (1) oxidative skeletal rearrangement of an aliphatic methyl group in the BPA molecule and (2) hydroxylation of the phenolic rings of BPA followed by ring cleavage [27], [42], [146]. In general, the most common bio-TPs are hydroxy BPA, 4-hydroxy acetophenone, 4-hydroxy benzaldehyde, 4-hydroxy benzoic acid, and the 4-hydroquinone [42], [147]. In addition, bacterial co-metabolism also plays an important role in the transformation of BPA as observed by McCormick et al. [148], where *Mycobacterium* species catalyzed the *O*-methylation of the parent compound resulting in the formation of the BPA mono- and dimethyl ether. AOPs also contribute towards the elimination of BPA from the aquatic environment, whereby the structural transformations mainly occur *via* oxidation or hydrolysis to form 4-isopropenyl phenol and 4-hydroxy-cumyl alcohol as the primary TPs [27].

Information regarding the degradation mechanisms and transformation products formed during water treatment of other bisphenols are limited but include 44BPF, BPS, BPAF, BPC, BPZ, and BPE (Table 8). Only four biotransformations and seven AOPs transformations are reported. Also, the majority of studies use a single bacterial strain instead of a bacterial consortium. Apart from 44BPF and BPS, their biodegradation pathways generally begin with the breakage of the alkyl groups that bind the two phenolic rings [146] resulting in the formation of similar TPs. Due to a well-characterized biodegradation pathway, namely a Baeyer Villiger reaction of 44BPF using the *Sphingobium yanoikuyae* FM-2 strain isolated from a river, a similar pathway is proposed during biological treatment [149]. Despite matching bio-TPs between BPA and other bisphenols formed during biological treatment, the *Bacillus amyloliquefaciens* was responsible for the formation of phosphate conjugates of BPA analogues [113]. Zdarta et al. [104] also demonstrated the formation of di- and trimers of BPF using the isolated laccase from fungi. Again BPS was recognized as a more biologically persistent BPA analogue since none of the isolated microbes were able to remove it from water, while exposure to a microbiological community led to the formation of 4-hydroxy(methyl) benzenesulfonic acid [112]. Hydroxylated, cleaved and polymerized TPs of BPA analogues were also formed during aggressive AOP treatment (Table 8).

Table 8: Transformation products formed during biological and advanced oxidation processes.

Treatment type/Bisphenol			**Transformation products**					**Reference**
Bio	*Bacillus amyloliquefaciens/* activated sludge	44BPF	phosphate conjugates					[113]
		BPZ BPE BPC						
	Sphingomonas sp. NP5/activated sludge	44BPF	1,4-hydroquinone		4-(hydroxymethyl)phenol			[114]
		BPS BPZ BPE			-			
		BPC	-					
	activated sludge	BPS	4-hydroxy(methyl) benzensulfonic acid					[112]
	Laccase/Fungi-immobilized on spongin	44BPF	4-hydroxy benzaldehyde	*ortho*-hydroxy-BPF	di- and trimer BPF	BPF+BPF smaller moieties		[104]
		BPS	-					
AOP	UV/β-CD	44BPF	*ortho*-hydroxy-BPF	*meta*-hydroxy-BPF		2,4,5-trihydroxy benzoic acid		[131]
	laser flash photolysis ($\lambda_{ex} = 266$ nm)	44BPF		4-(4-hydroxybenzyl) cyclohexa-3,5-diene-1,2-dione	4-hydroxy benzaldehyde	di-hydroxy-BPF	4-(hydroxymethyl) benzene-1,2-diol	[150]
	UV/montmorillonite KSF dispersion (Fe^{2+})	BPAF	*ortho*-hydroxy-BPAF					[127]
	photolysis/Fe^{3+}	BPS	*p*-hydroxybenzenesulfonic acid					[122]
	UV/β-CD	BPZ	*ortho*-hydroxy-BPAF	*meta*-hydroxy-BPAF	4-(2,4,5-trihydroxyphenyl)-4-(4-hydroxy phenyl) butanoic acid	5,5-bis(4-hydroxyphenyl) pentanoic acid	4-(1-(4-hydroxy phenyl)pentyl)phenol	[131]
	Fe^0/peroxymonosulfate	BPM	di- and trimer BPM	oligomers	hydroxylated derivatives	bond-cleavage (small) products		[151]
	MnO_2/I^-	BPS	mono-, di-, tri- hydroxylated BPS	p-hydroxybenzenesulfonic acid	dimer BPS	mono-, di-, tri-, tetra-iodinated BPS (7TPs)		[152]
		BPAF	mono-, tri- hydroxylated BPAF	2-hydroxy-2-(4-hydroxyphenyl)hexafluoropropane	dimer BPAF	mono-, di-, tri-, tetra-iodinated BPAF (7TPs)		

1.4 Bisphenols in Beverages and Food Simulants

The production of FCMs, i.e., all materials and articles intended to come into contact with food [29], comprises a significant segment of the food packaging market, which is becoming one of the largest global industrial sectors, amounting to around 839 billion USD in 2015 and set to reach 998 billion USD in 2020 [153], [154]. Along with becoming one of the largest global industrial sectors [155], it is also one of the leading sources of human exposure and eventually aqueous environment to bisphenols by contaminating food through leaching from packaging [156], [157]. In this context, European regulation No 10/2011 [35] on plastic materials and articles intended to come into contact with food has been established [155]. This regulation includes migration tests, where the simulation of a chemical leaching from plastic FCMs into food is modulated by various factors: 1) food composition, 2) whether or not the contact with food is direct, 3) contact time, 4) contact temperature and pH values, 5) thickness of packaging material; 6) chemical nature, and 7) amount of migrant compound [156]. Thus, the conventional approach for the FCMs safety assessment includes the monitoring of chemicals in materials [158] or leachable residues in food as well as in food simulants used as a surrogate for actual foodstuffs [159].

Migration of BPA into various foods and food simulants has been the focus of research since the early 1990s [160]–[163], although its analogues have received limited attention only recently [29]. So far, studies have found BPA, 44BPF, 22BPF, 24BPF, BPS, BPB, BPE, BPC, BPAP, BPP, BPZ, BPAF, and BPM in paper, lids and food from plastic containers and cans including vegetables, seafood, meat, milk products, oils, eggs, cereal, honey (1–959 µg kg^{-1}), and in beverages (14–3790 ng L^{-1}) [29], [163]–[167]. Their highest concentrations are reported from canned products, namely vegetables and seafood [167]. The migration studies analyzing BPA analogues from food simulants exposed to plastic containers or cans are even scarcer (Table 9). The majority of such studies use different aims as a justification for not following the EU testing guidelines, e.g., verification of migration test adequacy, the development of non-target approaches or short- and long-term migration testing for organic coating materials [168]–[170]. Moreover, the detected values in beverages and simulants are significantly lower compared to other food categories, and in many cases, not detectable (Table 9). This fact may be due to relatively high limits of quantification of the applied analytical methods as well as different production of materials intended to be used for beverages [29]. However, in some cases, the concentration of bisphenols, namely 44BPF and BPS, is also near or even higher to those of BPA (Table 9) [171], [172]. Currently, SML and migration test are established only for plastic FCMs. To protect human health, the migration of BPA analogues from FCMs into various foodstuffs and the establishment of migration tests, for other materials than plastics, are needed [29].

Table 9: Occurrence of bisphenols in beverages and aqueous-based food simulants.

Material	Matrix	Concentration as a range [µg L^{-1}]													Reference
		BPA	44BPF	22BPF	24BPF	BPS	BPAF	BPB	BPZ	BPE	BPAP	BPP	BPC	BPM	
plastic	carbonated beverages	0.51-2.97	0.27-1.44	-	-	3.21-13.43	<0.03-0.37	-	<0.02-2.41	<0.10-0.80	-	-	<0.03-0.41	-	[171]
	simulant*	<1.52	<3.09	-	-	<1.36	<0.53	<0.86	<1.26	<1.36	-	<1.38	-	-	[173]
		<0.81	<0.71	-	-	<0.88	<0.81	<0.98	<0.46	<0.90	<0.89	<0.55	<0.41	-	[159]
plastic baby bottles		n.d.	-	-	-	n.d.	-	-	-	-	-	-	-	-	[174], [175]
baby teethers		0.36-192	0.39-12.6	-	-	0.15-115	0.071-8.20	0.035-2.36	0.045-1.79	-	0.062-0.85	0.60-0.60	-	0.35-2.35	[176]
can	various beverages	0.044-0.607	<0.05	-	-	0.141-0.218	-	<0.05	-	<0.25	-	-	-	-	[166]
		30-4700	-	-	-	-	-	60-170	-	-	-	-	-	-	[165]
		<2.5-13.98	<0.9-4.44	-	-	-	-	-	<5.7-0.92	-	-	-	-	-	[30]
	simulant**	31.97-70.31	<0.082	-	-	-	-	<0.086	-	<0.085	-	-	-	-	[177]
	beer	1.26-3.79	<0.082	-	-	-	-	<0.086	-	<0.085	-	-	-	-	
	energy beverages	0.15-3.3	0.15-1.3	-	-	-	-	-	-	-	-	-	-	-	[178]
cans, paper, plastic, glass	various beverages	<0.002-1.26	<0.003-1	<0.003-0.12	<0.008-0.51	<0.018	<0.002	<0.003	<0.003	<0.002	<0.002	<0.002	-	-	[172]
	various milk products	<0.08-127.2	<0.08-1.8	-	-	<0.004-0.5	<0.0008-0.4	<0.08	<0-04-0.3	-	<0.02-0.4	<0.02	<0.02	-	[179]

n.d.: not detected (no LOD available) *ultrapure water, 50% (*v/v*) ethanol/water solution. **ultrapure water, 3% (*v/v*) acetic acid/water solution, 10% (*v/v*) ethanol/water solution and isooctane. *** given with different units µg dm^{-2}.

1.5 Methodological Approaches for the Determination of Bisphenol Residues in the Aqueous Environment

This chapter describes the analytical methods for the determination of bisphenol residues in aqueous matrices, including sample preparation, instrumental analysis, method validation, and data analysis. Quantitative analysis was exclusively carried out by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). The development of target analysis requires optimizing sample clean up, concentration, derivatization in the case of GC-MS, and method validation. For qualitative analysis, liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) has become the method of choice [16], [154]. These types of analyses include the identification of suspects or unknowns, e.g. metabolites/TPs of bisphenols, and require the development of non-target analytical tools (data processing) that can filter relevant mass features from a large number of possibly relevant mass features [159].

1.5.1 Sample preparation

1.5.1.1 Sampling and storage

Sampling is one of the first crucial steps in sample preparation since it may be the step which contributes the most to the overall uncertainty in the whole analytical procedure. The simplest, grab sampling, is also the most common sampling technique, by which the sample is taken at a specific point and time. Its main disadvantage is that it may miss events over time, which is taken into account by composite sampling that could be either time- or flow-proportional. The most advanced way of sampling is passive sampling, which allows for continuous sampling over time [180]. The selection of the most appropriate sampling technique depends on the given situation, e.g. purpose of the investigation, matrix type, and awareness of the advantages and disadvantages of each technique. Literature shows that composite sampling for monitoring of bisphenols residues in the aqueous environment, including WW, is replacing grab sampling since it provides more representative samples [56], [57], [64]. Its main drawback is the equipment cost, while passive sampling is still under development.

A suitable container and conditions for the collection, transport, and storage are based on desired sample volume, compound stability and potential contamination, e.g. bisphenols leaching from plastic containers. Among the studies investigating the occurrence of bisphenols (Tables 4 and 6), large sample volumes (100–400 mL) are usually collected in polypropylene (PP) bottles [16]. Although the stability of bisphenols during storage has not been assessed, samples are typically stored in the dark at 4 °C if processed in less than a week, else they are filtrated and stored frozen to prevent biodegradation.

1.5.1.2 Sample extraction

Simultaneous analyte concentration *via* sample extraction is a common step in sample preparation in most of the analytical methods [16]. In particular, liquid-liquid extraction (LLE), where the isolation of the analyte is achieved by its partition between two immiscible liquids and solid-phase extraction (SPE), where the analyte is partitioned between a solid and a liquid phase, are the most used extraction techniques. Solid-phase extraction, when compared to LLE, offers increased analyte recovery and a higher preconcentration factor with lower consumption of organic solvents. Thus it has become the primary choice for the extraction of organic contaminants like bisphenols from aqueous samples [181]. The general SPE procedure consists of four basic steps: conditioning, sample loading, washing, and elution. In the case of complex water samples, the available literature (Tables 4, 5 and 6) reports filtration prior to SPE to remove suspended material and prevent the SPE from clogging. Degasification before extraction by sonication is also necessary for the analysis of carbonated drinks [167]. Sometimes the adjustment of sample pH before being loaded on cartridges is reported to achieve better recoveries.

An important factor is the selection of a suitable SPE sorbent with appropriate selectivity, affinity, and capacity based on the attractive forces between the compound and its functional groups on the sorbent. Mixed-mode sorbents like OASIS HLB (hydrophilic and lipophilic interactions) [55], [182] and hemimicelles/admicelles (surfactant-coated mineral oxides) [183] are typically used for the extraction of bisphenols from aqueous matrices, including food, beverages, natural and residual waters. However, other sorbents in either on-line or off-line SPE have also been proposed, such as reversed-phase sorbent C18 [166]. Additionally, interferences (matrix effect) causing ion suppression in LC-MS analysis can be removed by using Oasis MAX, a mixed-mode anion-exchange sorbent [23]. The solid-phase extraction efficiency is closely connected with the eluent used to desorb target compounds from the SPE cartridge. The type and volume of the eluent used are dependent on the physico-chemical properties of the analytes and the elution strength of the solvent. Commonly used eluents are ethyl acetate, acetone, and methanol. Despite high extraction efficiencies, ranging from 61 to 128%, of bisphenols from aqueous samples reported in the review by Caballero et al. [16], this area was and could be further optimized especially towards developing a more selective and greener sample treatment (e.g. lower solvent consumption).

Interest into the fate of bisphenols and their occurrence in the environment has also led to the development of an effective sample pretreatment approach that has high selectivity, sensitivity, precision, and accuracy. On-line SPE can minimize sample manipulation compared to off-line SPE while providing higher recoveries [166]. Similarly, solid-phase microextraction (SPME) using, e.g. sorbents made of inverted hexagonal aggregates (SUPRAS), has been proposed as a robust, fast and straightforward technique for bisphenol extraction [50]. A rapid developing technique based on Molecularly Imprinted Polymers (MIPs) with excellent molecular recognition properties is Molecularly Imprinted Solid-Phase Extraction (MISPE) [184]. Moreover, the imprinted polymers that can selectively clean and concentrate BPA and closely related structures are already commercially available under the trade name AFFINIMIP® [185].

1.5.2 Quantitative analysis

The selection of the instrumental technique for quantitative analysis depends on the physico-chemical properties and concentration of target analytes. For the trace analysis of aqueous matrices and micropollutants including bisphenols, both GC and LC are the primary separation techniques used in combination with MS or MS/MS (tandem mass spectrometry) instrument. Mass spectrometry techniques coupled to high-performance/ultra-high performance (HP/UHP) LC are due to the rapid development of LC and especially MS system becoming methods of choice for multi-analyte analysis. These methods allow the detection of compounds with a wide range of polarities without the need for prior derivatization, including bisphenols. In this regard, the method of choice for bisphenol analysis is LC-MS/MS. However, GC-MS has certain advantages, e.g. spectral library, which can compare a compound of interest's structure based on the mass spectra matching and higher sensitivity resulting from a lower matrix effect, which continues to make it a valuable technique for bisphenol analysis.

1.5.2.1 Gas chromatography-mass spectrometry

In GC, separation is based on the volatility of the analyte and its interactions with liquid or solid-phase immobilized on the surface of an inert solid (stationary) phase. The GC-MS system consists of a carrier gas supply (source of chemically inert gas, e.g. helium), sample injector, separation (column and temperature oven) and a detection system, typically a mass spectrometer. Once the sample passes the heated injection port, compounds enter the chromatographic column in the gas phase. Thus this type of chromatography is best suited for organic/inorganic compounds that can be vaporized without decomposition (less polar, volatile and thermally stable compounds) [186]. Hence GC-based methods are limited to volatile or low molecular weight compounds. Analytes with high polarity need to be derivatized prior to analysis to increase their volatility for analysis, which is the main limitation of this technique [16], [187]. Accordingly, derivatization is also necessary after sample clean up and pre-concentration in the case of semi-volatile bisphenols. Most commonly, derivatization is conducted with the acetylation or silylation of the hydroxyl functional groups to yield thermally stable and highly volatile bisphenol derivatives (acetyl- and silyl-ethers). In particular, acetylation is achieved with acetic anhydride, while *N*-methyl-*N*-(trimethylsilyl trifluoroacetamide), MSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, BSTFA, and MTBSTFA (*N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide) are the most popular silylation reagents [16]. The MS spectra of acetyl or silyl derivatives with a correspondingly higher molecular mass show a characteristic MS fragmentation that improves the reliability of detection. Furthermore, the most commonly applied electron ionization (EI) technique and chemical ionization (CI) are less affected by the matrix suppression, compared to ionization modes commonly used in LC-MS, resulting in enhanced sensitivity and selectivity. Thus, ultra-trace levels of selected bisphenols can be quantified from complex matrices based on GC-MS methods with a lower limit of quantification (LOQ) compared to LC-MS [154].

Linear quadrupoles (Q) operating in the single ion monitoring (SIM) mode have been the most used analyzers for GC-MS analysis of organic contaminants, including bisphenols. For years, this approach has been one of the most studied and applied approaches because of its high sensitivity, good resolution and low cost [16]. Recently, GC with triple quadrupole MS analyzers capable of performing selected (SRM) and multiple reaction monitoring (MRM) has become popular because of its increased signal-to-noise (S/N) ratios and method sensitivity that can be achieved with MS/MS analysis [187].

1.5.2.2 Liquid chromatography-mass spectrometry

The most widely used type of LC is liquid-solid column chromatography in reverse-phase mode, where the liquid mobile phase and a liquid absorbed on a solid nonpolar stationary phase are used. The separation of the components is based on their different interactions with the reversed liquid stationary phase and mobile phase, causing different velocities of compounds. This technique is also suitable for analysis of nonvolatile polar compounds with lower thermal stability [186]. The latest development in the field of ion sources, namely the introduction of atmospheric pressure ionization (API) techniques, capable of handling a wide range of flow rates and mobile phases, enables direct coupling of LC to the mass spectrometer [188].

LC-MS instruments are generally composed of a mobile phase reservoir, pumping system, sample injection system, liquid chromatography column, and mass spectrometer [186]. The mobile phase is usually a mixture of water and organic solvents, e.g. acetonitrile or methanol that continue to be the most used mobile phases. As solvent modifiers, mainly formic or acetic acid and ammonia are added to enhance ionization efficiencies or improve peak separation or peak shape of target compounds [154]. Generally, the analysis is carried out using a reversed-phase, e.g. *n*-octadecyl or C18 column in the length range of 100–250 mm, with a particle size diameter 2-5 µm and < 2 µm in the case of HPLC and UHPLC, respectively [154]. In this way, the obtained higher peak resolving power leads to improved separation and sensitivity (narrower peaks) and reduced cost through shorter analysis time and lower solvent consumption.

Among API representatives, "soft" electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) can be applied to analyze a broad range of compounds and are the most widely used ion sources in the LC-MS system. Their efficient sample ionization and ion transmission to the mass analyzer contribute to high instrumental sensitivity, which is essential for the analysis of trace contaminants (ng L^{-1}) [189]. Nevertheless, the ESI mode is usually preferred over the APCI because it provides better sensitivity for the majority of organic molecules, including bisphenols and derivatives, whereas the APCI is more suitable for ionizing neutral and low polarity molecules [188], [190].

Single quadrupole instruments have also been the main choice in the early stage of LC-MS analysis, but its rapid progress caused the development of more sophisticated mass analyzers (e.g. triple quadrupole – QqQ, ion trap – IT, time-of-flight – TOF, and Orbitrap). Their coupling to LC enabled tandem MS operation, which helps to avoid false-positive determinations and enable structure elucidation of yet unknown compounds [188], [191]. The characteristics and applications of mass analyzers are discussed in detail in Section 1.5.3.1. However, because of its sensitivity, dynamic range and robustness, the quadrupole-linear ion trap (QqLIT) and mostly triple quadrupole (QqQ) analyzer have been the most applied mass detection techniques for the quantification of micropollutants at trace levels, including bisphenols. Their superior performance is enabled by operating in MRM mode, where at least two transitions between the precursor and product ions are obtained [191]. This operation greatly increased the specificity of the analysis over single-stage mass analysis.

LC-MS/MS technique is becoming the first choice for routine quantitative analysis of organic compounds present at trace levels, such as bisphenols because of its low method quantitation limits without derivatization. It usually involves the use of ESI in the negative mode, methanol/water as the mobile phase, reversed C18 columns and QqQ analyzer operating in the MRM mode [16], [190].

1.5.3 Qualitative analysis

In order to evaluate the fate and exposure risks to humans and environmental organisms, it is important to address not only the parent compounds but also qualitatively and quantitatively evaluate stable TPs formed during WWT and in the environment. To date, literature mostly ignores TPs, which can behave differently in terms of environmental persistence and toxicity [15]. The recent improvements in MS, notably the development of high-resolution mass spectrometry (LC-HRMS) analyzers working in the full-scan mode, represent the best option for identification of suspects or unknown compound, including metabolites/TPs using suspect and non-target screening approaches. In particular, two applicable strategies based on such instruments and library searching are suggested to fulfil the missing information: 1) the use of accurate-mass measurements, and 2) the use of multiple-mass fragmentations (MS^n) [191]. These non-target-based screening approaches can be applied to study bisphenol transformation.

1.5.3.1 Principles of mass spectrometry

The principle of MS is the conversion of the analyte to a charged state (e.g. ionization) followed by analysis based on the mass-to-charge (m/z) ratio and relative abundance of molecular or fragment ions formed during ionization processes [188]. Thus, the coupling of a chromatographic technique with a mass spectrometer into a GC-MS or LC-MS system requires the appropriate selection of its two major components, namely ion source and mass analyzer [189]. The EI and CI are the most commonly used techniques coupled with GC in which the vaporization of sample molecules before their ionization is required. Most instruments are equipped to switch between EI and CI conditions, both applicable for the volatile and thermostable compounds. The EI is one of the most widely used ionization methods and considered a hard ionization technique. Typically, EI accelerates one (seldom more) electron (e^-) out of the neutral molecule (M) with a beam power of 70 eV electrons, producing radical cation $M^{+\bullet}$ (molecular ion) and two e^-. The high ion currents give a good sensitivity leading to extensive fragmentation. Thus, molecular ions have very low intensities, while their fragmentation products have high intensities in the EI mass spectrum. Fortunately, the use of 70 eV enabled the creation of searchable EI mass spectral libraries that can compare the compounds qualitatively based on the mass spectra. Despite the isomers, each compound has an individual fragmentation pattern. Chemical ionization is a soft ionization technique, where a reagent gas, e.g., methane, is used. It is convenient for the detection of molecular mass by giving a well-defined molecular ion signal in the mass spectra [189].

The challenge of linking MS to LC was overcome by the development of API techniques that can dissolve sample molecules, ionize, and transmit them to the high-vacuum environment of the mass analyzer. Among all, the ESI and APCI are used predominantly [188]. Based on the available literature, the determination of unknown compounds (TPs) is mainly carried out with the ESI [192], especially for the analysis of polar or moderately polar molecules, while neutral or low polarity molecules are applied more to APCI. In both cases, a strong electrical field is responsible for the formation of protonated $[M+H]^+$ or deprotonated $[M-H]^-$ ions in positive or negative ion mode, respectively. While ESI favors the formation of multiply charged ions, with the APCI, the analyte is ionized *via* charge transfer of the primary ionized solvent spray producing dominantly singly-charged ions [186], [188], [189].

The four common mass analyzers are Q and IT that provide unit-mass resolution and TOF and Orbitrap that provide high-resolution accurate-mass analysis. The single-stage Q analyzer is one of the most widely used MS analyzers due to its simplicity, good quantitative analysis, and price. The core of the Q analyzer (Figure 3) consists of two pairs of rods, charged by either a positive or negative potential, where only a selection of ions with stable trajectories based on their m/z values and the applied voltage will pass the mass detector. The signal-to-noise ratio is improved when the Q mass analyzer operates in selected-ion monitoring (SIM), where only a limited m/z range is transmitted/detected by the instrument, as opposed to the full spectrum range. However, it still provides poor unit-mass resolution and poor selectivity. The latter is its main disadvantage, resulting in uncertain spectral data, but can be remedied by tandem mass analyzers, such as QqQ mass detector. The QqQ-MS is the representative of tandem-in-space instruments, in which the three steps of the MS/MS process: precursor-ion selection, CID and mass analysis of product ions are performed in spatially separated devices [188], [189] achieved by placing a quadrupole functioning as a collision cell (q) between two quadrupole mass analyzers (Q1 and Q2) acting as mass filters.

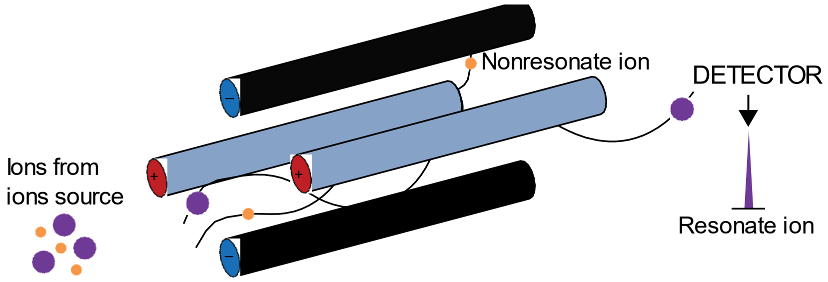


Figure 3: Schematic diagram of a quadrupole mass analyzer [189].

Precursor-ion scans, constant neutral-loss scans, and MRM *via* fragmentation by collision-induced dissociation (CID) greatly increase specificity over the single Q mass analysis. Nevertheless, the product ion scans of triple configuration when compared to IT or TOF instruments are less sensitive, which however is crucial for structural elucidation of TPs. The QqQ MS instruments are now being replaced by more powerful mass analyzers for qualitative analysis, while being the first choice in quantitative analysis, with excellent sensitivity, wide dynamic range and repeatability [191].

An important feature in terms of qualitative analysis is the IT mass analyzer (Figure 4), having the ability to fragment and isolate ions several times in succession before the final mass spectrum is obtained. Therefore, the IT-based instruments are an example of a tandem-in-time mass spectrometer, where the three steps (precursor-ion selection, CID, mass analysis of product ions) are performed sequentially in the same device. The overall effect is a significant improvement in the sensitivity of the scan mode, resolution and reproducibility compared to simple Q and most importantly ability to provide good MS^n [189]. The generation of spectra of the parent and product ions (and their fragment ions) contribute significantly towards the elucidation of the fragmentation mechanism of unknown species, consequently increasing the level of confidence in postulating the chemical structure of a tentative candidate [191]. The conventional IT (3D) uses three hyperbolic electrodes to trap ions in a three-dimensional space using static and RF voltages. In order to improve its scan rate and ion capacity, a new design was introduced, namely linear IT (LIT). The latter has a quadrupole made of four hyperbolic cross-sectional rods in a linear feature (2D), where the ions are radially trapped. This type of IT showed an enhancement of ion capacity, scan rate and detection (trapping) efficiency while quantification remains less reliable in comparison to MRM with triple quadrupole instruments [189], [193].

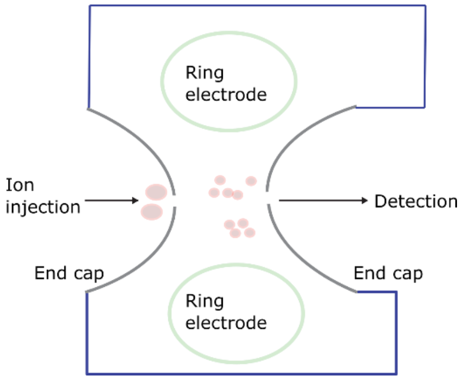


Figure 4: Scheme of IT mass analyzer [189].

The third mass analyzer is a TOF mass analyzer (Figure 5, right). Its basic structure consists of an accelerating grid and a flight tube. Here, ions are accelerated by a high voltage and begin their flight toward the detector all at the same time and with the same initial kinetic energy. Hence, TOF instruments utilize the time taken by ions to pass (fly) along an evacuated tube as a means of measuring m/z values, namely lower m/z ions will, due to their higher velocity, arrive at the detector before higher m/z ions [188]. A TOF-MS instrument is capable of 70,000 resolving power expressed in terms of full peak width at one-half maximum (FWHM); thus replacing sector field and Fourier-transform ion-cyclotron resonance mass analyzers [186]. It has a high acquisition speed with high full scan spectra sensitivity and provides accurate mass measurements (<3 ppm) accepted for the verification of the elemental composition of parent and fragment ions used for the identification of unknown species. With respect to monitoring practices which use SIM or MRM, TOF-MS offers identification and structural elucidation of target and non-target compounds in a sample. However, TOF is most commonly used as a second analyzer in the so-called tandem-in-space MS instruments having two independent mass analyzers with collision cell enabling fragmentation prior entering into the second TOF analyzer. Such a configuration enables the ultimate confirmation of analytes [194].

A stand-alone version of the most recent mass analyzer, Orbitrap, consists of one spindle-like central electrode and two cup-shaped outer electrodes facing each other. A radial electric field between electrodes leads to circular movements (oscillation) of ions around the central electrode proportional to $(m/z)^{-1/2}$ and independent of the ion velocity. An enhanced performance was provided by rapidly raping the RF voltage at the C-trap rods, delivering the adequate ion package in the Orbitrap, the so-called high-field compact Orbitrap. This mass analyzer provides HRMS above 10^5 FWHM and a mass accuracy ≤ 1 ppm, and even though a stand-alone Orbitrap has been produced, far better analytical capabilities are achieved with hybrid systems [189].

In this sense, tandem MS that uses a combination of different mass analyzers, namely hybrid analyzers, represents a powerful methodology to obtain the sufficient data for reliable structural elucidation of an unknown TP, i.e. combining TOF-MS and Orbitrap-MS accurate mass measurements to generate empirical formula and IT-MS^n providing additional fragmentation data [195]. Thereby, the advantages of mass analyzers are combined and integrated. Alternatively, two particular hybrid analyzers used for qualitative analysis are quadrupole time-of-flight (QqTOF) (Figure 5) and quadrupole – linear ion trap (QqLIT) mass spectrometer, where a TOF or a LIT analyzer replaces the third quadrupole. These types of hybrid instruments have several advantages, from which the main advantage of QqLIT is the ability of rapid switching between linear Q and LIT mode, achieving a combination of SRM and full-spectrum product analysis. The QqTOF instruments are outstanding in their ability to perform accurate-mass determination (<3 ppm) for both precursor and product ions, which is reflected in their wide use in structure elucidation and TP/metabolite identification. However, some deficiencies remain, notably lower sensitivity of QqTOF and lower accuracy of QqLIT [189].

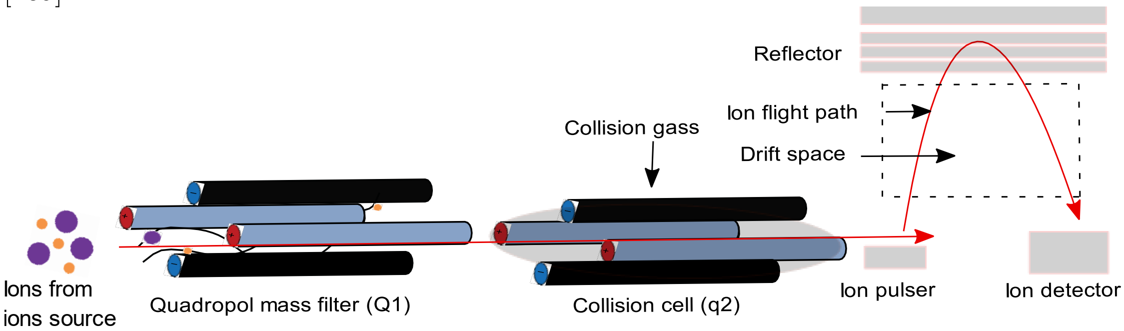


Figure 5: Scheme of QqTOF hybrid tandem mass analyzer: Quadrupole segment (left) and TOF analyzer (right) [189].

More recently, Orbitrap hybrid MS systems have been developed. The initial instrumental set-up consisted of the hybrid LIT-Orbitrap configuration, in which LIT controls the number of ions transferred to Orbitrap and performs MS^n, a C-trap to direct the ion package into the Orbitrap, and the Orbitrap itself. The introduction of the higher-energy collision-induced (HCD) cell next to the C-trap enabled the stand-alone Orbitrap, providing all-ion fragmentation without prior selection of precursor ion. The faster configuration, i.e. achieving FWHM 140,000 within 1 s was obtained when the LIT was replaced with Q into a Q–Orbitrap hybrid analyzer. The most advanced Orbitrap-based system, tribrid mass spectrometer (Figure 6), combines both LIT and Q mass analyzer, an HCD cell and C-trap next to the Orbitrap analyzer (LITQ- Orbitrap). The uniqueness of this configuration and eventually any Orbitrap hybrid system for MS/MS or MS^n is the integration of high sensitivity, mass accuracy (≤1 ppm) and resolution (10^5 FWHM) necessary to give exact-mass measurements in a single instrument [188], [189], [196]. Further improvements are expected as relatively young Orbitrap technology already plays an important role, e.g. metabolite identification, in peptide sequencing and has a great potential for the structure elucidation and identification of unknown compounds [189], [196].

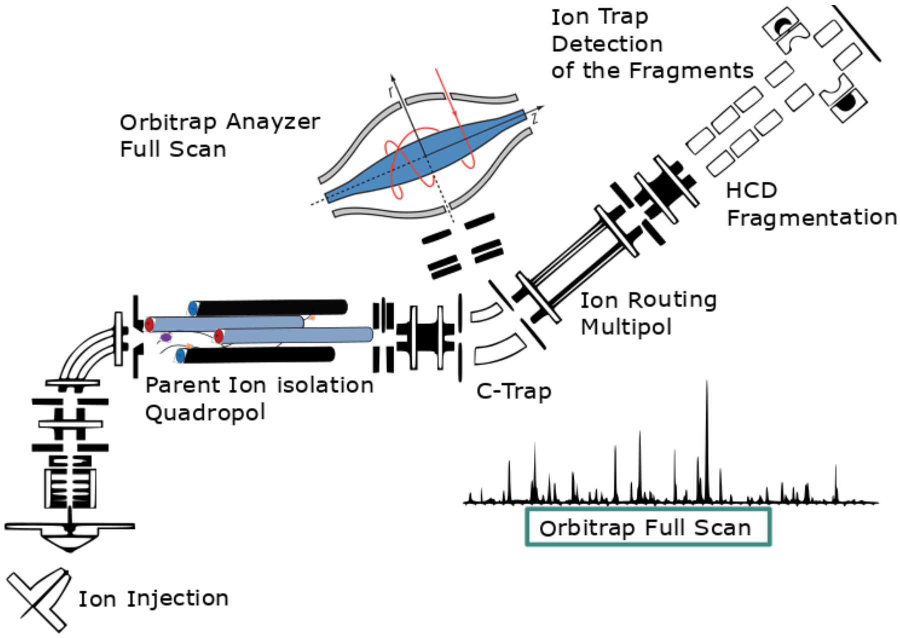


Figure 6: Scheme of tribrid LITQ-Orbitrap mass analyzer [196].

1.5.3.2 Identification

The rapid progress in the field of HRMS resulted in higher sensitivity and selectivity of instruments, which enables the identification of compounds without being pre-selected or commercially available, as required in the case of target identification. This ability triggered the introduction of various qualitative screening approaches that successfully extended the environmental monitoring of CECs [197]. The advantage of non-target screening performed with GC-HRMS are the extensive libraries (e.g., the NIST library) of comparable EI mass spectra and the direct comparison of any new-formed peaks in total ion chromatograms (TIC) of the sample compared to the control, whereas this is not feasible using LC. However, the range of this application is limited since the compounds must be volatile and thermally stable [198]. Instead, LC-HRMS allows the detection of a broader range of compounds but lacks the accessibility, easy-to-use spectral libraries, or databases for compound identification. This deficiency has been remedied by gaining parameters for structure elucidation, e.g. chromatographic retention, physico-chemical properties, and spectral data, etc. by which the suspect and unknown compounds can be detected using spectral and chromatographic search algorithms [199].

The two most used workflows for the identification of the compound metabolism/transformation are based on suspect or non-target screening approaches and are most commonly applied together. In this sense, several freely (MZmine, XCMS) or commercially (Compound Discoverer from Thermo Fisher Scientific, MassHunter$^{\mathrm{TM}}$ from Agilent, MetaboLynx$^{\mathrm{TM}}$ from Waters) available data processing software tools have been developed to handle the immense amount of data resulting from LC-MS mass scans recorded in short time frames. Most of them include similar steps of raw data preprocessing as a critical step in reducing data quantity and complexity, e.g. noise filtering, feature and peak detection, blank and control subtraction, replicate filtering, componentization by the grouping of isotopes, adducts, multi-charged ions and in-source fragments, alignment and normalization [197], [199], [200]. The obtained data from preprocessing is then reduced by using statistical methods such as principal component analysis, clustering and regression analysis, which are critical for prioritizing the most relevant and interesting components in a sample set [200]. Finally, the identification of prioritized components resulting from data analysis relies on the interpretation of the MS and MS/MS data (accurate mass, isotope and fragmentation pattern) using suspect and non-target-based approaches [200], [201].

Suspect analysis usually begins with building a database of compounds based on literature review and compounds that are suspected to be present or formed during metabolism/transformation. The tentative confirmation is based on accurate mass and isotope information provided in the generated list. If in suspect screening, mass spectral peaks are matched purposefully, the use of non-target-based screening also explores unexpected or unknown compounds, e.g. metabolites/TPs that are absent or exceed an intensity ratio compared to the control [148], [186], [188]. Thus, the tentative structure of potential metabolite/TP can be postulated based on the mass and retention time shift between the metabolite/TP and its respective parent compound, their elemental formula and the interpretation of MS/MS fragmentation based on the MS/MS spectrum of the parent compound (common fragment ions) [202]. In this step, information obtained using software to predict molecular formula (e.g., SIRIUS), chemical compound databases (e.g., ChemSpider, PubChem) and mass spectral database (e.g., mzCloud, MassBank, METLIN) are involved. The data from experimental MS/MS or database repositories can be compared with the results from in-silico fragmentation (e.g., MetFrag, CFM-ID) [200], [203]. Although these recently developed methods enable the tentative identification without requiring reference standards [159], the confidence level of the presented HRMS identifications varies between studies and substances. Based on a proposal by Schymanski et al. [204], they are classified into five levels, from which Level 1 (Confirmed structure) represents the ideal situation, where the proposed structure has been confirmed by a reference standard with MS, MS/MS and retention time matching. The Level 2 (Probable structure), indicates that it was possible to propose an exact structure using different evidence, namely Library (Level 2a), when matching literature or library spectrum data, and Diagnostic (Level 2b), when no other structure fits the experimental information (e.g., MS/MS fragments, ionization behavior), but no standard or literature information is available for confirmation. Level 3 is where there is evidence for possible structure(s) (Tentative candidate/s), but it is not sufficient to assign an exact structure (e.g., positional isomers). Obtaining an Unequivocal molecular formula (Level 4) is possible when a formula can be unambiguously assigned using the spectral information (e.g., adduct, isotope, fragment information), but there is a lack of the information to propose possible structures. Finally, only the Exact mass (Level 5), which can be of specific interest, can be measured, but data is not sufficient even for molecular formula assignment [204].

In line with rapidly growing mass spectral libraries and software packages that are capable of processing big data generated from LC-HRMS instruments, the chance for correctly and reliably identifying unknown spectra is also improving [200]. Since TPs and metabolites of contaminants tend to be more hydrophilic than their parent compounds, LC-HRMS has become the method of choice for the analysis of environmental, food and health-related samples (surface water, wastewater, drinking water, food simulants, biological fluids, etc.) [165]. Furthermore, the additional information can be provided by using other analytical techniques, such as LC with nuclear magnetic resonance (NMR) or hyphenated LC-NMR-MS systems in order to elucidate the structural ion, i.e. in the case of a structural isomer.

To date, the development and optimization of non-target workflow based on HRMS regarding bisphenol transformation during cycling in the environment are scarce [104], [154], [159]. This method opens the window for research to fill the missing gap over the identification of trace levels of unknown TPs and metabolites of bisphenol and their pathway.

1.5.4 Application of mass spectrometry in qualitative and quantitative analysis

The continuous improvement of hyphenated LC-MS instruments and especially the ongoing development of data processing make LC-HRMS methods the first choice for TPs and metabolite identification in the environment. However, the GC-HRMS is a necessary complementary method, where also nonpolar and volatile compounds are considered. Additionally, with such instruments, the t_Rs are easier to compare, allowing the confirmation of compound identity with high certainty but only a limited number of exact mass libraries are currently available [205]. As described in Section 1.5.3.1, different types of mass analyzers exist, able to provide either information about fragmentation patterns from MS/MS or MS^n spectra, high accurate masses of unknown compounds or even both. Accordingly, the most common application of specific mass analyses is a reflection of its performance (Table 10) [189], [206].

Table 10: Performance of various mass analyzers and their applicability adopted from Niessen & Correa [189] and Petrovic et al. [206].

MS analyzer	Sensitivity	Selectivity	Mass accuracy	Dynamic range	Fragmentation power	Application
Q	medium (SIM)	Low	low	high	MS	quantitative analysis
QqQ	medium (full scan)	High	low	high	MS/MS	
	high (SRM)					
TOF	high	Low	high	low	MS	partially qualitative for unknown compounds
QqTOF	medium (MS/MS)	High	high	medium	MS/MS	
IT	medium (MS/MS)	High	high	medium	MS^n	
QqLIT	high (SRM)	High	high	medium-high	MS^n	quantitative and qualitative analysis
LIT-Q-Orbitrap	high (SRM)	High	high	high	MS^n	

1.5.5 Validation of the analytical method

The confidence of any decision based on analytical results relies on their quality. Thus reliable analytical methods for obtaining accurate and comparable measurements are required [207]. This need means that in the case of non-standard methods, standard methods used outside their intended scope or in the case of amplification and modification of standard methods, the validation of method performance must be considered [208]. Typical method validation of target analysis involves the evaluation of a series validation parameters, i.e. stability, working and linear range, sensitivity, selectivity, trueness, precision (repeatability and reproducibility), robustness, limit of detection (LOD) and quantification (LOQ) and uncertainty of measurements, where the quantification of the compounds is the main goal [208]. Whereas validation procedure of qualitative analysis, in terms of identification of unknown compounds, is currently limited due to the absence of commercially available standards (e.g. TPs) [209]. Given the importance of method validation, requirements for how it should be carried out are specified in several guidelines [207], [208], [210]–[212], depending on the type of analysis, customer needs and regulations. Accordingly, the validation parameters are explained as follows:

Evaluation of analyte stability should be carried out as the first step in method validation to ensure that storage and processing conditions do not affect the determination of the analyte. In this context, the stability of analyte in the investigated matrix and stability of the analyte and internal standard in the stock and working solutions and in extracts under the entire period of sample storage, preparation and analysis should be ensured. Working and linear range is the interval over which the method provides results with an acceptable uncertainty, proportional to the amount (concentration) of analyte in the sample [208]. A minimum of six calibration concentration levels should be used to determine the relationship between the concentration of analyte and the corresponding response of the instrument with reported calibration curve parameters (the correlation coefficient (R^2), y-intercept and slope) [212]. Sensitivity relates to the degree to which a method responds uniquely to the required analyte expressed as the slope of the calibration curve. Selectivity is the ability of the analytical method to measure unequivocally and differentiate the analyte of interest from other components in the sample matrix, i.e. there is no signal interfering with the signal of the analyte or the internal standard. Trueness is an expression of how close the mean (expected) value of results, produced by the method, is to a reference (true) value. It is expressed quantitatively in terms of bias obtained by the three general approaches; 1) Analysis of certified reference material (CRM), 2) Recovery experiments using spiked samples, 3) Comparison with results obtained with another method. Precision is a measure of how close the individual results and a series of measurements are to one another. It is usually expressed by statistical parameters, which describe the spread of results, e.g., the variance, standard deviation, or coefficient of variation of a series of measurements. Normally, repeatability (the smallest), as a measure of the variability in results when performed by a single analyst using the same equipment over a short timescale, and reproducibility (the largest), as a measure of the variability in results between laboratories, represent the two extreme measures of precision. Between these two extremes, intermediate precision expresses the variation of measurements within-laboratories, but under conditions that are more variable than repeatability (e.g. new calibrations, different days, analysts, equipment). Robustness or ruggedness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters (e.g. extraction time, pH of the mobile phase, temperature, stability of analytical solutions). The LOD is the lowest amount of analyte in a sample that can be detected and reliably distinguished from zero, while not necessarily quantitated as an exact value. The LOQ is the lowest amount of analyte that can be determined quantitatively with an acceptable level of repeatability, precision, and trueness. Uncertainty is defined by the international vocabulary of metrology (VIM) as a “non-negative parameter characterizing the dispersion of the quantity values, being attributed to a measurand, based on the information use”. The measurand refers to the particular quantity (amount) of an analyte being measured. Estimating uncertainty became one of the most important metrological concepts in analytical science over the last years. It covers all sources of errors (systematic and random) and is expressed as a range [213]. According to the type of its quantification, standard or expanded uncertainty can be used. Standard uncertainty $u(x)$ refers to individual contributor to the uncertainty of the result, quantified as standard deviation. All possible correlation between separated $u(x)$ are considered by determining the covariance, and if being derived from different sources of uncertainty, this is expressed as combined standard uncertainty ($u_x(y_i)$). Thus, the analytical result is usually reported with the expanded uncertainty (U), which is obtained by multiplying the $u_x(y_i)$ of a result by the coverage factor k, based on the desired confidence level [207], [214].

Chapter 2

Aims and Hypotheses

Only a limited number of publications report the presence of BPA analogues in the environment and WW despite evidence that these compounds share a similar toxicity profile to BPA. However, efficient treatments preventing bisphenols from entering the environment have yet to be established. Greater insights into their occurrence, metabolism/transformation and migration from FCMs, an important link between environment and human, must be appropriately addressed in order to gain insight into their fate and the risk they pose to the environment. Equally, stability studies of bisphenols during sampling, storage and analysis have also been neglected. In order to fulfil these knowledge gaps, the aims of this thesis were the following:

1. To develop, optimize and validate analytical methods for the simultaneous determination of 18 bisphenol residues and to identify metabolites/TPs in aqueous samples based on GC-MS, GC-MS/MS and LC-MS/MS.
2. To evaluate the occurrence of selected bisphenols in Slovenian aqueous samples.
3. To examine the stability of bisphenols in aqueous matrices.
4. To assess the efficiency of hydrolysis, adsorption, biological treatment, and photochemical and advance oxidation processes for removing bisphenols from WW.
5. To investigate the breakdown and identify TPs of bisphenols during biodegradation, photodegradation and advance oxidation processes.
6. To evaluate the migration of bisphenols from FCMs into food simulants.

In order to achieve these aims, the following hypotheses were tested:

H1: Residues of bisphenols are present in Slovenian WWs.
H2: The stability of bisphenols depends on storage temperature and time, concentration, matrix, and compound properties.
H3: Single treatment (biodegradation, photodegradation or advance oxidation process) is not sufficient to completely mineralize bisphenols and TPs are formed during treatment.
H4: The migration of bisphenols from FCM depends on the material, food simulant and migration condition.

Chapter 3

Publications

The outcomes of the present work are presented in eight separate scientific papers divided into four parts:

i) Stability, occurrence, treatment and sources (Section 3.1);

   1. Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions.
   2. The removal of bisphenols and other contaminants of emerging concern.
   3. The occurrence and source identification of bisphenol compounds in wastewaters.

ii) Identification of transformation products (Section 3.2);

   1. Suspect and untargeted screening of bisphenol S metabolites produced by *in vitro* human liver metabolism.
   2. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics.
   3. Kinetics and biotransformation products of bisphenol F and S during aerobic degradation with activated sludge.

iii) Migration from food contact materials (Section 3.3);

   1. The migration of bisphenols from beverage cans and reusable sports bottles.

iv) Effects (Section 3.4);

   1. The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism.

3.1 Stability, Occurrence, Treatment and Sources

3.1.1 Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions

The paper “Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions” by A. Kovačič, M. Česen, M. L. Geraniou, D. Lambropoulou, T. Kosjek, D. J. Heath and E. Heath was published in *Environmental Research* in September 2019. I am solely responsible for the experimental design, method optimization, validation, and experimental work. I was also responsible for data analysis and visualization, and drafting the manuscript.

The paper examines the stability in different matrices as well as hydrolysis, adsorption, biological treatment and UV photolysis of eighteen bisphenols (BPA, 22BPF, 24BPF, 44BPF, BPS, BPB, BPAF, BPE, BPC, BPM, BPPH, BPP, BPBP, BP26DM, BPC2, BPZ, BPFL, and BPAP). All analyses were carried out by using an optimized and validated GC-MS method. The results revealed that bisphenols do not hydrolyze and are most stable in methanol, followed by ultra-pure water and WW. The main factor affecting their stability in WW samples was storage time, but all compounds were stable for up to 4 weeks at −20 °C or 4 °C. The study revealed that biological treatment and UV photolysis are efficient at removing bisphenols. In general, the behavior of bisphenols with higher K_{ow}, namely BPM, BPPH, BPP, BPBP, and BPFL, differs from that of other bisphenols, most likely due to rapid adsorption/desorption processes. For the majority of the studied bisphenols, the removal efficiency was $> 85\%$, using either suspended or attached-growth biomass. Adsorption to biomass was also recognized as an essential removal mechanism for bisphenols, and the difference in their adsorption affinities reflected the differences in their K_{ow} values. Unlike in biological treatment, greater variability in removal efficiencies was observed using UV photolysis that in terms of degradability within 4 h of irradiation divides bisphenols between three groups: (i) highly removable (RE $> 94\%$), (ii) moderately removable (RE 50–80%) and (iii) poorly removable (RE 25–45%). In nearly all cases degradation followed pseudo-first-order kinetics.

This study sets out to achieve three aims of this thesis, namely to (1) develop, optimize and validate analytical GC-MS method for simultaneous determination of 18 bisphenol residues; (2) examine bisphenols stability; and (3) assess the removal efficiency of bisphenols by hydrolysis, adsorption, biological treatment and photochemical processes. It is the first work to address the stability of bisphenols and the removal of their mixture by both bacterial consortia and photolysis and confirms their effectiveness. The outcome of this paper is crucial for understanding the behavior of bisphenols in the environment and during water treatment.

Environmental Research 179 (2019) 108738

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions

Ana Kovačič[a,b], Marjeta Česen[a,b], Maria Laimou-Geraniou[c], Dimitra Lambropoulou[c], Tina Kosjek[a,b], David Heath[b], Ester Heath[a,b,*]

[a] *Department of Environmental Sciences, Jožef Stefan Institute, Jamova Cesta 39, 1000, Ljubljana, Slovenia*
[b] *Jožef Stefan International Postgraduate School, Jamova Cesta 39, 1000, Ljubljana, Slovenia*
[c] *Department of Chemistry, Aristotle University of Thessaloniki, University Campus, 54124, Thessaloniki, Greece*

ARTICLE INFO

Keywords:
Bisphenol
Stability
Biological treatment
UV photolysis

ABSTRACT

The limited knowledge on the stability, removal, and the fate of bisphenol A analogues in the aqueous environment led us to assess the removal by hydrolysis, adsorption, biological treatment and UV photolysis of eighteen common bisphenol compounds (BPs). Hydrolysis of BPs does not occur. The main factor affecting their stability in wastewater samples is storage time, and safe storage conditions were found to be −20 °C or 4 °C for up to four weeks. The results also revealed no significant reduction in the levels of BPs standards when stored in either methanol or ultrapure water. BPE was found to be the most stable, followed by BPF isomers, BPS and BPF, while BP26DM was the least stable and BPM, BPPH, BPP, BPBP and BPFL were quickly adsorbed. For most BPs, the removal efficiency of biological treatment was > 85%, and there was no difference between the suspended activated sludge and moving bed bioreactors. Different adsorption affinities of the BPs to biomass were observed and reflect the differences in their K_{ow}. In terms of degradability, direct UV photolysis in water produced three groups of BPs: (A) highly removable (RE > 94%), (B) moderately removable (RE 50–80%) and (C) poorly removable (RE 25–45%). In nearly all cases degradation followed pseudo-first-order kinetics.

1. Introduction

Bisphenol A (BPA) is an industrial chemical used as a monomer in the manufacturing of polycarbonate plastic, epoxy resins, dental composites and thermal paper (Česen et al., 2018b; Jin and Zhu, 2016). The concern over the safety of BPA, a known endocrine disrupting compound (EDC), has resulted in its gradual replacement by other structurally similar bisphenol compounds (BPs). The most common are bisphenol F (BPF), bisphenol S (BPS), and bisphenol AF (BPAF), but over twenty other BPs are known (Ashfaq et al., 2018; Caballero-Casero et al., 2016; Carballa et al., 2008). They all have similar chemical structures (two hydroxyphenyl functional groups, Tables SI–1), which suggests that they are also endocrine disrupting and should be treated as contaminants of emerging concern (CECs) (Caballero-Casero et al., 2016; Gramec Skledar and Peterlin Mašič, 2016; Liu et al., 2010).

The primary source of BPs entering the aquatic environment is through wastewater (WW) effluent (Caballero-Casero et al., 2016). Unlike BPA (Ashfaq et al., 2018; Noszczyńska and Piotrowska-Seget, 2018; Petrie et al., 2019) there exist only a limited number of published studies addressing the fate of BPs, during wastewater treatment (WWT) and in the aqueous environment. These studies report levels of BPF, BPS, BPAF, BPE, BPP and BPB from 0.90–1170 ng L^{-1} in WW influents, 0.60–555 ng L^{-1} in WW effluents, 0.97–249 ng g^{-1} in sewage sludge, n.d.–2850 ng L^{-1} in natural waters, and 0.05–172 ng g^{-1} in sediments (Caballero-Casero et al., 2016; Česen et al., 2018b; Chen et al., 2016a; Gys et al., 2018; Jin and Zhu, 2016; Karthikraj and Kannan, 2017; Lee

---

Abbreviations: AS, activated sludge; AB, samples with activated biomass; BPA, bisphenol A; BPs, bisphenols; c_0, initial concentrations; c_t, residual concentrations; COD, chemical oxygen demand; DO, dissolved oxygen; EDC, endocrine disrupting compound; FDH, formaldehyde; FB, samples with deactivated biomass exposed to formaldehyde; GC-MS, gas chromatograph-mass sprectrometry; HRT, hydraulic retention time; K_d, sorption coefficients; K_{ow}, n-Octanol/Water Partition Coefficient; k_t, kinetic constants; LOD, limits of detection; LOQ, limits of quantification; MBBR, moving bed bioreactor; MeOH, methanol; MQF, samples with MilliQ-water and formaldehyde; MQ, samples with MilliQ-water; NH4–N, ammonia ion; NO3–N, nitrate ion; RB, samples with deactivated biomass exposed to γ-irradiation; RE, removal efficiency; RSD, relative standard deviation; SASR, suspended activated sludge reactor; SPE, solid-phase extraction; $t_{1/2}$, half-lives; UV, ultraviolet (irradiation); WW, wastewater; WWT, wastewater treatment; WWTP, wastewater treatment plant

[*] Corresponding author. Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000, Ljubljana, Slovenia.
E-mail address: ester.heath@ijs.si (E. Heath).

https://doi.org/10.1016/j.envres.2019.108738
Received 28 May 2019; Received in revised form 9 September 2019; Accepted 10 September 2019

et al., 2015; Sun et al., 2017; Wu et al., 2018; Xue and Kannan, 2019; Yamazaki et al., 2015; Yan et al., 2017; Yang et al., 2014; Yu et al., 2015; Zhang et al., 2018). The efficiency of WW treatment, therefore, plays an important role in the occurrence of BPs in the aqueous environment. Different techniques have been applied to remove BPs from WW, among which biodegradation seems to be the most effective process (Noszczyńska and Piotrowska-Seget, 2018), hence conventional biological treatment with activated sludge (AS) is the most widely studied (Ahmed et al., 2017). These studies show that microorganisms are also capable of removing (> 78%) BPs from WW, and that removal efficiency (RE) is influenced by factors such as amount of oxygen, microbiological composition, T, pH, photochemical properties, and adsorption onto activated sludge (Ahmed et al., 2017; Chen et al., 2016b; Karthikraj and Kannan, 2017; Noszczyńska and Piotrowska-Seget, 2018; Sun et al., 2017). To date, most investigations have been performed under different experimental conditions using isolated bacterial strains. In the majority of ecosystems, however, microorganism form species-rich communities that are able to degrade a wider range of substrates (Danzl et al., 2009; Noszczyńska and Piotrowska-Seget, 2018; Ogata et al., 2013; SAKAI et al., 2007; Zühlke et al., 2016). Recently, photochemical and photocatalytic based technologies have been shown to be efficient for treating water (Ahmed et al., 2017; Gmurek et al., 2017). In the case of BPs, studies report the rapid removal of BPF, BPS and BPZ while BPAF appears more recalcitrant (Cao et al., 2012; Kovačič et al., 2019; Liu et al., 2010; Salomatova et al., 2015; Wang et al., 2014). In addition, the stability of BPs during sampling and storage has been neglected in the literature.

This study has two aims: (1) to investigate the stability of 18 BPs in methanol (MeOH), ultrapure water and WW in order to determine optimum preparation and storage conditions of samples and laboratory standards; and (2) to determine the removal efficiency (RE) of biological treatment and UV photolysis (UV–C radiation) for BPs under controlled laboratory conditions.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards (minimum purity > 98%, Tables SI–1) of 22BPF, BPAF, 24BPF, BPF, BPE, BPA, BPC, BPM, BPPH, BPP, BPBP, BP26DM, BPC2, BPZ, BPFL, BPAP, BPS were obtained from Sigma-Aldrich (St. Louis, USA) while BPB was purchased from Dr Ehrenstoffer (Augsburg, Germany). Isotopically labelled [$^{13}C_{12}$] BPF ($^{13}C_{12}$-BPF), BPS ($^{13}C_{12}$-BPS), BPB ($^{13}C_{12}$-BPB) purchased from CanSyn Chem. Corp. (Toronto, Canada), and deuterated BPA (BPA-d_{16}) from Sigma Aldrich (USA) were used as internal standards. Compound specifications (Tables SI–1) and physicochemical properties (Tables SI–2) are given in SI-1. The derivatisation agent N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, ≥ 99.0%) and catalyst anhydrous pyridine (Pyridine, 99.8%) were purchased from Sigma-Aldrich (Schnelldorf, Switzerland and Steinheim, Germany). Acetonitrile (ACN), ethyl acetate (EtAc) and MeOH were purchased from J. T. Baker (Deventer, the Netherlands), while concentrated hydrochloric acid (37%, HCl) and formic acid (FA) used for solid-phase extraction (SPE) were purchased from Sigma-Aldrich (St. Louis, USA). Formaldehyde (FDH, 37%) was obtained from Merck (Darmstadt, Germany). All solvents were of analytical grade purity. Stock solutions (≈ 1 mg L^{-1}) of each compound were prepared in MeOH and ACN, while internal standards (≈ 10 mg L^{-1}) were dissolved in MeOH to give a final concentration of 1 µg mL^{-1}. Standards were prepared by serial dilution from the stock solutions. Ultrapure water was prepared with a MilliQ-water purification system (Millipore Merck Direct-Q^{TM}) to a specific resistance of > 18.0 MΩ cm $^{-1}$ at 25 C.

2.2. Sample preparation

Optimization of sample preparation was done in order to achieve high sensitivity and selectivity of the analytical method. The method used in this study was adapted from Česen et al. (2018a) with further optimization of the SPE procedure and the derivatisation protocol (data not shown). In the case of biological treatment, samples (250 mL) of WW influent and effluent were filtered first through glass fibre prefilters (0.5 µm, Machery Nagel, Dueren, Germany) and then through cellulose nitrate filters (1.2 µm, Sartorius, Goettingen, Germany) to prevent clogging of the SPE cartridges. Extraction was performed using divinylbenzene-*N*-vinylpyrrolidone copolymer SPE cartridges (60 mg, 3 mL; Oasis Prime HLB Waters, Massachusetts, USA). Samples were first acidified to pH 2 with HCl (0.5 mL of 37%) and loaded onto SPE cartridges at a flow rate of 3 mL min^{-1} using a Vacuum Manifold (Agilent Technologies). After loading, the sorbents were dried under vacuum (−1.33 kPa) for 45 min. The elution step was performed using 5% FA in EtAc (3 × 0.6 mL). The solvent was evaporated under a gentle stream of nitrogen at 40 °C. Derivatisation was achieved by adding 50 µL of MSTFA and 50 µL of pyridine to the dry residue, followed by mixing and heating at 80 °C for 1 h.

The same sample preparation was used in the adsorption experiments with the following modifications: samples (5.0 mL) were centrifuged at 9000 rpm for 20 min before SPE, the supernatant (3.0 mL) was then loaded onto the cartridges, and the sorbent dried under vacuum for 20 min. All UV light experiments were performed in MilliQ-water, and the samples (350 mL) were prepared as above, but without the filtration step. The stability tests required only a derivatisation step after complete evaporation of the sample for MeOH and water, and a filtration step for the WW samples.

2.3. Chemical analysis

All samples were analysed using a 7890B series gas chromatograph (GC) coupled to a 5977A single quadrupole mass selective detector (Agilent, United States). Chromatographic separation was achieved using a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 µm; Agilent, USA) with helium as the carrier gas (1 mL min^{-1}). Samples (1 µL) were injected in splitless mode at 250 °C. The GC oven temperature programme was as follows: an initial temperature of 120 °C was ramped at 20 °C min^{-1} to 250 °C and held for 6.0 min, then ramped to 300 °C at 10 °C min^{-1} and held for 3 min. Total runtime was 22.0 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. The target compounds were identified and quantified using selected ion monitoring (SIM mode). The monitored SIM ions and retention times (RTs) for the derivatized BPs and internal standards are presented in Table 1. Data were processed using MassHunter software (Agilent Technologies).

2.4. Method validation

Method performance for WW (Tables SI–3) and MilliQ-water (Tables SI–4) were assessed in terms of SPE recovery, linearity, accuracy as analyte recovery, limits of detection (LOD), limits of quantification (LOQ), sensitivity and precision expressed as instrumental repeatability. Full details are given in SI-2. Due to the widespread use of polymers in the laboratory, blanks were prepared to evaluate contamination during sample preparation (Caballero-Casero et al., 2016; Česen et al., 2016). All glassware was cleaned according to standard laboratory cleaning protocols for trace analysis. Procedural blanks were prepared for each experimental setup. Contamination was assessed by evaluating the ratio of the areas between the quantifier ions of the target BPs and the internal standards in the blanks (Česen et al., 2018b, 2016). Full details are given in SI-2.3, and Tables SI–5 shows the contribution that BPs make to background contamination. Additionally, solvent blanks (EtAc) were analysed after every tenth sample to

Table 1
Summary of derivatized compounds and internal standards measured as ions [*m/z*] (quantifier ion in bold and two qualifier ions), and their retention times (RT).

Compound	Monitored ion 1 [m/z]	Monitored ion 2 [m/z]	Monitored ion 3 [m/z]	RT [min]
22BPF-TMS	**344**	329	241	8.45
BPAF-TMS	480	465	**411**	8.95
24BPF-TMS	**344**	329	241	9.45
BPF-TMS	**344**	329	179	10.42
BPE-TMS	358	**343**	193	10.69
BPA-TMS	372	**357**	339	11.01
BPC-TMS	400	**385**	221	11.64
BPM-TMS	490	**475**	387	17.12
BPPH-TMS	524	**509**	283	20.47
BPP-TMS	490	**475**	387	19.58
BPBP-TMS	496	**419**	331	20.31
BPB-TMS	386	371	**357**	11.73
BP26DM-TMS	428	**413**	235	13.00
BPC2-TMS	**424**	374	259	13.17
BPZ-TMS	**412**	397	369	14.27
BPFL-TMS	**494**	329	239	21.63
BPAP-TMS	434	**419**	269	15.28
BPS-TMS	**394**	379	229	14.95
$^{13}C_{12}$-BPF-TMS	**356**	341	185	10.42
BPA-d_{16}-TMS	386	**368**	217	10.93
$^{13}C_{12}$-BPB-TMS	398	383	**369**	11.73
$^{13}C_{12}$-BPS-TMS	**406**	391	379	14.95

evaluate potential carryover between samples.

2.5. *Stability studies*

The study stability of the target BPs was studied by performing three separate experiments (Fig. 1):

2.5.1. *Exp 1. hydrolysis*

Hydrolysis was selected as the first step, as suggested by Franquet-Griell et al. (2017). A series of aluminium foil covered amber glass bottles (250 mL) were filled with MilliQ-water (100 mL) and spiked (4 μg mL^{-1}, 0.5 mL) with a mixture of BPs each at an initial concentration of 20 μg L^{-1}. The bottles were then sealed and incubated at room temperature (23 °C) for 48 h. Aliquots (0.5 mL) were taken at 0, 15, 30 min, 1, 2, 4, 8, 12, 24 and 48 h intervals.

2.5.2. *Exp 2. stability of BPs in the stock solution*

To a series of glass vials (n = 27) was added 1 mL of stock solution (1 mg L^{-1} in MeOH). The vials were then stored in the dark at 23, 4 and −20 °C for 24 weeks. For each experiment, a procedural blank was also prepared. At each sampling time (0, 4 and 24 weeks) an aliquot (0.5 mL) was spiked (25 μL) with the internal standard mixture ($^{13}C_{12}$-BPF, $^{13}C_{12}$-BPS, $^{13}C_{12}$-BPB and BPA-d_{16}). The samples and blanks were then reduced to dryness under a gentle stream of nitrogen at 40 °C and derivatized just prior to analysis by GC-MS. All samples were prepared and analysed in triplicate.

2.5.3. *Exp 3. stability in different matrices and at different temperatures*

In a similar experiment, to 60 glass vials was added 1 mL of each matrix (MilliQ-water or artificial WW from the RO bioreactor, Section 2.6). The experiment included three replicates for each matrix, a temperature setting and sampling time. The controls were prepared in triplicate. Each sample was then spiked (20 μL) with a mixture of the target compounds (1 μg mL^{-1}) to give a final concentration of 20 μg L^{-1} and stored in the dark at 4 and −20 °C for 24 weeks. In this case, an aliquot (0.5 mL) was sampled at 0, 1, 4, 14 and 24-week intervals and analysed as above (*Exp. 2*).

2.6. *Biological treatment*

Most studies report the near-complete removal of BPA by bacterial consortia in wastewater treatment plant (WWTP) and rivers. The most commonly used biological technology for treating WW is the suspended activated sludge (SAS) process (Noszczyńska and Piotrowska-Seget, 2018), while the attached-growth biomass process remains a popular option for upgrading conventional biological WWT (Gapes and Keller, 2009). For this reason, biological treatment was simulated in two different lab-scale flow-through bioreactors (Fig. SI-1): (1) a suspended activated sludge reactor (SASR) and (2) a moving bed bioreactor (MBBR) containing Mutag Biochip™ carriers (Multi Umwelttechnologie AG, Sachsen, Germany). The SASR consists of four different compartments: a selector, anoxic chamber, settling chamber and the main aeration chamber, while the MBBR has only an aerated compartment. In this study, three SASRs (RO, R1 and R2) and three MBBRs (M0, M1 and M2) bioreactors were used. The reactors, RO and MO, acted as controls. All the bioreactors were operated under identical conditions (hydraulic retention time (HRT): 48 h, working volume: 4 L) using synthetic WW (2 L day^{-1} (Tables SI–6)) (Česen et al., 2015). A mixture of 16 BPs (4 μg L^{-1}) was continuously added to each bioreactor (each BP: 200 ng L^{-1}). To the controls, MeOH was added in the same amount as in the spike. After the one-month adaptation period, influents and effluents were sampled at monthly intervals (n = 6) over six months. In

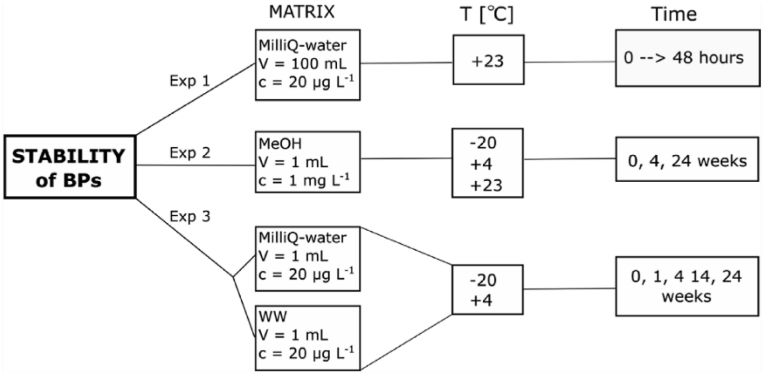


Fig. 1. The experimental design used for stability study of 18 BPs.

total, this amounted to 24 samples and 12 controls. Based on the HRT of the reactors, influent samples were taken 48 h before the effluent samples, which allowed the removal of each BP to be calculated. All samples were stored in the dark at −20 °C. The performance of the bioreactors was determined by measuring total and dissolved chemical oxygen demand (COD_t and COD_s) and nitrogen species (NO_3–N and NH_4–N) in the influents and effluents before, halfway and at the end of the experiment (Tables SI–7). At the same time, the biomass concentration was determined following the procedure of Zupanc et al. (2013). Additionally, dissolved oxygen (DO), T and pH in each were also monitored (Tables SI–8 and Tables SI–9). Details of the procedures and results are presented in the SI-3.

2.7. Adsorption to biomass

Batch adsorption experiments were performed in 250 mL amber glass bottles (total wetted volume of 100 mL) at room temperature (T = 23 °C) with constant shaking (200 rpm). The biomass was obtained from the blank RO bioreactors (Section 2.6). Samples with activated biomass (AB), deactivated biomass, controls and blanks were prepared. Biological inhibition was achieved using two different approaches: (1) exposing samples (RB) to γ-irradiation (25 kGray) and (2) adding 6% FDH (FB) and shaking for 12 h (Kosjek et al., 2016). Samples were irradiated at the TRIGA Mark II nuclear research reactor facility at the Jožef Stefan Institute (Ambrožič et al., 2017; Snoj et al., 2012). Control experiments used MilliQ-water with FDH (MQF) and without FDH (MQ). An aliquot (0.5 mL) of the solution of BPs in MeOH ($4\,\mu g\,L^{-1}$) was added to each of the test samples (100 mL) to give a final concentration of each BP of $20\,\mu g\,L^{-1}$. The blank (BL) consisted of 100 mL of MilliQ-water. The volume of sample was 5.0 mL. Each sample was then spiked (V = 25 µL) with the internal standard mixture. Sampling times were 0, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h. Samples were centrifuged (9000 rpm at 20 min) and the supernatant transferred to 10 mL glass vials and prepared according to Section 2.2. The standard WWT parameters (DO, T, pH, biomass concentration and COD) were measured as described in full in SI-3 (results not given).

2.8. UV photolysis

Laboratory scale UV light experiments were performed by irradiating aqueous solutions (MilliQ-water) of BPs for 10, 20, 40, 60, 120 and 240 min in a cylindrical glass immersion reactor (760 mL, Fig. SI-3). The source of UV irradiation was a monochromatic (λ_{max} = 254 nm) 6-W low-pressure Mercury UV lamp. The intensity of the emitted light, measured by ferrioxalate actinometry, was $8.91 \times 10^{-7}\,E\,s^{-1}$ (Union et al., 2005). The initial concentration of each BP was $200\,ng\,L^{-1}$, except for of BPA, which was added at a higher concentration ($1\,\mu g\,L^{-1}$) to reflect realistic WW concentrations (Chen et al., 2016b). Acetonitrile was chosen in place of MeOH as a co-solvent to avoid radical scavenging in the photolysis solution (Kosjek et al., 2013). The reactor solution was maintained at room temperature (T = 23 °C) under atmospheric pressure using a water-cooled quartz immersion well. The content of the immersion reactor was homogenised by continuous stirring using a magnetic stirrer (400 rpm). Additionally, two types of negative control samples were included in the experiment. Blank samples (without BPs) were exposed to UV irradiation in order to evaluate potential contamination. Zero samples were spiked with BPs and mixed for 10 min but were not exposed to UV irradiation.

2.9. Statistical analysis

Statistical calculations and visualization were made using the open source software package R, version 3.4.1. (R Development Core Team, 2011). A statistical comparison of two or more data samples was employed to assess the influence of the matrix, temperature and the storage time on the stability of the target compounds, and to reveal significant differences between the treatments. The Shapiro-Wilk test for checking data normality and the Levene's test for checking homoscedasticity of variance (significance level = 0.05) were used to select the most appropriate statistical test. In some instances, two data samples (paired) or more than two data samples (paired) satisfied the required conditions. Therefore a parametric test, paired *t*-test or repeated measures ANOVA and nonparametric test, a Wilcoxon signed-rank and the Friedman test were selected (Tables SI–10) (Eftimov et al., 2017). The statistical significance was evaluated at a level of 0.05. Ranking the stability of individual BPs was done by adopting a combination of a multi-criteria decision approach and an information theory approach, which ranks the best algorithm in numerical order according to stability, i.e., #1 is the most stable (Eftimov et al.,).

3. Results and discussion

3.1. Stability study

3.1.1. Exp 1 hydrolysis

The following BPs: 22BPF, 24BPF, BPA, BPAF, BPAP, BPB, BPC, BPC2, BPE, BPF, BP26DM, BPS, BPZ out of 18 BPs were shown to be stable (> 90% remaining) in MilliQ-water (Fig. 2). For BPA, this agrees

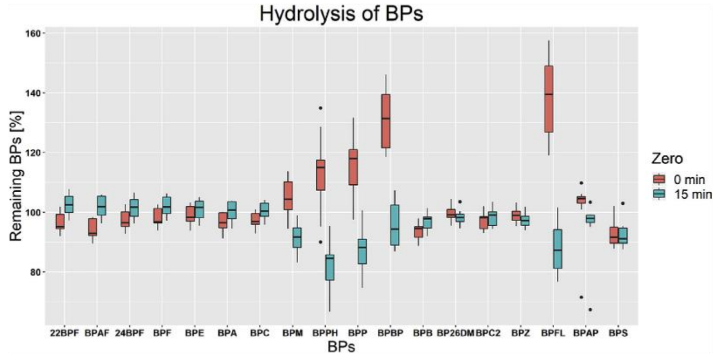


Fig. 2. Hydrolysis of BPs. The remaining BPs (%) were calculated using zero condition at 0 min or 15 min within 48 h (0, 15, 30 min, 1, 2, 4, 8, 12, 24 and 48 h intervals).

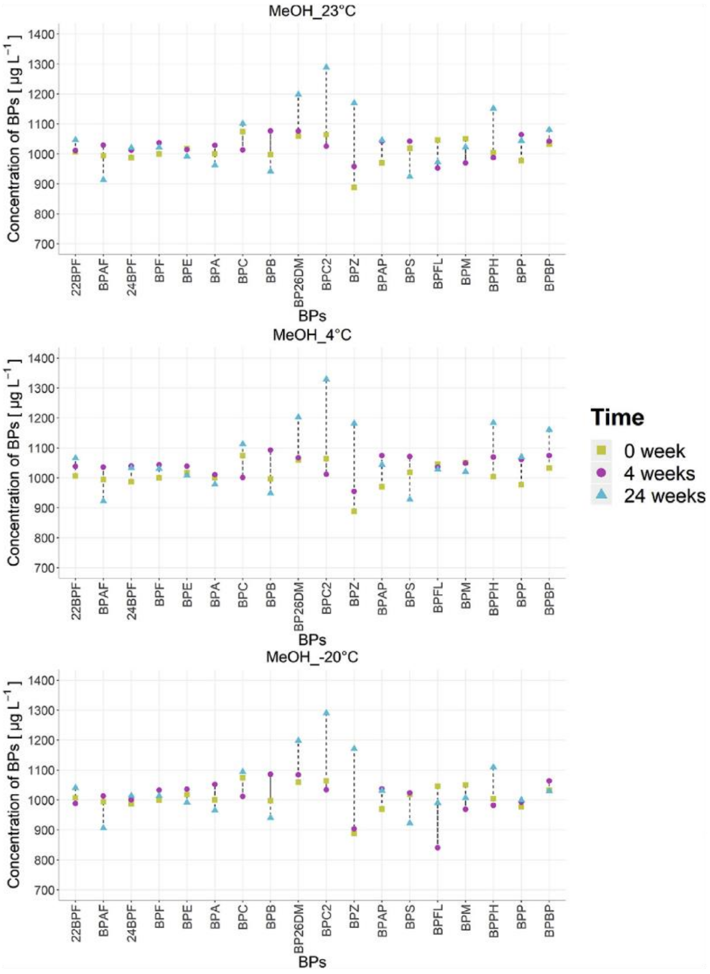


Fig. 3. Stability of 18 BPs in stock solution (MeOH, initial concentration 1.0 ± 0.1 mg L^{-1}) stored at 23, 4 and −20 °C for different lengths of time (up to 24 weeks).

with the findings of Late et al. (Lane et al., 2015). In the case of BPM, BPPH, BPP, BPBP and BPFL surprisingly > 100% remained after time zero if 0 min was used as the zero condition. These BPs contain a higher number of aromatic rings and have higher logK_{ow} (6.25–7.17, Tables SI–2). Although limited, the data shows that the BPs have a broad range of logK_{ow} values (1.25–7.17), suggesting that BPs with logK_{ow} < 3 will be in the aqueous phase while those with logK_{ow} > 3 are likely adsorbed to soil and sediment (Caballero-Casero et al., 2016; Tišler et al., 2016). A possible explanation for the observed results is the adsorption/desorption processes occurring in the samples. Initially, the concentrations of BPs with high K_{ow} decreased due to the rapid adsorption onto glass at time 0 min but then increased as the compounds desorbed. This effect was confirmed by using t = 15 min as the zero. In this case, the results were below 100% confirming that BPM, BPPH, BPP, BPBP and BPFL were rapidly absorbed (t = 0 min) and became desorbed after 15 min. When the zero condition was t = 15 min, these BPs are stable, and large fluctuations in their percentages remain, and for this reason, these BPs were excluded from further statistical analysis.

3.1.2. Exp 2. stability of BPs in the stock solution

Based on the estimated method relative standard deviation (RSD = ± 10%, data not shown), Fig. 3 confirms that all 18 of the investigated BPs are stable (measured concentration = ± 10% initial concentration) in MeOH at 23, 4 and −20 °C within 24 weeks. A slight increase (~20%) in concentration at t = 24 weeks in the case of BP26DM, BPC2 and BPZ was observed but statistical analysis (p-values > 0.05) shows no significant difference in the concentrations of BPs in MeOH with temperature (−20, 4 and 23 °C) and time (≤6

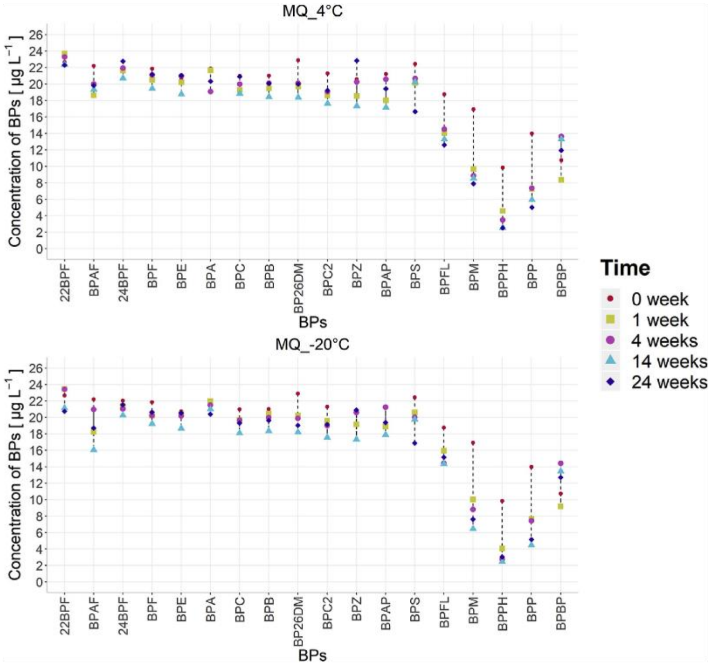


Fig. 4. Stability of the 18 BPs in MQ (initial concentration 20 ± 4 µg L^{-1}) stored at 4 and -20 °C for different lengths of time (up to 24 weeks).

months) (Tables SI–10).

3.1.3. Exp 3. stability in different matrices and temperatures

After 24 weeks at −20 and 4 °C, 12 out of the 18 BPs tested in MilliQ-water remained above 85% of the initial doses proving their stability. Even BPS, with > 75% remaining after 24 weeks are considered stable (Fig. 4). Statistical analysis shows no significant ($p > 0.05$) difference in concentration with time at each temperature, but there is a difference ($p < 0.05$) between −20 and 4 °C (Tables SI–10).

Nevertheless, 13 BPs are considered stable in MilliQ-water (Fig. 4) at tested conditions since their levels were within the estimated method RSD (MilliQ-water and WW: ± 20%, data not shown).

The BPs: BPM, BPPH, BPP, BPBP and BPFL, had significantly lower initial concentrations (9.9–18.8 µg L^{-1}) compared to the others (20 ± 4 µg L^{-1}). The findings agree with observations in Exp. 1. The initial concentrations of these five compounds reduced due to rapid adsorption and increased due to desorption with time (especially after one week). This effect occurred in MilliQ-water and WW, causing their concentrations to fluctuate (RSD > 20%), which meant it was not possible to comment on their stability and they were excluded from further statistical analysis.

In the case of WW, after 14 weeks at −20 °C, BPAF, BPE, BPC2 and BPS had RE of 75–100%, 22BPF, 24BPF, BPAP, BPB, BPF, BPZ had RE of 50–75%, and BPA, BPC and BP26DM between 26–33% (Fig. 5). Their concentrations after 14 and 24 weeks were similar and there is no significant difference between concentrations remaining after 1 and 4 weeks at −20 and 4 °C and 14 and 24 weeks at −20 and 4 °C. There was a significant difference between weeks 1 and 14, weeks 1 and 24, weeks 4 and 14, and weeks 4 and 24 (Tables SI–10) meaning that these 13 BPs can only be considered stable up to 4 weeks at −20 and 4 °C.

This study also carried out a statistical analysis of the different matrices (Tables SI–10). Analysis of the two data sets revealed a statistically significant difference between BP recoveries from MeOH and MQ, MeOH and WW and WW and MQ samples under all conditions. The only exceptions were between MeOH and MQ after 24 weeks at 4 °C and between WW and MQ after 1 week at −20 °C. Similarly, the Friedman test and repeated-measure ANOVA revealed a significant difference between the three matrices. This finding is mainly due to differences between WW and MQ. According to the algorithm rankings, and taking into account the limited number of data, and that BPM, BPPH, BPP, BPBP and BPFL were excluded, BPE was the most stable followed by the positional isomers of BPF (22BPF and 24BPF), BPS and BPF, while BP26DM ranked as the least stable (Tables SI–11).

3.2. Removal of BPs

3.2.1. Biological treatment

The performance of the laboratory bioreactors was checked by monitoring the following standard WWT parameters: NO_3–N, NH_4–N, COD_t, COD_s, DO, pH and biomass concentration. For full details, see SI-3. The removal efficiency was calculated as the difference between the dimensionless concentration of the target BP in the influent (c_{inf}) and the effluent (c_{eff}) using Eq. (1) (Zupanc et al., 2013).

$$RE\,[\%] = \left(1 - \frac{c_{eff}}{c_{\inf}}\right)x100 \tag{1}$$

Statistical analysis shows no significant correlation (Tables SI–12) between RE and sampling time over six months. The REs in Tables SI–12 are the average REs of BPs in each bioreactor during six

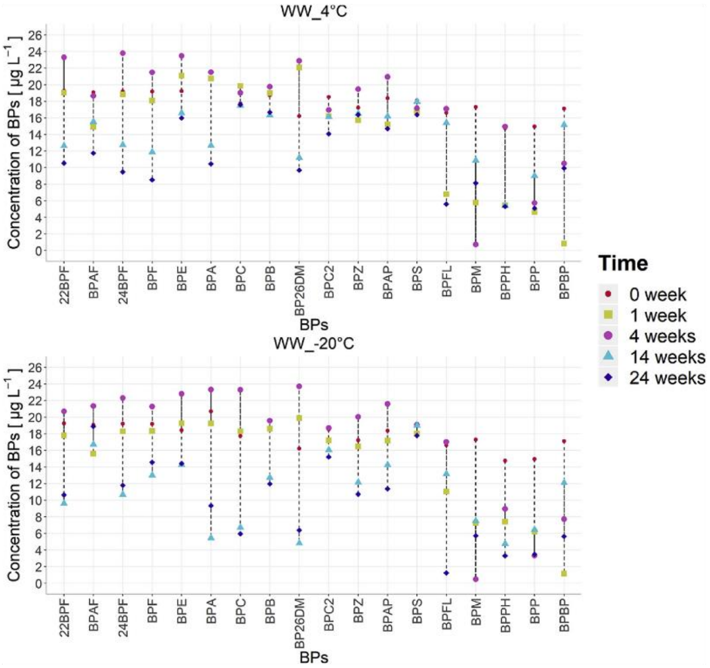


Fig. 5. Stability of the 18 BPs in WW (initial concentration 20 ± 4 μg L^{-1} μg L^{-1}) stored at 4 and -20 °C for different lengths of time (up to 24 weeks).

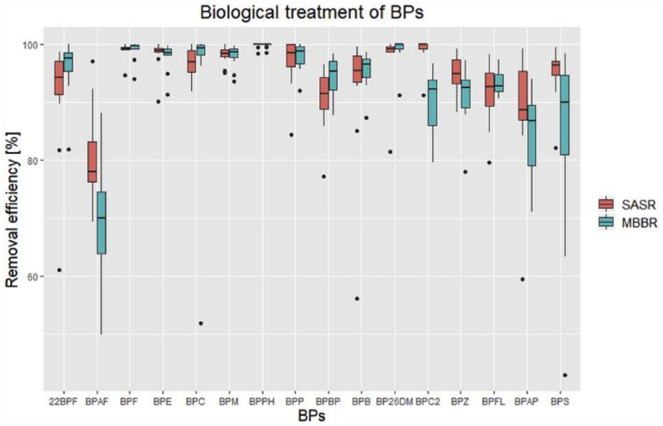


Fig. 6. The average removal efficiencies of two bioreactors for each type (SASR, MBBR) during 6 samplings (6 months).

samplings. Apart from BPAF and BPBP, no significant differences exist between the average RE of the SASR and MBBR neither for individual BPs nor for all BPs (Tables SI–12). Biological treatment resulted in REs > 85% for the majority of BPs (Fig. 6, Tables SI–12). The RE of BPAF was lower compared with the other compounds (Tables SI–12). The presence of C—F bonds in the structure of BPAF explains its low RE, since halogenated compounds are usually more resistant to biodegradation (Chen et al., 2016a).

To the authors' knowledge, except for BPA and one study of BPS (Huang et al., 2019), this is the first investigation of biological RE of BPs by bacterial communities in WW on a laboratory scale (Noszczyńska and Piotrowska-Seget, 2018). However, the findings can

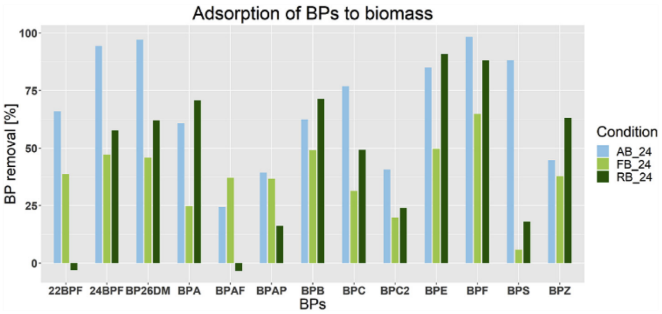


Fig. 7. Losses [%] of BPs after 24 h in the sample with activated (AB) or deactivated (RB – radiation, FB– formaldehyde) biomass.

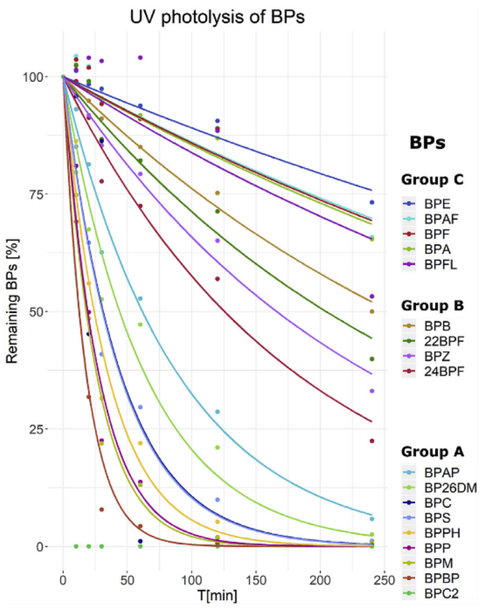


Fig. 8. Kinetic profiles for UV photolysis of 18 BPs.

be compared with RE obtained for WWTPs and previously published studies that focus on single bacterial strains, while the degradation of organic pollutants is a cooperative process involving various micro-organisms (Noszczyńska and Piotrowska-Seget, 2018). The high REs of these BPs are in line with the results reported by Drušković (2016), who studied BPA removal in the same SASR as used in this study. Drušković reported high REs at 50 μg L^{-1} (> 99.0% removed) and 1 μg L^{-1} (> 79.2% removed). The RE of BPS is similar to that reported in a recent study by Huang et al. (2019) who also used a microbial consortia. Sun et al. (2017) and Karthikraj et al. (Karthikraj and Kannan, 2017) also report high REs (RE: 76.9–98.9%) for BPA, BPF and BPS when comparing the levels in influents and effluents from actual WWTPs; only partial (if any) removal was observed for BPAF and BPE. The present work is a continuation of our previous "real" scale studies performed by Česen et al. (2018b) reporting a high RE (> 96.2%) for BPAF, BPAP, BPC, BPE, BPF, BPS, BPZ and BPB from WWs. The analogy with the results of the present laboratory-scale experiments confirms the effectiveness of biological treatment. The finding is also in agreement with the findings of Zühlke et al. (2016) who, using *Bacillus amyloliquefacien* isolated from sewage sludge, report a RE of 95%, 77%, and 69% for BPC, BPE = BPA and BPZ, respectively. Danzl et al. (2009) and Sakai et al. (SAKAI et al., 2007) both show the ability of an isolated seawater bacterial strain (BP-7) to remove BPF, BPE, BPZ and BPB from WW, while BPS remained recalcitrant. A more recent study by Ogata et al. (2013) found that *Sphingobium fuliginis* is the only bacterial strain able to degrade BPS. In general, biological treatment achieves high REs of BPs despite the differences in their chemical structures. Also, reactor design (SASR: ± 5%, MBBR: ± 7%) did not affect the REs of the different BPs. However, additional removal processes, like adsorption to biomass or material surfaces, may also be occurring.

3.2.2. *Adsorption to biomass*

The concentrations of target BPs in test solutions containing inhibited and activated biomass (AB) after 24 h and 48 h were compared to determine losses due to adsorption to biomass. A preliminary test found there was no significant difference in BP removal after 24 h and 48 h (Figs. SI-2.1, 2.2 and 2.3) in the deactivated biomass proving the system had reached equilibrium. In the case of activated biomass after 48 h, only BPAF remained in the solution (> 60%) confirming its recalcitrance when compared to other BPs. Like in the stability studies the levels of BPFL, BPM, BPPH, BPP, and BPBP varied to such an extent that a different approach is needed to evaluate their adsorption affinity to biomass (data not shown).

No significant losses (estimated method RSDs = 100 ± 20%) of the 13 BPs were observed in the controls MQ (< 10%) and MQF (< 20%)

Table 2

R-squared value (R^2), rate constants (k [min^{-1}]) and half-lives ($t_{1/2}$ [min]) for kinetic profiles of 18 BPs using UV irradiation.

BPs	22BPF	BPAF	24BPF	BPF	BPE	BPA	BPC	BPM	BPPH	BPP	BPBP	BPB	BP26DM	BPC2	BPZ	BPFL	BPAP	BPS
R^2	0.98	0.95	0.98	0.98	0.97	0.97	0.68	0.91	0.99	0.96	0.84	0.99	0.99	0.16	0.99	0.99	0.99	0.99
k_t [min^{-1}]	0.004	0.002	0.006	0.002	0.001	0.002	0.025	0.024	0.026	0.026	0.030	0.028	0.015	–	0.004	0.002	0.012	0.019
$t_{1/2}$ [min]	173.29	346.57	115.52	346.57	693.15	346.57	27.73	28.88	26.66	26.66	23.10	24.76	46.21		173.29	239.02	57.76	36.48

(Tables SI–13). The results show that removal of BPs in WW occurs through both biodegradation and adsorption to biomass. The results (Fig. 7, Tables SI–13) from the RB and FB samples show that 22BPF and BPS have the lowest adsorption affinity assuming that biodegradation is the primary removal process. The BPs: 24BPF, BPC2, BPC, BPAP and BP26DM showed modest adsorption relative to the others, i.e., rapid sorption occurs initially after which biodegradation prevails (Fig. SI-2). The RB results confirm adsorption as the primary mechanism for removing BPF, BPE, BPA, BPB and BPZ, while in the case of FB, the adsorption affinity of BPF, BPE, BPA and BPB was significantly lower (Fig. 7). The available literature on the adsorption of BPA to biomass is also contradictory (Keskinkan and Balci, 2016; Strenn et al., 2003; Zhao et al., 2008). The possible physical alteration of the biomass during exposure to γ-irradiation could explain the difference between the two sets of samples. This difference is because adsorption is mainly a physical process depending on the physicochemical properties of the adsorbent and the adsorbate (Carballa et al., 2008; Zhao et al., 2008). The tendency of BPs to adsorb to biomass can also be explained in terms of their K_{ow} values (Tables SI–2). In the RB, BPs with a high adsorption affinity have high K_{ow} values ($\log K_{ow} > 3.5$), e.g., BPZ has a $\log K_{ow} = 5$, while BPs with the lowest adsorption affinity have low K_{ow} values, e.g., BPS ($\log K_{ow} = 1.65$). There is also a difference between the adsorption affinities of individual BPs to biomass (Fig. 7.), which means that adsorption will be a dominant factor controlling the removal of certain BP in WWTPs. The results of this study allow not only the adsorption affinity of BPs towards biomass to be estimated but also form the basis for further studies to determine the sorption coefficients (K_d) of BPs.

3.2.3. *UV photolysis*

Calculating the kinetic constants k_t and substrate half-lives meant that it was possible to evaluate the RE and degradation kinetics of BPs by UV-light (λ = 254 nm). For the majority of BPs, regression analysis of the natural logarithm plots of the REs (Eq. (2)) *versus* exposure time yields an $R^2 > 0.95$ (Fig. 8). The results show that removal of the studied BPs follows pseudo-first-order kinetics (Eq. (3)).

$$RE\,[\%] = \frac{C_0 - C_t}{C_0} \times 100 \tag{2}$$

$$\frac{dc_0}{dt} = -k_t c_t \tag{3}$$

Here, c_0 and c_t are the initial and residual concentrations, and k_t is the observed pseudo-first-order rate constant. A summary of the rate constants and half-lives can be found in Table 2. The rapid removal of BPC2 (< 10 min), BPBP (< 40 min) and BPC (< 60 min), i.e., $R^2 < 0.95$, meant that their kinetic profile could not be determined and, in this case, shorter time intervals would be required. The rapid removal of BPC2 is likely due to the presence of chlorine atoms in its structure (Leydy Katherine Ardila et al., 2019; Zhang et al., 2019). The presence of aromatic rings (conjugated system) also explains the reason for the near-complete removal (Leydy Katherine Ardila et al., 2019; Yamaji et al., 2016) of BPM, BPP, BPPH and BPBP after 60 min (78–96%), and their complete removal after 4 h. The efficient removal of BPP agrees with Wang et al. (2008) who reported a RE of 94.8% for BPP in the presence of β-cyclodextrin, which they believe results from the formation of an inclusion complex with the β-cyclodextrin. All BPs showed varying degrees of photodegradation over time (4 h). After 4 h of irradiation, the BPs fall into three groups, based on their RE (Fig. 8). Group A consists of BPAP, BP26DM, BPC, BPS, BPPH, BPP, BPM, BPBP, and BPC2, with a RE > 94% and half-lives of < 60 min; Group B includes BPB, 22BPF, BPZ and 24BPF with a RE of 50–80% and half-lives > 100 min, where BPB is an exception ($t_{1/2} = 24.76$), but is included in this group due to its rapid decline in concentration after 1 h, and Group C that includes BPE, BPAF, BPF, BPA and BPFL, with a RE of 25–45% and half-lives > 300 min. Therefore, contrary to biological treatment, there are obvious differences in the RE of the different BPs when irradiated with UV light. In addition, BPAF is more resistant to both photolysis and biological treatment relative to the other BPs tested in this study. In general, photolysis is efficient at removing the majority of the tested BPs and after 4 h only five BPs remained between 65–75%. This is most likely due to the presence of aromatic moieties in their structure.

4. Conclusion

This study provides data on the stability of 18 BPs in MeOH, ultrapure water and WW and their fate during biological treatment and UV photolysis. It was found that hydrolysis of the investigated BPs does not occur, and were most stable in MeOH, followed by ultrapure water, followed by WW. For the majority of tested compounds, the safe storage conditions for WW samples are −20 °C or 4 °C for up to four weeks. The behaviour of BPM, BPPH, BPP, BPBP and BPFL differ from that of other BPs due to the rapid adsorption/desorption processes on material surfaces. The study also found that biological treatment, using both suspended or attached-growth biomass, is efficient at removing BPs (REs > 85%). Adsorption to biomass is also an important removal mechanism but varies depending on the K_{ow} of the compounds. UV photolysis also achieved high REs of BPs but only after a lengthy period (4 h) of irradiation. Based on their REs, the tested compounds can be classed into three groups: Group A (BPAP, BP26DM, BPC, BPS, BPPH, BPP, BPM, BPBP, and BPC2) with RE > 94%; Group B (BPB, 22BPF, BPZ and 24BPF) with REs = 50–80% and, Group C (BPE, BPAF, BPF, BPA and BPFL) with REs = 25–45%. Unlike in biological treatment, there is greater variability in the REs of BPs when irradiated with UV light. BPAF was less affected by both biological treatment (60–80%) and photolysis (Group C). This study is the first to address the removal of a mixture of BPs both by bacterial consortia and photolysis, and confirms their effectiveness. The lack of literature data on the fate of BPs reveals a need to assess their behaviour in the aqueous environment and the risk to biota and/or humans. Taken together, the findings of the present study contribute to a better understanding of the behaviour of BPs in the environment, which is gaining in importance.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the Slovenian Research Agency (Program Group P1-0143 and Projects L1-7544, N1-0047, J1-8147, J2-8162 and L1-9191) and the MASSTWIN (European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 692241). Ana Kovačič gratefully acknowledges the Young researcher grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.108738.

References

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J. Hazard Mater. 323, 274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045.

Ambrožič, K., Žerovnik, G., Snoj, L., 2017. Computational analysis of the dose rates at JSI TRIGA reactor irradiation facilities. Appl. Radiat. Isot. 130, 140–152. https://doi.org/10.1016/j.apradiso.2017.09.022.

Ashfaq, M., Sun, Q., Zhang, H., Li, Y., Wang, Y., Li, M., Lv, M., Liao, X., Yu, C.P., 2018. Occurrence and fate of bisphenol A transformation products, bisphenol A monomethyl ether and bisphenol A dimethyl ether, in wastewater treatment plants and surface water. J. Hazard Mater. 357, 401–407. https://doi.org/10.1016/J.JHAZMAT.2018.06.022.

Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53. https://doi.org/10.1016/j.aca.2015.12.034.

Cao, G., Lu, J., Wang, G., 2012. Photolysis kinetics and influencing factors of bisphenol S in aqueous solutions. J. Environ. Sci. 24, 846–851. https://doi.org/10.1016/S1001-0742(11)60809-7.

Carballa, M., Fink, G., Omil, F., Lema, J.M., Ternes, T., 2008. Determination of the solid–water distribution coefficient (Kd) for pharmaceuticals, estrogens and musk fragrances in digested sludge. Water Res. 42, 287–295. https://doi.org/10.1016/J.WATRES.2007.07.012.

Česen, M., Heath, D., Krivec, M., Ko, J., Kosjek, T., Heath, E., 2018a. Seasonal and Spatial Variations in the Occurrence , Mass Loadings and Removal of Compounds of Emerging Concern in the Slovene Aqueous Environment and Environmental Risk Assessment * 242. https://doi.org/10.1016/j.envpol.2018.06.052.

Česen, M., Kosjek, T., Laimou-geraniou, M., Kompare, B., Brane, Š., Lambropolou, D., Heath, E., 2015. Science of the Total Environment Occurrence of Cyclophosphamide and Ifosfamide in Aqueous Environment and Their Removal by Biological and Abiotic Wastewater Treatment Processes, vol. 528. pp. 465–473. https://doi.org/10.1016/j.scitotenv.2015.04.109.

Česen, M., Lambropoulou, D., Laimou-Geraniou, M., Kosjek, T., Blaznik, U., Heath, D., Heath, E., 2016. Determination of bisphenols and related compounds in honey and their migration from selected food contact materials. J. Agric. Food Chem. 64, 8866–8875. https://doi.org/10.1021/acs.jafc.6b03924.

Česen, M., Lenarčič, K., Mislej, V., Levstek, M., Kovačič, A., Cimrmančič, B., Uranjek, N., Kosjek, T., Heath, D., Dolenc, M.S., Heath, E., 2018b. The occurrence and source identification of bisphenol compounds in wastewaters. Sci. Total Environ. 616–617, 744–752. https://doi.org/10.1016/j.scitotenv.2017.10.252.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M., 2016a. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016b. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - a review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

Danzl, E., Sei, K., Soda, S., Ike, M., Fujita, M., 2009. Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater. Int. J. Environ. Res. Public Health 6, 1472–1484. https://doi.org/10.3390/ijerph6041472.

Druškovič, J., 2016. Odstranitev Organskih Onesnaževal Iz Odpadne Vode Z Uporabo Bioloških in Naprednih Oksidacijskih Postopkov Čiščenja.

Eftimov, T., Korošec, P., & Seljak, B.K., n.d. No titleriven preference-based deep statistical ranking for comparing multi-objective optimization algorithms. In International Conference on Bioinspired Methods and Their Applications.

Eftimov, T., Korošec, P., Potočnik, D., Ogrinc, N., Heath, D., Seljak, B.K., 2017. How to perform properly statistical analysis on food data? An e-learning tool: advanced Statistics in Natural Sciences and Technologies. Sci. within Food Up-to-date. Adv. Res. Educ. Ideas 144–151.

Franquet-Griell, H., Medina, A., Sans, C., Lacorte, S., 2017. Biological and photochemical degradation of cytostatic drugs under laboratory conditions. J. Hazard Mater. 323, 319–328. https://doi.org/10.1016/j.jhazmat.2016.06.057.

Gmurek, M., Olak-Kucharczyk, M., Ledakowicz, S., 2017. Photochemical decomposition of endocrine disrupting compounds – a review. Chem. Eng. J. 310, 437–456. https://doi.org/10.1016/j.cej.2016.05.014.

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014.

Gys, C., Kova, A., Huber, C., Yin, F., Heath, E., Covaci, A., 2018. Suspect and Untargeted Screening of Bisphenol S Metabolites Produced by in Vitro Human Liver Metabolism, vol. 295. pp. 115–123. https://doi.org/10.1016/j.toxlet.2018.05.034.

Huang, W.-C., Jia, X., Li, J., Li, M., 2019. Dynamics of microbial community in the bioreactor for bisphenol S removal. Sci. Total Environ. 662, 15–21. https://doi.org/10.1016/j.scitotenv.2019.01.173.

Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from liaohe river basin and taihu lake, China. Water Res. 103, 343–351. https://doi.org/10.1016/j.watres.2016.07.059.

Karthikraj, R., Kannan, K., 2017. Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere 181, 216–223. https://doi.org/10.1016/j.chemosphere.2017.04.075.

Keskinkan, O., Balci, B., 2016. Biotic and abiotic bisphenol-A removal from wastewater by activated sludge: effects of temperature, biomass, and bisphenol-A concentrations. Water Sci. Technol. 73, 317–328. https://doi.org/10.2166/wst.2015.495.

Kosjek, T., Negreira, N., Heath, E., de Alda, M.L., Barceló, D., 2016. Biodegradability of the anticancer drug etoposide and identification of the transformation products. Environ. Sci. Pollut. Res. 23, 14706–14717. https://doi.org/10.1007/s11356-016-6889-5.

Kosjek, T., Perko, S., Žigon, D., Heath, E., 2013. Fluorouracil in the environment: analysis, occurrence, degradation and transformation. J. Chromatogr. A 1290, 62–72. https://doi.org/10.1016/j.chroma.2013.03.046.

Kovačič, A., Gys, C., Kosjek, T., Covaci, A., Heath, E., 2019. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics. Sci. Total Environ. 664, 595–604. https://doi.org/10.1016/J.SCITOTENV.2019.02.064.

Lane, R.F., Adams, C.D., Randtke, S.J., Carter, R.E., 2015. Bisphenol diglycidyl ethers and bisphenol A and their hydrolysis in drinking water. Water Res. 72, 331–339. https://doi.org/10.1016/j.watres.2014.09.043.

Lee, S., Liao, C., Song, G.J., Ra, K., Kannan, K., Moon, H.B., 2015. Emission of bisphenol analogues including bisphenol A and bisphenol F from wastewater treatment plants in Korea. Chemosphere 119, 1000–1006. https://doi.org/10.1016/j.chemosphere.2014.09.011.

Leydy Katherine Ardila, P., da Silva, B.F., Spadoto, M., Clarice Maria Rispoli, B., Azevedo, E.B., 2019. Which route to take for diclofenac removal from water: hydroxylation or direct photolysis? J. Photochem. Photobiol. A Chem. 382, 111879. https://doi.org/10.1016/j.jphotochem.2019.111879.

Liu, Y., Zhang, X., Wu, F., 2010. Photodegradation of bisphenol AF in montmorillonite dispersions: kinetics and mechanism study. Appl. Clay Sci. 49, 182–186. https://doi.org/10.1016/j.clay.2010.05.002.

Noszczyńska, M., Piotrowska-Seget, Z., 2018. Bisphenols: application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers. Chemosphere 201, 214–223. https://doi.org/10.1016/j.chemosphere.2018.02.179.

Ogata, Y., Goda, S., Toyama, T., Sei, K., Ike, M., 2013. The 4-tert-butylphenol-utilizing bacterium sphingobium fuliginis OMI can degrade bisphenols via phenolic ring hydroxylation and meta-cleavage pathway. Environ. Sci. Technol. 47, 1017–1023. https://doi.org/10.1021/es303726h.

Petrie, B., Lopardo, L., Proctor, K., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2019. Assessment of bisphenol-A in the urban water cycle. Sci. Total Environ. 650, 900–907. https://doi.org/10.1016/J.SCITOTENV.2018.09.011.

R Development Core Team, R., 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://doi.org/10.1007/978-3-540-74686-7.

SAKAI, K., YAMANAKA, H., MORIYOSHI, K., OHMOTO, T., OHE, T., 2007. Biodegradation of bisphenol a and related compounds by *sphingomonas* sp. strain BP-7 isolated from seawater. Biosci. Biotechnol. Biochem. 71, 51–57. https://doi.org/10.1271/bbb.60351.

Salomatova, V., Pozdnyakov, I., Sherin, P., Grivin, V., Plyusnin, V., 2015. Photochemistry of bisphenol F in aqueous solutions: a mechanistic study. J. Photochem. Photobiol. A Chem. 305, 45–50. https://doi.org/10.1016/j.jphotochem.2015.03.008.

Snoj, L., Žerovnik, G., Trkov, A., 2012. Computational analysis of irradiation facilities at the JSI TRIGA reactor. Appl. Radiat. Isot. 70, 483–488. https://doi.org/10.1016/j.apradiso.2011.11.042.

Strenn, B., Clara, M., Kreuzinger, N., 2003. Degradation and Adsorption to Activated Sludge of Bisphenol-A. 17-P-Estradiole and 61.

Sun, Q., Wang, Y., Li, Y., Ashfaq, M., Dai, L., Xie, X., Yu, C.P., 2017. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ. Pollut. 225, 542–549. https://doi.org/10.1016/j.envpol.2017.03.018.

Tišler, T., Krel, A., Gerželj, U., Erjavec, B., Dolenc, M.S., Pintar, A., 2016. Hazard identification and risk characterization of bisphenols A, F and AF to aquatic organisms. Environ. Pollut. 212, 472–479. https://doi.org/10.1016/j.envpol.2016.02.045.

Union, I., Pure, O.F., Chemistry, A., 2005. International union of pure and applied chemistry chemical actinometry. pp. 1–47.

Wang, G., Huang, L., Yu, R., Deng, N., 2008. Photocatalytic degradation of 2,2-bis(4-hydroxy-3-methylphenyl) propane (BPP) based on molecular recognition interaction. J. Chem. Technol. Biotechnol. https://doi.org/10.1002/jctb.1837.

Wang, X., Ma, J., Wang, Z., Guo, R., Hu, X., 2014. Aqueous phototransformation of bisphenol S: the competitive radical-attack pathway to *p* -hydroxybenzenesulfonic acid. Water Sci. Technol. 70, 540. https://doi.org/10.2166/wst.2014.257.

Wu, L.-H., Zhang, X.-M., Wang, F., Gao, C.-J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci. Total Environ. 615, 87–98. https://doi.org/10.1016/J.SCITOTENV.2017.09.194.

Xue, J., Kannan, K., 2019. Mass flows and removal of eight bisphenol analogs, bisphenol A diglycidyl ether and its derivatives in two wastewater treatment plants in New York State, USA. Sci. Total Environ. 648, 442–449. https://doi.org/10.1016/j.scitotenv.2018.08.047.

Yamaji, M., Fujino, S., Horimoto, A., 2016. Photophysical and photochemical properties of an aromatic carbonyl compound for a moderate triplet energy sensitizer studied by steady state and laser flash photolysis. J. Photochem. Photobiol. A Chem. 317, 9–11. https://doi.org/10.1016/j.jphotochem.2015.10.016.

Yamazaki, E., Yamashita, N., Taniyasu, S., Lam, J., Lam, P.K.S., Moon, H.-B., Jeong, Y., Kannan, P., Achyuthan, H., Munuswamy, N., Kannan, K., 2015. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol. Environ. Saf. 122, 565–572. https://doi.org/10.1016/J.ECOENV.2015.09.029.

Yan, Z., Liu, Y., Yan, K., Wu, S., Han, Z., Guo, R., Chen, M., Yang, Q., Zhang, S., Chen, J., 2017. Bisphenol analogues in surface water and sediment from the shallow Chinese freshwater lakes: occurrence, distribution, source apportionment, and ecological and human health risk. Chemosphere 184, 318–328. https://doi.org/10.1016/j.chemosphere.2017.06.010.

Yang, Yunjia, Lu, L., Zhang, J., Yang, Yi, Wu, Y., Shao, B., 2014. Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A 1328, 26–34. https://doi.org/10.1016/j.chroma.2013.12.074.

Yu, X., Xue, J., Yao, H., Wu, Q., Venkatesan, A.K., Halden, R.U., Kannan, K., 2015. Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the U . S . EPA targeted national sewage sludge survey. J. Hazard Mater. 299, 733–739. https://doi.org/10.1016/j.jhazmat.2015.07.012.

Zhang, H., Zhang, Y., Li, J., Yang, M., 2018. Occurrence and Exposure Assessment of Bisphenol Analogues in Source Water and Drinking Water in China. https://doi.org/10.1016/j.scitotenv.2018.11.053.

Zhang, Yiqing, Xiao, Y., Zhang, Yicheng, Lim, T.-T., 2019. UV direct photolysis of halogenated disinfection byproducts: experimental study and QSAR modeling. Chemosphere 235, 719–725. https://doi.org/10.1016/j.chemosphere.2019.06.167.

Zhao, J., Li, Y., Zhang, C., Zeng, Q., Zhou, Q., 2008. Sorption and degradation of bisphenol A by aerobic activated sludge. J. Hazard Mater. 155, 305–311. https://doi.org/10.1016/j.jhazmat.2007.11.075.

Zühlke, M.K., Schlüter, R., Henning, A.K., Lipka, M., Mikolasch, A., Schumann, P., Giersberg, M., Kunze, G., Schauer, F., 2016. A novel mechanism of conjugate formation of bisphenol A and its analogues by Bacillus amyloliquefaciens: detoxification and reduction of estrogenicity of bisphenols. Int. Biodeterior. Biodegrad. 109, 165–173. https://doi.org/10.1016/j.ibiod.2016.01.019.

Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., Širok, B., Blažeka, Ž., Heath, E., 2013. Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment. Ultrason. Sonochem. 20, 1104–1112. https://doi.org/10.1016/j.ultsonch.2012.12.003.

3.1.2 The removal of bisphenols and other contaminants of emerging concern

The paper "The removal of bisphenols and other contaminants of emerging concern", authored by A. Kovačič, D. Škufca, M. Zupanc, J. Gostiša, B. Bizjan, N. Krištofelc, M. Sollner Dolenc, E. Heath was published in *Science of the Total Environment* in July 2020. With the assistance of N. Krištofelc, I performed the lab-scale experiment, including experimental work, method optimization, and instrumental and data analysis. In terms of pilot-scale experiments, I was responsible for experimental design (removal of bisphenols), sample preparation, instrumental analysis, and data evaluation. I also collated and combined the results and authored the manuscript.

The rapid growth in the variety and quantity of CEC in WW requires efficient and environmentally friendly methods for their removal [77]. This study investigated the removal efficiency of 46 CEC, including 12 bisphenols from WW using a lab and pilot-scale hydrodynamic cavitation (HC) generator alone and in combination with UV illumination (pilot-scale). The paper aimed to (1) answer whether bisphenols are susceptible to HC and how different HC conditions affect the removal of bisphenols from artificial WW; (2) appraise the effectiveness of pilot-scale HC, UV, and HC/UV treatment for removing CECs from WW, and (3) evaluate the potential of hydrodynamic cavitation for a full-scale application as a pre-treatment technology. During lab-scale cavitation, the highest removal efficiencies of bisphenols (15–63%) were obtained at a rotational frequency (ν_{cav}) = 9500 rpm and time (t_{cav}) = 10 min. Although an increase in ν_{cav} had little effect, temperature, and physico-chemical properties (e.g. K_{ow}) had a significant effect on the removal efficiency of the studied compounds. At the pilot-scale, 11 CECs (including BPA, 44BPF, and BPS) were quantifiable in the WW influent. Their highest removal efficiencies (15–90%) were obtained at a lower ν_{cav} = 2290 rpm with lower energy consumption, while neither an increase in ν_{cav}, t_{cav} nor the presence of UV light improved treatment efficiency.

The results of this work fulfil a part of the thesis aims, namely to develop, optimize and validate analytical GC-MS method for simultaneous determination of 12 bisphenol residues and assess the removal efficiency of bisphenols by UV photolysis and AOPs. The suitability of the method for the determination of other micro-pollutants was also confirmed. This paper is the first to examine the possibility of HC as a treatment technology for removing bisphenols from WW at the lab-scale and CECs from WW at the pilot-scale. Overall, the results of this paper show the potential of HC as an AOP representative, which can be used for the full-scale application as a (pre)treatment technology and pave the way for future improvements in the design of cavitation reactors.

Science of the Total Environment 743 (2020) 140724

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

The removal of bisphenols and other contaminants of emerging concern by hydrodynamic cavitation: From lab-scale to pilot-scale

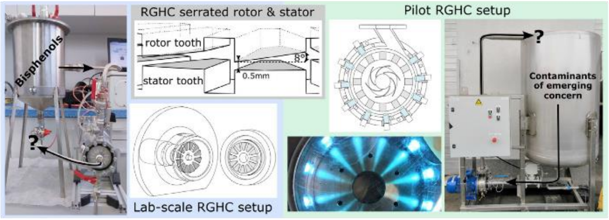
Ana Kovačič [a,b], David Škufca [a,b], Mojca Zupanc [c], Jurij Gostiša [c], Benjamin Bizjan [c], Nina Krištofelc [d], Marija Sollner Dolenc [d], Ester Heath [a,b,*]

[a] Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[b] International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia
[c] University of Ljubljana, Faculty of Mechanical Engineering, Aškerčeva 6, 1000 Ljubljana, Slovenia
[d] University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

HIGHLIGHTS

- Removal efficiencies for bisphenols observed in lab-scale HC set-ups were 15–63%.
- Temperature and K_{ow} effect HC removal efficiency.
- A lower v_{cav} resulted in higher removal of CECs in the pilot-scale HC system.
- Increasing t_{cav} or combined HC and UV did not improve removal efficiencies.
- HC shows potential for large-scale application as a pre-treatment technology.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 May 2020
Received in revised form 20 June 2020
Accepted 2 July 2020
Available online 4 July 2020

Editor: Paola Verlicchi

Keywords:
Contaminants of emerging concern
Bisphenol
Hydrodynamic cavitation
UV
Lab-scale
Pilot-scale

ABSTRACT

The rapid growth in the variety and quantity of contaminants of emerging concern (CEC) in wastewater indicates the necessity for developing efficient and environmentally friendly methods for their removal. This study investigates the removal efficiency of 46 CEC, including 12 bisphenols, from wastewater using a lab and pilot-scale hydrodynamic cavitation generator alone and in combination with UV illumination (pilot-scale). During lab-scale cavitation, the highest removal efficiencies of bisphenols (15–63%) for this specific design of cavitator were obtained at a rotational frequency (v_{cav}) = 9500 rpm and time (t_{cav}) = 10 min. Temperature and the physicochemical properties (e.g. K_{ow}) of the studied compounds also had a significant effect on removal efficiency. At the pilot-scale, 11 CECs were quantifiable in the wastewater influent, and the generator operated at v_{cav} = 2290 and 2700 rpm. The highest removal efficiencies (15–90%) were obtained at a lower v_{cav} = 2290 rpm while neither an increase in v_{cav}, t_{cav} or the presence of UV-C light increased the removal efficiency. A lower v_{cav} also reduced the hydrodynamic power of the cavitator from 477 W to 377 W, resulting in reduced energy consumption. Overall, the results show the potential of hydrodynamic cavitation for a large-scale application as a pre-treatment technology and pave the way for future improvements in the design of cavitation reactors.

1. Introduction

Personal care products, active pharmaceutical ingredients, industrial chemicals, and pesticides are an indispensable part of modern life (Česen et al., 2019). However, the presence of certain compounds in

---

[*] Corresponding author at: Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
E-mail address: ester.heath@ijs.si (E. Heath).

https://doi.org/10.1016/j.scitotenv.2020.140724
0048-9697/

surface and groundwaters is of concern to scientists because of their possible adverse effects on non-target organisms and human health (He et al., 2019), and scientists collectively refer to these compounds as contaminants of emerging concern (CEC) (Sauvé and Desrosiers, 2014). To date, treated wastewater (WW) remains the primary source of CEC in the environment resulting from the fact that conventional (biological) wastewater treatment plants (WWTPs) are not designed to remove all of them (Soriano-Molina et al., 2019).

Recently, several non-biological treatment technologies have been proposed to reduce organic pollutants in WW, such as advanced oxidation processes, which show significant potential for removing recalcitrant organic compounds (Zupanc et al., 2013). These processes are characterised by the in situ generation of reactive oxygen species, such as hydroxyl or perhydroxyl radicals, with a high oxidation potential that enables a rapid and non-selective attack on structurally diverse organic micropollutants (Dietrich et al., 2017; Zupanc et al., 2014), preferably resulting in their complete mineralisation (Zupanc et al., 2013). Cavitation is another promising advanced oxidation process that results in a significant enhancement in compound removal when combined with other techniques (Bagal and Gogate, 2014a; Dular et al., 2016) and recently showed the potential to be scaled up to WWTP level making it suitable technology for the degradation of organic pollutants (Gągol et al., 2018; Zupanc et al., 2019).

In hydrodynamic cavitation (HC), cavities are formed as a result of a sudden pressure drop, caused by local velocity fluctuations, induced by the geometry of the flow channel (Braeutigam et al., 2012). The collapse of these cavities causes extreme energies, e.g., localized areas of high temperature and pressure resulting in the homolytic cleavage of vaporous water molecules into reactive oxygen species (Zupanc et al., 2014), able to oxidise organic compounds (Zhang and Li, 2014). Ultraviolet (UV) photolysis is also commonly used to disinfect water (Kovačič et al., 2019b). Photolysis of a compound can proceed either by direct photolysis, where a compound is transformed by absorbing photons directly, or by indirect photolysis, where degradation occurs via the reaction of a compound with a reactive species (Ahmed et al., 2017). Currently, the use of HC, either alone, or when combined with other oxidation processes, mainly addresses the removal of individual compounds performed in the laboratory and do not take into account mixtures of compounds, complex matrices like WW, and removal at the pilot and full-scale (Bagal and Gogate, 2014b; Braeutigam et al., 2012; Çalışkan et al., 2017; Joshi and Gogate, 2012, 2019; Wang and Zhang, 2009). In particular, studies investigating the removal efficiency (RE) of CECs mixture, especially from WW performed on a pilot-scale at a real WWTP are missing, despite the fact that HC is a promising solution for WW treatment (Joshi and Gogate, 2019). Hydrodynamic cavitation has also been shown to have the potential not only to remove organic contaminants but also for bacteria eradication and virus inactivation (Albanese et al., 2015; Kosel et al., 2017; Zupanc et al., 2019).

In this study we aim to 1) determine whether bisphenols are susceptible to HC and 2) investigate the effect of different HC conditions on the removal of bisphenols from artificial WW; 3) appraise the effectiveness of HC, UV and HC/UV at a pilot-scale for removing CECs from real WW, and 4) evaluate the potential of this technology as a pre-treatment to conventional biological treatment.

2. Materials and methods

2.1. Reagents, materials and standards

A list of all reagents and details about the preparation of stock, working and calibration standards and internal standards are given in detail in the Supplementary Information (SI-I). Details about the studied compounds are given in Table SI-1.

2.2. Sample preparation and analysis

The methods used for the determination of 46 CECs, including 12 bisphenols were adapted from Kovačič et al. (2019a) and Česen et al. (2019, 2018), respectively. All glassware was cleaned and pyrolysed at 400 °C for 4 h. Samples of artificial WW were prepared, as shown in Table SI-2. Briefly, extraction was performed using Oasis HLB Prime cartridges (3 cm^3, 60 mg, Waters, Massachusetts, USA). After loading and washing the sorbents were dried under vacuum (-1.33 kPa) for 45 min. The analytes were then eluted with the optimal solvent, dried (N_2), derivatised (Table SI-2), and analysed using gas chromatography with mass selective detection (GC–MS, Agilent 7890B/5977A, United States). Separation was achieved using an Agilent Technologies DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) with helium (Purity 6.0) as the carrier gas at a flow rate of 1 $mL\ min^{-1}$. Compounds were identified and quantified using selected ion monitoring (SIM) (Table SI-3 and SI-4). Data acquisition and processing were performed using MassHunter software (Agilent Technologies).

2.3. Method validation

Matrix-matched validation for WW samples was performed with artificial WW effluent from lab-scale (control) bioreactors (Kovačič et al., 2019a). Validation parameters included: linearity, sensitivity, accuracy as analyte recovery, precision and extraction recovery determined. Full details are given in Kovačič et al. (2019a) and Česen et al. (2018), with the following modifications, namely sensitivity was evaluated in terms of limit of detection and the limit of quantification (LOQ) calculated by multiplying the standard deviation of the background (six distinct matrix blanks) by a factor of three and ten, respectively (Magnusson and Örnemark, 2014). The LOQ also had to fulfil the criteria that the analyte signal was at least five times the analyte signal in the blank. The calibration range consisted of five to ten calibration points (1–23,000 $ng\ L^{-1}$) using the LOQ as the lowest calibration level, and the highest calibration level selected according to expected concentrations in WW (Česen et al., 2019, 2018). All validation parameters were measured over two calibration ranges depending on the applied method: low (5, 17 or 50 $ng\ L^{-1}$) and high (130, 600 or 667 $ng\ L^{-1}$) level, as described in SI-III. To account for contamination, controls and procedural blanks were prepared and systematically included in each batch of analyses. Quality control samples (lower calibration range) were included after every 20th sample to monitor the performance of the instrument, while solvent blanks were analysed to evaluate potential carry-over.

2.4. Experimental design

Lab-scale HC experiments were performed using cavitation generators based on a rotating generator of hydrodynamic cavitation (RGHC) which was on a pilot-scale fitted with an additional UV-C source. Flow rate (Q) was measured with an electromagnetic flowmeter (ABB WaterMaster DN40), pressure rise across the RGHC with a differential pressure transducer (ABB 2600T series) and the static pressure at the pressure side using an absolute pressure transducer (ABB 2600T series). Pressure fluctuations resulting from cavitation were measured using a hydrophone (Teledyne Reason, TC4013). Cavitation was visualized through a transparent window using a high-speed camera (Fastec HiSpec 4 mono) and LED fibre as a light source for illumination. In order to determine the most effective HC regime, two rotational frequencies, the maximum and correspondingly lower rotational frequency allowed by the lab- or pilot-scale design (higher ν_{cav} = higher power and Q) and different times of cavitation (t_{cav}) were investigated. When adjusting the parameters, the authors focused on Q in order to

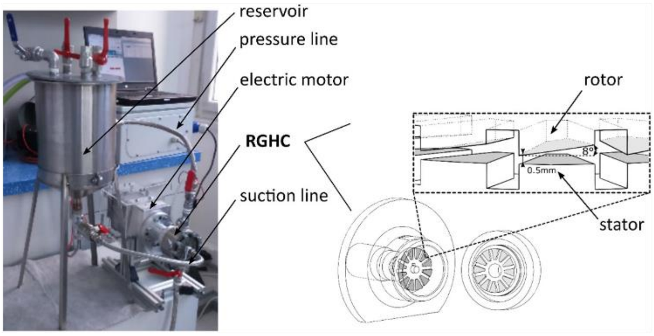


Fig. 1. Lab-scale HC set-up (left) with RGHC (right) designed by Stepišnik-Perdih et al. (Perdih et al., 2017). A close up of the rotor is shown in the insert.

compare different treatments for the same number of cavitation passes (N_p) calculated using Eq. (1), where V_s is the sample volume

$$N_p = \frac{t_{cav} \times \theta}{V_s} \tag{1}$$

2.4.1. Lab-scale HC set-up

The lab-scale HC set-up (Fig. 1) consisted of a reservoir, the RGHC, a temperature sensor installed directly into the reservoir and a cooling coil. The HC generator was a centrifugal pump fitted with a specially designed rotor and stator with 12 distinct teeth which produce the cavitation effect. The rotor (Ø 50 mm) was driven by an electrical motor and acted as both a pump and HC generator.

Experiments were performed on 2 L samples of artificial WW effluent (see Section 2.3) with the addition of 12 bisphenols (BPA analogues: 200 ng L^{-1}, bisphenol A (BPA): 1000 ng L^{-1}) under both controlled and uncontrolled temperature conditions. The temperature (T = 30, 40, 50 and 60 °C) was maintained using a cooling coil for t_{cav} = 15 min with ν_{cav} = 9500 rpm. The effect of operating t_{cav} (5, 10, 15, 30 and 60 min) and ν_{cav} (6000 and 9500 rpm) on HC was evaluated under uncontrolled temperature conditions but did not exceed a T_{max} = 68 °C. Each experiment was repeated in triplicate within one day and during two consecutive days. Samples (250 mL, n = 3) were prepared and analysed according to Section 2.2.

2.4.2. Pilot-scale HC/UV set-up

Pilot-scale experiments were performed using a larger version of the lab-scale RGHC, but with additional UV illumination (Fig. 2). Both cavitation generators were run under comparable hydrodynamic conditions (i.e. Reynolds number). UV illumination was provided by a series of ten × 3 W UV-C (λ = 254 nm) germicidal sterilisation bulbs[1] fitted into the casing surrounding the cavitation chamber. The pilot rig also included a 1000 L reservoir and a closed-circuit pipeline equipped with throttling valves used to set the desired cavitation conditions (Fig. 2).

The pilot-scale HC/UV rig was set-up at a local WWTP with a population equivalent of 5000. Five experiments (Exp. A–E) were performed using 800 L of WW influent. The efficiency of UV (Exp. A) and HC for two different ν_{cav} (2290 and 2700 rpm) alone (Exp. B and D) and in combination with UV (HC/UV; Exp. D and E) were examined by studying the RE of 46 CECs including 12 bisphenols. The same Q (4.4 L s^{-1}) and N_p after 30 min (10) and 90 min (30) in all experiments (UV, HC and HC/UV) were maintained by adjusting the pressures on the suction and pressure side of the RGHC. Treatment parameters and sample descriptions are given in Table SI-9. The samples comprised of primary treated WW. After each experiment, a new sample (800 L) of WW was introduced into the reservoir. The characteristics of the WW and the performance of the pilot-plant were monitored using standard WW treatment parameters: pH, dissolved oxygen, temperature, total chemical oxygen demand (COD_t), dissolved biological oxygen demand (BOD_5), total nitrogen and phosphorus (SI-IV). All experiments were conducted over 48 h. Samples (V = 500 mL, n = 3) were collected at time = 0, 30 and 90 min and stored in dark at −20 °C ready for analysis (Section 2.2).

3. Results and discussion

3.1. HC characteristics

Two measures best represent the characteristics of HC: differential pressure gain (Δp) and the relative optical intensity of cavitation, defined as brightness (grey level) of flow structures recorded by high-speed imaging. Fig. 3 (left) shows Δp vs Q for the RGHC. The characteristic curves for pressure side throttling (i.e. manipulating Q by adjusting the valve while keeping the pump speed constant) show the performance of the pump in the absence of cavitation since there is no cavitation when the valve is fully open. Alternatively, manipulating the Q by throttling the suction side valve produces intense cavitation, which is shown by a marked reduction in Δp for a given Q. Fig. 3 (right) shows how the optically measured intensity of cavitation (proportional to the vapour fraction in the Q visualization area) increases as the Q is reduced by throttling on the suction side. The nominal (open valve) value of Q at 2700 rpm (8 L s^{-1}) was significantly higher than at 2290 rpm (6.4 L s^{-1}), which meant reducing the suction valve throttling to 55% of the nominal Q as opposed to 70% for measurements at 2290 rpm. Consequently, a more substantial valve-induced pressure drop at the pump inlet resulted in more intense cavitation when v_{cav} = 2700 rpm (Exp. D and E).

3.2. Method validation

Calibration curves for all bisphenols (R^2 = 0.98) and CECs (R^2 > 0.97 and >0.90 for bisphenol C, 2-ethylhexyl 4-methoxycinnamate and 4,4′-dihydroxybiphenyl) in WW show adequate linearity when using a weighted (1/x or 1/y) linear curve (Table SI-7 and SI-8). The LOQs were between 1 and 66.7 ng L^{-1}. Method accuracy (85–115%) was lower over the lower calibration range, and only BPA, bisphenol C, bisphenol PH and 4,4′-dihydroxydiphenyl ether, had the method and instrumental repeatability >15% (lower calibration range). The relative standard deviations (RSD, %) of repeat injections of the quality control samples were <15% for the majority of compounds. Also, the average concentrations of

---

[1] https://www.aliexpress.com/item/32665910312.html.

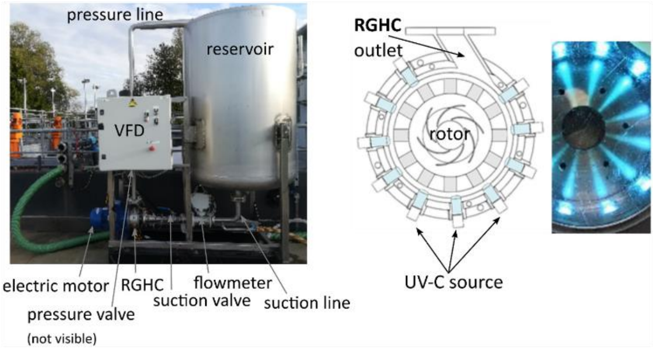


Fig. 2. Pilot-scale HC (left) with pilot-scale RGHC with UV illumination (right) designed by Laboratory for Hydraulic Machines, Department of Power Engineering (Faculty of Mechanical Engineering, Ljubljana, Slovenia).

analytes in the procedural blanks were <LOQ for each analyte. Full details about the validation parameters are given SI-III.

3.3. Removal efficiency of bisphenols: lab-scale HC set-up

Twelve lab-scale experiments were performed. The relative standard deviation between average REs obtained by repeated experiments ($n = 6$) was for the majority of bisphenols <20%. In line with published studies (Braeutigam et al., 2012; Choi et al., 2018; Joshi and Gogate, 2012; Wang and Zhang, 2009), the temperature had a significant effect on HC efficiency. For instance, in temperature-controlled experiments (Exp. 9–12; 9500 rpm, 15 min) REs increased when the temperature was increased from 30 to 50 °C, but then significantly decreased when it increased from 50 to 60 °C (Table SI-12). The same optimal $T = 50$ °C for the elimination of BPA was reported by Choi et al. (2018). As expected, when a constant temperature was not maintained, an increase in t_{cav} did not result in a higher HC efficiency (Table 1). In these experiments, the temperature increased to 40 °C at a $\nu_{cav} = 9500$ rpm within 15 min and to 30 °C at $\nu_{cav} = 6000$ rpm after 60 min. Higher REs were obtained after 10 min compared to 5 min (lower ΔT) or 15 min (higher ΔT) at a $\nu_{cav} = 9500$ rpm, and after 5, 10 or 15 min (lower ΔT) compared to 30 and 60 min (higher ΔT) at a $\nu_{cav} = 6000$ rpm. A comparison between experiments with a similar N_p (Exp. 1 and 5, Exp. 2 and 6 and Exp. 3 and 7; Table 1) but with different ν_{cav} showed that the RE for the majority of bisphenols was higher at higher ν_{cav}. Also, similar REs were obtained in a shorter time when $\nu_{cav} = 9500$ rpm, although the energy consumption is much lower at lower ν_{cav}. Generally, lab-scale HC experiments produced poor REs, i.e., from 0 to 43% and from 0 to 63% under uncontrolled temperature conditions. To better compare RE, the results were also expressed as cumulative removal efficiency (ΣRE). The highest REs (ΣRE = 533%) for all bisphenols, which ranged from 15 to 63% were obtained when $\nu_{cav} = 9500$ rpm and $t_{cav} = 10$ min and the temperature was not controlled (Exp. 2). Experiments 1, 4–6 produced slightly lower REs (ΣRE = 264–286%) compared to Exp. 2. Different REs for different bisphenols under the same conditions indicate that removal by HC depends on their physicochemical properties. Based on all tested conditions, the highest overall REs (ΣRE = 303%) were obtained for bisphenol AF, followed by bisphenol C2, Z, and AP (ΣRE = 229–272%), followed by 4,4′-bisphenol F (44BPF), bisphenol B, C, and E (ΣRE = 134–180%). The lowest REs were obtained for 2,2′- and 2,4′-bisphenol F, bisphenol S (BPS), and BPA (ΣRE = 88–109%). The results for both controlled and uncontrolled temperature conditions suggest that a correlation exists between REs of bisphenols and their $\log K_{ow}$ since higher REs were obtained for bisphenols with $\log K_{ow} > 3.5$ and conversely lower REs in the case of $\log K_{ow} < 3.5$. One reason for this is the higher tendency of compounds with a higher $\log K_{ow}$ to reach the radical rich sites, i.e., the gas-liquid interface (Zupanc et al., 2014). Alternatively, bisphenols with lower $\log K_{ow}$ values remain in the bulk water phase, where there is a lower concentration of radicals.

3.4. The occurrence and removal efficiency of 46 CECs: pilot-scale HC/UV set-up

Table SI-13 gives the average concentrations of CECs in WW influents before (X_0) and after 30 or 90 min (X_1 and X_2, respectively) of treatment (UV, HC or HC/UV). Variations in levels of the analytes are likely a result of sampling, namely different sampling days, sample heterogeneity, changing WW characteristics (Table SI-10), analyte

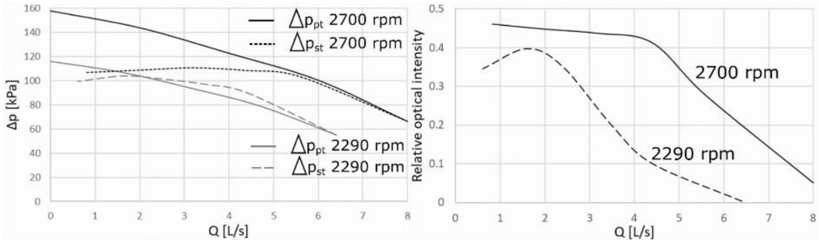


Fig. 3. Hydrodynamic characteristics of the cavitation pump for Q throttling on the pressure (Δp_{pt}) and suction side (Δp_{st}) (left) and optically measured relative intensity of cavitation (right).

Table 1
The average ($n = 6$) RE and ΣRE [%] of bisphenols[a] with lab-scale experiments under uncontrolled temperature at different HC parameters: ν_{cav}, power, Q and t_{cav}.

HC parameters	V_s [L]	2								ΣRE [%]	$\log K_{ow}$[b]
	ν_{cav} [rpm]	9500			6000						
	Power [W]	990			340						
	Q [L min^{-1}]	7.7			4.8						
	N_p	19	39	58	12	24	36	72	144		
	Experiment	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5	Exp.6	Exp.7	Exp.8		
	t_{cav} [min]	5	10	15	5	10	15	30	60		
	ΔT [°C]	18	23	40	6	10	14	20	30		
RE [%]	BPS	8	15	4	23	20	5	6	8	88	1.65
	22BPF	21	24	6	14	16	13	9	6	109	3.06
	24BPF	17	38	2	15	17	7	6	–	104	3.06
	44BPF	21	63	6	37	32	14	7	–	180	3.06
	BPE	13	49	8	16	16	13	11	11	137	3.19
	BPA	14	38	2	11	14	13	2	–	94	3.43
	BPC2	35	48	14	41	33	39	31	32	272	3.75
	BPB	18	54	9	18	18	17	18	17	169	4.13
	BPAF	49	45	14	48	41	49	33	24	303	4.47
	BPC	26	57	12	–	5	36	19	42	197	4.74
	BPAP	32	43	11	36	29	35	25	22	233	4.86
	BPZ	31	58	11	28	24	33	22	22	229	5
ΣRE [%]	12 bisphenols	286	533	98	288	264	275	189	183		

[a] Abbreviations of bisphenols: 2,2′-bisphenol F (22BPF), 2,4-bisphenol F (24BPF), 4,4′-bisphenol F (44BPF), bisphenol AF (BPAF), bisphenol E (BPE), bisphenol A (BPA), bisphenol C (BPC), bisphenol B (BPB), bisphenol C2 (BPC2), bisphenol Z (BPZ) bisphenol AP (BPAP) and bisphenol S (BPS).

[b] Estimated $\log K_{ow}$ by the EPI Suite™ method.

concentration, treatment efficiency and method uncertainty (RSD). Except for naproxen (RSD < 49%), all other compounds had RSDs <20% ($n = 3$). Results for naproxen with an RSD >30% were excluded from further RE calculations. Of the 46 CECs, only 12: caffeine, 2-hydroxy-4-methoxybenzophenone, dihydroxy-4-methoxybenzophenone, BPS, 44BPF, BPA, methylparaben, ethylparaben, propylparaben, ibuprofen, naproxen and ketoprofen, were above the LOQ (8–14,000 ng L^{-1}). Compared to previous studies (Česen et al., 2019, 2018) on the occurrence of CECs in Slovenian WWs, the absence of industries connected to the municipal WWTP and additional dilution of WW due to significant rainfall can explain the low levels and the number of compounds detected. Caffeine was the most abundant compound (8,200–14,000 ng L^{-1}) followed by naproxen (<10,000 ng L^{-1}), whereas 44BPF had the lowest concentration (12–26 ng L^{-1}). Also, caffeine and naproxen both required a higher calibration range (167–23,000 ng L^{-1}); however, due to the nonlinearity of the caffeine calibration curve, only its approximate concentrations are reported, and the RE could not be calculated. However, similar levels of caffeine in the influent before and after HC and HC/UV treatment implies a poor RE. The RE was calculated using Eq. (2) (Zupanc et al., 2014)

$$RE\ [\%] = \left(1 - \frac{c_{X\_1,2}}{c_{X\_0}}\right) \times 100, \tag{2}$$

where C_{X_0} is the concentration of the analyte at time zero and C_{X_1} and C_{X_2} is the concentration after 30 or 90 min of treatment.

The results (Table 2) include only REs obtained after 30 min, as there was no positive effect from increasing $t_{cav} = 90$ min. In general, all treatments resulted in low REs and dihydroxy-4-methoxybenzophenone was not removed by any of the treatments. A combination of HC and UV degradation did not increase treatment efficiency while other studies report a synergistic enhancement in the degradation of the parent compounds when combining HC with different advanced oxidation processes (Bagal and Gogate, 2014b; Gągol et al., 2018; Joshi and Gogate, 2019). The poor removal of BPS (14%) when exposing WW only to UV irradiation, was opposite to its complete removal observed in the previous UV based lab-scale experiments (Kovačič et al., 2019a, 2019b). A possible explanation is that the UV light does not penetrate sufficiently through the WW in the cavitation chamber, but becomes dissipated by the cavitation cloud. Variations in temperature, composition and dilution of WW influent (uncontrolled parameters) during the experiments also limited the comparability between laboratory and pilot-scale experiments. However, in line with lab-scale experiments, bisphenols were eliminated: REs = 8–25% for HC, 10–43% for UV and REs = 10–29% for HC/UV. Also, similar to lab-scale observations, increasing ν_{cav} (HC or HC/UV) does not significantly improve RE. Moreover, the REs (B_1 and C_1) obtained at lower ν_{cav} were generally higher (<90%) than REs obtained at higher ν_{cav} (<34%). The results also suggest that more aggressive cavitation in terms of pressure fluctuations does not necessarily favour the formation of reactive oxygen species. A $\nu_{cav} = 2290$ rpm (Sample B_1) gave the highest REs (15–90%) for all of the compounds tested, namely

Table 2
REs [%] of the determined CECs[a] in WW influents by UV, HC or HC/UV process after 30 min with lower and higher ν_{cav}.

Exp.	Treatment conditions			N_p	Power [kW]	Sample name	RE [%]										
							HM-BP	DH-BP	44BPF	BPA	BPS	MePB	EtPB	IB	PrPB	NP	KP
A	–	UV	30 min	10	0.03	A_1	–	–	25	43	14	79	73	47	49	22	–
B	2290 rpm	HC			4.60	B_1	25	–	25	20	15	78	29	52	90	[b]	15
C		UV/HC			4.63	C_1	–	–	–	10	16	15	–	–	–	50	16
D	2700 rpm	HC			7.50	D_1	–	–	15	8	–	17	21	–	9	34	–
E		UV/HC			7.53	E_1	–	–	11	13	29	20	51	13	13	[b]	24

[a] Abbreviations of CECs: 2-hydroxy-4-methoxybenzophenone (HM-BP), dihydroxy-4-methoxybenzophenone (DH-BP), 2,4′-bisphenol F (44BPF), bisphenol A (BPA), bisphenol S (BPS), methyl paraben (MePB), ethyl paraben (EtPB), propyl paraben (PrPB), ibuprofen (IB), naproxen (NP) and ketoprofen (KP).

[b] RSD ($n = 3$) > 30%.

2-hydroxy-4-methoxybenzophenone, 44BPF, BPA, BPS, methylparaben, ethylparaben, propylparaben, ibuprofen, naproxen and ketoprofen, followed by UV alone (Sample A_1: 14–79%). Similar REs (15–52%) of ibuprofen, naproxen and ketoprofen obtained by HC under lab-scale experiments were also reported in a previous study by Zupanc et al. (2014). These findings point to the possibility that HC is suitable for real applications and have the potential to improve the total treatment efficiency when used before, e.g., biological treatment. Also, the occurrence of CECs at low concentration and lower values of WW treatment parameters compared to an average WWTP indicate the relevance of performing improved pilot-scale experiments during longer periods of less rain. In the case of WW treatment parameters (Tables SI-10, SI-11), there was a negligible reduction in COD_t and BOD_5 when being lower before treatment, whereas a 13–29% reduction was observed at their higher values (>300 and 100 mg L^{-1}, respectively) with increased t_{cav}. In addition, a 12% reduction in total nitrogen was observed, while there was no effect on total phosphorus. The pH, dissolved oxygen and temperature increased to 8.29, 8 mg L^{-1} and 31 °C, respectively, with increasing time. To fully understand which fraction of the organic constituents (soluble, particulate or volatile part) was removed with HC and HC/UV, a more detailed analysis is needed.

4. Conclusions

A significant effect of temperature on RE using HC was observed, although an increase in ν_{cav} had little effect on REs of studied CECs. The highest REs for the studied bisphenols (15–63%) were obtained at 9500 rpm for 10 min. Under pilot-scale HC conditions with simultaneous UV treatment (performed for the first time on a WWTP), the highest REs (15–90%) at $\nu_{cav} = 2290$ rpm for all 11 quantifiable CECs (including BPA, 44BPF and BPS) were achieved with lower energy consumption. The increase of t_{cav} and the combination of HC and UV did not improve treatment efficiency; however, a longer t_{cav} reduced COD_t, BOD_5 and total nitrogen, which justifies further investigations. The results of this study confirm the suitability of HC for large scale applications as a pretreatment technique. In future applications, achieving more intensive cavitation by redesigning the rotor/stator, and moving the UV source either before or after the sample reaches the treatment chamber has the potential to improve the efficiency of the HC and HC/UV generator.

CRediT authorship contribution statement

Ana Kovačič: Investigation, Formal analysis, Data curation, Visualization, Writing - original draft. **David Škufca:** Formal analysis, Data curation. **Mojca Zupanc:** Investigation, Methodology, Conceptualization, Writing - original draft. **Jurij Gostiša:** Investigation, Data curation, Writing - original draft. **Benjamin Bizjan:** Investigation, Data curation, Writing - original draft. **Nina Krištofelc:** Investigation, Formal analysis. **Marija Sollner Dolenc:** Supervision. **Ester Heath:** Supervision, Conceptualization, Writing - review & editing, Project administration, Resources, Funding acquisition.

Declaration of competing interest

None.

Acknowledgements

Ana Kovačič acknowledges the Young researcher grant (Slovenian Research Agency). The study was performed with the financial assistance of the Slovenian Research Agency, namely Program Group P1-0143 projects L1-7544, L1-9191, N1-0047, J1-8147, J2-8162 and Slovenia's Smart Specialisation Strategy Cel. Cycle: "Potential of biomass for development of advanced materials and bi for funding the Research, development and innovation project (RDI)o-based products" (contract number: OP20.00365), co-financed by the Ministry of Education, Science and Sport of the Republic of Slovenia and the European Union as part of the European Regional Development Fund 2016–2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140724.

References

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J. Hazard. Mater. 323, 274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045.

Albanese, L., Ciriminna, R., Meneguzzo, F., Pagliaro, M., 2015. Energy efficient inactivation of saccharomyces cerevisiae via controlled hydrodynamic cavitation. Energy Sci. Eng. 3, 221–238. https://doi.org/10.1002/ese3.62.

Bagal, M.V., Gogate, P.R., 2014a. Wastewater treatment using hybrid treatment schemes based on cavitation and Fenton chemistry: a review. Ultrason. Sonochem. https://doi.org/10.1016/j.ultsonch.2013.07.009.

Bagal, M.V., Gogate, P.R., 2014b. Degradation of diclofenac sodium using combined processes based on hydrodynamic cavitation and heterogeneous photocatalysis. Ultrason. Sonochem. 21, 1035–1043. https://doi.org/10.1016/j.ultsonch.2013.10.020.

Braeutigam, P., Franke, M., Schneider, R.J., Lehmann, A., Stolle, A., Ondruschka, B., 2012. Degradation of carbamazepine in environmentally relevant concentrations in water by Hydrodynamic-Acoustic-Cavitation (HAC). Water Res. 46, 2469–2477. https://doi.org/10.1016/j.watres.2012.02.013.

Çalışkan, Y., Yatmaz, H.C., Bektaş, N., 2017. Photocatalytic oxidation of high concentrated dye solutions enhanced by hydrodynamic cavitation in a pilot reactor. Process. Saf. Environ. Prot. 111, 428–438. https://doi.org/10.1016/j.psep.2017.08.003.

Česen, M., Heath, D., Krivec, M., Ko, J., Kosjek, T., Heath, E., 2018. Seasonal and Spatial Variations in the Occurrence, Mass Loadings and Removal of Compounds of Emerging Concern in the Slovene Aqueous Environment and Environmental Risk Assessment. , p. 242 https://doi.org/10.1016/j.envpol.2018.06.052.

Česen, M., Ahel, M., Terzić, S., Heath, D.J., Heath, E., 2019. The occurrence of contaminants of emerging concern in Slovenian and Croatian wastewaters and receiving Sava river. Sci. Total Environ. 650, 2446–2453. https://doi.org/10.1016/j.scitotenv.2018.09.238.

Choi, J., Cui, M., Lee, Y., Kim, J., Son, Y., Khim, J., 2018. Hydrodynamic cavitation and activated persulfate oxidation for degradation of bisphenol A: kinetics and mechanism. Chem. Eng. J. 338, 323–332. https://doi.org/10.1016/j.cej.2018.01.018.

Dietrich, M., Franke, M., Stelter, M., Braeutigam, P., 2017. Degradation of endocrine disruptor bisphenol A by ultrasound-assisted electrochemical oxidation in water. Ultrason. Sonochem. 39, 741–749. https://doi.org/10.1016/j.ultsonch.2017.05.038.

Dular, M., Griessler-Bulc, T., Gutierrez-Aguirre, I., Heath, E., Kosjek, T., Krivograd Klemenčič, A., Oder, M., Petkovšek, M., Rački, N., Ravnikar, M., Šarc, A., Širok, B., Zupanc, M., Žitnik, M., Kompare, B., 2016. Use of hydrodynamic cavitation in (waste)water treatment. Ultrason. Sonochem. 29, 577–588. https://doi.org/10.1016/j.ultsonch.2015.10.010.

Gągol, M., Przyjazny, A., Boczkaj, G., 2018. Wastewater treatment by means of advanced oxidation processes based on cavitation – a review. Chem. Eng. J. 338, 599–627. https://doi.org/10.1016/j.cej.2018.01.049.

He, K., Hain, E., Timm, A., Tarnowski, M., Blaney, L., 2019. Occurrence of antibiotics, estrogenic hormones, and UV-filters in water, sediment, and oyster tissue from the Chesapeake Bay. Sci. Total Environ. 650, 3101–3109. https://doi.org/10.1016/j.scitotenv.2018.10.021.

Joshi, R.K., Gogate, P.R., 2012. Degradation of dichlorvos using hydrodynamic cavitation based treatment strategies. Ultrason. Sonochem. 19, 532–539. https://doi.org/10.1016/j.ultsonch.2011.11.005.

Joshi, S.M., Gogate, P.R., 2019. Intensification of industrial wastewater treatment using hydrodynamic cavitation combined with advanced oxidation at operating capacity of 70 L. Ultrason. Sonochem. 52, 375–381. https://doi.org/10.1016/j.ultsonch.2018.12.016.

Kosel, J., Gutiérrez-Aguirre, I., Rački, N., Dreo, T., Ravnikar, M., Dular, M., 2017. Efficient inactivation of MS-2 virus in water by hydrodynamic cavitation. Water Res. 124, 465–471. https://doi.org/10.1016/j.watres.2017.07.077.

Kovačič, A., Česen, M., Laimou-Geraniou, M., Lambropoulou, D., Kosjek, T., Heath, D., Heath, E., 2019a. Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions. Environ. Res. 179, 108738. https://doi.org/10.1016/J.ENVRES.2019.108738.

Kovačič, A., Gys, C., Kosjek, T., Covaci, A., Heath, E., 2019b. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics. Sci. Total Environ. 664, 595–604. https://doi.org/10.1016/J.SCITOTENV.2019.02.064.

Magnusson & Örnemark, 2014. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 2nd ed. .

Perdih, T.S., Širok, B., Dular, M., 2017. Influence of hydrodynamic cavitation on intensification of laundry aqueous detergent solution preparation. J. Mech. Eng. 63, 83–91. https://doi.org/10.5545/sv-jme.2016.3970.

Sauvé, S., Desrosiers, M., 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8, 1–7. https://doi.org/10.1186/1752-153X-8-15.

Soriano-Molina, P., García Sánchez, J.L., Malato, S., Plaza-Bolaños, P., Agüera, A., Sánchez Pérez, J.A., 2019. On the design and operation of solar photo-Fenton open reactors for the removal of contaminants of emerging concern from WWTP effluents at neutral pH. Appl. Catal. B Environ. 256, 117801. https://doi.org/10.1016/j.apcatb.2019.117801.

Wang, X., Zhang, Y., 2009. Degradation of alachlor in aqueous solution by using hydrodynamic cavitation. J. Hazard. Mater. 161, 202–207. https://doi.org/10.1016/j.jhazmat.2008.03.073.

Zhang, A., Li, Y., 2014. Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H2O2, and UV/H2O2oxidation processes: effects of reaction conditions and sludge matrix. Sci. Total Environ. 493, 307–323. https://doi.org/10.1016/j.scitotenv.2014.05.149.

Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., Širok, B., Blažeka, Ž., Heath, E., 2013. Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment. Ultrason. Sonochem. 20, 1104–1112. https://doi.org/10.1016/j.ultsonch.2012.12.003.

Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., Širok, B., Stražar, M., Heath, E., 2014. Shear-induced hydrodynamic cavitation as a tool for pharmaceutical micropollutants removal from urban wastewater. Ultrason. Sonochem. 21, 1213–1221. https://doi.org/10.1016/j.ultsonch.2013.10.025.

Zupanc, M., Pandur, Ž., Stepišnik Perdih, T., Stopar, D., Petkovšek, M., Dular, M., 2019. Effects of cavitation on different microorganisms: the current understanding of the mechanisms taking place behind the phenomenon. A review and proposals for further research. Ultrason. Sonochem. 57, 147–165. https://doi.org/10.1016/j.ultsonch.2019.05.009.

3.1.3 The occurrence and source identification of bisphenol compounds in wastewaters

The paper "*The occurrence and source identification of bisphenol compounds in wastewaters*" by M. Česen, K. Lenarčič, V. Mislej, M. Levstek, A. Kovačič, B. Cimrmančič, N. Uranjek Ževart, T. Kosjek, D. J. Heath, M. Sollner Dolenc, and E. Heath was published in *Science of the Total Environment* in March 2018. Dr Česen and K. Lenarčič performed the main work under the supervision of Prof Dr Heath. In this work, I contributed to method validation and manuscript preparation.

The concern over the adverse effects of BPA analogues is increasing, while their presence in surface waters and WWs remains poorly investigated [52], [215], [216]. This study aimed (1) to assess the occurrence and removal of BPAF, BPAP, BPF, BPE, BPB, BPC, BPS, and BPZ in Slovene WWTPs employing different treatment technologies and (2) to identify their potential sources. Wastewater influent and effluent samples from five WWTPs and WW inflows from industrial, commercial, and residential sources entering the sewerage systems of two different catchments were collected. All chemical analyses were based on optimized and validated GC-MS method with LOQ in the ng L^{-1} range. The investigated bisphenols were measured in WW within the EU region for the first time. Only BPF (36.7 ng L^{-1}) and BPS (40.6 ng L^{-1}) were detected exclusively in WW effluents, whereas other bisphenols were present in both influents ($<$ LOD–403 ng L^{-1}) and effluents ($<$ LOD–85.7 ng L^{-1}). Bisphenol Z was found in the highest concentration (up to 403 ng L^{-1}), and levels of BPC $>$ LOD were reported for the first time (1.01–11.8 ng L^{-1}). Although the removal of bisphenols could not be directly compared between WWTPs, except for BPAP and BPB (6.39%–43.2%), bisphenols were removed by $\geq$ 96.2%. The results also show that food processing and textile cleaning company discharges are a significant source (concentrations up to 3030 ng L^{-1} with high detection frequency) of the targeted bisphenols.

This paper fulfils several aims of the thesis, namely (1) development, optimization, and validation of a GC-MS method for the simultaneous determination of bisphenol residues, (2) evaluation of the bisphenols occurrence in Slovenian aqueous samples and (3) assessment of the removal efficiency of bisphenols by biological treatment. Meeting the above objectives contributes knowledge on the occurrence and cycling of bisphenols during WWT and in the environment.

Science of the Total Environment 616–617 (2018) 744–752

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The occurrence and source identification of bisphenol compounds in wastewaters

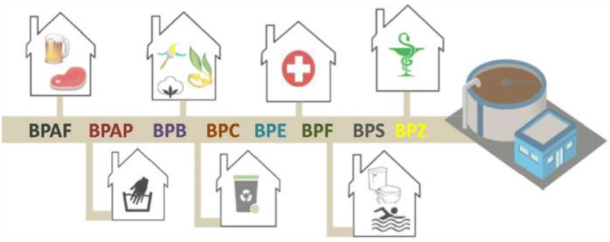
Marjeta Česen [a,b], Kaja Lenarčič [c], Vesna Mislej [d], Meta Levstek [e], Ana Kovačič [a,b], Bernardka Cimrmančič [f], Nataša Uranjek [g], Tina Kosjek [a,b], David Heath [b], Marija Sollner Dolenc [c], Ester Heath [a,b,*]

[a] *Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia*
[b] *Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia*
[d] *JP VODOVOD-KANALIZACIJA d.o.o., Central Wastewater treatment plant Ljubljana, Vodovodna c. 90, 1000 Ljubljana, Slovenia*
[e] *Wastewater Treatment Plant Domžale-Kamnik, Študljanska cesta 91, 1230 Domžale, Slovenia*
[f] *Wastewater Treatment Plant Novo mesto, Šmarješka cesta 10, Novo Mesto, Slovenia*
[g] *Wastewater Treatment Plant Šaleška dolina, Primorska cesta 8, Šoštanj, Slovenia*

HIGHLIGHTS

- BPs were measured in different WWs including inflows, influents and effluents.
- Most BPs were >LOD in analysed WW.
- BP removal efficiencies were in general >96%.
- BPZ was most commonly detected and BPC was quantified in WW for the first time.
- Food processing and textile cleaning facilities represent major sources of BPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 23 October 2017
Accepted 24 October 2017
Available online 31 October 2017

Editor: D. Barcelo

Keywords:
Bisphenols
Wastewater
Wastewater treatment plant
Source identification
Removal efficiency

ABSTRACT

This study reports the occurrence of eight bisphenols (BPs): bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol B (BPB), bisphenol C (BPC), bisphenol E (BPE), bisphenol F (BPF), bisphenol S (BPS) and bisphenol Z (BPZ) in wastewaters (WWs). Sample preparation involved pre-concentration with SPE cartridges (Oasis HLB), followed by derivatization using *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide with 1% *tert*-butyldimethylchlorosilane. Chemical analysis was based on gas chromatography–mass spectrometry. A validated method with limits of detection (LODs) at ng L^{-1} range was applied to WWs collected at five Slovene wastewater treatment plants (WWTPs) and WW inflows from industrial, commercial and residential sources entering the sewerage systems of two catchments (Domžale-Kamnik (DK) and Ljubljana (LJ)). The presence of all BPs was confirmed in three inflows in DK and two inflows in the LJ catchments. High cumulative concentrations of all BPs were determined in WW from food processing facilities (LJ: 3030 ng L^{-1} and DK: 599 ng L^{-1}). A high detection frequency was observed in the WW from two textile cleaning companies (6 BPs for LJ and 8 BPs for DK). The analysis of WW from WWTPs revealed that only BPF (36.7 ng L^{-1}) and BPS (40.6 ng L^{-1}) were >LODs in the influents, whereas other BPs were detected also in the effluents. BPZ was found in the highest concentration (403 ng L^{-1} at WWTP-DK). WW collected at this WWTP also contained the highest amount of BPE (238 ng L^{-1}). Although BPs removal could not be directly compared between the WWTPs, with the exception

---

* Corresponding author at: Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia.
E-mail address: ester.heath@ijs.si (E. Heath).

https://doi.org/10.1016/j.scitotenv.2017.10.252

of BPAP and BPB in the case of two smaller WWTPs (6.39%–43.2%) bisphenols were in general highly removed (≥ 96.2%). Finally, levels of BPC > LOD are reported for first time (WWTP in the DK catchment: 1.01 ng L^{-1}–11.8 ng L^{-1}; LJ inflow from food processing plant up to 2560 ng L^{-1}).

1. Introduction

Bisphenol A (BPA) is a monomer used in the production of polycarbonates and epoxy resins commonly applied in food contact materials, digital media (CDs and DVDs), electronic equipment, medical devices, dental fillings, thermal receipts, water pipes and toys, etc. (Chen et al., 2016). The potential adverse effects on human health and regulation of BPA production led to the use of structurally related BPA alternatives (BPs) (Belfroid et al., 2002; Goldinger et al., 2015; Usman and Ahmad, 2016).

Data on the physico-chemical properties of BPs is limited (see Supplementary Information 1; SI-1; Table S2), but still could be used to explain their environmental fate. Based on their Henry's Law constants (K_H), their presence in the gas phase is negligible (Chen et al., 2016). The octanol-water partition coefficient (log K_{ow}) of BPs is from 1.25–7.17. Bisphenols with log $K_{ow} \leq 4$, such as bisphenol B (BPB), bisphenol E (BPE), bisphenol F (BPF) and bisphenol S (BPS) are expected mostly in the water phase. Other BPs such as bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol C (BPC), bisphenol P (BPP) and bisphenol Z (BPZ) have log K_{ow} of 4–6 and have the potential to adsorb to soil and sediment and to accumulate into the fat tissues of living organisms (Caballero-Casero et al., 2016; Chen et al., 2016).

A review by Jiang et al. (2013) reports numerous studies on the global occurrence of BPA in surface waters (SWs) and wastewaters (WWs), whereas there is only limited information available about other BP compounds. For example, only five published studies describe the presence of BPAF, BPAP, BPC, BPB, BPF, BPFL, BPS, BPZ, tetrachlorobisphenol A (TCBPA) and tetrabromobisphenol A in SWs in China and BPS and BPF in SWs from Japan, Korea and India (Jin and Zhu, 2016; Liu et al., 2016; Song et al., 2012; Yamazaki et al., 2015; Yang et al., 2014). Also, WW as a matrix is poorly investigated in terms of the presence of BPs. BPAF, BPB, BPE, BPF, BPS and BPZ were targeted in WW at wastewater treatment plants (WWTPs) in China, BPAF, BPAP, BPB, BPF, BPP, BPS and BPZ in WWs at WWTPs in India and chlorobisphenol A (CBPA), dichlorobisphenol A (DCBPA), trichlorobisphenol A (TrCBPA) and TCBPA in WW effluents in Spain (Ballesteros et al., 2006; Karthikraj and Kannan, 2017; Sun et al., 2017). To our knowledge, Ballesteros et al. (2006) is the only published study addressing the presence of chlorinated BPs in Europe, however their limits of quantification (LOQs) were so high (100–250 ng L^{-1}), that the reported values of all BPs were < LOQs. In addition to this study, a local screening project conducted in Norway was also reported, where the occurrence of BPA, BPAF, two structural isomers of BPF (2,2- and 4,4-dihydroxy bisphenols), BPS and bisphenol BP (BPBP) were determined in WWTP effluents, leachates, sediments and biota from Oslofjord and Mjøsa (Thomas et al., 2014).

Besides the occurrence of BPs in the WWTP influents and effluents, it is also important to assign point sources that contribute to the overall contamination with these compounds. Sun et al. (2017) reported BPAF and BPE as having low removal during conventional WW treatment. In such cases, it seems reasonable to search for the main inputs of contamination, which can be achieved by the collection and analysis of WWs originating from various facilities (e.g. inflows from industrial facilities and hospitals) entering the sewerage system. The aim of this study was to assess the occurrence and removal of BPAF, BPAP, BPF, BPE, BPB, BPC, BPS and BPZ in WWs collected at five Slovene WWTPs with varying treatment technologies. In addition, we attempted to identify possible sources of target BPs in two different WWTP catchments in Slovenia: Ljubljana (LJ) and Domžale-Kamnik (DK).

2. Materials and methods

2.1. Materials

BPAF (>99%), BPAP (>99%), BPC (>99%), BPE (>98%), BPF (>98%), BPS (>98%) and BPZ (>98%) were obtained from Sigma-Aldrich (St. Louis, USA), while BPB (>99.8%) was purchased from Dr Ehrenstorfer (Augsburg, Germany). Structural formulas, CAS numbers and structural names are presented in SI-1; Table S1. BPF refers to 4,4-dihydroxy substituted BP. Deuterated bisphenol A (BPA-d16), purchased from Sigma Aldrich (USA), was used as a surrogate standard. The silylating agent *N*-(*tert*-butyldiethylsilyl)-*N*-methyltrifluoroacetamide with 1% *tert*-butyldimethylchlorosilane (MTBSTFA with 1% TBDMCS; 95%) was purchased from Sigma Aldrich (St. Louis, USA). Dichloromethane (DCM), ethyl acetate (EtAc), methanol (MeOH) and purified water were obtained from J. T. Baker (Deveter, Netherlands) and were all of analytical grade purity. Stock solutions (≈ 10 μg L^{-1}) of each compound were prepared in MeOH, while calibration standards were prepared through serial dilutions of the stock solutions.

2.2. Sampling

Eighteen samples of WW influents and effluents (V = 2 L; n = 1) were collected at five WWTPs during August and October 2015 (Table 1). At the WWTP Domžale-Kamnik (WWTP-DK), an additional four samples containing WW influent mixed with the WW from cistern trucks from surrounding industries were collected. Full details are provided in with additional information given in SI-2 (Tables S4–S5).

In addition, twenty-five samples (V = 2 L; n = 1) of various potential source WWs were also sampled, i.e. the inflows of various industrial and other facilities entering into the sewerage system of the LJ and DK treatment plant catchments (SI-2; Fig. S1 and Table S3). The identity of these sources cannot be disclosed, therefore only the type of WW is shown (Table 2). These sources included a pre-treated WW (e.g. neutralisation, settlement, chemical precipitation) from two food and beverage producers (F), various industrial sources (I), health centres, hospitals and retirement homes (H), pharmaceutical companies (P), textile cleaning companies (C), landfill sites (L), swimming pool and communal sewerage canals (other sources, O; SI-2; Fig. S1 and Table S3). All samples were stored at −20 °C prior to analysis. Additional sample information is given in SI-2; Table S3.

2.3. Sample preparation

Samples were first filtered through glass-microfibre filters (Macherey Nagel, Düren, Germany) and then through cellulose nitrate filters (0.45 μm, Sartorius Stedim Biotech, Göttingen, Germany). A surrogate standard was then added to give a final concentration of 25 ng L^{-1} and the samples extracted using solid-phase extraction (SPE) cartridges with divinylbenzene-*N*-vinylpyrrolidone copolymer sorbent (Oasis HLB Waters, Massachusetts, USA; 60 mg, 3 mL). The cartridges were conditioned with 3 mL of DCM, EtAc and MeOH and equilibrated with 3 mL of purified water. The samples were then extracted at a flow-rate of 3 mL min^{-1} using a Supelco Vacuum Manifold (Bellefonte, USA). After loading, the sorbents were dried under vacuum (−1.33 kPa, 30 min). Elution was performed with 3 mL of MeOH (3 aliquots of 1 mL). The remaining solvent was removed under a gentle stream of nitrogen. Derivatization was performed with 30 μL of MTBSTFA with 1% TBDMCS in 220 μL EtAc for 16 h at 60 °C.

Table 1
Sampling details including sample names, location and time, type of WWTP treatment and sample and population equivalents for each WWTP.

WWTP location	Type of WWTP/WW	Population equivalent (PE)	Date of sampling	Sample type	Abbreviation
Ljubljana (WWTP-LJ)	Mechanical-biological with anaerobic stabilisation of excess sludge/mixed sewer system (hospital, domestic and industrial waste; storm runoff)	360,000	10.9.2015	Grab:	
				Influent	LJinf_1
				Effluent	LJeff_1
			27.10.2015[a]	Time-prop.:	
				Influent	LJinf_2
				Effluent	LJeff_2
Domžale-Kamnik (WWTP-DK)	Mechanical-biological with anaerobic stabilisation of sludge/infiltration, municipal, industrial	200,000	22.7.2015	Time-prop.:	
				Influent	DKinf_1
				Influent + inflows	DKinf+_1
			23.7.2015	Time-prop.:	
				Effluent	DKeff_1
			1.9.2015	Grab:	
				Influent	DKinf_2
				Influent + inflows	DKinf+_2
			2.9.2015	Time prop.:	
				Effluent	DKeff_2
			10.9.2015	Grab:	
				Influent	DKinf_3
			9.9.2015	Time-prop.:	
				Influent + inflows	DKinf+_3
			10.9.2015	Time-prop.:	
				Effluent	DKeff_3
			22.9.2015	Time-prop.:	
				Influent	DKinf_4
				Influent + inflows	DKinf+_4
			23.9.2015	Time-prop.:	
				Effluent	DKeff_4
Novo mesto (WWTP-NM)	Membrane-biological (MBR)/industrial, hospital, municipal, precipitation	55,000	6.8.2015	Time-prop.:	
				Influent	NMinf
			7.8.2015	Time-prop.:	
				Effluent	NMeff
Velenje (WWTP-VE)	Biofiltration (MBBR)/municipal, industrial	50,000	3.9.2015	Time-prop.:	
				Influent	VEinf
				Effluent	VEeff
Golnik (WWTP-GO)	Mechanical-biological + constructed wetland/hospital, municipal, precipitation	900	26.8.2015	Time-prop.:	
				Influent	GOinf
			27.8.2015	Time-prop.:	
				Effluent	GOeff

[a] Samples were collected on the same day (not taking into account hydraulic retention time), therefore removal was not determined on this occasion.

2.4. GC–MS analysis

Extracts were analysed using an Agilent 7890B series gas chromatograph with a 5977A single quadrupole mass spectrometer (Agilent, USA). Separation was achieved with a HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA). Carrier gas was helium. One microliters of sample extract was injected in splitless mode at 250 °C. For optimal chromatographic separation, the temperature program was as follows: an initial temperature 120 °C was ramped at 20 °C min^{-1} to 200 °C and held for 2 min, then at 10 °C min^{-1} to 280 °C (held for 5 min) and finally at 20 °C min^{-1} to 310 °C (held for 10 min). Post-run was 3 min. Total GC–MS runtime was 20 min. The mass spectrometer was operated in EI mode at 70 eV. Selected compounds were determined using selected ion monitoring (SIM mode). The fragment ions (m/z) and retention times (Rt) of the derivatized compounds that were used for identification and quantification are given in SI-3 (Table S6). Data was processed using MassHunter Workstation - Quantitative Analysis software (Agilent Technologies).

2.5. Method validation

Method performance was assessed in terms of linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), sensitivity and precision expressed as method and instrumental repeatability. An 8-point calibration curve was prepared using a least square linear regression analysis over a concentration range from 4 ng L^{-1} to 100 ng L^{-1}. The coefficient of determination (R^2) was used to express linearity. Sensitivity was expressed as the slope of the calibration curves. Since the initial analysis revealed the concentration of several BPs above the calibration range, an additional calibration curve from 4 ng L^{-1} to 1000 ng L^{-1} was prepared. Method accuracy was expressed as [(experimental value − spiked value) / spiked value] ($n = 3$; at 8 and 75 ng L^{-1}). WW collected from laboratory-scale bioreactors fed with artificial WW (at Faculty of Civil and Geodetic Engineering, University of Ljubljana, Ljubljana) was used for determination of LODs and LOQs (blank samples; $n = 6$), which were calculated as 3-times and 10-times the standard deviation (SD) of the baseline of the blanks, respectively, divided by the slopes of their calibration curves. Method repeatability was calculated as the relative standard deviation (RSD) of three replicate samples (at 25 ng L^{-1} and 100 ng L^{-1}), while instrumental repeatability was determined as the RSD of three consecutive injections of the same sample at 25 ng L^{-1} and 100 ng L^{-1}. SPE recovery was assessed at 20 ng L^{-1} and 80 ng L^{-1} ($n = 6$). At each concentration, the standards of bisphenols were added before SPE to three samples, while the other three samples were blanks without the addition of standards. After elution, a surrogate standard was added to all samples and standards of BPs to the blank samples. Recovery was calculated as a quotient of the analytes spiked in a sample prior to SPE ($n = 3$) and the same amount of analytes added post SPE to the extracted blanks ($n = 3$). Background contamination blanks ($n = 1$) revealed the presence of BPAF, BPF and BPS and samples containing measurable amounts of these compounds were blank-corrected. Details are given in SI-3 (Table S7).

Table 2
Description of facilities (inflows) sampled including sample names, sampling time and location.

Location	Abbreviation	Facility	Date of sampling
DK	DK-F01	Meat processing plant	28.7.2015
	DK-I02	Cleaning products manufacturer	24.7.2015
	DK-I03	Hygienic and health personal care products manufacturer	23.7.2015
	DK-I04	Biogas producer	22.7.2015
	DK-I05	Paint and lacquer manufacturer	28.7.2015
	DK-H06	Retirement home	30.9.2015
	DK-H07	Health care centre	30.9.2015
	DK-P08	Pharmaceutical factory	22.7.2015
	DK-C09	Textile cleaning company	21.7.2015
	DK-L10	Landfill	27.7.2015
	DK-O11	Communal sewage	20.8.2015
	DK-O12	Communal sewage	27.7.2015
LJ	LJ-F01	Dairy industry	9.9.2015
	LJ-F02	Poultry producer	10.9.2015
	LJ-F03	Meat processing plant	10.9.2015
	LJ-F04	Brewery and beverage producer	9.9.2015
	LJ-I05	Paint and lacquer manufacturer	8.9.2015
	LJ-I06	Gasket seal manufacturer for the application in potable water systems and in food industry	8.9.2015
	LJ-I07	Polyamide filaments and granulate producer	9.9.2015
	LJ-H08	Hospital	9.9.2015
	LJ-H09	Health care centre	10.9.2015
	LJ-P10	Pharmaceutical company	9.9.2015
	LJ-C11	Textile cleaning company	10.9.2015
	LJ-L12	Landfill (public municipal waste management company)	10.9.2015
	LJ-O13	Recreational centre with public swimming pool	28.8.2015

3. Results and discussion

3.1. Validation of method

Validation parameters of the method are given in SI-3 (Table S8). In general, method repeatability was between 1.16% (BPS at 25 ng L^{-1}) to 23.9% (BPC at 100 ng L^{-1}), while instrumental repeatability ranged between 3.65% for BPB (25 ng L^{-1}) and 18.0% for BPF (100 ng L^{-1}). Although it was expected that the accuracy would be higher at the higher concentration for all compounds, BPAP and BPS had higher percentage accuracy at the lower concentration (Table S8). Adequate linearity was confirmed by the R^2 values being >0.989 for BPS and BPZ. In terms of recovery, BPC had the lowest recovery (56.1%) at 80 ng L^{-1}. For all other compounds, recovery was >79.1% (BPAP at 20 ng L^{-1}). LODs and LOQs were in ng L^{-1} range. The lowest LOD and LOQ were determined for BPE (0.207 ng L^{-1} and 0.465 ng L^{-1}, respectively), whereas the highest LOD (1.24 ng L^{-1}) and LOQ (4.13 ng L^{-1}) were obtained for BPZ.

3.2. Sample analysis

3.2.1. Occurrence of BPs in WW inflows at WWTP-DK and WWTP-LJ region

To our knowledge, this is the first study attempting to determine BP sources to WW, hence there are no other literature data to compare. However, we do compare determined BP concentrations between analysed WW and potential sources later on (Comparison of BP concentrations in WW inflows to WWTP influents at DK and LJ region). The calculation of mass loads was omitted for these samples since data on WW flows were not available.

3.2.1.1. DK WWTP catchment. All samples contained at least one compound >LOQ (DK-P08; BPB), whereas DK-F01, DK-C09 and DK-L10 revealed the presence of all analytes at concentrations >LOD (Fig. 1). Interestingly, these three samples contained the highest cumulative concentrations (BPs$_{\text{total}}$) among all samples (DK-C09: 1310 ng L^{-1}; DK-L10: 1010 ng L^{-1} and DK-F01: 599 ng L^{-1}). In addition, apart from BPS, all BPs were present in the highest concentrations in one of these three samples, i.e. BPAP and BPE in DK-F01, BPAF and BPF in DK-C09 and BPB, BPC and BPZ in DK-L10 (Fig. 1). Concentrations of all the BPs are given in SI-4 (Table S9).

3.2.1.2. LJ WWTP catchment. The analysis of WW inflows from LJ catchment (Fig. 2) revealed that samples contained from one (LJ-I05) up to eight analytes >LOD (LJ-F03 and LJ-H09). Similar to DK catchment, two samples (LJ-F03 and LJ-H09) contained the highest cumulative concentrations of BPs (3030 ng L^{-1} and 531 ng L^{-1}, respectively) as well as the highest individual BP concentrations (BPAF, BPB, BPC, BPE and BPS in LJ-F03 and BPAP, BPF and BPZ in LJ-H09; Fig. 2). Sample LJ-F03 contained the highest concentration of BPC (2560 ng L^{-1}), which was the highest among all the BPs in the analysed samples. The concentrations of BPs are presented in full in Table S10.

Interestingly, the comparison of WW inflows in terms of BPs presence in the DK and LJ catchments revealed that BPs were present in both at the highest concentrations in the samples originating from food processing plants (DK-F01 and LJ-F03). This suggests that this type of facility represents a significant potential "point source" of BPs.

3.2.1.3. Detection frequency of BPs in DK and LJ WWTP catchments. A comparison of DK and LJ catchments was performed also in terms of BPs' detection frequency (Table 3), where the presence of BPs in WW inflows was separated into three groups: (I) expected due to their application of BPs at the facilities, (II) expected at other facilities due to the elimination from higher number of people situated at the facilities during the sampling campaign, and (III) other facilities, where BPs were least expected. Group I was further classified into four groups according to their application (Table 3).

Results confirmed that out of the seven suspected WW samples that might contain BPs due to their migration from food contact materials (FCMs) at facilities within this group, all samples contained between two to seven of the target analytes (Table 3; Group I.a). Although a higher number of samples from the LJ catchment were associated with potential contamination with BPs originating from food production industry, when compared to the DK catchment, only one sample for LJ and two samples for DK catchment contained all seven BPs. Among them, DK-L10 is a WW sampled at a landfill site, where detected compounds can originate from sources other than FCMs, e.g. from dyestuffs (Table 3; Groups I.b, I.c and I.d). Similarly, sample LJ-L12 is a WW from a landfill site in LJ catchment, where the detected BPs cannot be attributed to a specific source. Another source of contamination with BPAP and BPS in landfill WW is waste paper material, whose production employs both compounds. In addition, BPC is also placed in Group I.b and since no other use of this BP has been reported to the author's knowledge, we assume, that the detected BPC in samples DK-L10 and LJ-L12 originates from waste paper products.

Interestingly, as BPF and BPS are used for the production of lacquers and dyestuffs (Table 5), one might expect their presence in samples DK-I05 and LJ-I05, which are WWs originating from paint industry. However, the concentrations in these samples were <LOD for both compounds, which may be a result of nature of sampling (grab sampling), dilution or simply absence of application of BPF and BPS at these two facilities. Partially, this issue would have been clarified if composite samples were collected at different times.

Among the samples within this group, DK-I04 is the only WW sample originating from biogas production, where biodegradable waste (including wasted and spoiled food) is mainly used to produce electrical energy. Since BPs migrate into foodstuffs from FCMs, they were expected to be present in WW originating from this facility (Table 3). Indeed, 4 BPs (BPAF, BPAP, BPB and BPE), which are known food contaminants, were detected in this sample, among which BPE was present in relatively high concentration (Fig. 1; Table S9).

The samples in group II contained from two (LJ-O13) to all of the target BPs (LJ-H09). Any firm conclusions regarding the presence of the

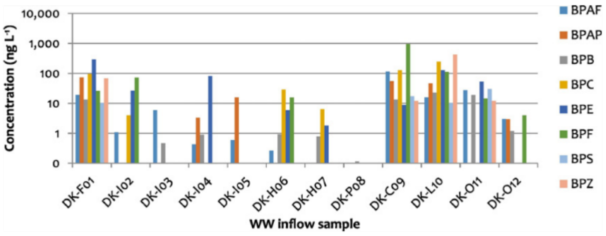


Fig. 1. Concentrations of BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ in WW inflows collected in the DK catchment.

detected BPs are hard to make. It is plausible that their source are excretions from human body after ingestion of contaminated foodstuff at the stated facilities, e.g. retirement home in the case of sample DK-H06 (Gramec Skledar and Peterlin Mašič, 2016). This observation partially agrees with the fact that in the case of WW coming from a recreational centre with a swimming pool (mixed communal WW and water from swimming pool, LJ-O13) only two BPs were detected, i.e. BPAF and BPE, whereas other samples (which derived only from municipal WW) contained more BPs.

Group III consisted of samples containing BPs that were not expected to be present based on the source of the collected WWs and taking into account their application stated in the literature for Group I (Table 3). Surprisingly, in both catchments, samples from textile cleaning facilities (DK-C09 and LJ-C11) contained the highest number of BPs, i.e. eight and six, respectively. Although LJ-C11 contained all six BPs at very low concentrations, high detection frequency suggests that companies use cleaning agents, which contain BPs that migrate from packaging and are washed during textile cleaning into the sewerage system. Another explanation could be the use of BPs in the textile production, where BPA is reported as an intermediate in the manufacture of textile dyes (Xue et al., 2017). Xue et al. (2017) targeted 9 BPs (BPA, BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ) in various textile products for infants <1 yr (e.g., diapers, blankets, clothes, and socks) and confirmed the presence of BPA, BPF, BPS and BPP in at least one type of sample. This agrees with the outcomes of the present study, where BPs (BPAF, BPAP, BPB, BPC and BPE in DK-C09 and BPAF, BPAP, BPB, BPC, BPE and BPF in LJ-C11) were also detected. Regardless, a detailed study on their origin should be performed in order to confirm their sources.

Interestingly, while WW from the pharmaceutical company in DK region (sample DK-P08) contained only BPB, the other facility in LJ area (sample LJ-P10) contained BPC, BPE, BPF and BPZ. The latter is a larger pharmaceutical industry with more employees, which could partially explain more contaminated WW in sample LJ-P10.

Samples LJ-I06 and LJ-I07 contained three and two BPs, respectively. Based on their type of production (Table 2), we did not expect the presence of the target BPs in high amounts. In case these industries would use any of the targeted BP for the production of their products, the detected concentrations would be significantly higher than those that were detected (see Section 3.2.1 and SI-IV; Table S10).

A further comparison among both catchments is hard due to the varying types of WW and different number of samples in each group. Overall, we can conclude that the presence of BPs was detected more frequently in samples from the DK catchment (Table 3).

3.2.1.4. Comparison of BP concentrations in WW inflows with WWTP influents in the DK and LJ catchments. Since not all the samples were not collected simultaneously (Tables 1 and 2), a comparison between WW inflows and WWTP influents was assessed only for DK-P08 and DKinf_1 (22.7.2015) and LJ-F03, LJ-H09, LJ-C11, LJ-L12 and LJinf_1 (10.9.2015; Table 4). The comparison was based on concentrations rather than mass loads as no data on flows are available for WW inflows.

DK-P08 originates from a pharmaceutical industry, where all BPs were <LOD (Table 4). On the contrary, DKinf_1 contained BPAF, BPB, BPE, BPF and BPZ in concentrations from 11.4 ng L^{-1} to 120 ng L^{-1}, which implies that these contaminants most probably derived from WW that was released into the sewerage system from other facilities within this region.

The opposite phenomenon was observed for the samples collected in the LJ catchment, where LJ-F03 and LJ-H09 contained all BPs at concentrations higher than those detected in LJinf_1. Similarly, LJ-C11 contained higher concentrations of BPB, BPC and BPE and LJ-L12 BPAF, BPC and BPE (Table 4). This indicates that these WW inflows represent a "point source" of BPs and that their concentration decreases on the way to the WWTP by dilution with WW coming from other facilities.

3.2.2. Occurrence of BPs in WWTP's influents and effluents

All BPs were >LOD in at least one sample of either WWTP influent or effluent (Table 5). In the case of WWTP-DK, WW samples that were delivered to the WWTP by cistern truck (DKinf+_1 – DKinf+_4) were compared with other WWTPs, whereas a comparison between them

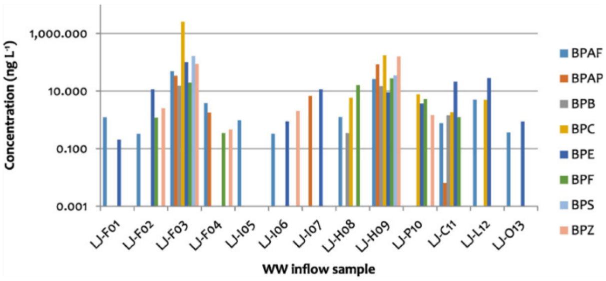


Fig. 2. Concentrations of BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ in WW inflows collected in LJ catchments.

Table 3
Expected and confirmed presence of BPs in WW inflows separated into three groups (Group I: expected presence of BPs due to their application/use; Group II: expected BPs presence at other facilities due to the elimination from higher number of people situated at the facility during sampling campaign; Group III: other facilities where BPs are not/less expected).

I. Expected presence of BPs due to their application/use

Application	Compound	Presence in WW inflows		References
		Expected	Positive hits	
a) FCM production (reported use/presence in a foodstuff)	BPAF BPAP BPB BPE BPF BPS BPZ	DK-F01 DK-I04 DK-L10 LJ-F01 LJ-F02 LJ-F03 LJ-F04 LJ-L12	DK-F01 – all (7/7) DK-I04 – BPAF, BPAP, BPB, BPE (4/8) DK-L10 – all (7/7) LJ-F01 – BPAF, BPE (2/7) LJ-F02 – BPAF, BPE, BPF, BPZ (4/7) LJ-F03 – all (7/7) LJ-F04 – BPAF, BPAP, BPF, BPZ (4/7) LJ-L12 – BPAF, BPC, BPE (3/7)	(Chunyang and Kurunthachalam, 2013; Gramec Skledar and Peterlin Mašič, 2016; Usman and Ahmad, 2016; Česen et al., 2016)
b) Paper products production, e.g. thermal paper	BPAP BPC BPS	DK-L10 LJ-L12	DK-L10 – all (3/3) LJ-L12 – BPC (1/3)	(Gramec Skledar and Peterlin Mašič, 2016; United States Environmental Protection Agency, 2012)
c) Production of lacquers, varnishes, adhesives, plastics and preservation of bamboo shoots	BPF	DK-I05 DK-L10 LJ-I05 LJ-L12	DK-I05 – / (0/1) DK-L10 – BPF (1/1) LJ-I05 – / (0/1) LJ-L12 – / (0/1)	(Toxnet, 2005)
d) Additive in pesticides, production of dyestuffs, colourfast agents, leather tanning agents and dye dispersants	BPS	DK-I05 DK-L10 LJ-I05 LJ-L12	DK-I05 – / (0/1) DK-L10 – BPS (1/1) LJ-I05 – / (0/1) LJ-L12 – / (0/1)	(Cao et al., 2012)

II. Expected BPs presence at other facilities due to the elimination from higher number of people situated at the facility during sampling campaign (hospitals, retirement homes, health care centres etc.)

Compound	Presence in WW inflows	
	Expected	Confirmed
BPAF	DK-H06	DK-H06 – BPAF, BPB, BPC, BPE, BPF (5/8)
BPAP	DK-H07	DK-H07 – BPB, BPC, BPE (3/8)
BPB	DK-O11	DK-O11 – BPAF, BPB, BPE, BPF, BPS, BPZ (6/8)
BPC	DK-O12	DK-O12 – BPAF, BPAP, BPB, BPF (4/8)
BPE	LJ-H08	LJ-H08 – BPAF, BPB, BPC, BPF (4/8)
BPF	LJ-H09	LJ-H09 – all (8/8)
BPS	LJ-O13	LJ-O13 – BPAF, BPE (2/8)
BPZ		

III. Other facilities where BPs are not/less expected

Compound	Presence in WW inflows	
	Not expected	Confirmed
BPAF	DK-I02	DK-I02 – BPAF, BPC, BPE, BPF (4/8)
BPAP	DK-I03	DK-I03 – BPAF, BPB (2/8)
BPB	DK-P08	DK-P08 – BPB (1/8)
BPC	DK-C09	DK-C09 – all (8/8)
BPE	LJ-I06	LJ-I06 – BPAF, BPE, BPZ (3/8)
BPF	LJ-I07	LJ-I07 – BPAP, BPE (2/8)
BPS	LJ-P10	LJ-P10 – BPC, BPE, BPF, BPZ (4/8)
BPZ	LJ-C11	LJ-C11 – BPAF, BPAP, BPB, BPC, BPE, BPF (6/8)

and DKinf_1 – Dkinf_4 is discussed as a Section "Comparison of DKinf+_1 – DKinf+_4 and DKinf_1 – DKinf_4" with exact data given in Table 6. The detection frequencies per compound in all samples from WWTP were as follows: BPS (2) < BPC (4) < BPF (5) < BPAF and BPAP (7) < BPZ (8) < BPE (10) < BPB (12). Moreover, BPF and BPS were >LOD only in the influents, whereas other BPs were detected at least once (BPC) in influents and effluents (Table 5).

The detected concentrations of BPs and the cumulative concentrations values *per* compound and *per* sample are given in Table 5. Except for BPAF on three occasions, BPs were generally higher in influents than in effluents which is most likely a result of sampling (i.e., grab samples and HRT not taken into account). This is also discussed in a recent paper by Sun et al. (2017), where they assigned this phenomenon. However, as the authors collected 24 h time-proportional samples, where HRT was taken into account, they believe the higher amounts of BPAF in the effluent is due to low biodegradation during WW treatment.

The highest detection frequency of BPs *per* sample was observed for DKinf+_1 (5), DKinf+_3 (5) and DKinf+_4 (7), which also contained the highest cumulative values (40.9–698 ng L^{-1}; Table 5). This is not surprising since WWTP-DK receives WW that is ≈11% industrial. These samples contain the highest levels of individual compounds,

Table 4
Concentrations of BPs in WW inflows and WWTP influents in the DK and LJ catchments.

DK catchment	DK-P08 (ng L^{-1})	DKinf_1 (ng L^{-1})
BPAF	<LOD	11.4
BPAP	<LOD	<LOD
BPB	<LOD	16.3
BPC	<LOD	<LOD
BPE	<LOD	77.8
BPF	<LOD	15.1
BPS	<LOD	<LOD
BPZ	<LOD	120

LJ catchment	LJ-F03 (ng L^{-1})	LJ-H09 (ng L^{-1})	LJ-C11 (ng L^{-1})	LJ-L12 (ng L^{-1})	LJinf_1 (ng L^{-1})
BPAF	49.0	26.2	<LOD	4.99	4.97
BPAP	33.8	84.6	<LOD	<LOD	<LOD
BPB	15.2	14.9	1.44[a]	<LOD	<LOD
BPC	2560	175	1.84[a]	4.97	<LOD
BPE	101	8.90	21.3	28.3	4.74
BPF	19.4	27.6	1.24[a]	<LOD	5.083
BPS	164	34.6	<LOD	<LOD	<LOD
BPZ	87.1	159	<LOD	<LOD	1.64[a]

[a] The values are > LOD, but below first point in calibration curve.

with exception of BPC, which was present in the highest concentration in NMinf (11.8 ng L^{-1}; Table 5). The cumulative value for BPZ in all samples (994 ng L^{-1}) was significantly higher than for the rest of the compounds implying its high usage compared to other BPs in Slovenia. BPC and BPS had the lowest cumulative values in all samples (18.4 ng L^{-1} and 42.6 ng L^{-1}, respectively).

The literature data on the occurrence of investigated BPs is scarce as only two other studies report measurable concentrations of BPs in WWs (Table 5). Comparison with available literature data shows that BPC was investigated in terms of occurrence in WW and also determined above the LOD for the first time in this study. Furthermore, a comparison of the average BP concentrations reported by Sun et al. (2017), who addressed the occurrence of BPs in Chinese WWs and Karthikraj and Kannan (2017) that studied Indian WWs, found 3 BPs (BPAF, BPAP and BPZ) in considerably higher concentrations in both, influents and effluents, than analysed within this study. On the contrary, Sun et al. (2017) reported higher average concentrations of BPF and BPS in WW influents and effluents. Within Europe, only two studies addressed this issue. Ballesteros et al. (2006) searched for CBPA, DCBPA, TrCBPA and TCBPA in WW effluents in Spain, however due to their high LODs none of the analytes were quantified. Thomas et al. (2014), reported measurable concentrations of BPA, two structural isomers of BPF, BPS and BPBP in Norwegian WWTP effluents, whereas BPAF was <LOD. BPF (4,4-dihydroxy substituted, the same as in this study) was present in the highest concentration (6200 ng L^{-1}) in one WWTP effluent, which is significantly higher than the concentrations of BPF detected in Slovene WWs (Table 5).

3.2.2.1. Calculation of BP mass loads in WWTP's influents and effluents. Mass loads were calculated for the collected influents and effluents in order to compare the studied WWTPs in terms of BP pollution. The concentrations were multiplied by flow rate and divided by PE$_{COD}$ (organic load) at the time of sampling. Where these data were not available at the time of sampling, the average values for 2015 were used for calculations (SI-2: Tables S4–S5).

In general, these results showed that the DK catchment appears to be more polluted with target BPs than the WWTP LJ catchment, which has the highest PE and flow rates among the studied WWTPs. Regardless, as discussed in Section 3.2.3 and shown in SI-5 (Table S11), the removal efficiency of WWTP-DK is high since the effluents DKeff_1, DKeff_2, DKeff_3 and DKeff_4 mainly contain BPs at low levels or even <LODs. At other WWTPs with a lower PE and flow rates, the majority of BPs were not detected in influents which suggests only minor/negligible pollution of these catchments with the studied BPs.

Table 5
Concentrations of BPs in five Slovene WWTP influents and effluents.

Comp.	Concentration (ng L^{-1})[a]											
	LJinf_1	LJeff_1	LJinf_2	LJeff_2	DKinf+_1	DKeff_1	DKinf+_2	DKeff_2	DKinf+_3	DKeff_3	DKinf+_4	DKeff_4
BPAF	4.97	7.04	<LOD	6.66	23.0	<LOD	<LOD	9.53	29.8	<LOD	8.20	<LOD
BPAP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	35.6	9.49	83.7	3.17*	177	<LOD
BPB	<LOD	<LOD	1.13*	<LOD	15.1	0.566*	0.439	<LOD	27.7	0.318*	19.9	0.624*
BPC	<LOD	<LOD	4.18	<LOD	<LOD	1.45*	1.01*	<LOD	<LOD	<LOD	<LOD	<LOD
BPE	4.74	<LOD	<LOD	<LOD	91.6	<LOD	3.17*	0.326*	<LOD	1.30*	238	1.28*
BPF	5.08	<LOD	<LOD	<LOD	18.7	<LOD	<LOD	<LOD	36.7	<LOD	15.2	<LOD
BPS	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	40.6	<LOD
BPZ	1.64*	<LOD	6.95	<LOD	403	<LOD	<LOD	<LOD	290	2.70*	199	85.7
Sum per sample	16.4	7.04	12.26	6.66	551	2.02	40.2	19.3	468	7.49	698	87.6

Comp.	Concentration (ng L^{-1})[a]											
	NMinf	NMeff	VEinf	VEeff	GOinf	GOeff	Sum per compound	Average (influents)	Average (effluents)	Average (influents)	Average (effluents)	Average (influents)	Average (effluents)
										(Sun et al., 2017)[b]		(Karthikraj and Kannan, 2017)[b]	
BPAF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	89.2	16.5	7.74	0.901	1.16	1.1	<LOD
BPAP	<LOD	<LOD	<LOD	<LOD	3.26*	2.08*	314	74.9	4.91	n.a.	n.a.	0.3	<LOD
BPB	0.295*	<LOD	0.431*	<LOD	2.65*	1.51*	70.7	8.46	0.755	n.a.	n.a.	2.5	0.6
BPC	11.8	<LOD	<LOD	<LOD	<LOD	<LOD	18.4	5.66	1.45	n.a.	n.a.	n.a.	n.a.
BPE	<LOD	2.83*	<LOD	0.276*	<LOD	5.62	349	84.4	1.94	4.51	4.07	n.a.	n.a.
BPF	6.15	<LOD	<LOD	<LOD	<LOD	<LOD	81.8	16.4	<LOD	35.8	1.67	10.4	0.6
BPS	<LOD	<LOD	1.95*	<LOD	<LOD	<LOD	42.6	21.3	<LOD	55.7	0.596	14.7	2.4
BPZ	4.66	<LOD	<LOD	<LOD	<LOD	<LOD	994	151	44.2	n.a.	n.a.	0.6	<LOD
Sum per sample	22.9	2.83	2.38	0.276	5.91	9.21							

[a] Concentrations marked with * were >LOD but below the first calibration point (i.e. 4 ng L^{-1}).

[b] n.a. - BPs that were not analysed within the study by Sun et al. (2017) and Karthikraj and Kannan (2017).

Table 6
Concentrations of BPs in influents, influents with cistern trucks WW and amounts attributed to cistern truck WW calculated as a difference among each pair (DK region).

	DKinf_1 (A)	DKinf+_1 (B)	*B-A*	DKinf_2 (C)	DKinf+_2 (D)	*D-C*	DKinf_3 (E)	DKinf+_3 (F)	*F-E*	DKinf_4 (G)	DKinf+_4 (H)	*H-G*
BPAF	11.4	23.0	11.6	19.4	<LOD	−18.6	5.08	29.8	24.7	0.0187	8.20	8.18
BPAP	<LOD	<LOD	/	0.00220	35.6	35.6	99.9	83.7	−16.2	0.00220	177	177
BPB	16.3	15.1	−1.16	17.8	0.439[a]	−17.4	15.1	27.6	12.6	0.277[a]	19.9	19.6
BPC	<LOD	<LOD	/	388	1.01[a]	−387	<LOD	<LOD	/	0.743[a]	0.00115	−0.742
BPE	77.8	91.6	13.8	215	3.17[a]	−212	<LOD	<LOD	/	31.9	238	206
BPF	15.1	18.7	3.59	25.8	0.00162	−25.8	23.7	36.7	13.0	7.08	15.2	8.07
BPS	<LOD	<LOD	/	41.5	0.000486	−41.5	<LOD	<LOD	/	0.000486	40.6	40.6
BPZ	120	403	283	<LOD	<LOD	/	0.00765	290	290	91.2	199	108

[a] The values are >LOD, but below first point in calibration curve.

3.2.2.2. Comparison of DKinf+_1 – DKinf+_4 and DKinf_1 – DKinf_4. Table 6 gives the determined concentrations of BPs in four pairs of WWTP-DK influents and influents including WW brought in by cistern trucks. In addition, differences among each pair were calculated to assess the amount of contamination deriving from the cistern truck WW. When concentrations were <LOD in one of the sample pair, one half of the compounds' LOD was used to calculate the difference between the samples (Verbovšek, 2011). When both samples contained BPs < LOD, the difference was not calculated. The majority of BPs were present in higher concentrations in samples from cistern truck WWs, which was expected as WW from cistern trucks represent WW collected exclusively at nearby industries, which have the potential to be contaminated with BPs. Regardless, the second sampling campaign revealed the opposite phenomenon, where samples without cistern truck WW contained all quantifiable BPs except BPAP at higher concentrations. These elevated concentrations of especially BPC, BPE and BPS are difficult to explain and may arise from uncontrolled emission of contaminated WW into the sewerage system by certain industrial facilities in the DK catchment. Similarly, high concentrations of BPAP, BPE and BPZ were observed in DKinf+_4 (177 ng L^{-1}, 206 ng L^{-1} and 108 ng L^{-1}, respectively). Interestingly, BPZ was observed in the high concentrations in DKinf+_1, DKinf+_3 and DKinf+_4 suggesting that BPZ is constantly present in a WW of a certain point source, which is driven to WWTP-DK by cistern trucks. To further locate its source, the WW from each cistern truck should be analysed.

3.2.3. Estimation of BP removal at WWTPs

Removal efficiency of tested BPs was estimated by dividing mass loads of target BPs in WW influents and effluents only when samples were collected as composite samples taking into account WWTP HRT (Table 5). When concentrations in effluents were <LOD, one half of the compounds' LOD was used for the calculation of mass loads and removal, whereas when influents contained the values <LOD, removal was not determined (Verbovšek, 2011). Removal estimation (in %) is given in SI-5 (Table S11).

Removal efficiency was calculated on 25 occasions. Results showed that BPs were in general successfully removed (>96.2%). When comparing our results with the literature data, we observed two anomalies. For example, BPAF was removed >99.8% during three sampling campaigns at WWTP-DK, whereas Sun et al. (2017) report poor biological removal of BPAF due to the lack of degrading bacteria (Table 7). Sun et al. (2017) also report negative removal for BPE, unlike in the present study, where >99.5% removal was determined on two occasions at WWTP-DK (Table 7 and Table S11). The discrepancy among both studies probably derives form a different approach used for the removal calculation since Sun et al. (2017) applied concentrations in influents and effluents rather than mass loads of the studied BPs (Table 7). Adsorption onto sludge is, besides biodegradation, one of the most crucial parameters, affecting removal efficiency of organic contaminants. Based on reported log K_{OW} (Table S2 and Table 7) for the studied BPs, it is assumed that BPAF, BPAP, BPB, BPC and BPZ (log $K_{OW} > 4$) have a tendency to adsorb onto sludge. By this assumption and in accordance with the confirmed adsorption onto sludge for BPAF, BPE, BPF and BPS by Sun et al. (2017), the removal obtained in this study was related to either biodegradation or to a combination of biodegradation and adsorption onto sludge (Table 7). Only one discrepancy between log K_{OW} values and adsorption by Sun et al. (2017) was revealed, i.e. for BPF (Table 7). Based on gathered data, we concluded that BPAF, BPAP, BPB, BPC and BPZ are most likely removed by their adsorption and biodegradation, whereas BPE is mainly biodegraded and BPF is predominantly removed by adsorption. Further studies are needed to confirm these assumptions since other processes like back-transformation of human metabolites might also play an important role in the WWTP efficiency.

In addition, a direct comparison of removal efficiency between different treatment technologies of the studied WWTPs was not possible due to the presence of different BPs at each site. Regardless, poor removal of BPAP and BPB (<50%) was observed for WWTP-GO, the smallest and oldest WWTP (900 PE). Moreover, although removal at WWTP-NM and WWTP-VE was in general >99.8% for the BPs detected, BPB was poorly removed at these two WWTPs. These observations suggest that BPAP and BPB were poorly removed in the case of smaller WWTPs (900–55,000 PE), where MBR, MBBR or conventional treatments with constructed wetlands technologies are used. On the other hand, conventional treatment at WWTP-DK resulted in high removal of BPAP and BPB (Table S11), suggesting this type of treatment as a more suitable alternative for their removal.

Table 7
The removal efficiency of studied BPs related to log K_{OW} and comparison with the data by Sun et al. (2017).

	BPAF	BPAP	BPB	BPC	BPE	BPF	BPS	BPZ
Expected adsorption to biomass based on log K_{OW}	✓ 4.47	✓ 4.86	✓ 4.13	✓ 4.74	✘ 3.19	✘ 3.06	✘ 1.65	✓ 5.00
Reported adsorption to sludge by Sun et al. (2017)	✓	n.a.	n.a.	n.a.	✘	✓	✘	n.a.
Reported removal by Sun et al. (2017)	−153%	n.a.	n.a.	n.a.	−82.5%	≈100%	≈100%	n.a.
Calculated removal in this study	≈100% (n = 3)	36.1% (n = 1); 96–100% (n = 2)	6.39–98.9% (n = 6)	100% (n = 1)	≈100% (n = 2)	100% (n = 4)	100% (n = 2)	57% (n = 1); ≈100% (n = 3)
Removal due to suspected adsorption (A) and/or biodegradation (B)	A + B	A + B	A + B	A + B	B	A	B	A + B

4. Conclusions

The present work investigated the occurrence of BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ in WWs from five Slovene WWTPs (WWTP-LJ, WWTP-DK, WWTP-NM, WWTP-VE and WWTP-GO). The main WW inflows in DK and LJ catchments were also analysed, where all target BPs were detected in three (in DK catchment) and two inflows (in LJ catchment). Among these samples, high cumulative concentrations of all BPs were observed in WW from two food production/processing plants located in both catchments. A high detection frequency was observed also in WW from textile cleaning companies also located at both locations (DK: all BPs; LJ: BPAF, BPAP, BPB, BPC, BPE and BPF). This suggests that food processing and textile cleaning company discharges represent a significant source of the target BPs.

Among the BPs in WWs from the studied WWTPs, only BPF and BPS were detected exclusively in effluents, whereas other BPs were present in both, influents and effluents. With the exception of BPAP, BPB and BPZ removal of the remaining target BPs was high on five occasions. Based on the obtained results, BPAP and BPB removal is also higher in large conventional treatment plants with higher PE capacity than other treatment techniques, however further studies are needed to confirm this assumption.

To the authors' knowledge, BPC was determined >LOD in WW samples for the first time. This is also the first study addressing “point source” contamination of WW with BPs and the occurrence of the investigated BPs in WWTP influents and effluents in Europe. The lack of literature data addressing their presence in both, SWs and WWs, clearly shows the need for further assessment of their presence in the aqueous environment and the need to evaluate the potential risk they may pose to aquatic biota and/or humans. In addition, structural differences in BP alternatives are to be addressed in regards to their presence in different environmental compartments as well as their removal by different treatment technologies.

Acknowledgments

The authors would like to thank all collaborating WWTPs and partners for their support within the sampling campaigns and for providing the supplementary data on WWTPs.

Funding

This work was financially supported by the Slovenian Research Agency (Program groups P1-0143, P-0208 and Projects L1-5457, J1-6744, L1-7544 and N1-0047), the EC 7th Framework Programme Funding under Grant agreement no. 603629-ENV-2013-6.2.1-Globaqua, ISO-FOOD project (Era chair for isotope techniques in food quality, safety, and traceability, Grant 621329) and the MASSTWIN project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 692241.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.252.

References

Ballesteros, O., Zafra, A., Navalón, A., Vílchez, J.L., 2006. Sensitive gas chromatographic–mass spectrometric method for the determination of phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives in wastewater samples. J. Chromatogr. A 1121, 154–162.
Belfroid, A., van Velzen, M., van der Horst, B., Vethaak, D., 2002. Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. Chemosphere 49, 97–103.
Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53.
Cao, G., Lu, J., Wang, G., 2012. Photolysis kinetics and influencing factors of bisphenol S in aqueous solutions. J. Environ. Sci. 24, 846–851.
Česen, M., Lambropoulou, D., Laimou-Geraniou, M., Kosjek, T., Blaznik, U., Heath, D., Heath, E., 2016. Determination of bisphenols and related compounds in honey and their migration from selected food contact materials. J. Agric. Food Chem. 64, 8866–8875.
Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review. Environ. Sci. Technol. 50, 5438–5453.
Chunyang, L., Kurunthachalam, K., 2013. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J. Agric. Food Chem. 61, 4655–4662.
Goldinger, D.M., Demierre, A.-L., Zoller, O., Rupp, H., Reinhard, H., Magnin, R., Becker, T.W., Bourqui-Pittet, M., 2015. Endocrine activity of alternatives to BPA found in thermal paper in Switzerland. Regul. Toxicol. Pharmacol. 71, 453–462.
Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199.
Hazardous SubstancesData Bank TOXNET, 2005. available from. https://toxnet.nlm.nih.gov/, Accessed date: 18 April 2017.
Jiang, J.-Q., Zhou, Z., Sharma, V.K., 2013. Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water — a review from global views. Microchem. J. 110, 292–300.
Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351.
Karthikraj, R., Kannan, K., 2017. Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere 181, 216–223.
Liu, D., Liu, J., Guo, M., Xu, H., Zhang, S., Shi, L., Yao, C., 2016. Occurrence, distribution, and risk assessment of alkylphenols, bisphenol A, and tetrabromobisphenol A in surface water, suspended particulate matter, and sediment in Taihu Lake and its tributaries. Mar. Pollut. Bull. 112, 142–150.
Song, S., Ruan, T., Wang, T., Liu, R., Jiang, G., 2012. Distribution and preliminary exposure assessment of bisphenol AF (BPAF) in various environmental matrices around a manufacturing plant in China. Environ. Sci. Technol. 46, 13136–13143.
Sun, Q., Wang, Y., Li, Y., Ashfaq, M., Dai, L., Xie, X., Yu, C.-P., 2017. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ. Pollut. 225, 542–549.
Thomas, K., Schlabach, M., Langford, K., Fjeld, E., Øxnevad, S., Rundberget, T., Bæk, K., Rostkowski, P., Harju, M., 2014. Screening program 2013. New bisphenols, organic peroxides, fluorinated siloxanes, organic UV filters and selected PBT substances. Oslo, Norwegian Environment Agency (NIVA rapport, 6696-2014) (Miljødirektoratet rapport, M-176/2014) (NILU OR, 26/2014).
United States Environmental Protection Agency, 2012. Preliminary Screen for Possible Future Consideration as Potential Designated Chemicals for Biomonitoring California. available from: https://biomonitoring.ca.gov/sites/default/files/downloads/031612PrelimScreen2.pdf, Accessed date: 18 April 2017.
Usman, A., Ahmad, M., 2016. From BPA to its analogues: is it a safe journey? Chemosphere 158, 131–142.
Verbovšek, T., 2011. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods. Mater. Geoenviron. 58, 393–404.
Xue, J., Liu, W., Kannan, K., 2017. Bisphenols, benzophenones, and bisphenol A diglycidyl ethers in textiles and infant clothing. Environ. Sci. Technol. 51, 5279–5286.
Yamazaki, E., Yamashita, N., Taniyasu, S., Lam, J., Lam, P.K.S., Moon, H.-B., Jeong, Y., Kannan, P., Achyuthan, H., Munuswamy, N., Kannan, K., 2015. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol. Environ. Saf. 122, 565–572.
Yang, Y., Lu, L., Zhang, J., Yang, Y., Wu, Y., Shao, B., 2014. Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography–electrospray tandem mass spectrometry. J. Chromatogr. A 1328, 26–34.

3.2 Identification of Transformation Products

3.2.1 Suspect and untargeted screening of bisphenol S metabolites produced by *in vitro* human liver metabolism

The paper "*Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism*" is authored by C. Gys, A. Kovačič, C. Huber, F. Yin Lai, E. Heath, A. Covaci. The paper was published in *Toxicology letters* in October 2018. The study was performed in collaboration with the Toxicological Center at the University of Antwerp, Belgium, under the supervision of Prof Dr Covaci. The experiment was led by C. Gys from the University of Antwerp. My role in this joint effort included performing experimental work, instrumental analysis, data evaluation, and preparation of the manuscript.

As pointed out in this thesis, available studies focus on the measurement of the parent BPS molecule, which can lead to an underestimation of human exposure to this emerging contaminant [22]. The aims of this work were (1) to elucidate the *in vitro* metabolic pathways of BPS using human liver microsomes as well as cytosol fractions and (2) by identifying metabolites contribute to the structural identification of TPs formed during WWT and in the environment. In these terms, a full screening, identification and structural elucidation of Phase I (oxidative) and II (conjugative) metabolites of BPS were for the first time executed using high-resolution LC-QTOF-MS. Data analysis included two complementary workflows. The first involved a suspect screening employing a database based on literature research. The second involved a non-target screening using the open-source software packages MZmine and R. This study design resulted in the identification and elucidation of two Phase I and four Phase II in vitro human metabolites for BPS, formed through hydroxylation of phenolic ring or conjugation with glucuronic acid or sulfate, respectively. Three metabolites, namely dihydroxy-BPS, hydroxy-BPS-glucuronide and hydroxy-BPS-sulfate, were identified for the first time.

The obtained results fulfil two aims of the thesis, namely to develop and optimize analytical methods and data analysis for the identification of metabolites of bisphenols and to provide insights into the identification of novel TPs during WWT and in the environment.

Toxicology Letters 295 (2018) 115–123

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Suspect and untargeted screening of bisphenol S metabolites produced by *in vitro* human liver metabolism

Celine Gys[a,*], Ana Kovačič[b,c], Carolin Huber[d,1], Foon Yin Lai[a], Ester Heath[b,c], Adrian Covaci[a,*]

[a] *Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium*
[b] *Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia*
[c] *International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000, Slovenia*
[d] *Norwegian University of Science and Technology, Høgskoleringen 1, 7042 Trondheim, Norway*

ARTICLE INFO

Keywords:
Bisphenol S
In vitro metabolism
Human liver microsomes
High-resolution mass spectrometry
MZmine
Emerging contaminants

ABSTRACT

Bisphenol S (BPS) is increasingly used as substitute for bisphenol A, resulting in higher potential of human exposure to this compound. Yet, information on the human metabolism of BPS is limited. Hence, current biomonitoring studies rely only on the measurement of BPS itself, leading to a potential underestimation of assessing human exposure to this emerging contaminant. The aims of this study were to investigate the *in vitro* metabolic pathways of BPS using human liver microsomes and cytosol fractions and propose *in vitro* metabolites for evaluation in pharmacokinetics studies. Liquid chromatography coupled to quadrupole time-of-flight high-resolution mass spectrometry was used for the screening, identification, and structural elucidation of Phase I and II metabolites of BPS for the first time. Metabolite identification was performed using two complementary workflows: suspect and untargeted screening. Two Phase I metabolites were formed through hydroxylation of the phenolic rings. Four Phase II metabolites were formed through conjugation with glucuronic acid or sulfate. Three of these metabolites, namely dihydroxy-BPS, hydroxy-BPS-glucuronide and hydroxy-BPS-sulfate were identified and structurally elucidated for the first time. As such, we provide an expanded set of *in vitro* biotransformation products of BPS, which can potentially support a reliable assessment of BPS exposure in future biomonitoring studies.

1. Introduction

Bisphenol A (4-[2-(4-hydroxyphenyl)propan-2-yl]phenol, BPA), a widely used industrial chemical, is being produced in high quantities over five decades, with yearly global production of 8 million tonnes, and is mainly used as a monomer in the production of polycarbonate plastic and epoxy resins (Asimakopoulos et al., 2016; Brandon et al., 2003). Many studies have associated exposure to BPA with adverse effects in humans (Eladak et al., 2015). Due to its potential harm as an endocrine disrupting chemical, more stringent regulations on the production and application of BPA are in force (EFSA, 2015; EU, 2011). To comply with legislation, BPA has been gradually replaced with other structurally related compounds, including bisphenol S (4,4′-sulfonyldiphenol, BPS).

BPS is one of the main alternatives to BPA in a number of applications such as the manufacturing of plastic, epoxy resins and as a developer in thermal paper (Skledar et al., 2016). Recent studies have shown its presence in indoor dust, foodstuff, paper products, sludge, sediments (Wu et al., 2018), wastewater influent and effluent (Sun et al., 2017) and surface water (Chen et al., 2016). In accordance with BPA, the mechanism by which the population is exposed to BPS is mainly by ingestion (food, dust) and in lower extent through inhalation (air, particles) and dermal adsorption (dust) (Caballero-Casero et al., 2016; Wu et al., 2018). Handling thermal paper is a possible additional way of exposure (Liao et al., 2012b). In 2012, the study by Liao et al. (Liao et al., 2012b) reported the presence of BPS in human urine for the first time, in a concentration range from < 0.02 to 21.0 ng/mL, with a detection frequency of 81%. Recent biomonitoring studies confirmed the presence of BPS in human urine at similar (Wu et al., 2018) or even higher concentrations of BPS in comparison to BPA (Asimakopoulos et al., 2016).

Since international production and application of BPS are not regulated, BPS levels in the environment and therefore human exposure to this compound are steadily increasing. While human metabolism for BPA has been studied extensively (Gramec Skledar and Peterlin Mašič, 2016; Thayer et al., 2015; Völkel et al., 2002), only a few studies have

---

* Corresponding authors.
E-mail addresses: celine.gys@uantwerpen.be (C. Gys), adrian.covaci@uantwerpen.be (A. Covaci).

[1] Present address: Helmholtz Centre for Environmental Research-UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany.

https://doi.org/10.1016/j.toxlet.2018.05.034
Received 6 April 2018; Received in revised form 22 May 2018; Accepted 29 May 2018
Available online 18 June 2018

used *in vitro* and *in silico* experiments to provide a preliminary evaluation of human metabolism of BPS (Gramec Skledar et al., 2015; Grignard et al., 2012; Le Fol et al., 2015; Skledar et al., 2016). Two *in vitro* studies have described the influence of metabolism on the endocrine activity of BPS, reporting divergent results (Kang et al., 2014; Skledar et al., 2016). So far, no study has performed a full screening of Phase I and Phase II *in vitro* metabolites of BPS using accurate mass high-resolution mass spectrometry, which allows chemical identification and characterisation. Well-designed *in vitro* experiments followed by the analysis using liquid chromatography combined with accurate mass spectrometry could enhance confidence in detecting and identifying metabolites formed in the *in vitro* metabolism (Erratico et al., 2015; Lai et al., 2015; Mortelé et al., 2018; Negreira et al., 2015; Van den Eede et al., 2013).

Pooled human liver microsomes (HLMs) contain a wide variety of drug metabolising enzymes and are commonly used for *in vitro* human metabolism studies. They particularly contain the cytochrome P450 s (CYPs) and UDP-glucuronosyltransferases (UGTs), which are the major enzyme systems responsible for the metabolism of exogenous compounds (Brandon et al., 2003; Zhang et al., 2016). In a previous *in vitro* study, the hepatic human UGT enzyme UGT1A9 was found to conjugate BPS with glucuronic acid more extensively than the homologous intestine enzyme (Gramec Skledar et al., 2015). Conjugation with glucuronic acid or sulfate is generally regarded as the main detoxification pathway for most bisphenols (Gramec Skledar and Peterlin Mašič, 2016). Although BPS is structurally related to other bisphenols, it cannot be assumed that their metabolism is interchangeable (Oh et al., 2018).

The aims of this study were to elucidate the *in vitro* metabolic pathway of BPS using HLMs and human liver cytosol fractions (HLCYT) to produce Phase I and Phase II metabolites and to propose a set of potential additional biomarkers for use in future biomonitoring studies. To our best knowledge, this study was the first to use liquid chromatography coupled to high-resolution quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) to perform a full screening, identification, and structural elucidation of *in vitro* BPS metabolites.

2. Materials and methods

2.1. Chemicals and reagents

The analytical reference standard of BPS (4,4′-sulfonyldiphenol, purity 98%) was purchased from Sigma-Aldrich (Missouri, USA). The analytical reference standards of valsartan-d3 and BPS-glucuronide were acquired from Toronto Research Chemicals (Toronto, Canada). Pooled human liver microsomes (HLMs, mixed gender, n = 50) were obtained from Tebu-Bio (Boechout, Belgium). Pooled human liver cytosol (HLCYT), 2,5-uridinediphosphate glucuronic acid (UDPGA), adenosine-3′-phosphate 5′-phosphosulfate (PAPS, > 60%) lithium salt hydrate, alamethicin (neat, > 98%), dimethyl sulfoxide (DMSO), and 4-nitrophenol (4-NP) were acquired from Sigma-Aldrich (Missouri, USA). NADPH tetrasodium salt hydrate (> 96%) was purchased from Acros (Geel, Belgium). Acetonitrile (ACN, HPLC-grade) was obtained from Fisher Chemical (Loughborough, United Kingdom), formic acid (> 98%) from Merck KGaA (Darmstadt, Germany). A 100 mM TRIS-buffer was prepared by dissolving 12.1 g Trizma base (Janssen Chimica, Beerse, Belgium) and 1.0 g $MgCl_2$ (Merck KGaA, Darmstadt, Germany) in 1 L ultrapure water. The pH was adjusted to 7.4 by adding 1 M HCl solution. Ultra-pure water was produced in-house with a PURELAB-purifier system of Elga Labwater (Tienen, Belgium).

2.2. In vitro metabolism assay

This study employed the *in vitro* human metabolism assay optimised and used in our previous studies (Erratico et al., 2015; Lai et al., 2015; Mortelé et al., 2018; Negreira et al., 2015; Van den Eede et al., 2013). The experimental setup in a schematic summary can be found in the Supporting Information (Fig. SI-1.1). All sample sets consisted of three replicates. Briefly, Phase I metabolites were generated using pooled HLMs. The reaction mixture (1 mL), which consisted of 945 μL of TRIS-buffer (pH 7.4, 100 mM), 25 μL of HLM (20 mg/mL) and 10 μL of BPS stock solution (0.5 mM), was incubated in a 1.5 mL Eppendorf tube at 37 °C for 1 and 3 h. At 5, 60 and 120 min of incubation, 10 μL of NADPH (0.1 M in buffer) was added. Three negative control samples (*i.e.*, either without BPS, HLM or NADPH) were included in the experiment. Also, a positive control sample, using 10 μL phenacetin (5 μg/mL) as substrate, was prepared and incubated. The reaction was stopped after 1 and 3 h incubation by adding 250 μL ice-cold ACN with 1% (v/v) formic acid and the internal standard valsartan-d3, at a concentration of 1 μg/mL.

Following the *in vitro* Phase I metabolism, samples were subject to Phase II conjugation *via* either glucuronidation (GLU) or sulfation (SUL). Phase I samples were centrifuged for 5 min at 8000 rpm. For the GLU samples, 935 μL of the supernatant was transferred and incubated with 25 μL of HLM and 10 μL of alamethicin (1 mg/mL in DMSO) at 37 °C. At 5, 60 and 120 min of incubation, 10 μL of UDPGA (100 mM in buffer) was added. For the SUL samples, 965 μL of the supernatant originating from the Phase I samples was transferred and incubated with 25 μL of HLCYT (20 mg/mL). At 5, 60 and 120 min of incubation, 10 μL of PAPS (10 mM in buffer) was added. Negative control samples were prepared for both sets of samples by omitting BPS and the cofactor (UDPGA and PAPS). Another sample was prepared by subjecting BPS only to Phase II metabolism (GLU or SUL respectively). Positive control samples were prepared by spiking the reaction mixture with 4-NP (10 mM) as the substrate. The reaction was stopped after 3 h incubation by adding 250 μL of ice-cold ACN with 1% (v/v) formic acid and the internal standard valsartan-d3, at a concentration of 1 μg/mL.

Before the concentration step, all the samples were centrifuged for 5 min at 8000 rpm. The supernatant was then transferred to a clean glass tube and evaporated under nitrogen at 37 °C. Subsequently, samples were reconstituted in 200 μL of a 10:90 (v/v) MeOH/water solution.

2.3. LC-QTOF-MS analytical method

Analysis of the samples was conducted using an Agilent 1290 Infinity UPLC coupled to an Agilent 6530 Accurate-Mass QTOF (Agilent, Santa Clara, US). Chromatographic separation was achieved on a Poroshell 120 C18 column (50 x 3 mm; 2.7 μm particle size, Agilent), using a mobile phase composed of ultra-pure water with 1 mM NH_4F (A) and MeOH with 1 mM NH_4F (B). The injection volume was 5 μL and column temperature was kept constant at 40 °C. All samples were analysed in positive and negative ionisation mode. Both methods used the same chromatographic conditions; the run started with an isocratic part for 2 min at 10% B, then a gradient was applied to raise the percentage of B to 50% at 12.8 min. B remained at this percentage until 13 min. A second gradient was used to increase the level of B to 95% at 14 min. Subsequently, the column was rinsed with 95% B for six minutes and re-equilibrated at 10% B for five minutes. The flow rate was 0.4 mL/min.

For all analyses, the QTOF-MS instrument was operated in the 2 GHz (extended dynamic range) mode, providing a Full Width at Half Maximum (FWHM) resolution of approximately 5400 at m/z 112.9856 and 11,000 at m/z 1033.9881. The ions 121.0508 and 922.0097 for positive mode and 112.9856 and 980.0164 for negative mode were selected for a continuous recalibration during the run to ensure mass accuracy. The eluting compounds were ionised using Agilent Jet-Stream electrospray ionisation (AJS-ESI) under the following parameters for both positive and negative ionisation. Drying gas temperature was set at 300 °C and the flow was 8 L/min. The sheath gas temperature was 400 °C at a flow of 11 L/min. Nebulizer pressure was set at 25 psig. Capillary, nozzle and fragmentor voltages were set at 2000 V, 0 V and 120 V, respectively. Acquisition parameters were set for the m/z values

to range from 50 to 1000 at a scan rate of 4.00 spectra/s for MS and 6.67 spectra/s for MS/MS spectra, respectively. Signals were detected using a data-dependent acquisition method (auto MS/MS) with an isolation width of 4 amu. Collision energy was applied at 10 and 20 V to the selected precursor ions. An active exclusion of 0.1 min was set to prevent repetitive acquisition of MS/MS spectra for the same precursor ion. Additional injections in targeted MS/MS mode using selected precursor ions were executed to obtain fragmentation spectra of a higher quality for some metabolites. Collision energies and isolation width were the same as for the data-dependent acquisition method. All data were stored in centroid mode.

2.4. Data processing and analysis

This study used two complementary workflows for an enhanced confidence in the detection and identification of the *in vitro* metabolites (Mortelé et al., 2018). Firstly, a suspect screening approach was applied. A list of possible metabolites with their molecular formula, exact mass and structure was stored as a csv-database. This suspect list was generated by performing a literature search for *in vitro* metabolism and degradation of BPS (Gramec Skledar et al., 2015; Skledar and Mašič, 2016; Grignard et al., 2012; Le Fol et al., 2015; Skledar et al., 2016). Further BPS metabolites were predicted by analogy with the metabolites or degradation products of other bisphenols described in literature (Cao et al., 2016, 2013; Gramec Skledar and Peterlin Mašič, 2016; Salomatova et al., 2015; Wang et al., 2014). The *m/z* values resulting from the suspect screening approach were extracted using the *Find by Formula* algorithm in the MassHunter Qualitative Analysis Workstation Software (version B.07.00) (Agilent Technologies).

Secondly, an untargeted screening approach was conducted using the open-source software packages MZmine (version 2.30) and R (version 3.4.1.) (Pluskal et al., 2010; R Development Core Team, 2011). MZmine allowed a comparison between different sample sets for detection and differentiation of MS features in the generated *in vitro* samples from the control samples through different algorithms. Initially, *m/z* features were detected using the centroid algorithm, subsequently followed by a chromatogram-building step. The resulting chromatograms were deconvoluted employing the noise amplitude algorithm. Next, chromatograms were deisotoped, with the lowest *m/z* value remaining as the representative isotope. During this step, peaks were also filtered according to their peak width: only peaks with a width within the range of 0.05 to 1 min were retained. Thereafter, the retained peaks were aligned across all samples using the Random Sample Consensus (RANSAC) algorithm. Ultimately, any possibly missed peaks were re-iteratively extracted using the *Same RT and m/z range gap filler* algorithm. The obtained *m/z* features were further processed using R, visualising them in a volcano plot (see Fig. SI-2.1). Based on this plot, *m/z* values were selected based on a fold-change of the intensity of the chromatographic peak in the replicate samples compared to their respective negative controls. A student *t*-test was employed to test the statistical significance of the fold-change. All features showing a fold-change > 10 and a p-value < 0.05 were selected. The *m/z* values resulting from the non-target screening workflow were extracted using the *Formula Prediction* algorithm in MZmine.

Identification of the potential metabolites resulting from both workflows was based on accurate mass and isotopic pattern in MS mode and the fragmentation pattern and accurate mass of the respective product ions. The following criteria were applied: (a) a maximal mass variation of ± 10 ppm between the measured and theoretical parent ions; (b) a maximal mass variation of ± 25 ppm for product ions; (c) the measured isotope pattern matched the predicted one with an isotope pattern score of at least 70%; (d) the measured double bond equivalent (DBE) values matched the postulated structure; (e) identified metabolites were not present in any of the respective negative control samples; and (f) identified metabolites had to be present in at least two out of three replicates. Where possible, identification of the metabolites was confirmed by injection of the analytical reference standard on the LC-QTOF-MS. Identified metabolites were assigned a certain level of confidence in this identification, following the scale and requirements as proposed by Schymanski et al., in order to communicate this confidence clearly (Schymanski et al., 2014).

3. Results and discussion

3.1. Experimental quality controls

For Phase I experiments, phenacetin was used as a positive control. Two metabolites of phenacetin were monitored; resulting from oxidative dealkylation (N-(4-hydroxyphenyl)-acetamide (P1)) and hydroxylation (N-(4-ethoxy-2-hydroxyphenyl)-acetamide (P2)), respectively (chromatograms and MS/MS spectra can be found in SI-3.1). As a positive control for Phase II experiments, the formation of 4-nitrophenol glucuronide and 4-nitrophenol sulfate was monitored (see SI-3.2 and SI-3.3). The detection and identification of these metabolites in the respective positive control samples confirmed the successful performance of the experimental assays used in our study. Using the response of the internal standards throughout the analyses, the instrumental variation was assessed at about 18.5% (relative standard deviation on the area of the chromatographic peak in all samples). The identified metabolites were crosschecked with their respective negative control samples to eliminate false positive results.

3.2. Bisphenol S

BPS was detected in the negative ionisation mode as the deprotonated molecular ion ($[M-H]^-$, *m/z* 249.0235) and eluted at 9.16 min (Fig. 1-I and -II). The fragmentation pattern of BPS is shown in Fig. 1-III. The product ion with the highest abundance was *m/z* 108.0232, corresponding to the $[M-H\text{-}C_6H_5O_2S]$- ion (Zhao et al., 2016). Other fragments were *m/z* 155.9891, *m/z* 139.9932, *m/z* 92.0283 and 63.9600, corresponding to the ions $[M-H\text{-}C_6H_5O]$-, $[M-H\text{-}C_6H_5O_2]$-, $[M-H\text{-}C_6H_5O_3S]$ and $[SO_2]$-, respectively (Holcapek et al., 2010; Zhao et al., 2016). Identification of BPS was confirmed with the injection of an analytical reference standard.

Incubation of BPS with HLMs and HLCYT has resulted in the formation of two Phase I metabolites and four Phase II metabolites (Table 1). All identified metabolites were detected in negative ionisation mode, in all relevant replicates. Their tentative structures were postulated by the mass shift of the biotransformation product from the mass of the parent compound BPS. Interpretation of the MS/MS spectra of the metabolites was based on the MS/MS spectrum of BPS, in particular on the appearance of common fragment ions in both spectra, making the fragment identification in the spectra of the metabolites more reliable. In positive ionisation mode, only $[NH_4^+]$ adducts were identified of metabolites that were already detected in negative ionisation mode. All metabolites were identified both by the suspect and the untargeted screening approach. A summary of all identified metabolites with their corresponding confidence levels as proposed by Schymanski et al. can be found in Table 1 (Schymanski et al., 2014). All metabolites included in this table met the requirements listed under 2.4. Chromatograms and MS/MS spectra of all identified metabolites can be found in Fig. 2.

3.3. Phase I metabolite identification

3.3.1. Hydroxy-BPS (M1)

The hydroxylated BPS (M1) eluted at 7.42 min and was detected as the deprotonated molecular ion ($[M-H]^-$, *m/z* 265.0158). The fragment ions *m/z* 63.9641, 93.0350, 108.0226 and 156.9963 were observed both for hydroxyl-BPS and BPS. The *m/z* 171.9819 corresponds to fragment ion $[C_6H_4O_4S]^-$, indicating a hydroxylation of one of the phenolic rings. Based on the acquired MS/MS data, the exact position of

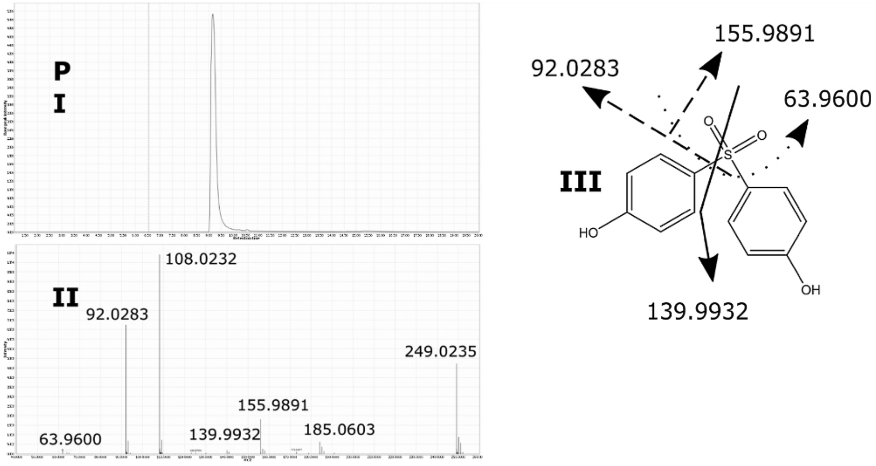
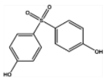
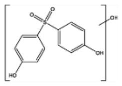
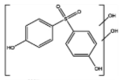
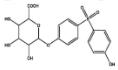
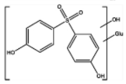
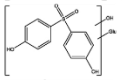
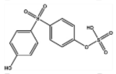
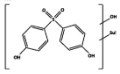


Fig. 1. Chromatogram (I), MS/MS spectrum at collision energy of 20 V (II) and fragmentation pattern (III) of BPS.

Table 1
Summary of all identified features (P – parent compound; M1-6 – metabolites). Included are confidence levels, tentative formulas, double bond equivalent (DBE), measured *m/z*, theoretical *m/z*, retention times (RT), mass errors (in ppm), diagnostic product ions and proposed structures for BPS and its *in vitro* metabolites.

Metabolite ID	Confidence level	RT [min]	Precursor ion (MS) Measured *m/z* $[M-H]^-$	Precursor ion (MS) Theoretical *m/z* $[M-H]^-$	Error [ppm]	Tentative formula	DBE	Diagnostic product ions (MS/MS) *m/z* $[M\text{-}H]^-$	Proposed structure
P	L1	9.16	249.0235	249.0227	3.21	$C_{12}H_{10}O_4S$	8	63.9600, 92.0283, 108.0232, 139.9932, 155.9891, 185.0603	
M1	L3	7.42	265.0158	265.0176	−6.79	$C_{12}H_{10}O_5S$	8	63.9641, 93.0350, 108.0226, 156.9963, 171.9819	
M2	L3	5.95	281.0102	281.0125	−8.18	$C_{12}H_{10}O_6S$	8	108.0215, 172.9952	
M3	L1	3.91	425.0538	425.0548	−2.35	$C_{18}H_{18}O_{10}S$	10	44.9977, 175.02782, 249.0266	
M4-A	L3	5.92	441.0500	441.0497	0.68	$C_{18}H_{18}O_{11}S$	10	44.9982, 175.0233, 265.0173	
M4-B	L4	3.71	441.0502	441.0497	1.13	$C_{18}H_{18}O_{11}S$	10	/	
									
M5	L2b	6.62	328.9763	328.9795	−9.73	$C_{12}H_{10}O_7S_2$	8	108.0238, 155.9910 185.0555, 249.0269	
M6-A	L3	6.22	344.9774	344.9744	8.70	$C_{12}H_{10}O_8S_2$	8	108.0229, 156.9971, 265.0205	
M6-B	L3	5.93	344.9773	344.9744	8.41	$C_{12}H_{10}O_8S_2$	8	108.0236, 156.9978, 265.0231	

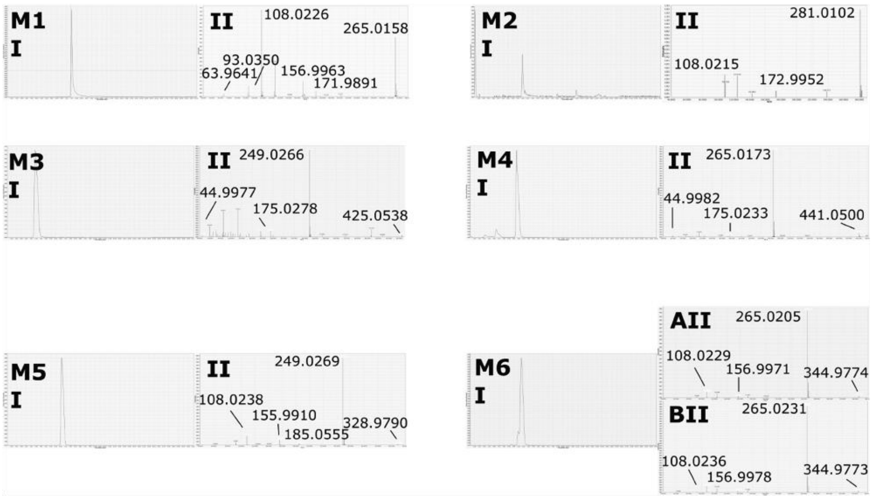


Fig. 2. Chromatograms (I) and MS/MS spectra at collision energy of 20 V (II) of BPS metabolites (M1-M6).

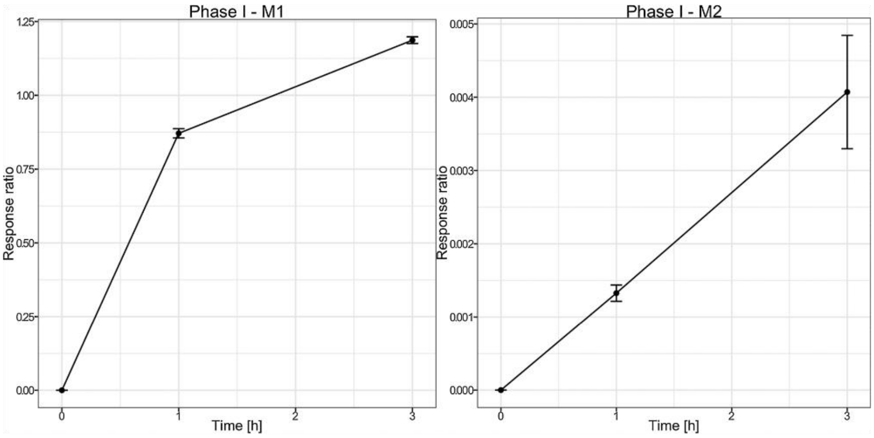


Fig. 3. Plots of the response ration *versus* time [h] for the Phase I oxidative metabolites (M1, M2) of BPS.

the hydroxyl-group could not be determined, but regarding the chemical structure of BPS and published literature, it can be assumed that hydroxylation is taking place in the *ortho*-position of one of the phenolic rings (Gramec Skledar and Peterlin Mašič, 2016; Grignard et al., 2012; Skledar et al., 2016). When plotting the response ratio of the signal (the peak area of the metabolite divided by the peak area of the internal standard valsartan-d3) *versus* the time (see Fig. 3), there is a strong increase of hydroxy-BPS after 1 h of Phase I incubation. After 3 h, there is still an increase visible, but the slope of the curve is lower. In the Phase II tier (both GLU and SUL); M1 was detected in all samples that were subjected to Phase I in advance, while in the samples that were only subjected to Phase II, M1 was not present.

3.3.2. *Dihydroxy-BPS (M2)*

Dihydroxy-BPS (M2) was detected as the deprotonated molecular ion ($[M-H]^-$, *m/z* 281.0152) at 5.95 min. The fragment ion *m/z* 108.0215 was observed both for this metabolite and BPS, corresponding to $[M-H\text{-}C_6H_5O_2S]^-$. Similar to M1, the fragment ion *m/z* 172.9952 was observed for M2, indicating mono-hydroxylation of both phenolic rings. No fragment was detected showing two hydroxylations taking place on the same phenolic ring. Based on the acquired MS/MS data, it was not possible to define the exact position of hydroxylation on the phenolic rings. In the plot showing the response ratio of the signal of M2 *versus* the time (see Fig. 3), a linear rising trend during Phase I incubation is visible. In Phase II incubation however, M2 was not detected.

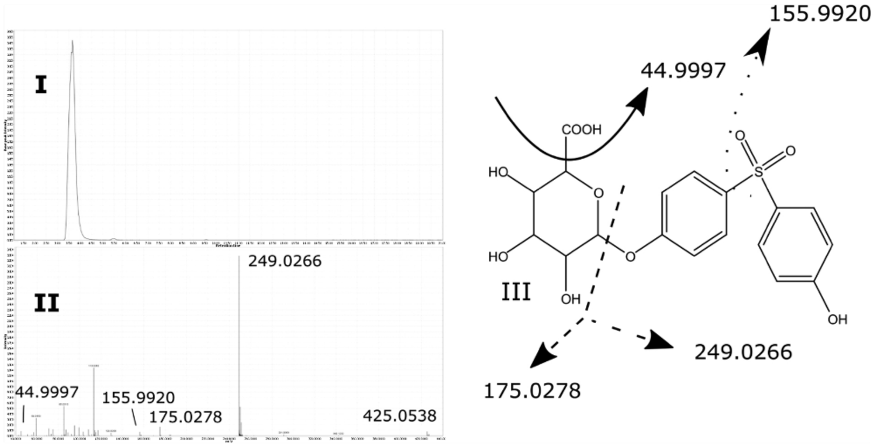


Fig. 4. Chromatogram (I), MS/MS spectrum at collision energy of 20 V (II) and fragmentation pattern (III) of BPS-glucuronide.

3.4. Phase II metabolite identification

3.4.1. BPS-glucuronide (M3)

M3 eluted at 3.91 min and was identified as the deprotonated molecular ion ($[M-H]^-$, m/z 425.0538). The mass difference of 176.0303 Da from the parent compound corresponds to the conjugation with glucuronic acid. The ion with m/z 249.0266 was observed in the MS/MS spectrum of M3 and corresponds to the deprotonated molecular ion $[M-H]^-$ of BPS. Based on the fragmentation pattern, the mass accuracy (0.94 ppm mass error), an obtained DBE of 10 and the isotope pattern, M3 was identified as BPS-glucuronide. This identification was confirmed by analysis of a BPS-glucuronide analytical reference standard (chromatogram, MS/MS spectrum and fragmentation pattern can be found in Fig. 4).

3.4.2. Hydroxy-BPS-glucuronide (M4)

There were two peaks found for the hydroxylated glucuronidation products (M4-A and M4-B) of BPS, probably due to different isomers ($[M-H]^-$, m/z 441.0500). The peak at 5.92 min (M4-A) showed the highest intensity and was the only peak for which MS/MS spectra were available. In correspondence with the deprotonated molecular ion of M1, a fragment ion with m/z 265.0173 was detected for M4-A, indicating hydroxylation of BPS. The fragments with m/z 44.9982 and 175.0233 were observed in the MS/MS spectra of both M4-A and M3, thus confirming the presence of the glucuronide entity for M4-A. The peak at 3.71 min (M4-B) was not fragmented, but based on the mass error, isotope pattern and the obtained DBE of 10, this peak was identified as an isomer of M4-A at a lower confidence level, L4. Hydroxy-BPS-glucuronide was only detected in the replicates for GLU that were first subjected to Phase I metabolism. Although M4 is a more polar molecule than M3, one isomer, M4-A (RT 5.92 min), elutes later in comparison to M3. This phenomenon does not take place for the other isomer, M4-B (RT 3.71 min) and could be explained by intramolecular hydrogen bonding (Grosse and Letzel, 2007; Skledar et al., 2016). Since only one peak was detected for hydroxy-BPS, regarding the structure of BPS and literature, it was assumed that hydroxylation was taking place on the *ortho*-position of the phenolic rings. This implies that there are two possible isomers for M4; one is conjugated with glucuronic acid on the same phenolic ring that was hydroxylated, the second has the glucuronide entity on the other phenolic ring. As such, only the first isomer is capable of forming an intramolecular hydrogen bond and thus shows a higher retention time.

3.4.3. BPS-sulfate (M5)

M5 eluted at 6.62 min and was detected as the deprotonated molecular ion ($[M-H]^-$, m/z 328.9763). The fragment m/z 249.0269 was observed in the MS/MS spectrum of M5 and corresponds to the deprotonated molecular ion of BPS. The mass difference between the deprotonated molecule of M5 and that of BPS corresponds to the neutral loss of $[SO_3]$, which is diagnostic for a molecule containing a sulfate group (Holcapek et al., 2010). Also fragment ions m/z 108.0238 and 155.9910 were detected in the MS/MS spectra of both M5 and BPS. Based on the MS/MS spectrum, mass accuracy (8.21 ppm mass error), an obtained DBE of 9 and the isotope pattern, M5 was identified as BPS-sulfate at confidence level L2b (Schymanski et al., 2014).

3.4.4. Hydroxy-BPS-sulfate (M6)

Two hydroxylated and sulfated metabolites (M6-A and M6-B) were detected as the deprotonated molecular ion ($[M-H]^-$, m/z 344.9774 and m/z 344.9773 respectively). Retention times were 6.22 min and 5.93 min for M6-A and M6-B, respectively. These peaks were not baseline-separated. The m/z 108.0229 was a common fragment ion observed for M6 and for BPS. In correspondence with the deprotonated molecule of M1, a fragment ion with m/z 265.0205 was observed for M6, indicating hydroxylation. The mass difference between this fragment and the deprotonated molecule of M6 corresponds to the neutral loss of $[SO_3]$. In analogy to M4, the M6 metabolite was also only detected in the SUL replicates that were first subjected to Phase I metabolism.

3.5. Biotransformation of BPS

In the present study, Phase I and Phase II *in vitro* human metabolism of BPS was examined. The detection of hydroxy-BPS (M1), BPS-glucuronide (M3) and BPS-sulfate (M5) is in line with previous studies (Le Fol et al., 2015; Skledar et al., 2016). However, our study, using Q-TOF high mass accuracy screening with two complementary workflows for data processing, has identified and structurally elucidated three additional metabolites for the first time, namely dihydroxy-BPS (M2), two isomers of hydroxy-BPS-glucuronide (M4) and two isomers of hydroxy-BPS-sulfate (M6). Possible cleavage products of BPS were included in the aforementioned suspect list, but none of these products were

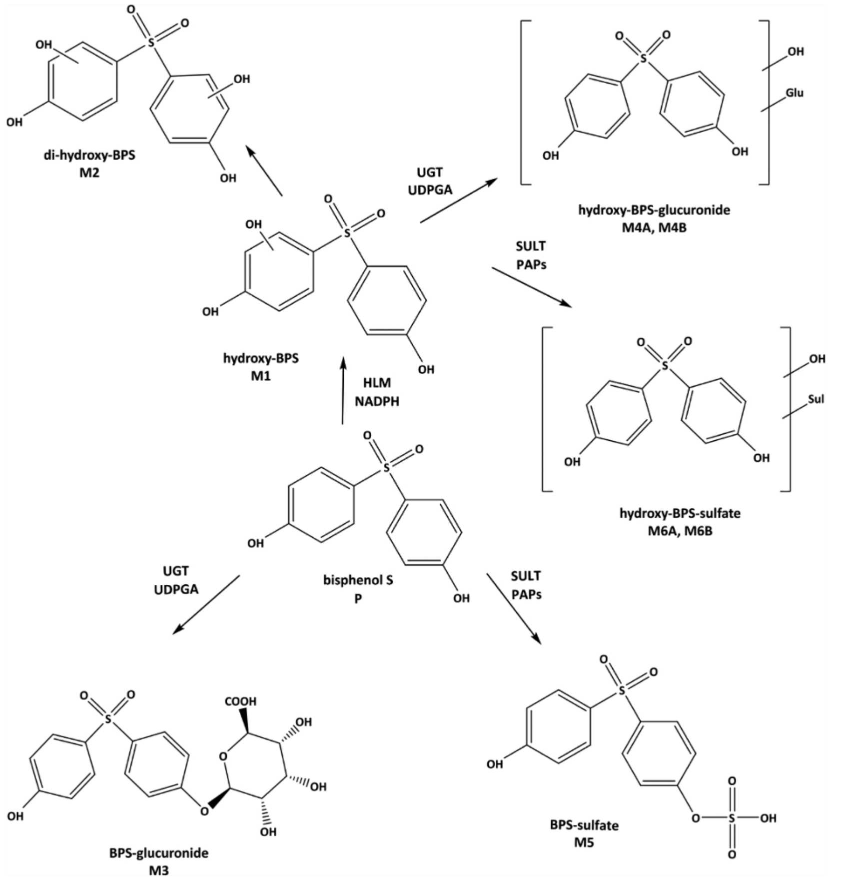


Fig. 5. Proposed *in vitro* metabolic pathway for BPS. The arrows are marked with the enzymes and cofactors responsible for the reaction.

detected in the samples generated in this experiment.

Comparable to other studies, the hydroxylated BPS (M1) is the major oxidative metabolite of our *in vitro* Phase I system. This *in vitro* metabolism experiment also generated a minor oxidative metabolite with *m/z* 281.0152 (M2). This *m/z* has been observed in a study by Skledar et al., but the identification and elucidation of this metabolite was impossible due to the use of low resolution MS (Skledar et al., 2016). Based on the acquired MS/MS data in this study, this feature could be confirmed for the first time as the dihydroxy-BPS metabolite, at a confidence level L3 (Schymanski et al., 2014).

The Phase II tier of this study yielded the glucuronidated and sulfated conjugation products of BPS, in accordance with other *in vitro* metabolism studies for this compound (Gramec Skledar et al., 2015; Le Fol et al., 2015; Skledar et al., 2016). Identification of BPS-glucuronide was confirmed by the analysis of an analytical reference standard. Moreover, also hydroxylated conjugated metabolites were detected for both GLU and SUL. For M4, two peaks appeared at different retention times. Identification of the largest peak, M4-A, could be supported by acquired MS/MS data. Despite of the absence of fragmentation data for M4-B, the pattern of the peaks is the same as was reported in Skledar et al. This thus suggested that glucuronidation could occur on different hydroxyl-groups (Skledar et al., 2016). Also M6 gave rise to two peaks at different retention times, suggesting formation of two isomers. For both features, MS/MS data was acquired and confirmed the postulated structures, which are reported here for the first time. A di-glucuronide BPS metabolite has been reported by Le Fol et al., generated by a zebrafish hepatic cell model (Le Fol et al., 2015). However, both our study and another human *in vitro* metabolism study did not detect any di-conjugated metabolites (Skledar et al., 2016).

The calculated response ratios of the two hydroxylated metabolites throughout the incubation in the Phase I *in vitro* system are showing that M1 is the major Phase I metabolite. The lower slope of the plot of the response ratio *versus* the time for M1 between 1 h and 3 h may suggest that M1 could act also as the intermediate for formation of M2. Given that the curve for M1 is persistently rising despite the lower slope, it implies that M2 is formed to a minor extent. When BPS was subsequently subjected to Phase I and Phase II *in vitro* metabolism, hydroxy-BPS-glucuronide and hydroxy-BPS-sulfate were detected. In samples that were subjected only to Phase II metabolism, these metabolites were absent. Since the signal for M1 was low in Phase II samples (both GLU and SUL), it can be assumed that the hydroxylated metabolite is likely to be conjugated. In line with other bisphenols, BPS also appears to be subjected to direct conjugation extensively (Gramec Skledar and Peterlin Mašič, 2016; Li et al., 2013). Since BPS itself is a quite polar molecule and it possesses two phenol groups susceptible to conjugation, it seems rather unlikely for hydroxylation to take place after conjugation of BPS. The design of this study allowed the proposal for an expanded human *in vitro* metabolism pathway, which can be found in Fig. 5.

Based on established methods developed for other bisphenols, it is common in current biomonitoring studies to measure the total amount of the parent compound BPS in urine after deconjugation (Liao et al., 2012a; Thayer et al., 2016; Yang et al., 2014; Zhou et al., 2014). The *in vivo* pharmacokinetics of free BPS and total BPS (after deconjugation) in humans have been investigated recently by Oh et al. (Oh et al., 2018). Another study has evaluated the metabolism of BPS in mice after oral administration (Song et al., 2017). However, the latter targeted only BPS-glucuronide and BPS-sulfate. Additionally, a difference in metabolism between species might be possible. Our study revealed that hydroxylated BPS and the glucuronidated or sulfated conjugates of hydroxylated BPS should also be considered as potential additional biomarkers. They could be an addition to the monitoring of the total amount of the parent compound BPS after deconjugation, which can be prone to external contamination. In order to reliably assess BPS exposure in the future, *in vivo* pharmacokinetics of the *in vitro* metabolites proposed in this study should be investigated.

4. Conclusions

This study identified and elucidated two Phase I *in vitro* human metabolites for BPS and four Phase II metabolites. The major Phase I metabolite was found to be hydroxy-BPS. Three metabolites were identified and structurally elucidated for the first time: dihydroxy-BPS, two isomers of hydroxy-BPS-glucuronide and two isomers of hydroxy-BPS-sulfate. As such, our study can contribute to a reliable assessment of BPS exposure in future biomonitoring studies by proposing these new identified *in vitro* metabolites as potential additional biomarkers, for which *in vivo* pharmacokinetics should be further evaluated.

Conflicts of interest

The authors declare they have no conflicts of interest.

Acknowledgements

This work was supported by project MASSTWIN (European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 692241). Celine Gys acknowledges a PhD fellowship from Research Foundation Flanders (project G0E5216N). Ana Kovačič acknowledges the Young researcher grant and the Slovenian Research Agency (Program Group P1-0143 and Projects L1-7544, N1-0047, J1-8147 and J2-8162). Carolin Huber acknowledges the travel fund granted by NTNU. Foon Yin Lai acknowledges an EU H2020 Marie Skłodowska-Curie Individual Fellowship (project no. 749845 APOLLO).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2018.05.034.

References

Asimakopoulos, A.G., Xue, J., De Carvalho, B.P., Iyer, A., Abualnaja, K.O., Yaghmoor, S.S., Kumosani, T.A., Kannan, K., 2016. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. Environ. Res. (150), 573–581. http://dx.doi.org/10.1016/j.envres.2015.11.029.

Brandon, E.F.A., Raap, C.D., Meijerman, I., Beijnen, J.H., Schellens, J.H.M., 2003. An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. Toxicol. Appl. Pharmacol. 1 (89), 233–246. http://dx.doi.org/10.1016/S0041-008X(03)00128-5.

Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53. http://dx.doi.org/10.1016/j.aca.2015.12.034.

Cao, G., He, R., Cai, Z., Liu, J., 2013. Photolysis of bisphenol S in aqueous solutions and the effects of different surfactants. React. Kinet. Mech. Catal. 1 (09), 259–271. http://dx.doi.org/10.1007/s11144-013-0553-6.

Cao, G., Zhang, J., Liu, B., 2016. Effects of nitrite anions and ammonium cations on the photolysis of bisphenol S in nitrate solution. Desalin. Water Treat. 57, 25686–25695. http://dx.doi.org/10.1080/19443994.2016.1157044.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - a review. Environ. Sci. Technol. 50, 5438–5453. http://dx.doi.org/10.1021/acs.est.5b05387.

EFSA Panel on Food Contact Materials, 2015. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13, 3978. http://dx.doi.org/10.2903/j.efsa.2015.3978.

Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol a story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril. 103, 11–21. http://dx.doi.org/10.1016/j.fertnstert.2014.11.005.

Erratico, C., Negreira, N., Norouzizadeh, H., Covaci, A., Neels, H., Maudens, K., van Nuijs, A.L.N., 2015. *In vitro* and *in vivo* human metabolism of the synthetic cannabinoid AB-CHMINACA. Drug. Test. Anal. 7, 866–876. http://dx.doi.org/10.1002/dta.1796.

European Commission Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A (BPA) in plastic infant feeding bottles. Text with EEA relevance, 2011. 3–4. https://doi.org/http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R0321.

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. http://dx.doi.org/10.1016/j.etap.2016.09.014.

Gramec Skledar, D., Troberg, J., Lavdas, J., Peterlin Mašič, L., Finel, M., 2015. Differences in the glucuronidation of bisphenols F and S between two homologous human UGT enzymes, 1A9 and 1A10. Xenobiotica (45), 511–519. http://dx.doi.org/10.3109/00498254.2014.999140.

Grignard, E., Lapenna, S., Bremer, S., 2012. Weak estrogenic transcriptional activities of bisphenol A and bisphenol S. Toxicol. In Vitro 26, 727–731. http://dx.doi.org/10.1016/j.tiv.2012.03.013.

Grosse, S., Letzel, T., 2007. Liquid chromatography/atmospheric pressure ionization mass spectrometry with post-column liquid mixing for the efficient determination of partially oxidized polycyclic aromatic hydrocarbons. J. Chromatogr. A 1139, 75–83. http://dx.doi.org/10.1016/j.chroma.2006.10.086.

Holcapek, M., Jirásko, R., Lísa, M., 2010. Basic rules for the interpretation of atmospheric pressure ionization mass spectra of small molecules. J. Chromatogr. A 1217, 3908–3921. http://dx.doi.org/10.1016/j.chroma.2010.02.049.

Kang, J.S., Choi, J.-S., Kim, W.-K., Lee, Y.-J., Park, J.-W., 2014. Estrogenic potency of bisphenol S, polyethersulfone and their metabolites generated by the rat liver S9 fractions on a MVLN cell using a luciferase reporter gene assay. Reprod. Biol. Endocrinol. 12, 102. http://dx.doi.org/10.1186/1477-7827-12-102.

Lai, F.Y., Erratico, C., Kinyua, J., Mueller, J.F., Covaci, A., van Nuijs, A.L.N., 2015. Liquid chromatography-quadrupole time-of-flight mass spectrometry for screening in vitro drug metabolites in humans: investigation on seven phenethylamine-based designer drugs. J. Pharm. Biomed. Anal. 114, 355–375. http://dx.doi.org/10.1016/j.jpba.2015.06.016.

Le Fol, V., Aït-Aïssa, S., Cabaton, N., Dolo, L., Grimaldi, M., Balaguer, P., Perdu, E., Debrauwer, L., Brion, F., Zalko, D., 2015. Cell-specific biotransformation of benzophenone-2 and bisphenol-s in zebrafish and human in vitro models used for toxicity and estrogenicity screening. Environ. Sci. Technol. 49, 3860–3868. http://dx.doi.org/10.1021/es505302c.

Li, M., Yang, Y., Yang, Y., Yin, J., Zhang, J., Feng, Y., Shao, B., 2013. Biotransformation of bisphenol AF to its major glucuronide metabolite reduces estrogenic activity. PLoS One (8), 1–11. http://dx.doi.org/10.1371/journal.pone.0083170.

Liao, C., Liu, F., Alomirah, H., Loi, V.D., Mohd, M.A., Moon, H.B., Nakata, H., Kannan, K., 2012a. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ. Sci. Technol. 46, 6860–6866. http://dx.doi.org/10.1021/es301334j.

Liao, C., Liu, F., Kannan, K., 2012b. bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol A residues. Environ. Sci. Technol. 46, 6515–6522. http://dx.doi.org/10.1021/es300876n.

Mortelé, O., Vervliet, P., Gys, C., Degreef, M., Cuykx, M., Maudens, K., Covaci, A., van Nuijs, A.L.N., Lai, F.Y., 2018. In vitro phase I and phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. J. Pharm. Biomed. Anal. http://dx.doi.org/10.1016/j.jpba.2018.02.032.

Negreira, N., Erratico, C., Kosjek, T., van Nuijs, A.L.N., Heath, E., Neels, H., Covaci, A., 2015. In vitro phase I and phase II metabolism of α-pyrrolidinovalerophenone (α-PVP), methylenedioxypyrovalerone (MDPV) and methedrone by human liver microsomes and human liver cytosol. Anal. Bioanal. Chem. 407, 5803–5816. http://dx.

doi.org/10.1007/s00216-015-8763-6.

Oh, J., Choi, J.W., Ahn, Y.-A., Kim, S., 2018. Pharmacokinetics of bisphenol S in humans after single oral administration. Environ. Int. 112, 127–133. http://dx.doi.org/10.1016/j.envint.2017.11.020.

Pluskal, T., Castillo, S., Villar-Briones, A., Orešič, M., 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinform. 11. http://dx.doi.org/10.1186/1471-2105-11-395.

R Development Core Team, R., 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computinghttp://dx.doi.org/10.1007/978-3-540-74686-7.

Salomatova, V., Pozdnyakov, I., Sherin, P., Grivin, V., Plyusnin, V., 2015. Photochemistry of bisphenol F in aqueous solutions: a mechanistic study. J. Photochem. Photobiol. A Chem. 305, 45–50. http://dx.doi.org/10.1016/j.jphotochem.2015.03.008.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. (48), 2097–2098. http://dx.doi.org/10.1021/es5002105.

Skledar, D.G., Schmidt, J., Fic, A., Klopčič, I., Trontelj, J., Dolenc, M.S., Finel, M., Mašič, L.P., 2016. Influence metabolism on endocrine activities of bisphenol S. Chemosphere 157, 152–159. http://dx.doi.org/10.1016/j.chemosphere.2016.05.027.

Song, Y., Xie, P., Cai, Z., 2017. Metabolism of bisphenol S in mice after oral administration. Rapid Commun. Mass Spectrom. http://dx.doi.org/10.1002/rcm.8051.

Sun, Q., Wang, Y., Li, Y., Ashfaq, M., Dai, L., Xie, X., Yu, C.P., 2017. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ. Pollut. 225, 542–549. http://dx.doi.org/10.1016/j.envpol.2017.03.018.

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ. Int. (83), 107–115. http://dx.doi.org/10.1016/j.envint.2015.06.008.

Thayer, K.A., Taylor, K.W., Garantziotis, S., Schurman, S.H., Kissling, G.E., Hunt, D., Herbert, B., Church, R., Jankowich, R., Churchwell, M.I., Scheri, R.C., Birnbaum, L.S., Bucher, J.R., 2016. Bisphenol a, bisphenol s, and 4-hydro xyphenyl 4-isoprooxyphenyl sulfone (bpsip) in urine and blood of cashiers. Environ. Health Perspect. 124, 437–444. http://dx.doi.org/10.1289/ehp.1409427.

Van den Eede, N., Maho, W., Erratico, C., Neels, H., Covaci, A., 2013. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. Toxicol. Lett. 223, 9–15. http://dx.doi.org/10.1016/j.toxlet.2013.08.012.

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287. http://dx.doi.org/10.1021/tx025548t.

Wang, X., Ma, J., Wang, Z., Guo, R., Hu, X., 2014. Aqueous phototransformation of bisphenol S: the competitive radical-attack pathway to p-hydroxybenzenesulfonic acid. Water Sci. Technol. 70, 540–547. http://dx.doi.org/10.2166/wst.2014.257.

Wu, L.H., Zhang, X.M., Wang, F., Gao, C.J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Sci. Total Environ. 615, 87–98. http://dx.doi.org/10.1016/j.scitotenv.2017.09.194.

Yang, Y., Guan, J., Yin, J., Shao, B., Li, H., 2014. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 112, 481–486. http://dx.doi.org/10.1016/j.chemosphere.2014.05.004.

Zhang, H., Gao, N., Tian, X., Liu, T., Fang, Y., Zhou, J., Wen, Q., Xu, B., Qi, B., Gao, J., Li, H., Jia, L., Qiao, H., 2016. Content and activity of human liver microsomal protein and prediction of individual hepatic clearance in vivo. Sci. Rep. 5, 17671. http://dx.doi.org/10.1038/srep17671.

Zhao, H., Xiang, L., Li, J., Yang, Z., Fang, J., Zhao, C., Xu, S., Cai, Z., 2016. Investigation on fragmentation pathways of bisphenols by using electrospray ionization orbitrap mass spectrometry. Rapid Commun. Mass Spectrom. 1901–1913. http://dx.doi.org/10.1002/rcm.7666.

Zhou, X., Kramer, J.P., Calafat, A.M., Ye, X., 2014. Automated on-line column-switching high performance liquid chromatography isotope dilution tandem mass spectrometry method for the quantification of bisphenol A, bisphenol F, bisphenol S, and 11 other phenols in urine. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 944, 152–156. http://dx.doi.org/10.1016/j.jchromb.2013.11.009.

3.2.2 Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics

The paper "*Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics*" by A. Kovačič, C. Gys, T. Kosjek, A. Covaci, E. Heath, was published in S*cience of the Total Environment* in February 2019. The study was performed in collaboration between two research groups, e.g. Department of Environmental Sciences, Jožef Stefan Institute, and Toxicological Center at the University of Antwerp, Belgium, under the supervision of Prof Dr E. Heath and Prof Dr Covaci. All the work regarding the paper, from conducting the experimental design to writing the manuscript, was equally divided between C. Gys (co-author) and myself.

The work aimed to investigate the photochemical degradation of BPF, BPS, and BPZ and determine their degradation kinetics and characterization of their TPs. Three independent experimental set-ups were performed based on (1) direct photolysis using UV irradiation, 2) cyclodextrin-enhanced photolysis, and (3) photo-Fenton reaction. This approach enabled for the first time a comparison of degradation efficiency and TP formation of under various conditions. The analyses of degradation kinetics (quantification) were carried out using GC-MS, while the LC-QTOF-MS was used for the detection and identification of TPs. The results showed the efficient removal of BPF, BPS, and BPZ from aqueous samples (>90%) of the photo-assisted treatment technologies. They also show that although degradation followed pseudo-first-order kinetics in all cases, the removal efficiency was dependent on the applied process, among which photo-Fenton resulted in the shortest half-lives and generated the highest number of TPs. The study identified 11 new and confirmed eight previously reported TPs.

The paper addresses three aims of the thesis; (1) to develop, optimize and validate analytical methods for simultaneous determination of bisphenol residues and identification of their TPs in aqueous samples (GC-MS and LC-MS/MS), (2) to assess the removal efficiency of bisphenols by photochemical and advance oxidation processes, and (3) to investigate bisphenol breakdown during photodegradation and advance oxidation process and identify their TPs. Findings presented in this paper contribute to a better understanding of the bisphenols fate during WWT and in the environment.

Science of the Total Environment 664 (2019) 595–604

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Photochemical degradation of BPF, BPS and BPZ in aqueous solution: Identification of transformation products and degradation kinetics

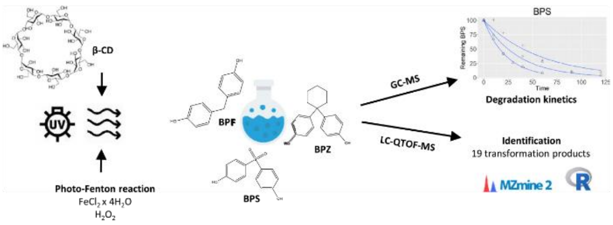
Ana Kovačič [a,b,1], Celine Gys [c,1], Tina Kosjek [a,b], Adrian Covaci [c,*], Ester Heath [a,b,**]

[a] Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[b] International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia
[c] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

HIGHLIGHTS

- Photodegradation of BPS, BPF, and BPZ follows a pseudo-first order kinetic profile.
- Removal efficiency of the bisphenol depends on the applied process.
- The photo-Fenton reaction results in the shortest half-lives.
- Eleven novel transformation products are identified.
- Insights into the photochemical behaviour of bisphenols are obtained.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 December 2018
Received in revised form 3 February 2019
Accepted 4 February 2019
Available online 05 February 2019

Editor: Damia Barcelo

Keywords:
Bisphenol
UV irradiation
Kinetics
Photodegradation
Transformation product

ABSTRACT

Bisphenols (BPs) are industrial chemicals that are used as monomers in the production of polycarbonate plastics and epoxy resins. These compounds can leach into the aqueous environment, where they can potentially have toxic effects. The aim of this study was to assess the photochemical degradation of three common bisphenols: BPF, BPS and BPZ in aqueous solution and determine their degradation kinetics and characterise their transformation products. Three independent experiments were performed based on: 1) direct photolysis using UV irradiation, 2) cyclodextrin-enhanced photolysis and 3) the photo-Fenton reaction. Analysis was performed using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography coupled to high-resolution quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). This approach enabled for the first time a comparison between various conditions of photochemical degradation, revealing to be an effective way of removing (>90%) BPF, BPS and BPZ from aqueous samples. In all cases, degradation followed a pseudo-first order kinetic profile, while removal efficiency and formation of transformation products depended on the applied process. The photo-Fenton process resulted in the shortest half-lives (16.1– 21.7 min) and generated the highest number of transformation products. Overall, in this study we identified 11 novels and eight previously reported TPs.

1. Introduction

The scientific and public concern over the risk posed by endocrine disrupting chemicals (EDCs) has increased significantly due to possible damage to reproductive systems in living organisms (Behr et al., 2011; Bittner et al., 2014; Gmurek et al., 2017; Salomatova et al., 2015). One of the most studied EDCs is bisphenol A (4-[2-(4-hydroxyphenyl)

---

[*] Corresponding author.
[**] Correspondence to: E. Heath, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
E-mail addresses: adrian.covaci@uantwerpen.be (A. Covaci), ester.heath@ijs.si (E. Heath).
[1] Joint first authors.

https://doi.org/10.1016/j.scitotenv.2019.02.064

propan-2-yl]phenol, BPA), which not only affects reproduction and development, but also has the potential to damage the neural, cardiovascular, metabolic and immune systems (Gramec Skledar and Peterlin Mašič, 2016; Le Fol et al., 2017; Siracusa et al., 2018; Skledar et al., 2016). BPA is a prominent industrial chemical used as a monomer in the production of polycarbonate plastics and epoxy resins, which are used in many applications, including food contact materials. Concern over exposure to BPA has led to restrictions and regulations regarding its use (Chen et al., 2016; EFSA, 2015; European Commission Regulation (EU), No 321/2011). Since BPA is currently the only regulated bisphenol, it is being replaced by BPA-alternatives in various applications; i.e. other bisphenols (BPs) such as bisphenol F (4,4′-dihydroxydiphenyl-methane, BPF), bisphenol S (4,4′-sulphonyldiphenol, BPS) and bisphenol Z (1,1-bis(4-hydroxyphenyl)cyclohexane, BPZ). These compounds have been detected in numerous human and environmental samples (Caballero-Casero et al., 2016; Chen et al., 2016; Liao et al., 2012a, 2012b). Studies show that the main source of BPs in the aqueous environment is an industrial effluent discharge. A number of publications report elevated concentrations of BPs in sewage sludge, municipal waste water influents and effluents, sediments and surface water (Caballero-Casero et al., 2016; Chen et al., 2016; Gmurek et al., 2017; Jin and Zhu, 2016; Karthikraj and Kannan, 2017; Lee et al., 2015; Sun et al., 2017). Despite these reports, limited information is available on their behaviour and toxicity although their structural similarity to BPA suggests they have the potential to exert similar effects. This has been observed in a number of studies (Bittner et al., 2014; Caballero-Casero et al., 2016; Gramec Skledar and Peterlin Mašič, 2016; Horan et al., 2018; Molina-Molina et al., 2013; Salomatova et al., 2015; Siracusa et al., 2018; Wang, 2007; Wu et al., 2018).

Removal of BPA by physical, (photo-)chemical and biological processes has been widely investigated (Daskalaki et al., 2011; Eio et al., 2014; Katsumata et al., 2004; Kondrakov et al., 2014; Li et al., 2006; Maroga Mboula et al., 2013; Neamţu and Frimmel, 2006; Zhang and Li, 2014) and the data suggest that photochemical and photocatalytic processes are more effective than biological treatments (Salomatova et al., 2015). These photochemical reactions involve exposure to artificial light (e.g. UV light generated by a low-pressure (LP) lamp) and are classified into two categories; i.e., direct photolysis, a process in which absorption of a photon causes structural changes in a target compound, and indirect photolysis, where the structural degradation of contaminants is facilitated by other sensitizing species (Wang et al., 2017). Photodegradation has been shown to be especially enhanced by the in situ formation of powerful oxidizing agents like hydroxyl radicals (•OH) or other reactive oxygen species (ROS) (De la Cruz et al., 2013). These processes are generally referred to as advanced oxidation processes (AOPs) and serve the purpose of destructing aqueous pollutants, preferably achieving complete mineralization to non-toxic products like carbon dioxide, water and mineral acids (Deng and Zhao, 2015; Erjavec et al., 2016; Liu et al., 2010; Zhang and Li, 2014). To date, there are only a few studies published that examined the photochemistry of BPs in water by direct photolysis or AOPs. Cyclodextrin (CD) enhanced photolysis was evaluated by adding the cyclic oligosaccharide to the reaction medium. This additive can catalyze the degradation due to the complexation of BPs within its cavity, while producing •OH radicals more readily (Cao et al., 2016; Horikoshi et al., 2008; Liu et al., 2010; Pozdnyakov et al., 2011; Salomatova et al., 2015; Wang, 2008; Wang et al., 2007; Xiao et al., 2007). Until now, a combination of UV irradiation with addition of H_2O_2 and Fe(II), the so-called photo-Fenton reaction, known to significantly enhance the decomposition of BPA (De la Cruz et al., 2013; Katsumata et al., 2004), has not been applied to BPA-alternatives. In addition, there are very few studies reporting the identification of transformation products (TPs) generated by these processes (Chen et al., 2016). This is important because TPs could potentially be more toxic than their parent compound.

The goals of this study were to determine the photochemical degradation kinetics of selected BPs using gas chromatography–mass spectrometry (GC–MS), and to identify their main TPs using liquid chromatography-high-resolution quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The experimental set-up involved exposing aqueous solutions (in ultra-pure water) of BPF, BPS and BPZ to UV irradiation, β-CD enhanced photolysis, and the photo-Fenton reaction. The findings of this study are expected to bring about a greater understanding of the behaviour of these BPs during photochemical degradation in water.

2. Materials and methods

2.1. Reagents and chemicals

The reagents BPF, BPS and BPZ (all >98%) were obtained from Sigma-Aldrich, (St. Louis, USA). Isotopically labelled $[^{13}C_{12}]$ BPF ($^{13}C_{12}$-BPF), BPS ($^{13}C_{12}$-BPS) and BPB ($^{13}C_{12}$-BPB) were purchased from CanSyn Chem. Corp. (Toronto, Canada). The chemical structures, IUPAC names, molecular weights, and typical applications are given in Table SM-1 of the Supplementary Material (SM). The derivatizing agent *N*,*O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA, ≥99.0%) and anhydrous pyridine (99.8%) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (ACN), ethyl acetate (EtAc) and methanol (MeOH) were purchased from J.T. Baker (Deventer, the Netherlands). All solvents were of analytical grade purity. Hydrochloric acid (37%, HCl) and formic acid (FA) were purchased from Sigma-Aldrich (St. Louis, USA) and hydrogen peroxide (H_2O_2, 30%) from AppliChem (Darmstadt, Germany). β-Cyclodextrin (β-CD, ≥99%) was obtained from Sigma-Aldrich (Steinheim, Germany) and ferrous chloride tetrahydrate ($FeCl_2 \times 4H_2O$, 98%) from Merck KGaA (Darmstadt, Germany). Stock solutions (5 mg mL^{-1}) of each compound were prepared in ACN, while internal standards were dissolved in MeOH at a final concentration of 1 μg mL^{-1}. Working solutions were prepared by appropriate dilution of the stock. All solutions were stored at 4 °C in the dark.

2.2. Set-up of the photochemical reactor

Laboratory scale photochemical degradation experiments were performed in a cylindrical glass immersion reactor (Fig. SM-1) and involved exposing 760 mL of an aqueous solution of each individual bisphenol in ultra-pure water. To study degradation kinetics and TP formation, initial concentrations of the BPs were 200 ng L^{-1} and 5 mg L^{-1}, respectively. Stock solutions were prepared using acetonitrile to avoid radical scavenging (Kosjek et al., 2013). Degradation of BPs was carried out applying three different conditions: UV light exposure (UV), UV with added β-cyclodextrin (UV/β-CD, which consists of seven D-glucose entities) and the photo-Fenton reaction (UV with added Fenton's reagent (H_2O_2/Fe^{2+}), PF). Where applicable, β-CD was added in a 1:1 M ratio according to the respective BP (Pozdnyakov et al., 2011; Wang, 2007; Wang et al., 2007; Xiao et al., 2007), while for the photo-Fenton reaction, Fe(II) and H_2O_2 were added to the reaction mixture in a 0.01:1 and 10:1 M ratio (Ruppert et al., 1993).

A monochromatic low-pressure mercury UV lamp ($\lambda = 254$ nm; 6 W) was used as the source of UV irradiation. The intensity of the emitted light, measured by ferrioxalate actinometry, was 8.91×10^{-7} Einstein s^{-1} (Union et al., 2005). The reactor solution was maintained at 23 °C (1 atm) using a water-cooled quartz immersion well. The content of the immersion reactor was continuously stirred using a magnetic stirrer bar (400 rpm).

For the UV and UV/β-CD experiments, samples were taken at 20, 40, 60 and 120 min; while for PF experiments, the sampling times were 10, 20, 30, 40 and 60 min. Two types of control samples were included in the experiment: blank and zero samples. Blanks were prepared in the same way as the exposed samples, only omitting the target compounds. Zero samples were prepared for each experiment in the same way as the samples but were not exposed to the UV light.

2.3. Degradation kinetics

2.3.1. Sample preparation

The kinetics of the degradation of the three BPs were determined by analysing two 350 mL aliquots that were collected after exposure (see 2.2). Upon sampling, a mixture of internal standards (V = 25 μL; 1 μg mL^{-1} of $^{13}C_{12}$-BPF, $^{13}C_{12}$-BPS and $^{13}C_{12}$-BPB) was added. The analytical method was adapted from the study by Česen et al. (2018) and involved acidifying the samples to pH 2 using 0.5 mL HCl (37%). The samples were then loaded onto Oasis Prime HLB SPE cartridges (divinylbenzene-N-vinylpyrrolidone copolymer sorbent, 60 mg, 3 mL; Waters, Massachusetts, USA) at a flow rate of 3 mL min^{-1} using a Vacuum Manifold (Agilent Technologies, Santa Clara, USA). After loading, the sorbent was dried under vacuum (−1.33 kPa) for 45 min. The elution step was performed using 5% FA in EtAc (3 × 0.6 mL). The solvent was removed under a gentle stream of nitrogen at 40 °C. Derivatization was performed with 50 μL of BSTFA and 50 μL of pyridine for 16 h at 80 °C prior to analysis.

Bisphenols are inherently ubiquitous in the laboratory environment due to their widespread application in various materials used in laboratory equipment (Caballero-Casero et al., 2016). Since BPF and BPS have been also observed as a contaminants in other studies, determining and minimising background contamination is essential (Caballero-Casero et al., 2016; Česen et al., 2016). For this reason, all glassware was cleaned following general procedures and a series of experimental blanks were prepared for each experiment. Procedural blanks were systematically included in each batch of analyses. Solvent blanks were analysed every 10th sample to evaluate potential carry-over. Since contamination with the analytes of interest was negligible, blank correction was not performed.

2.3.2. GC–MS analysis

Extracts were analysed on an Agilent 7890B series GC coupled to a 5977A single quadrupole MS (Agilent Technologies). 1 μL of sample was injected in the splitless mode at 250 °C. Separation was achieved using an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies) with helium as the carrier gas. The GC oven temperature program was as follows: 120 °C for 2 min, 120–250 °C at 25 °C min^{-1}, 250–300 °C at 10 °C min^{-1}, and 300 °C for 3 min. The total runtime was 16.2 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV and the analytes of interest were detected using selected ion monitoring (SIM). The retention time (RT) of the analytes and the *m/z* values of the monitored ions are given in Table 1. Data was processed using MassHunter Workstation software (version B.07.00, Agilent Technologies).

Method performance was assessed in terms of linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ) and sensitivity. Precision was expressed as method and instrumental repeatability, at 0.70 ng L^{-1} and at 114.3 ng L^{-1}. The calibration curve consisted of seven points ranging from 0.14 ng L^{-1} to 228.6 ng L^{-1}. The linearity was expressed as the coefficient of determination (R^2) and the sensitivity as the slope (k) of the calibration curves. Method accuracy was expressed as [(experimental value - spiked value)/spiked value] (n = 3). Ultra-pure water spiked with internal standards before SPE (blank samples, n = 6) was used for determining of the LOD and LOQ, which were calculated as 3-times and 10-times the standard deviation (SD) of the baseline divided by the slope of the calibration curves, respectively. Method repeatability was calculated as the relative standard deviation (RSD) of the procedural blanks (n = 3), while instrumental repeatability was determined as the RSD of three consecutive injections of the same sample. Analyte recovery was calculated as a quotient of the response areas of the analytes spiked in the validation standards prior to SPE and the same amount of analytes added to the extracts after SPE.

Table 1
Summary of derivatised compounds and internal standards; their monitored ions [*m/z*]: quantifier ion (bold) and two qualifier ions, and their retention times [min].

Compound	Monitored ion 1 [*m/z*]	Monitored ion 2 [*m/z*]	Monitored ion 3 [*m/z*]	RT [min]
BPF-TMS	**344**	329	179	8.61
$^{13}C_{12}$-BPF-TMS	**356**	341	185	8.61
BPS-TMS	**394**	379	229	13.20
$^{13}C_{12}$-BPS-TMS	**406**	391	379	13.20
BPZ-TMS	**412**	397	369	12.49
$^{13}C_{12}$-BPB-TMS	398	383	**369**	9.83

2.4. Identification of transformation products

2.4.1. Sample preparation

For the second tier of this study, the determination of major TPs, 1 mL aliquots were sampled in duplicate at the same previously mentioned time points and spiked immediately with the same mixture of internal standards as used for determining the degradation kinetics. All samples were stored at −20 °C prior to analysis. The samples were analysed directly, using LC-QTOF-MS, without additional sample preparation.

2.4.2. LC-QTOF-MS analysis

Samples used for identifying TPs were analysed using an Agilent 1290 Infinity UPLC hyphenated to an Agilent 6530 QTOF (Agilent Technologies). Chromatographic separation was carried out on a Poroshell 120 C18 column (50 × 3 mm; 2.7 μm particle size, Agilent Technologies) using a mobile phase which consisted of ultra pure water (A) and 90/10 (v/v) MeOH/ultra pure water (B). The injection volume was 5 μL. All samples were analysed both in positive and negative ionisation mode. Both ionisation modes used the same chromatographic conditions; the run started isocratically for 1 min at 10% B, then the gradient was applied to raise the percentage of B to 50% at 5 min, a second gradient was used to increase this to 85% B at 10 min and finally, the percentage of B was increased to 95% at 11 min. Subsequently, the column was rinsed at 95% B for four minutes and equilibrated at 5% B for 5 min before the next injection. The flow rate was 0.4 mL min^{-1} and the column temperature was kept constant at 40 °C.

The QTOF-MS instrument was operated in the 2 GHz (extended dynamic range) mode, providing a Full Width at Half Maximum (FWHM) resolution of approximately 5100 at *m/z* 112.9856 and 10,300 at *m/z* 1033.9881. The ions 112.0508 and 922.0097 for positive mode and 112.9856 and 980.0164 for negative mode were selected for continuous recalibration during the run to ensure mass accuracy. The eluting compounds were ionised by an Agilent Jet-Stream electrospray ionisation (AJS-ESI) source applying both positive and negative polarity at a flow rate of 8 L min^{-1}. The sheath gas temperature was 350 °C at a flow of 11 L min^{-1}. Nebulizer pressure was set at 25 psi. Capillary, nozzle and fragmentor voltages were set at 3500 V, 1500 V and 120 V, respectively. Acquisition parameters were set for the *m/z* values to range from 50 to 1000 at a scan rate of 2 spectra s^{-1} for MS and 5 spectra s^{-1} for MS/MS spectra, respectively. Signals were detected using a data-dependent acquisition method with and absolute intensity threshold set at 2000 counts and an isolation width of 1.3 amu. Collision energy was applied at 10 and 20 V to the selected precursor ions. An active exclusion of 0.15 min was set after the acquisition of one MS/MS spectrum to prevent repetitive acquisition for the same precursor ion. All data were stored in centroid mode.

2.4.3. Data processing and analysis

This study used two complementary workflows for data analysis, optimised and used in our previous studies (Gys et al., 2018; Mortelé et al., 2018). The use of these two workflows resulted in an enhancement of confidence in the detection and identification of the phototransformation products. Briefly, in the first workflow, suspect screening was used. A csv-database was made up of a list of possible

TPs with their molecular formula, exact mass and structure. This suspect list was generated from a literature search for TPs of BPF, BPS and BPZ, generated during photochemical degradation and other treatments or metabolism experiments (Cao et al., 2016; Gys et al., 2018; Inoue et al., 2008; Le Fol et al., 2015; Pozdnyakov et al., 2011; Salomatova et al., 2015; Xiao et al., 2007). Further possible TPs were added to the list by analogy with the TPs of other BPs described in the literature (Horikoshi et al., 2008; Katsumata et al., 2004; Wang et al., 2007). The suspect list is included in SM-3. The features resulting from this suspect screening were extracted using the MassHunter Qualitative Analysis Workstation Software (version B.07.00, Agilent Technologies).

The second workflow consisted of an untargeted screening approach and was conducted using the open-source software packages MZmine (version 2.30) and R (version 3.4.1.) (Gys et al., 2018; Mortelé et al., 2018; Pluskal et al., 2010; R Development Core Team, 2011; Vervliet, 2018). Detection and differentiation of MS features in the samples was carried out in the following steps: peak detection, chromatogram building, chromatogram deconvolution, deisotoping, filtering according to peak width (0.05 to 1 min) and peak alignment. Ultimately, any missed peaks were re-iteratively extracted based on the RT. The retained *m/z* features were further processed using R, applying a student *t*-test to test the statistical significance of the increase in intensity of a feature in an exposed sample compared to its respective blank and zero samples. Only features that showed a statistically significant difference between the sample and the controls ($p\text{-value} < 0.05$), were selected. The resulting *m/z* values from this untargeted workflow were extracted using the *Formula Prediction* algorithm in MZmine.

The identification of potential TPs resulting from both workflows was based on accurate mass and isotopic pattern in MS mode and the fragmentation pattern and accurate mass of the respective product ions. Their tentative structures were postulated based on the mass shift of the TP in comparison to the mass of their respective parent compound, their elemental formula and the MS/MS fragmentation spectrum. Interpretation of the MS/MS spectra of the TPs was based on the MS/MS spectrum of the parent compound, in particular on the appearance of common fragment ions, making the fragment identification in the spectra of the TPs more reliable. The following selection criteria were applied: (a) a maximal mass variation of ±10 ppm between the measured and theoretical parent ions; (b) a maximal mass variation of ±25 ppm for product ions; (c) the measured isotope pattern matched the predicted one with an isotope pattern score of at least 70%; (d) the measured double bond equivalent values (DBE) matched the postulated structure; (e) identified TPs were not present in the respective blank samples; and (f) identified TPs had to be present in both replicates.

3. Results and discussion

3.1. Degradation kinetics

To determine the kinetic profiles of BPF, BPS and BPZ during photochemical degradation (UV, UV/β-CD and PF), the respective kinetic constants and substrate half-lives were calculated. The plots of the natural logarithm of the remaining BP concentration versus the time of exposure yielded a linear correlation with $R^2 > 0.96$. This means that all the degradation kinetics for each of the applied processes followed pseudo first-order kinetics, described by the following equation:

$$\frac{dc_0}{dt} = -k_t c_t$$

In this equation, c_0 is the initial BP concentration in the aqueous solution, c_t is the remaining BP concentration after exposure during time t and k_t is the observed pseudo first-order rate constant. The rate constants and half-lives of the photochemical degradation of BPF, BPS and BPZ can be found in Table 2. In Fig. 1, the matching graphs are displayed.

For BPF, the k_t values were of the same order of magnitude for UV and UV/β-CD. The kinetic constant for PF was the highest and the fraction of remaining BPF after 1 h of applying the PF process was <10%. Rate constants for BPS with UV and UV/β-CD photochemical degradation were found to be higher than those for BPF; but similar for the PF application. Consequently, <10% of BPS remained after 1 h of the PF process. The photochemical degradation half-lives of BPS showed less variation between the three treatments compared to those of BPF. For BPZ, notably, the rate constant was lower when β-CD was added in comparison to exposure to UV irradiation alone. Similar to the other two compounds, the highest degradation efficiency was obtained when applying PF.

3.2. Identification of transformation products

3.2.1. Experimental quality control

All identified TPs were crosschecked with their respective negative control samples to eliminate false positive results. Using the response of the internal standards throughout the analysis, the instrumental variation was approximately 20% (relative standard deviation of the area of the chromatographic peak of the internal standards across all samples). All identified TPs were detected in the negative ionisation mode. In positive ionisation mode, only adducts of TPs were identified that had already been detected in the negative ionisation mode. A summary of their postulated structures, diagnostic product ions and corresponding confidence levels as proposed by Schymanski et al. (2014) is presented in Table 3, listed according to their respective parent compounds and condition applied (Schymanski et al., 2014). All TPs included in this table met the criteria listed under 2.4.3. A confidence level L2b was assigned to each TP which had been previously reported in the literature. These were identified using both the suspect and the untargeted screening workflows. A confidence level L3 was assigned to novel TPs, identified only by the untargeted screening workflow or to those with isomers at different retention times that could not be resolved based on the acquired data of this study. Chromatograms and MS/MS spectra of parent compounds and their photochemical TPs can be found in SM-3. We estimated the relative quantities of formed TPs (major and minor) by calculating the ratio of the peak area of the TPs divided by the peak area of the internal standard in the respective sample (SM-5) and proposed a tentative photodegradation pathway for each compound, presented in Figs. 2, 3 and 4.

3.2.2. BPF

The parent compound BPF was detected in negative ionisation mode as the deprotonated molecule ($[M\text{-}H]^-$, 199.0781) at 7.11 min. The

Table 2
Rate constants k [min^{-1}] and half-lives (t1/2) [min] for BPF, BPS and BPZ using three different photodegradation treatments: exposure to UV irradiation (UV), exposure to UV with addition of β-cyclodextrin (β-CD) and exposure to UV with addition of H_2O_2 and Fe^{2+} (photo-Fenton reaction; PF).

BPs	BPF			BPS			BPZ		
Treatment	UV	β-CD	PF	UV	β-CD	PF	UV	β-CD	PF
Rate constant k [min^{-1}]	0.005	0.006	0.043	0.021	0.026	0.042	0.017	0.008	0.052
$t_{1/2}$ [min]	138.63	115.52	16.12	33.01	26.66	16.50	40.77	86.64	21.66

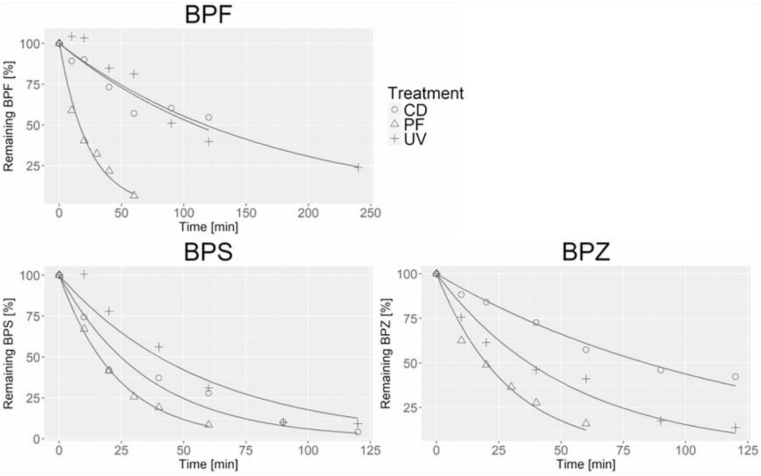


Fig. 1. Kinetic profiles for BPF, BPS and BPZ using three different photodegradation treatments: exposure to UV irradiation (UV), exposure to UV with addition of β-cyclodextrin (β-CD) and exposure to UV with addition of H_2O_2 and Fe^{2+} (photo-Fenton reaction; PF).

chromatogram and fragmentation pattern are shown in Fig. SM-2. The product ion with the highest abundance was *m/z* 93.0346, corresponding to the $[C_6H_5O]^-$ ion. Other fragment ions were *m/z* 108. 0575 and 77.0416. Degradation of BPF resulted in the detection of four identified TPs, their chromatograms are shown in Fig. SM-3. BPF-P1 is a product of the cleavage and oxidation of the methylene bond of BPF and was detected as the deprotonated molecule ([M-H]$^-$, 121.0299) at RT 3.84 min. The formula $C_7H_6O_2$ was proposed based on its exact mass and isotope pattern. The postulated structure, included in Table 3, is suggested by the acquired DBE and the presence of the fragment ion with *m/z* 92.0258. This TP has been reported as a product of the photochemical process of BPF by Salomatova et al. (2015).

Based on the relative quantities of the formed TPs, hydroxylation is the main mechanism of BPF transformation when exposed to UV or CD, while cleavage seems to dominate when PF is applied (Fig. SM-11), complemented by further oxidation of hydroxylated TPs (Fig. 2). For the hydroxylated BPF-P2, three peaks were detected in the chromatograms at different retention times, suggesting three positional isomers. Each photochemical process produced a different isomeric pattern. BPF-P2A ([M-H]$^-$, 215.0712 at RT 4.06 min) was only detected in case of application of the PF reaction. BPF-P2B ([M-H]$^-$, 215.0709 at RT 5.27 min) and BPF-P2C ([M-H]$^-$, 215.0728 at RT 6.25 min) however, appeared in chromatograms of samples generated by all three experiments. The MS/MS fragmentation spectra of BPF-P2B and BPF-P2C show a strong similarity; the detected product ions mostly overlap and indicate positional isomers resulting from phenolic hydroxylation, either at the ortho- or at the meta-position. Based on the acquired MS/MS data, it was not possible to define the exact positions of the hydroxylation on the phenolic ring. For BPF-P2A, different product ions were detected compared to BPF-P2B and BPF-P2C. The fragment ion with *m/z* 197.0582 suggests the neutral loss of water, which is indicative of aliphatic hydroxylation (Ramanathan et al., 2000). The postulated structure for BPF-P2A is shown in Table 3.

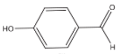
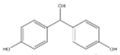
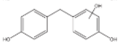
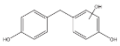
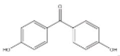
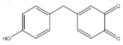
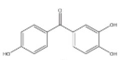
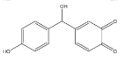
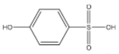
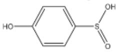
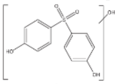
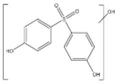
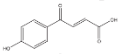
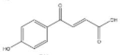
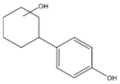
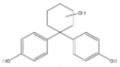
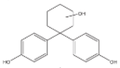
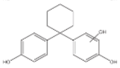
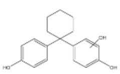
The third TP, an advanced oxidation product, showed two peaks at RT 6.02 min and 5.27 min for the positional isomers BPF-P3A and BPF-P3B, respectively. BPF-P3A ([M- H]$^-$, 213.0555) only appeared in chromatograms of samples generated by the PF reaction and is probably formed by oxidation of BPF-P2A. A single peak for BPF-P3B ([M-H]$^-$, 213.0572) occurred in case of all three exposures formed by oxidation or rearrangement of BPF-P2B or BPF-P2C. Common fragment ions are *m/z* 93.03, corresponding to $[C_6H_5O]^-$, and *m/z* 185.06, suggesting the neutral loss of CO (Holcapek et al., 2010a). Similarly to the parent compound, the product ion with the highest abundance for BPF-P3A was *m/z* 93.03, indicating the presence of two phenol groups and oxidation of the methylene bridge. The product ion *m/z* 107.0149 appeared only in the fragmentation spectrum of BPF-P3B, representing [C6H3O2]$^-$. Based on this product ions, which differed from each other, their postulated structures are shown in Table 3. The fourth TP was only detected when BPF was exposed to the PF reaction and appeared in the chromatogram at two retention times (5.34 min and 4.56 min). These peaks likely represent two positional isomers with the postulated formula $C_{13}H_{10}O_4$ and are minor TPs, assumed to have been generated by the additional hydroxylation of the TPs described above. In the MS/MS spectrum of BPF-P4A the neutral loss of water was observed, generating the product ion with *m/z* 211.0378; while for BPF-P4B, the product ion with *m/z* 201.0559 resulted from the neutral loss of CO.

3.2.3. BPS

The parent compound BPS was detected as the deprotonated molecule ([M-H]$^-$, 249.0227) at 5.39 min. The chromatogram and fragmentation spectrum can be found in Fig. SM-5. The product ion with the highest abundance was *m/z* 108.0225, corresponding to $[M\text{-}H\text{-}C_6H_5O_3S]^-$. Other fragment ions were *m/z* 156.9959 and 93.0346. Three different TPs of BPS were detected and identified (Table 3, SM-6 and -7). BPS-P1 eluted at RT 0.67 min and was detected as its deprotonated molecule ([M-H]$^-$, 172.9926). It was generated by all three processes and appeared as the major TP when exposed to UV or CD process (Fig. SM-12). Based on the accurate mass, isotope pattern and detected product ions, this TP was identified as $C_6H_6O_4S$, a product that was previously identified and reported (Wang et al., 2014). Its product ions *m/z* 93.0357 and 108.0233 are overlapping with the fragments from the parent compound. The *m/z* 79.9586 corresponds to the fragment $[SO_3]^-$ (Holcapek et al., 2010a).

BPS-P2 is a cleavage product of BPS that was generated by UV and β-CD conditions, and is to our best knowledge, reported here for the first time. It appeared in the chromatogram at RT 0.73 min ([M-H]$^-$,

Table 3
Summary of the identified TPs, which parent compound they originate from and by which treatment they were generated, the confidence level, RT [min], measured [M-H]-for the precursor ions, theoretical [M-H]-, mass error [ppm], tentative formula, double bond equivalent (DBE), diagnostic product ions and proposed structure.

Parent compound	Treatment	Product ID	Confidence level	RT [min]	Deprotonated molecule (MS) Measured $[M\text{-}H]^-$	Deprotonated molecule (MS) Theoretical $[M\text{-}H]^-$	Error [ppm]	Tentative formula	DBE	Diagnostic product ions (MS/MS) Measured *m/z*	Proposed structure
BPF	UV CD PF	BPF-P1	L2b	3.84	121.0299	121.0295	−3.30	$C_7H_6O_2$	5	92.0258	
	PF	BPF-P2A	L3	4.06	215.0712	215.0714	0.92	$C_{13}H_{12}O_3$	8	169.0646; 197.0582	
	UV CD PF	BPF-P2B	L3	5.27	215.0709	215.0714	2.32	$C_{13}H_{12}O_3$	8	77.0406; 93.0374; 108.0227	
	UV CD PF	BPF-P2C	L2b	6.25	215.0728	215.0714	−6.51	$C_{13}H_{12}O_3$	8	77.0426; 93.0367; 108.0197; 121.0267	
	PF	BPF-P3A	L3	6.02	213.0555	213.0557	0.93	$C_{13}H_{10}O_3$	9	93.0371; 169.0687; 185.0596	
	UV CD PF	BPF-P3B	L2b	5.27	213.0572	213.0557	−6.97	$C_{13}H_{10}O_3$	9	93.0372; 107.0149; 185.0633	
	PF	BPF-P4A	L3	5.34	229.0505	229.0506	0.44	$C_{13}H_{10}O_4$	9	108.0141; 155.0516; 211.0378	
	PF	BPF-P4B	L3	4.56	229.0517	229.0506	−4.8	$C_{13}H_{10}O_4$	9	93.0368; 108.0175; 185.0571; 201.0559	
BPS	UV CD PF	BPS-P1	L2b	0.67	172.9926	172.9914	6.94	$C_6H_6O_4S$	4	79.9586; 93.0357; 108.0233	
	UV CD	BPS-P2	L3	0.73	156.9963	156.9965	−1.27	$C_6H_6O_3S$	4	63.9624; 93.0339; 108.0229	
	UV CD	BPS-P3A	L2b	1.21	265.0169	265.0176	−2.59	$C_{12}H_{10}O_5S$	8	79.9589: 156.0568; 185.0554	
	UV CD PF	BPS-P3B	L2b	4.32	265.0191	265.0176	5.47	$C_{12}H_{10}O_5S$	8	93.0338 108.0256; 156.9916 171.9852	
BPZ	UV CD	BPZ-P1A	L3	1.59	191.0344	191.0350	3.14	$C_{10}H_8O_4$	7	93.0322; 121.0293; 147.0418	
	PF	BPZ-P1B	L3	2.35	191.0338	191.0350	6.28	$C_{10}H_8O_4$	7	93.0359; 121.0297 147.0448	
	UV CD PF	BPZ-P2	L3	7.33	191.1083	191.1078	−2.6	$C_{12}H_{17}O_2$	5	93.0355; 173.0940	
	PF	BPZ-P3A	L3	5.57	283.1320	283.1340	7.06	$C_{18}H_{20}O_3$	9	93.0387; 189.0906; 198.0624	
	PF	BPZ-P3B	L3	6.51	283.1349	283.1340	−3.78	$C_{18}H_{20}O_3$	9	93.0312; 198.0667; 265.1293	
	UV CD PF	BPZ-P3C	L2b	8.73	283.1344	283.1340	−1.41	$C_{18}H_{20}O_3$	9	93.0364 173.0946; 214.0662; 239.0704; 255.1261	
	UV CD PF	BPZ-P3D	L2b	9.18	283.1324	283.1340	5.65	$C_{18}H_{20}O_3$	9	93.0362; 189.0947; 214.0708 239.0691	

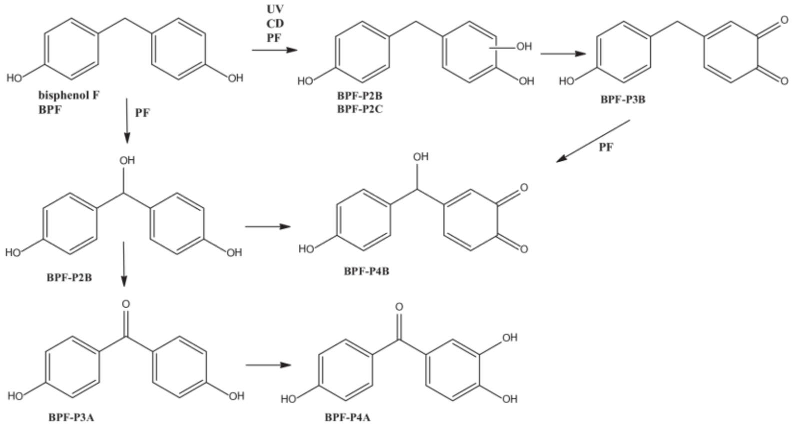


Fig. 2. Proposed tentative photodegradation pathway for BPF.

156.9963) and the postulated formula for this TP is $C_6H_6O_3S$. Its product ions *m/z* 108.0229 and 93.0357 are matching with the product ions of the parent compound; *m/z* 63.9624 represents $[SO_2]^-$ (Holcapek et al., 2010a).

Hydroxy-BPS (BPS-P3) was detected as its deprotonated molecular ion ($[M\text{-}H]^-$, 265.0169) at two different retention times: 1.21 min and 4.32 min for BPS-P3A and BPS-P3B, respectively. Our data suggest that BPS-P3B is the major product formed during the PF process, as displayed in SM-5. For BPS-P3A we observed the same fragment with *m/z* 79.9589 as for BPS-P1. Common product ions appeared in the spectrum of the parent compound and BPS-P3B, namely: *m/z* 93.0338, 108.0256 and 156.9916. Additionally, the fragment with *m/z* 171.9852 corresponds to $[C_6H_4O_4S]^-$ (Gys et al., 2018). Based on the acquired MS/MS data, the exact position of the hydroxyl groups could not be determined.

3.2.4. BPZ

The parent compound BPZ was detected as the deprotonated molecule ($[M\text{-}H]^-$, 267.1398) at 10.09 min, and also appeared as the base peak. The chromatogram and fragmentation spectrum can be found in Fig. SM-8. The detected fragment ions were *m/z* 198.0670, 173.0955 and 93.0363. Degradation of BPZ resulted in three TPs, by the proposed tentative photodegradation pathway displayed in Fig. 4. Their chromatograms and MS/MS spectrum can be found in Figs. SM-9 and -10. The product ion with *m/z* 93.0362 was observed for all products, corresponding to the fragment $[C_6H_6O]^-$. The cleaved product BPZ-P1 was detected as its deprotonated molecule ($[M\text{-}H]^-$, 191.0344) in all three exposures, at RT 1.59 min in samples generated by the UV and β-CD set-ups and at 2.35 min for PF as the major TP. The observed fragment ion with *m/z* 147.0466 indicated a neutral loss of CO_2 (Holcapek et al., 2010b).

The product BPZ-P2 suggests the loss of one phenolic ring and hydroxylation of the cyclohexane ring, generated as the major TP by UV and CD conditions (SM-13) from the parent compound (Fig. 4). It was detected as the deprotonated molecular ion ($[M\text{-}H]^-$, 191.1043) at 7.33 min. The observed ion with *m/z* 173.0940 represents a loss of H_2O, again indicating that hydroxylation took place on an aliphatic carbon atom, in this case on the cyclohexane ring more specifically (Ramanathan et al., 2000). Based on the acquired MS/MS data, it was not possible to determine the exact position of this hydroxylation.

The third product BPZ-P3 was identified as hydroxy-BPZ ($[M\text{-}H]^-$, 283.1324) and showed peaks at four different retention times: 5.57, 6.51, 8.73, and 9.18 min, suggesting the occurrence of positional isomers. All four isomers are formed during PF reaction, while in case of UV and CD conditions, only peaks at RT 8.73 min and 9.18 min were observed (Fig. 4). Again, the exact position of the hydroxyl-group could not be determined. From the chemical structure of BPZ and the published literature (Wang, 2007), it is assumed that minor hydroxylation taking place on the cyclohexane ring results in a relatively lower RT

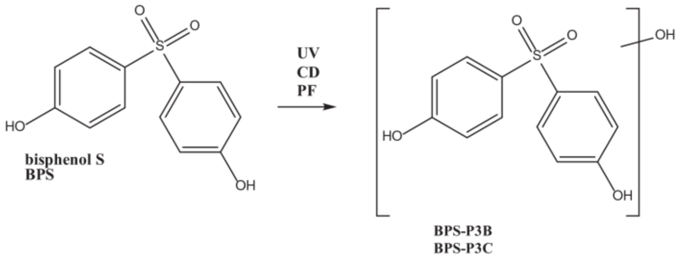


Fig. 3. Proposed tentative photodegradation pathway for BPS.

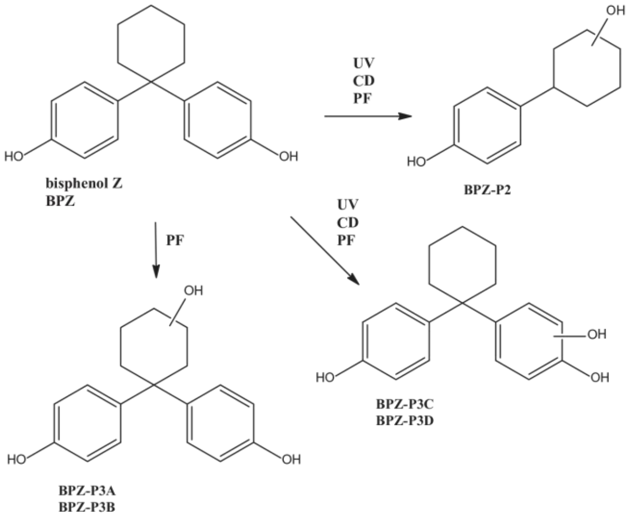


Fig. 4. Proposed tentative photodegradation pathway for BPZ.

(BPZ-P3A and BPZ-P3B), while the major share of the hydroxylation reaction, on one of the phenolic rings, results in a product with a higher RT (BPZ-P3C and BPZ-P3D). Additionally, for products BPZ-P3A and BPZ-P3B, the fragment ion with *m*/*z* 173.0940 indicates the loss of water, which is favoured in the presence of a non-phenolic hydroxyl group (Holcapek et al., 2010b; Ramanathan et al., 2000). For BPZ-P3C and BPZ-P3D, fragment ions with *m*/*z* 255.1261 and 239.0704 appear in the MS/MS spectrum, indicating the neutral loss of CO and CO_2, respectively (Holcapek et al., 2010b).

3.3. Degradation and transformation of BPs

To date, a limited number of studies have evaluated the photochemical degradation of only one BP at a time. The design of this study enabled, for the first time, a comparison between the photochemical degradation profiles of BPF, BPS and BPZ, exposed to three bench-scale processes and proposal of their tentative photodegradation pathways.

All three procedures resulted in the degradation of all three BPs, and all followed a pseudo-first order kinetic profile. The expected increase in the removal efficiency by adding β-CD, was observed only for BPS. The removal efficiency of BPZ was even lower in the presence of β-CD and in contrast to the findings of Wang et al. (Wang, 2007), who reported increased photodegradation of BPZ in its presence. The use of UV light with lower intensity in our study could explain this disagreement. The PF reaction generated the highest k_t and consequently the shortest half-lives for all three compounds. After 1 h of exposure <10% of BPF and BPS, and <20% of BPZ remained. These results agree with published studies that addressing the removal of contaminants of emerging concern in water. Clearly, the PF conditions are the most aggressive of the three by generating a higher amount of ROS (Andreozzi et al., 1999; Esplugas et al., 2002; Klamerth et al., 2010; Machulek et al., 2012). In the future, similar procedures as used in the present study, could be combined with biodegradation to attempt to completely remove potentially hazardous substances such as the examined BPs from environmental matrices. To our knowledge, this has so far been studied for BPA only, but should be evaluated for alternative BPs as well (Chen et al., 2016; Wilkinson et al., 2017; Zhang and Li, 2014).

In accordance with previous studies (Cao et al., 2013; Salomatova et al., 2015; Wang, 2007; Wang et al., 2014), hydroxylated, cleaved and oxidized TPs of all three BPs were identified. While some of these TPs matched those previously published results, several new TPs were identified by employing high resolution LC-Q-TOF screening with two complementary workflows for data processing. It also appears that different exposure promotes the formation of different TPs of a certain BP. Some TPs were identified in samples generated by all three processes, i.e. the cleaved products BPF-P1, BPS-P1 and BPZ-P2, the phenolic hydroxylated products BPF-P2B and BPF-P2C, BPS-P3A and BPS-P3B, BPZ-P3C and BPZ-P3D and the advanced oxidation products BPF-P3B. Others appeared to be condition-dependent, i.e. the aliphatic hydroxylation products BPF-P2A and BPF-P4B, BPZ-P3A and BPZ-P3B, the advanced oxidation products BPF-3A and BPF-P4A as well as the cleaved products BPS-P2, BPZ-P1A and BPZ-P1B. For UV and CD conditions the same TPs were identified, whereas the PF reaction promoted the formation of different products for all three exposed compounds. For example, the hydroxylation of available aliphatic carbon atoms in BPF or BPZ only occurred when exposed to the PF reaction. In addition, generation of oxidative TPs of BPF and BPZ appeared to dominate when UV or CD conditions were applied, while cleaved TPs in case of PF conditions. The opposite is true for BPS. In most cases all identified TPs followed an increasing trend over the measured time at UV or CD conditions while there was a visible decrease in formation of TPs in case of PF (see SM-5).

So far, the phototransformation of BPF has only been reported by Salomatova et al. (Salomatova et al., 2015), who used laser flash photolysis and by Xiao et al. (2007), who compared the formation of TPs during UV irradiation with and without β-CD. In line with both studies, isomers of hydroxy-BPF were detected; only one isomer was reported by Salomatova et al. (2015), two phenolic isomers of hydroxy-BPF by Xiao et al. (Wang et al., 2007) and, additionally, the aliphatic isomer in the present study. The TP with the formula $C_7H_6O_2$ (BPF-P1) was also described by Salomatova et al. (2015) as well as the products $C_{13}H_{10}O_3$ (BPF-3A and BPF-3B). However, in their study, only one isomer was reported, while in this study two isomers of the product $C_{13}H_{10}O_4$ (BPF-P4A and BPF-4B) were identified for the first time. Dihydroxy-BPF and 4-(hydroxymethyl)benzene-1,2-diol were not detected in our study which can be explained by the more aggressive photochemical degradation (laser-flash photolysis) used by Salomatova et al. (2015). Similar to our findings, Xiao et al. reported no difference in the formation of TPs with or without β-CD, considering BPF. However, 2,4,5-trihydroxybenzoic acid was not detected in the present study, which may be due to the use of a UV lamp with a higher intensity by Xiao et al. (2007).

The photochemical degradation of BPS using UV irradiation was studied by Cao et al. (2013) and Wang et al. (2014). Both studies reported *p*-hydroxybenzensulfonic acid (BPS-P1) as the major TP, which was also formed during all of the three experiments in this study. The formation of hydroxy-BPS was proposed by Cao's group (Cao et al., 2013) and by us, but the formation of two isomers (BPS-P3A and BPS-P3B) were only observed in this study, when the PF reaction was applied. The present study also reports the formation of a novel TP with the formula $C_6H_6O_3S$ (BPS-P2), when BPS was exposed to UV both with or without the addition of β-CD.

So far, photochemical degradation of BPZ was studied only by Wang et al. (Wang, 2007). Similarly, we exposed BPZ to UV irradiation with the addition of β-CD. They report two phenolic isomers of hydroxy-BPZ (BPZ-P3C and BPZ-P3D) that were also formed during all three exposures included in this study. However, they missed the formation of two isomers of aliphatic hydroxy-BPZ (BPZ-P3A and BPZ-P3B), two cleaved isomers (BPZ-P1A and BPZ-P1B) as well as the cleaved and hydroxylated product with the formula $C_{12}H_{17}O_2$ (BPZ-P2). Thus, these TPs were identified for the first time in our study.

Since the increasing application of bisphenol analogues and their potential human and ecological hazard, their fate in the environment should be studied more extensively. The presence of the BPs that were examined in this study in different environmental matrices has been documented multiple times (Caballero-Casero et al., 2016; Chen et al., 2016; Fromme et al., 2002; Noszczyńska and Piotrowska-Seget, 2018). In these matrices, such as surface water, sediment and wastewater sludge, photochemical transformation, whether direct or facilitated via reaction with photo-sensitized species, can occur and possibly generate the TPs that were identified in our study (Wilkinson et al., 2017; Zhang and Li, 2014). More research is needed to evaluate the environmental fate and effects of the alternative BPs and their TPs (Chen et al., 2016). Our findings can contribute to the monitoring of these compounds and their TPs in the environment in the future and the examination of their potential toxicity.

4. Conclusion

The results of this study show that the three processes had different efficiencies for degrading BPF, BPS and BPZ while also promoting the formation of different transformation products of each compound. The experiments revealed that UV exposure is an effective way of removing BPF, BPS and BPZ from water, showing the fastest degradation rate in case of the photo-Fenton reaction. The study identified 11 novels and eight previously reported transformation products, a finding that provides insight into the photochemical behaviour of the investigated bisphenols and contributes to a better understanding of their environmental fate.

Acknowledgements

This work was supported by MASSTWIN (European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 692241). Celine Gys acknowledges a PhD fellowship from Research Foundation Flanders (project G0E5216N). Ana Kovačič acknowledges the Young researcher grant and the Slovenian Research Agency (Program Group P1-0143 and Projects L1-7544, L1-9191, N1-0047, J1-8147 and J2-8162). The authors gratefully acknowledge Dr. David Heath for his help in optimising the manuscript.

Conflicts of interest

The authors declare they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.064.

References

Andreozzi, R., Caprio, V., Insola, A., Marotta, R., 1999. Advanced oxidation processes (AOP) for water purification and recovery. 53, 51–59.

Behr, M., Oehlmann, J., Wagner, M., 2011. Estrogens in the daily diet: in vitro analysis indicates that estrogenic activity is omnipresent in foodstuff and infant formula. Food Chem. Toxicol. 49, 2681–2688. https://doi.org/10.1016/J.FCT.2011.07.039.

Bittner, G.D., Yang, C.Z., Stoner, M.A., 2014. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environ. Health 13, 41. https://doi.org/10.1186/1476-069X-13-41.

Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53. https://doi.org/10.1016/j.aca.2015.12.034.

Cao, G., He, R., Cai, Z., Liu, J., 2013. Photolysis of bisphenol S in aqueous solutions and the effects of different surfactants. React. Kinet. Mech. Catal. 109, 259–271. https://doi.org/10.1007/s11144-013-0553-6.

Cao, G., Zhang, J., Liu, B., 2016. Effects of nitrite anions and ammonium cations on the photolysis of bisphenol S in nitrate solution. Desalin. Water Treat. 57, 25686–25695. https://doi.org/10.1080/19443994.2016.1157044.

Česen, M., Lambropoulou, D., Laimou-Geraniou, M., Kosjek, T., Blaznik, U., Heath, D., Heath, E., 2016. Determination of bisphenols and related compounds in honey and their migration from selected food contact materials. J. Agric. Food Chem. 64, 8866–8875. https://doi.org/10.1021/acs.jafc.6b03924.

Česen, M., Heath, D., Krivec, M., Ko, J., Kosjek, T., Heath, E., 2018. Seasonal and Spatial Variations in the Occurrence, Mass Loadings and Removal of Compounds of Emerging Concern in the Slovene Aqueous Environment and Environmental Risk Assessment *. 242. https://doi.org/10.1016/j.envpol.2018.06.052.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - a review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

Daskalaki, V.M., Frontistis, Z., Mantzavinos, D., Katsaounis, A., 2011. Solar light-induced degradation of bisphenol-A with TiO_2 immobilized on Ti. Catal. Today 161, 110–114. https://doi.org/10.1016/j.cattod.2010.09.018.

De la Cruz, N., Esquius, L., Grandjean, D., Magnet, A., Tungler, A., de Alencastro, L.F., Pulgarín, C., 2013. Degradation of emergent contaminants by UV, UV/H_2O_2 and neutral photo-Fenton at pilot scale in a domestic wastewater treatment plant. Water Res. 47, 5836–5845. https://doi.org/10.1016/j.watres.2013.07.005.

Deng, Y., Zhao, R., 2015. Advanced oxidation processes (AOPs) in wastewater treatment. Curr. Pollut. Rep. 1, 167–176. https://doi.org/10.1007/s40726-015-0015-z.

EFSA, 2015. EFSA Panel on Food Contact Materials, Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13, 3978. https://doi.org/10.2903/j.efsa.2015.3978.

Eio, E.J., Kawai, M., Tsuchiya, K., Yamamoto, S., Toda, T., 2014. Biodegradation of bisphenol A by bacterial consortia. Int. Biodeterior. Biodegrad. 96, 166–173. https://doi.org/10.1016/j.ibiod.2014.09.011.

Erjavec, B., Hudoklin, P., Perc, K., Tišler, T., Dolenc, M.S., Pintar, A., 2016. Glass fiber-supported TiO_2 photocatalyst: efficient mineralization and removal of toxicity/estrogenicity of bisphenol A and its analogs. Appl. Catal. B Environ. 183, 149–158. https://doi.org/10.1016/j.apcatb.2015.10.033.

Esplugas, S., Gimenez, J., Contreras, S., Pascual, E., Rodrıuez, M., 2002. Comparison of different advanced oxidation processes for phenol degradation. 36, 1034–1042.

European Commission Regulation (EU), n.d. European Commission Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A (BPA) in plastic infant feeding bottles. Text With EEA Relevance. 3–4. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R0321

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., Wenzel, A., 2002. Occurrence of phthalates and bisphenol A and F in the environment. Water Res. 36, 1429–1438. https://doi.org/10.1016/S0043-1354(01)00367-0.

Gmurek, M., Olak-Kucharczyk, M., Ledakowicz, S., 2017. Photochemical decomposition of endocrine disrupting compounds – a review. Chem. Eng. J. 310, 437–456. https://doi.org/10.1016/j.cej.2016.05.014.

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014.

Gys, C., Kova, A., Huber, C., Yin, F., Heath, E., Covaci, A., 2018. Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism. 295, 115–123. https://doi.org/10.1016/j.toxlet.2018.05.034.

Holcapek, M., Jirásko, R., Lísa, M., 2010a. Basic rules for the interpretation of atmospheric pressure ionization mass spectra of small molecules. J. Chromatogr. A 1217, 3908–3921. https://doi.org/10.1016/j.chroma.2010.02.049.

Holcapek, M., Jirásko, R., Lísa, M., 2010b. Basic rules for the interpretation of atmospheric pressure ionization mass spectra of small molecules. J. Chromatogr. A 1217, 3908–3921. https://doi.org/10.1016/j.chroma.2010.02.049.

Horan, T.S., Pulcastro, H., Lawson, C., Gerona, R., Martin, S., Gieske, M.C., Sartain, C., Vnn Hunt, P.A., 2018. Replacement bisphenols adversely affect mouse gametogenesis with consequences for subsequent generations. Curr. Biol. 28, 2948–2954.e3. https://doi.org/10.1016/j.cub.2018.06.070.

Horikoshi, S., Miura, T., Kajitani, M., Horikoshi, N., Serpone, N., 2008. Photodegradation of tetrahalobisphenol-A (X = Cl, Br) flame retardants and delineation of factors affecting the process. Appl. Catal. B Environ. 84, 797–802. https://doi.org/10.1016/j.apcatb.2008.06.023.

Inoue, D., Hara, S., Kashihara, M., Murai, Y., Danzl, E., Sei, K., Tsunoi, S., Fujita, M., Ike, M., 2008. Degradation of bis(4-hydroxyphenyl)methane (bisphenol F) by Sphingobium yanoikuyae strain FM-2 isolated from river water. Appl. Environ. Microbiol. 74, 352–358. https://doi.org/10.1128/AEM.01708-07.

Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351. https://doi.org/10.1016/j.watres.2016.07.059.

Karthikraj, R., Kannan, K., 2017. Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere 181, 216–223. https://doi.org/10.1016/j.chemosphere.2017.04.075.

Katsumata, H., Kawabe, S., Kaneco, S., Suzuki, T., Ohta, K., 2004. Degradation of bisphenol A in water by the photo-Fenton reaction. 162, 297–305. https://doi.org/10.1016/S1010-6030(03)00374-5.

Klamerth, N., Rizzo, L., Malato, S., Maldonado, M.I., Ferna, A.R., 2010. Degradation of Fifteen Emerging Contaminants at m g L L 1 Initial Concentrations by Mild Solar Photo-Fenton in MWTP Effluents. 44, pp. 545–554. https://doi.org/10.1016/j.watres.2009.09.059.

Kondrakov, A.O., Ignatev, A.N., Frimmel, F.H., Bräse, S., Horn, H., Revelsky, A.I., 2014. Formation of genotoxic quinones during bisphenol A degradation by TiO_2 photocatalysis and UV photolysis: a comparative study. Appl. Catal. B Environ. 160–161, 106–114. https://doi.org/10.1016/j.apcatb.2014.05.007.

Kosjek, T., Perko, S., Žigon, D., Heath, E., 2013. Fluorouracil in the environment: analysis, occurrence, degradation and transformation. J. Chromatogr. A 1290, 62–72. https://doi.org/10.1016/j.chroma.2013.03.046.

Le Fol, V., Aït-Aïssa, S., Cabaton, N., Dolo, L., Grimaldi, M., Balaguer, P., Perdu, E., Debrauwer, L., Brion, F., Zalko, D., 2015. Cell-specific biotransformation of benzophenone-2 and bisphenol-s in zebrafish and human in vitro models used for toxicity and estrogenicity screening. Environ. Sci. Technol. 49, 3860–3868. https://doi.org/10.1021/es505302c.

Le Fol, V., Aït-Aïssa, S., Sonavane, M., Porcher, J.-M., Balaguer, P., Cravedi, J.-P., Zalko, D., Brion, F., 2017. In vitro and in vivo estrogenic activity of BPA, BPF and BPS in zebrafish-specific assays. Ecotoxicol. Environ. Saf. 142, 150–156. https://doi.org/10.1016/j.ecoenv.2017.04.009.

Lee, S., Liao, C., Song, G.J., Ra, K., Kannan, K., Moon, H.B., 2015. Emission of bisphenol analogues including bisphenol A and bisphenol F from wastewater treatment plants in Korea. Chemosphere 119, 1000–1006. https://doi.org/10.1016/j.chemosphere.2014.09.011.

Li, F., Chen, J., Liu, C., Dong, J., Liu, T., 2006. Effect of iron oxides and carboxylic acids on photochemical degradation of bisphenol A. Biol. Fertil. Soils 42, 409–417. https://doi.org/10.1007/s00374-006-0084-7.

Liao, C., Liu, F., Alomirah, H., Loi, V.D., Mohd, M.A., Moon, H.-B., Nakata, H., Kannan, K., 2012a. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ. Sci. Technol. 46, 6860–6866. https://doi.org/10.1021/es301334j.

Liao, C., Liu, F., Guo, Y., Moon, H.-B., Nakata, H., Wu, Q., Kannan, K., 2012b. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. Environ. Sci. Technol. 46, 9138–9145. https://doi.org/10.1021/es302004w.

Liu, Y., Zhang, X., Wu, F., 2010. Photodegradation of bisphenol AF in montmorillonite dispersions: kinetics and mechanism study. Appl. Clay Sci. 49, 182–186. https://doi.org/10.1016/j.clay.2010.05.002.

Machulek, A., Quina, F.H., Gozzi, F., Silva, V.O., Friedrich, L.C., Moraes, J.E., 2012. Fundamental mechanistic studies of the photo-fenton reaction for the degradation of organic pollutants. Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical Update. InTech https://doi.org/10.5772/30995.

Maroga Mboula, V., Héquet, V., Andrès, Y., Pastrana-Martínez, L.M., Doña-Rodríguez, J.M., Silva, A.M.T., Falaras, P., 2013. Photocatalytic degradation of endocrine disruptor compounds under simulated solar light. Water Res. 47, 3997–4005. https://doi.org/10.1016/j.watres.2013.01.055.

Molina-Molina, J.-M., Amaya, E., Grimaldi, M., Sáenz, J.-M., Real, M., Fernández, M.F., Balaguer, P., Olea, N., 2013. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272, 127–136. https://doi.org/10.1016/j.taap.2013.05.015.

Mortelé, O., Vervliet, P., Gys, C., Degreef, M., Cuykx, M., Maudens, K., Covaci, A., van Nuijs, A.L.N., Lai, F.Y., 2018. In vitro Phase I and Phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. J. Pharm. Biomed. Anal. https://doi.org/10.1016/j.jpba.2018.02.032.

Neamţu, M., Frimmel, F.H., 2006. Degradation of endocrine disrupting bisphenol A by 254 nm irradiation in different water matrices and effect on yeast cells. Water Res. 40, 3745–3750. https://doi.org/10.1016/j.watres.2006.08.019.

Noszczyńska, M., Piotrowska-Seget, Z., 2018. Bisphenols: application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers. Chemosphere 201, 214–223. https://doi.org/10.1016/J.CHEMOSPHERE.2018.02.179.

Pluskal, T., Castillo, S., Villar-Briones, A., Orešič, M., 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinforma. 11. https://doi.org/10.1186/1471-2105-11-395.

Pozdnyakov, I.P., Guo, L., Glebov, E.M., Wu, F., Plyusnin, V.F., Grivin, V.P., Deng, N., 2011. Aqueous photochemistry of bisphenol E in the presence of β cyclodextrin. 45, 214–221. https://doi.org/10.1134/S001814391103012X 1.

R Development Core Team, R, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing https://doi.org/10.1007/978-3-540-74686-7.

Ramanathan, R., Su, A., Alvarez, N., Blumenkrantz, N., Chowdhury, S., Alton, K., Patrick, J., 2000. Liquid chromatography/mass spectrometry methods for distinguishing N-oxides from hydroxylated compounds. Anal. Chem. 72, 1352–1359. https://doi.org/10.1021/ac9911692.

Ruppert, G., Heisler, G., Bauer, R., 1993. The Photo-Fenton Reaction - Wastewater Treatment Process an Effective Photochemical. 73.

Salomatova, V., Pozdnyakov, I., Sherin, P., Grivin, V., Plyusnin, V., 2015. Photochemistry of bisphenol F in aqueous solutions: a mechanistic study. J. Photochem. Photobiol. A Chem. 305, 45–50. https://doi.org/10.1016/j.jphotochem.2015.03.008.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105.

Siracusa, J.S., Yin, L., Measel, E., Liang, S., Yu, X., 2018. Effects of bisphenol A and its analogs on reproductive health: a mini review. Reprod. Toxicol. 79, 96–123. https://doi.org/10.1016/j.reprotox.2018.06.005.

Skledar, D.G., Schmidt, J., Fic, A., Klopčič, I., Trontelj, J., Dolenc, M.S., Finel, M., Mašič, L.P., 2016. Influence of metabolism on endocrine activities of bisphenol S. Chemosphere 157, 152–159. https://doi.org/10.1016/j.chemosphere.2016.05.027.

Sun, Q., Wang, Y., Li, Y., Ashfaq, M., Dai, L., Xie, X., Yu, C.P., 2017. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ. Pollut. 225, 542–549. https://doi.org/10.1016/j.envpol.2017.03.018.

Union, I., Pure, O.F., Chemistry, A., 2005. Chemical Actinometry. International Union of Pure and Applied Chemistry, pp. 1–47.

Vervliet, P., 2018. invitRo-master. https://doi.org/10.5281/zenodo.1252144 (WWW Document).

Wang, G., 2007. Photodegradation of bisphenol Z by UV irradiation in the presence of b-cyclodextrin. 67, 762–769. https://doi.org/10.1016/j.chemosphere.2006.10.041.

Wang, G., 2008. Photocatalytic degradation of 2,2-bis(4-hydroxy-3-methylphenyl) propane (BPP) based on molecular recognition interaction. J. Chem. Technol. Biotechnol. https://doi.org/10.1002/jctb.1837.

Wang, G., Xue, X., Li, H., Wu, F., Deng, N., 2007. □-Cyclodextrin-enhanced photodegradation of bis (4-hydroxyphenyl) ethane under UV irradiation. 276, 143–149. https://doi.org/10.1016/j.molcata.2007.06.024.

Wang, X., Ma, J., Wang, Z., Guo, R., Hu, X., 2014. Aqueous phototransformation of bisphenol S: the competitive radical-attack pathway to p-hydroxybenzenesulfonic acid. Water Sci. Technol. 70, 540–547. https://doi.org/10.2166/wst.2014.257.

Wang, Y., Roddick, F.A., Fan, L., 2017. Direct and indirect photolysis of seven micropollutants in secondary effluent from a wastewater lagoon. Chemosphere 185, 297–308. https://doi.org/10.1016/J.CHEMOSPHERE.2017.06.122.

Wilkinson, J., Hooda, P.S., Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: an overarching review of the field. Environ. Pollut. 231, 954–970. https://doi.org/10.1016/J.ENVPOL.2017.08.032.

Wu, L.H., Zhang, X.M., Wang, F., Gao, C.J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci. Total Environ. 615, 87–98. https://doi.org/10.1016/j.scitotenv.2017.09.194.

Xiao, J., Wang, G., Xue, X., Wu, F., Luan, H., Deng, N., 2007. Enhanced photodegradation behavior of bisphenol F in the presence of □-cyclodextrin under UV light. 24, 812–820. https://doi.org/10.1089/ees.2006.0107.

Zhang, A., Li, Y., 2014. Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H_2O_2, and UV/H_2O_2 oxidation processes: effects of reaction conditions and sludge matrix. Sci. Total Environ. 493, 307–323. https://doi.org/10.1016/j.scitotenv.2014.05.149.

3.2.3 Kinetics and biotransformation products of bisphenol F and S during aerobic degradation with activated sludge

The paper "*Kinetics and biotransformation products of bisphenol F and S during aerobic degradation with activated sludge*" co-authored by A. Kovačič, C. Gys, M. R. Gulin, T. Gornik, T. Kosjek, D. Heath, A. Covaci and E. Heath is scheduled to be published in the *Journal of Hazardous Materials* in February 2021. The study was performed in collaboration between two research groups Department of Environmental Sciences, Jožef Stefan Institute, Slovenia, and Toxicological Center at the University of Antwerp, Belgium. Together with C. Gys, we conducted the experimental design under the supervision of Prof Dr E. Heath, Assist Prof Dr Kosjek, and Prof Dr Covaci. I led and performed the majority of the experimental work, data analysis and visualization and writing the manuscript.

This study is the first to determine the biodegradation kinetics of BPF and BPS during biological treatment by bacterial consortia (AS) using GC-MS/MS, and accurate mass high-resolution mass spectrometry LC-QTOF-MS for the identification and characterization of their bio-TPs. The studied conditions better reflect the natural environment or conditions in a WWTP, where microbial consortia degrade organic pollutants rather than using single bacterial strains. The results suggest that BPF and BPS do not accumulate in biosolids or WW effluent. The first-order kinetic model revealed the faster degradation of BPF compared to BPS as well as the effect of a compound's initial concentration on degradation rate, in particular for BPS. The optimized suspect and non-target screening approach, using a machine-learning algorithm, enabled the identification of one and ten novel bio-TPs and confirmed three and one previously reported bio-TPs of BPF and BPS, respectively. Based on these data, possible novel biotransformation pathways were postulated, namely sulfation, methylation, cleavage of the $S-C$ bond between the phenyl rings and the joining of smaller moieties.

The paper fulfilled the following aims of the thesis: (1) development, optimization and validation of GC-MS/MS for simultaneous determination of bisphenol residues and identification of their TPs using LC-QTOF-MS analytical method, (2) assessment of the removal efficiency of bisphenols by biological treatment, and (3) investigation of the bisphenol breakdown during biodegradation and identification of their TPs. Significantly, new insights into the non-target-based approach for the detection and identification of TPs and knowledge of the novel biodegradation pathways were provided within this paper. The findings contribute to a better understanding of the fate of bisphenols in the environment and during biological water treatment.

Journal of Hazardous Materials 404 (2021) 124079

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Kinetics and biotransformation products of bisphenol F and S during aerobic degradation with activated sludge

Ana Kovačič[a,b,1], Celine Gys[c,1], Martin Rafael Gulin[a], Tjaša Gornik[a,b], Tina Kosjek[a,b], David Heath[a], Adrian Covaci[c], Ester Heath[a,b,*]

[a] Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[b] International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia
[c] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

ARTICLE INFO

Keywords:
Bisphenol
Biodegradation
Kinetic
Biotransformation product
Pathway

ABSTRACT

Bisphenol F (BPF) and bisphenol S (BPS) are becoming widespread in the environment despite the lack of information regarding their fate during wastewater treatment and in the environment. This study assessed the biodegradation kinetics of BPF and BPS during biological wastewater treatment with activated sludge using GC-MS/MS, and the identification of biotransformation products (BTPs) using LC-QTOF-MS. The results showed that BPF and BPS degrade readily and unlikely accumulate in biosolids or wastewater effluent ($c_i = 0.1$ mg L^{-1}, half-lives <4.3 days). The first-order kinetic model revealed that BPF ($k_t = 0.20$–0.38) degraded faster than BPS ($k_t = 0.04$–0.16) and that degradation rate decreases with an increasing initial concentration of BPS (half-lives 17.3 days). The absence of any additional organic carbon source significantly slowed down degradation, in particular, that of BPS (lag phase on day 18 instead of day 7). The machine-learning algorithm adopted as part of the non-targeted workflow identified three known BTPs and one novel BTP of BPF, and one known and ten new BTPs of BPS. The data from this study support possible new biodegradation pathways, namely sulphation, methylation, cleavage and the coupling of smaller bisphenol moieties.

1. Introduction

Bisphenol A (4-[2-(4-hydroxyphenyl)propan-2-yl]phenol, BPA) has been in commercial use since 1957 as a monomer in the production of polycarbonate plastics and epoxy resins. Concerns over its toxicity, particularly its endocrine disrupting activity, have led many to call for an outright ban on its use, specifically in food contact materials (Choi and Lee, 2017; Chen et al., 2016). Besides BPA, 4,4′-dihydroxydiphenyl-methane (BPF) and 4,4′-sulphonyldiphenol (BPS) are also widely used in the production lacquers, varnishes, liners, adhesives plastics, and water pipes, as well as in dental sealants, oral prosthetic devices, tissue substitutes, coatings for food packaging and thermal receipt papers (Caballero-Casero et al., 2016). Both BPF and BPS remain unregulated while present in consumer products and various environmental (Liao et al., 2012a, 2012b) and human media (Chen et al., 2016; Liao et al., 2012c) and are known to be endocrine disrupting (Moreman et al., 2017). A significant source of bisphenols in the environment are wastewater treatment plant (WWTP) effluents (Kovačič et al., 2019a) and the land application of sewage sludge (Wang et al., 2019). Concentrations of BPA range from 3–84,110 ng L^{-1} in influent, n.d.–3100 ng L^{-1} in the effluent, and 3–3670 ng g^{-1} dw^{-1} in sludge, while BPF ranges from n.d.–117 ng L^{-1}, n.d.– 65 ng L^{-1} and n.d.–384 ng g^{-1} dw^{-1}, and BPS from n.d.–435 ng L^{-1}, n.d.–27 ng L^{-1} and n.d.–600 ng g^{-1} dw^{-1}, respectively (Wang et al., 2019; Česen et al., 2018, 2019). In some cases, the levels of BPF and BPS are similar or even exceed that of BPA in wastewaters, while data regarding their fate during WWTP is scarce (Xue and Kannan, 2019). Among the different technologies used to treat wastewater, limited data showed the potential of the biological treatment with activated sludge (AS) to be used for the removal of BPF and BPS (<99%) (Sun et al., 2017; Karthikraj and Kannan, 2017). Thus, a considerable effect on the environmental fate of BPF and BPS by bacterial communities is expected and should also be studied, including identification of degradation mechanisms and biotransformation products (BTPs).

Biodegradation of BPA involves oxidative skeletal rearrangement and hydroxylation of the aromatic ring following ring cleavage

---

* Corresponding author at: Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
E mail address: ester.heath@ijs.si (E. Heath).

[1] Joint first authors.

https://doi.org/10.1016/j.jhazmat.2020.124079
Received 12 August 2020; Received in revised form 10 September 2020; Accepted 21 September 2020

(Noszczyńska and Piotrowska-Seget, 2018; Eio et al., 2014), and similar biotransformation of BPF and BPS is expected. However, the different linkage of the BPF and BPS phenolic rings may affect their biodegradability (Ogata et al., 2013). To date, only a few studies have focused on the biodegradation of BPF and BPS, mainly using single bacterial strains (Table SI-1). However, these conditions are not representative of the natural environment or conditions in WWTPs, where microbial consortia degrade organic pollutants. While well-designed experimental set-ups have been conducted on other organic contaminants (Wu et al., 2020), only one study looks at the biodegradation of BPF in soil (Lu et al., 2017) and one study of BPF and BPS, by a microbiological community in seawater (Danzl et al., 2009), and two studies looking at the fate of BPS in soil (Choi and Lee, 2017) and AS (Huang et al., 2019) have been published. Also, specific biodegradation pathways have been proposed only for BPF using bacteria consortium from soil (Danzl et al., 2009) and Sphingobium yanoikuyae strain FM-2 isolated from river water, in which degradation proceeds via the Baeyer−Villiger reaction (Toyama et al., 2009; Sakai et al., 2007), and ring fission followed by ring hydroxylation in the case of *Cupriavidus basilensis* isolated from a composite soil (Zühlke et al., 2020). Zühlke et al. (2016) suggest that the formation of phosphate conjugates by *Bacillus amyloliquefaciens* (isolated from AS) is another possible detoxification mechanism for BPF. *Sphingobium fuliginis* OMI (isolated from the rhizosphere of the giant duckweed *Spirodela polyrrhiza*) is also capable of degrading BPF and BPS via hydroxylation and *meta*-cleavage and is the first report of the aerobic biodegradation of BPS (Ogata et al., 2013). Other studies show that BPS degrades in forest and farm soils as a result of *ortho*-cleavage (Choi and Lee, 2017) and recent kinetic studies of BPS indicate it is susceptible to degradation in AS (Huang et al., 2019) or by a *Sphingomonas* sp. strain NP5 isolated from AS (Takeo et al., 2020). Overall, BPS appears to be more resistant than BPF, especially when only isolated strains were studied (Table SI-1).

Despite progress, the fate of BPF and BPS in WWTPs remains poorly understood (Huang et al., 2019), and no studies have used non-target screening for BTPs of BPF or BPS using bacterial consortia (AS) and accurate mass high-resolution mass spectrometry for their identification and characterisation. This paper seeks to address this knowledge gap by determining the biodegradation kinetics of BPF and BPS during biological treatment with AS using gas chromatography-tandem mass spectrometry (GC-MS/MS), and the identification of BTPs using liquid chromatography-high-resolution quadrupole time-of-flight mass spectrometry (LC-QTOF-MS).

2. Materials and methods

2.1. Reagents and chemicals

General information about BPF and BPS, reagents and materials, and a description of standards preparation, is presented in the Supplementary information (SI): Chapters SI-2 and SI-3.

2.2. Batch biodegradation experiments

Aerobic biodegradation was conducted using a modified Zahn–Wellens test (Kosjek et al., 2015; Gornik et al., 2020). Briefly, batch biodegradation experiments (Table 1) were performed in the dark in 0.5 L glass bottles with a total volume of 400 mL. Each test was aerated using an aquarium pump (4 W, 4.0 L min^{-1}). Compounds were added at 0.1, 1, 2, 5 and 10 mg L^{-1} for the kinetics experiment and 10 mg L^{-1} for studying BTP formation. Series A, B, C, D, BL-A and BL-C contained 10 mL of freshly settled AS obtained from an operating mechanical-biological sequencing batch reactor of a nearby WWTP designed to treat 65,000 population equivalents. Two experimental conditions were applied: (1) using 330 mL of nutrient-mineral rich medium (A, B and BL-A), and (2) using 330 mL mineral rich medium (C, D and BL-C) without any source of organic carbon other than the tested compound (Table SI-3). Controls E and F contained only deionised water. Biological activity was inhibited by adding 6% formaldehyde, a method proven successful in our previous experiments (Kosjek et al., 2015, 2018) and as reported in the literature (Popova and Baykov, 2014). Each batch (B, D and E) was then shaken for 4 h before the addition of the analytes. These batches act as controls for A and C, to account possible abiotic losses, e.g. sorption. Except for these controls and blanks, all series were prepared in parallel (A1/2 and C1/2). At each sampling event, the ambient temperature was kept at 20 °C and monitored together with pH using a WTW pH330i (Weilheim, Germany) probe. Biomass concentrations were determined at the beginning of the experiment in three separately prepared flasks containing 10 mL of AS

Table 1
Experimental design.

Sample type[a]	AS [mL]	Nutrient-mineral rich medium [mL]	Mineral rich medium [mL]	Deionized water [mL]	Formaldehyde [mL]	Initial concentration of BP[b] [mg L^{-1}]	Total volume [mL]
Degradation kinetics							
BP-A1/2-CON1	10	330	0	60	0	0.1	400
BP-A1/2-CON2	10	330	0	60	0	1	400
BP-A1/2-CON3	10	330	0	60	0	2	400
BP-A1/2-CON4	10	330	0	60	0	5	400
BP-A1/2-CON5	10	330	0	60	0	10	400
BP-B-CON2	10	330	0	0	60	1	400
BL-A	10	330	0	60	0	0	400
Biotransformation product identification							
BP-A1/2	10	330	0	60	0	10	400
BP-B	10	330	0	0	60	10	400
BP-C1/2	10	0	330	60	0	10	400
BP-D	10	0	330	0	60	10	400
BP-E	0	0	0	340	60	10	400
BP-F	0	0	0	400	0	10	400
BL-A	10	330	0	60	0	0	400
BL-C	10	0	330	60	0	0	400

[a] BP = BPF or BPS.

[b] The initial concentration achieved by adding BP solution in DMSO, whereas the concentration of DMSO did not exceed 0.2% of the total volume of the test solution to maintain biomass viability (Neumegen et al., 2005).

Table 2

Summary of the identified metabolites, ordered by increasing *m/z*. The table displays which parent compound they originate from and by which biodegradation condition they were generated, their confidence level, RT [min], measured and theoretical [M-H]$^-$ for the precursor ion mass with calculated mass error [ppm], tentative formula, double bond equivalent (DBE), measured and theoretical product ion mass with a corresponding mass error [ppm].

Parent comp.	Biodeg. condition	Comp. ID	Conf. level[a]	RT [min]	Measured mass [M-H]$^-$	Theoretical mass [M-H]$^-$	Error precursor ion [ppm]	Elemental formula	Isotope ratio score [%]	DBE	Diagnostic product ion mass	Error product ion [ppm]	Theoretical product ion mass	Elemental composition of product ions
BPF		BPF		9.20	199.0759	199.0765	–3.01	$C_{13}H_{12}O_2$	96.1	8	77.0398	1.30	77.0397	C_6H_5
											93.0343	–3.22	93.0346	$C_6H_5O^-$
											105.0340	–5.71	105.0346	$C_7H_5O^-$
	A	BPF-213	L2b	7.98	213.0558	213.0557	0.45	$C_{13}H_{10}O_3$	96.2	9	93.0346	0	93.0346	$C_6H_5O^-$
	C										121.0270	–16.52	121.0290	$C_7H_5O_2^-$
											169.0683	14.20	169.0659	[M-CO$_2$]$^-$
	A	BPF-215	L2b	5.60	215.0719	215.0714	2.32	$C_{13}H_{12}O_3$	98.0	8	169.0644	–8.87	169.0659	$C_{12}H_9O^-$
	C										180.0566	–8.33	180.0581	$C_{13}H_8O^{\bullet -}$
											197.0616	–4.06	197.0608	[M-H$_2$O]$^-$
	A	BPF-279	L3	7.10	279.0349	279.0333	–8.24	$C_{13}H_{12}O_5S$	97.5	8	79.9560	–17.51	79.9574	SO_3^-
	C										105.0348	1.9	105.0346	$C_7H_5O^-$
											199.0760	–2.51	199.0765	$C_{13}H_{11}O_2^-$
	C	BPF-229	L4	8.40	229.0525	229.0506	8.29	$C_{13}H_{10}O_4$	78.8	NA	NA			
BPS		BPS		7.10	249.0227	249.0229	–0.80	$C_{12}H_{10}O_4S$	91.8	8	63.9635	–10.94 11.95 0.92 –0.64	63.9642	SO_2^-
											92.0273		92.0262	$C_6H_4O^{\bullet -}$
											108.0218		108.0217	$C_6H_4O_2^-$
											155.9886		155.9887	$C_6H_4O_3S^{\bullet -}$
	A C	BPS 93	L3	1.40	93.0342	93.0346	4.30	C_6H_6O		4	65.0404	10.76	65.0397	[M-CO]$^-$
											75.0253	17.33	75.0240	[M-H$_2$O]$^-$
	A	BPS-157	L3	1.36	156.9967	156.9965	1.27	$C_6H_6O_3S$	99.1	4	63.9636	–9.38	63.9642	SO_2^-
	C										93.0351	5.37	93.0346	$C_6H_5O^-$
											109.0299	3.67	109.0295	$C_6H_5O_2^-$
	A	BPS 173	L3	1.20	172.9915	172.9914	0.58	$C_6H_6O_4S$	N	4	79.9581	8.75	79.9574	SO_3^-
	C										93.0340	–6.45	93.0346	$C_6H_5O^-$
											109.0290	–4.59	109.0295	$C_6H_5O_2^-$
	C	BPS 189	L3	1.42	188.9854	188.9863	–4.76	$C_6H_6O_5S$	N	4	79.9577	3.75	79.9574	SO_3^-
											109.0290	–4.59	109.0295	$C_6H_5O_2^-$
											156.9981	10.19	156.9965	$C_6H_5O_3S^-$
											170.9729	–16.19	170.9757	[M-H$_2$O]$^-$
	A	BPS 263	L3	8.87	263.0383	263.0384	4.94	$C_{13}H_{12}O_4S$	98.6	8	92.0270	8.69	92.0262	$C_6H_4O^{\bullet -}$
	C										108.0221	3.70	108.0217	$C_6H_4O_2$
											155.9881	–3.85	155.9887	$C_6H_4O_3S^{\bullet -}$
											248.0157	3.22	248.0149	$C_{12}H_8O_4S^{\bullet -}$
	A	BPS-265A	L2b	5.90	265.0181	265.0176	0.79	$C_{12}H_{10}O_5S$	98.2	8	123.0091	2.44	123.0088	$C_6H_3O_3$
	C										108.0228	10.18	108.0217	$C_6H_4O_2^-$
											156.9949	–10.19	156.9965	$C_6H_5O_3S^-$
											171.9846	5.81	171.9836	$C_6H_4O_4S^-$
	A	BPS 265B	L3	8.60	265.0186	265.0176	3.91	$C_{12}H_{10}O_5S$	93.8	8	93.0347	1.07	93.0346	$C_6H_5O^-$
	C										108.0208	–8.33	108.0217	$C_6H_4O_2^-$
											141.0025	6.38	141.0016	$C_6H_5O_2S^-$

(*continued on next page*)

Table 2 (*continued*)

Parent comp.	Biodeg. condition	Comp. ID	Conf. level[a]	RT [min]	Measured mass [M-H]$^-$	Theoretical mass [M-H]$^-$	Error precursor ion [ppm]	Elemental formula	Isotope ratio score [%]	DBE	Diagnostic product ion mass	Error product ion [ppm]	Theoretical product ion mass	Elemental composition of product ions
											156.9962	−1.91	156.9965	$C_6H_5O_3S^-$
	A	BPS-279A	L3	7.24	279.0342	279.0333	3.22	$C_{13}H_{12}O_5S$	98.6	8	108.0229	11.11 5.69 −14.49 −3.82 1.51	108.0217	$C_6H_4O_2^-$
	C										123.0095		123.0088	$C_6H_3O_3^-$
											138.0302		138.0302	$C_6H_3O_3^-$
											156.9959		156.9965	$C_6H_5O_3S^-$
											264.0102		264.0098	$C_{12}H_8O_5S^{\bullet-}$
	A	BPS-279B	L3	8.11	279.0350	279.0333	6.09	$C_{13}H_{12}O_5S$	N	8	123.0066	−17.88	123.0088	$C_6H_3O_3^-$
	C										138.0319	−2.27	138.0322	$C_6H_3O_3^-$
											185.9988	12.37	185.9992	$C_7H_6O_4S^{\bullet-}$
											264.0059	−14.77	264.0098	$C_{12}H_8O_5S^{\bullet-}$
	A	BPS-329	L3	5.30	328.9819	328.9795	7.29	$C_{12}H_{10}O_7S_2$	85.5	8	108.0210	−6.48	108.0217	$C_6H_4O_2^-$
	C										249.0192	−12.05	249.0227	$C_9H_{12}O_4S^-$
	A	BPS-341	L3	10.00	341.0499	341.0489	3.05	$C_{18}H_{14}O_5S$	91.7	12	93.0362	17.20	93.0346	$C_6H_5O^-$
	C										108.0217	0.00	108.0217	$C_6H_4O_2^-$
											156.9985	12.74	156.9965	$C_6H_5O_3S^-$
											313.0498	13.42	313.0540	[M-CO]$^-$

[a] A confidence level L2b was assigned to BTPs based on MS, MS^2 and were previously reported in the literature, L3 was assigned to novel BTPs based on MS, MS/MS with insufficient information for one exact structure (e.g., positional isomers) and L4, was assigned to BTPs when an unequivocal molecular formula is possible using the MS and literature data.

and 390 mL of deionised water and after the closure of the experiment in flasks with AS (A-D, BL). Three parallels of 15 mL homogenised sample were collected from each flask and filtered through pre-dried and weighted GF-2 filters.

2.3. *Sample collection and preparation*

During the kinetic experiments, samples (4-mL) were collected at t = 15 min (to assure homogeneity) and then at 24-hour intervals over 14-days. Based on kinetic experiments BTP samples were collected at t = 15 min and then at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 9 days for BPF and at 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18 days for BPS. Each sample was centrifuged at 9000 rpm for 20 min and the supernatant filtered through 0.2 μm regenerated cellulose filters (Phenomenex, USA). Aliquots of individual extracts were transferred to GC (0.1 or 0.5 mL) or LC (0.5 mL) vials and spiked with isotope-labelled internal standards: $^{13}C_{12}$-BPF and $^{13}C_{12}$-BPS (V = 25 μL; 10 μg mL^{-1}) or BPA-d_{16} (V = 25 μL; 20 μg mL^{-1}). For quantification purposes, the solvent was removed with N_2 (40 °C), derivatised with 50 μL of MSTFA and 50 μL of pyridine for 1 h at 80 °C and diluted with 0.4 mL of ethyl acetate prior to GC-MS/MS analysis. For BTPs screening, the extracts were stored at −20 °C and analysed directly by LC-QTOF-MS.

2.4. *GC-MS/MS analysis for degradation kinetics*

Kinetic experiment samples were analysed using a 7890B series GC coupled to an Agilent 7000 series triple quad MS/MS, (Agilent Technologies, USA). The method was modified from our previous study (Kovačič et al., 2020). Briefly, the chromatographic separation was achieved using 30 m × 0.25 mm Ø x 0.25 μm i.d. μm DB-5 MS capillary column (Agilent Technologies). The carrier gas was He, operated in constant flow mode (1 mL min^{-1}), and the collision gas was N_2 (1.5 mL min^{-1}). Samples were injected (1 μL) in the splitless mode at 270 °C, and the oven program was run isothermally (270 °C) with a total runtime of 9.33 min. The mass spectrometer was operated in EI mode at 70 eV in the multi-reaction monitoring mode. Retention times (RT) and MS/MS optimised conditions are summarised in Table SI-4. MassHunter software (Version B.07.00, Agilent Technologies) was used for data analysis. Matrix-matched validation was performed with treated artificial WW collected from a laboratory-scale WW bioreactor for WW samples (Kovačič et al., 2019b). The linearity of the method was evaluated using isotopically labelled internal standards and matrix-matched calibration curves. The concentration of BPF and BPS in the extract subsamples (0.1 mL (higher) and 0.5 mL (lower) concentration) increased from 0.1 to 10 mg L^{-1}, whereas the concentration of the internal standards was fixed (2.5 μg mL^{-1}). The R^2 was >0.99 for each BP when using a weighted (1/x) linear calibration curve. Each sample batch consisted of duplicate calibrants, samples collected during three consecutive days, controls (sample B), blanks (sample BL) and solvent blanks. The relative standard deviations of the calibrants between batches were <10% except for the lowest calibration level (BPF: RSD = 36%, BPS: RSD = 15%). Limit of detection and quantification (LOQ) were determined (Magnusson and Örnemark, 2014) as three and ten times the standard deviation of the background (defined by the signal in ten distinct matrix blanks), respectively. The LOQ for BPF and BPS were 0.02 or 0.1 mg L^{-1}. The lowest calibration point, depending on the initial concentration of the investigated compound, covered 80–99% bisphenol removal. Concentrations in blanks were <LOQ. No potential carry-over was observed.

2.5. *LC-QTOF-MS analysis for biotransformation product identification*

The non-target screening was conducted using an Agilent 1290 Infinity UPLC coupled to an Agilent 6530 Accurate-Mass QTOF (Agilent Technologies, USA). The separation was achieved at 40 °C using a Poroshell 120 EC-C18 column (100 ×3 mm; 2.7 μm particle size, Agilent

Technologies, USA). The mobile phase was ultra-pure water with 1 mM NH_4F (A) and methanol with 1 mM NH_4F (B) at a flow rate of the 0.4 mL min^{-1}. The injection volume was 5 µL. The run started isocratically for 1 min at 10% B then raised to 40% in 4 min, to 75% in 5 min, and finally to 95% in 1 min. The column was rinsed with 95% B for 4 min and re-equilibrated for 5 min at 10% B. The QTOF-MS was operated in the 2 GHz mode (Extended Dynamic Range), at a resolution of 3800 at m/z 112.9856 and 8500 at m/z 1033.9881. The ions m/z 119.0363 and m/z 940.0009 were selected for continuous in-run calibration to ensure mass accuracy. For ionisation, an Agilent Jet-Stream electrospray source was operating in negative ionisation mode only, as non of the reported transformation products of bisphenols from our previous studies were identified in positive mode (Kovačič et al., 2019a; Gys et al., 2018). Drying gas temperature was 300 °C at a flow of 8 L min^{-1}, respectively. The sheath gas temperature was 350 °C (11 L min^{-1}) with a nebuliser pressure of 25 psig. Capillary, nozzle and fragmentor voltages were 3500, 1500, and 150 V, respectively. The instrument was run in data-dependent acquisition mode with a mass range of 50–1000 m/z and a scan rate of 2 scans s^{-1} for MS and 5 scans s^{-1} for MS/MS. Collision energies were 10 and 20 V. Instrumental variation was <10%.

2.6. Data processing and analysis

Data processing, including statistical calculations and visualisation, were performed using Python (version 3.6) (Python) and R-based software (version 3.6.1) (RStudio Team). All data were fitted using a first-order kinetic model by nonlinear least-squares analysis. Correspondingly, half-lives and degradation rates (k_t = day^{-1}) were calculated by the following equation: $c_t/c_i = e\hat{}(-k_t \times t)$, where c_i and c_t are initial and concentration at a certain time (t = days) (Perrin, 2017). A statistical analysis of the data samples was performed to assess the influence of c_i and medium type on the degradation kinetics. Based on the Shapiro–Wilk test for normality and the Levene's test for homoscedasticity, Wilcoxon signed-rank and the Friedman test for nonparametric two data or more than two data samples were selected. The statistical significance was evaluated at a level of 0.05 (Eftimov et al., 2017). The suspect and non-target screening workflows used in this study were modified from previous studies (Kovačič et al., 2019a; Gys et al., 2018; Mortelé et al., 2018; Vervliet, 2018). The optimised approach resulted in enhanced confidence in the detection and identification of BTPs. Initially, suspect screening was performed using an expanded csv-database of possible BTPs (Kovačič et al., 2019a), and the features were extracted using MassHunter Qualitative Analysis Software (version B.07.00, Agilent Technologies). A non-targeted approach was conducted and optimised using MZmine (version 2.52) (Pluskal et al., 2010) and R (Beirnaert, 2018). Briefly, MZmine was used to detect and differentiate MS features as follows: mass detection (centroid algorithm), chromatogram building, chromatogram deconvolution (noise amplitude algorithm), deisotoping, filtering according to peak width (0.03–2 min), peak alignment (RANSAC algorithm) and gap-filling (the same m/z and RT). For each investigated compound, two lists of mass features, one with broader (lower noise level and peak height) and one with restricted limits (higher noise level and peak height) were generated and processed in R using the modified Shiny app tindeResting, which uses a Random Forest machine-learning algorithm (Beirnaert, 2018). This approach enabled the ability to filter the relevant mass features from a smaller or larger number of false features. Predictions were based on mass feature intensity (>1000), RT (>0.5 min), presence/absence in the first sample, controls and blanks, presence in both aliquots, the response ratio between peak height in control/peak height in the sample (<1), and the prediction value (>0.1). The resulting m/z values were extracted using the *Formula Prediction* algorithm in MZmine. The identification of potential BTPs resulting from both workflows were based on accurate mass and isotopic patterns in MS mode. For each match, the MS/MS fragmentation pattern and accurate mass of the respective product ions were analysed. Tentative structures were postulated based on the mass shift of the BTP compared to the parent compound, elemental formula and by comparing MS/MS fragmentation spectra with the parent compounds and other BTPs. Identification was based on the following criteria: (a) a maximal mass variation of ±10 ppm between the measured and theoretical parent ions; (b) a maximal mass variation of ±20 ppm for product ions; (c) an isotope pattern score of at least 70%; (d) the measured double bond equivalent values (DBE) matched the postulated structure; (e) absence/presence in control and blank samples; (f) absence/presence in both aliquots and parallels; and (g) by following the biotransformation time profile. Levels of confidence were assigned to identified BTPs following the scale and requirements, as proposed by Schymanski et al. (2014).

3. Results and discussion

3.1. Degradation kinetics

Results on degradation kinetics are fully presented in Table SI-6 and Fig. 1. In controls, no significant changes in levels of BPF (87 ± 8%) and BPS (89 ± 10%) were observed during the first ten days. After ten days, a 30% loss of parent compound was observed, indicating abiotic removal. Neither BPF nor BPS was present in the blanks. Experimental parameters are given in Chapter SI-4. At time zero (t = 0) biomass concentrations were 0.44 ± 0.05 g L^{-1} and 0.35- 1.43 g L^{-1} at the end of the experiment (t = 14 days). Biological growth and sample evaporation during the incubation period could be two of the reasons for the observed increased

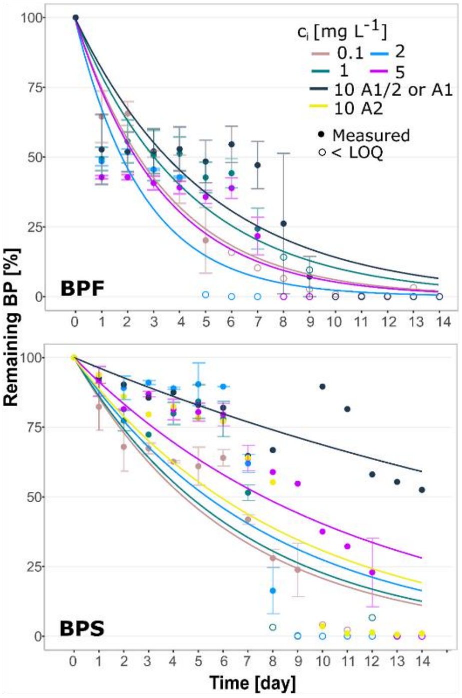


Fig. 1. Aerobic degradation kinetics (single first-order) of BPF (above) and BPS (below) in AS. Vertical bars are standard deviations of parallels A1 and A2, except for BPS at 10 mg L^{-1}. Open circles are <LOQ.

in biomass concentration (Table SI-5). Therefore, the amount of evaporated sample was expressed as the daily factor of evaporation (F_{evap}), calculated as the total loss of sample at the end of the set-up divided by the incubation period and was included in the calculations of quantitative analysis. The temperature (19.9 ± 0.17 °C) and pH (7.26 ± 0.13) remained similar between all batches of A and BL, whereas in batch B, the addition of formaldehyde reduced the pH to 4.73 ± 0.07. Fig. 1 shows the degradation kinetics for BPF and BPS in nutrient-mineral rich media (A). A nonlinear least-squares analysis produced reasonably good fits for BPF, namely $R^2 = 0.72–0.97$ while in most cases of BPS lower $R^2 = 0.68–0.88$ were observed, especially in case of its highest c_i (Table SI-7). The results showed that BPF degrades faster ($k_t = 0.20–0.38$, half-lives = 1.8– 3.5 days) than BPS ($k_t = 0.04–0.16$, half-lives 4.3–17.3 days) and that their c_i affects degradation kinetics. The latter was also confirmed by the statistical analysis using the Friedman test that found a significant difference ($p < 0.05$) between degradation kinetic of both compounds at their c_i. For BPF, 35- 64% was degraded within 24 h for all tested c_i, then after a plateau phase and depending on c_i a complete removal was observed. Levels of BPF were <LOQ (1–20% remaining) by day 5 at $c_i = 2$ mg L^{-1}, by day 8 at $c_i = 0.1$ and 5 mg L^{-1} and by day 10 at $c_i = 1$ and 10 mg L^{-1} (Fig. 1), in accordance with the $k_t = 0.20$- 0.38 (Table SI-7). Zühlke et al. (2016) also found that at a certain c_i, the formation of BTP could activate bacteria responsible for compound degradation. The degradation rate for BPF in this study is slower compared to those reported in the literature (Table SI-1), where complete degradation was achieved within 36 h at a $c_i \leq 300$ mg L^{-1}. One explanation for this discrepancy could be the authors' use of optimal, isolated bacteria compared to a more realistic microbiological community used in this study..

In line with our study, the opposite effect is shown for the more persistent BPS, for which the removal is higher in bacterially rich communities. At a $c_i = 0.1–5$ mg L^{-1}, the level of BPS was <LOQ at day 10, as was the case in one parallel batch ($k_t = 0.12$, half-lives = 5.8 days) at $c_i = 10$ mg L^{-1}, whereas in a second parallel >50% remained at day 13 ($k_t = 0.04$, half-lives = 17.3 days). Therefore, c_i had a greater effect on BPS degradation compared to BPF (Fig. 1). The observed discrepancy between the parallels at $c_i = 10$ mg L^{-1} could result from the increased presence of BPS that could have toxic effects on bacteria responsible for its degradation. Moreover, the same phenomenon was observed in the case of biotransformation product identification. These results are in agreement with Zdarta et al. (2018), who also noted a decrease in the degradation rate of BPF and BPS with increasing c_i by the fungal enzyme *Trametes Versicolor* laccase, immobilised on spongin. In contrast, Huang et al. (2019), report the complete removal of BPS from AS in a continuous flow bioreactor at a $c_i = 50$ mg L^{-1} after 6 days. However, strong variations in experimental conditions and employed organisms make comparing the data from different studies difficult.

3.2. Biotransformation products identification

Bisphenol F and S BTPs formed in nutrient-mineral (A), and mineral (C) rich medium at $c_i = 10$ mg L^{-1} by aerobic AS microbes were identified and compared. The results are presented in Chapter SI-4. No significant losses were observed for BPF (<10%) over 11 days and BPS (< 20%) over 18 days in the controls (BP-B, -D, -E and -F). The exception is control F, where >30% BPS was degraded within 14 days. The experimental parameters are presented in Table SI-8 and are in line with the experiment of degradation kinetics. The identified BTPs were not detected in the controls and blanks, showing that the removal of BPF and BPS in conditions A and C was the result of biodegradation. Their postulated formulas and corresponding confidence levels, as proposed by Schymanski et al., (2014), are presented in Table 2.

All proposed BTPs that met the necessary identification criteria and were detected in samples from at least one sampling time; chromatograms and MS/MS spectra are given in Subchapter SI-4.2.2. Broader limits in MZmine resulted in a higher number of identified BTPs when the relevant mass features were mainly filtered using the machine-learning algorithm. A semi-quantitative analysis, carried out by calculating the ratio of the peak areas of the parent compound and BTPs to that of the internal standard, provided information on the time trend of parent degradation and BTPs formation (Fig. 2) and allowed biodegradation pathways to be postulated (Figs. 3 and 4). In this case, the averages of both parallels are not presented; rather, Fig. 2 shows parallels with the highest relative quantities of BTPs. All other relevant information is shown in Figs. SI-5 and SI-6.

Bisphenol F eluted at RT 9.20 min as the deprotonated molecule ($[M-H]^-$, 199.0765) and was completely degraded by day 5. Statistical analysis showed a significant difference ($p < 0.05$) between degradation kinetics of parent compound in the different media. Three BTPs (BPF-215, BPF-213 and BPF-279) were identified in nutrient-mineral (A) rich media and four (BPF-215, BPF-213, BPF-279 and BPF-229) in mineral (C) rich media (Table 2). BTPs appeared between day 2 and 3, reached a maximum by day 4 and were undetectable by day 6 (Fig. 2). The spectrum of BPF-215 ($[M-H]^-$, 215.0719) shows the neutral loss of water and is indicative of aliphatic hydroxylation. Based on the calculated relative quantities, BPF-213 ($[M-H]^-$, 213.0558) most likely forms by the oxidation of BPF-215. Its transformation to BPF-229 ($[M-H]^-$, 229.0525), identified at a confidence level L4 (Fig. 3), possibly forms via a Baeyer-Villiger reaction, as suggested by Inoue et al. (2008) and Lu et al. (2017). A small peak at RT 7.90 min had the *m/z* ($[M-H]^-$, 215.0758) characteristic of a hydrolysed BTP. However, MS/MS fragmentation was not triggered, and the ppm error was high (>10). A novel minor BTP, BPF-279 ($[M-H]^-$, 279.0349) was also identified and the presence of a sulphate (SO_3^-) functional group suggests a sulphate-conjugated BTP.

Bisphenol S was detected as the deprotonated molecule ($[M-H]^-$, 249.0227) at RT 7.10 min. In this case, degradation was complete by day 7 under condition A and by day 18 under condition C (Fig. 2). A significant difference between degradation kinetic of BPS under condition A and C was also shown by the statistical analysis ($p < 0.05$). In line with the degradation kinetics, a similar discrepancy between two parallel batches at $c_i = 10$ mg L^{-1} (Figs. 2 and SI-6) were observed, which further suggests that this c_i may represent the concentration, beyond which BPS could have a toxic effect on the bacteria. Although the lag phase for BPS (day 7 or 18) in the case of faster parallel batch (Fig. 2) was longer than for BPF (day 5), the concentration of both compounds declined significantly within two days in both nutrient-mineral (A) and mineral (C) rich media. Interestingly, despite the sudden and complete reduction in the amounts of BPF and BPS, BTP formation varied depending on degradation kinetics, applied conditions and exposure time. Of the eleven BTPs identified (Table 2), only hydroxylated BPS-265A is consistent with that reported for degradation with the *Sphingobium fuliginis OMI* (Ogata et al., 2013), while BTPs with characteristic ring cleavage was not observed (Choi and Lee, 2017). Between RT 1.20–1.42 min, four S–C cleavage BTPs were identified: three (BPS-93, BPS-157, and BPS-173) in nutrient-mineral and four (BPS-93, BPS-157, BPS-173 and BPS-189) in mineral rich media (Table 2). The first three had similar kinetic profiles under both conditions reaching a maximum concentration at day 7 in Series A and day 17 in Series C, followed by their gradual decomposition. The inclusion of oxygen was observed in two compounds: BPS-265A ($[M-H]^-$, 265.0181) at RT 5.90 min and BPS-265B ($[M-H]^-$, 265.0186) at RT 8.60 min. Their RT and MS/MS spectra suggest that they are not positional isomers, but rather, BPS-265A is a hydroxylated form of BPS and is probably the major BTP formed under both conditions. However, it was detected only during slower transformation (C) of BPS and was completely degraded within 15 days. Alternatively, the formation of BPS-265B follows that of cleaved BTPs and is likely formed by coupling of smaller BPS moieties. The presence of BPS-263 ($[M-H]^-$, 263.0383) suggests the linking of a methyl group, as one of the possible biotransformation reaction (George and Häggblom, 2008) from the methylation of BPS, while BPS-279A ($[M-H]^-$, 279.0342) and BPS-279B ($[M-H]^-$, 279.0350) are isomers

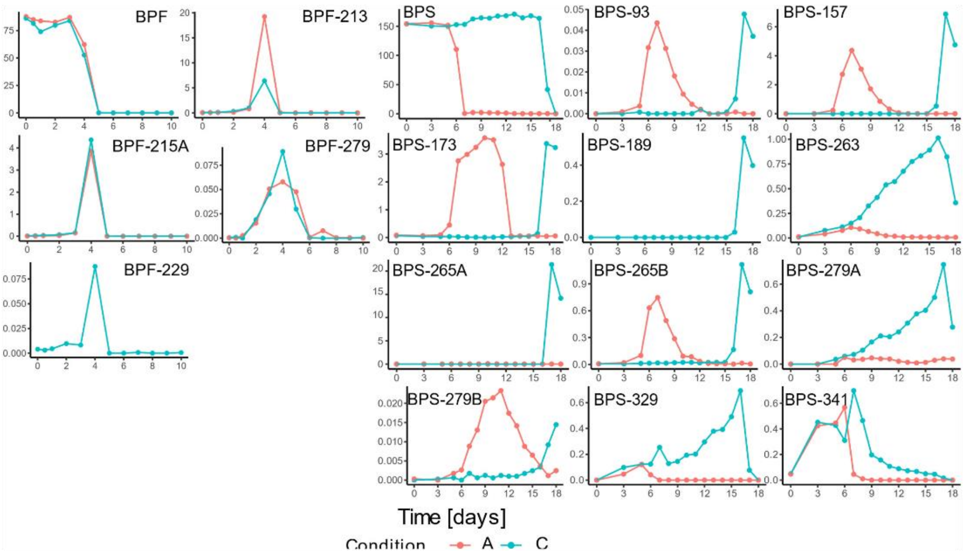


Fig. 2. Degradation kinetics of identified biotransformation products of BPF (left) and BPS (right) formed in AS under conditions A (red line) and C (blue line).

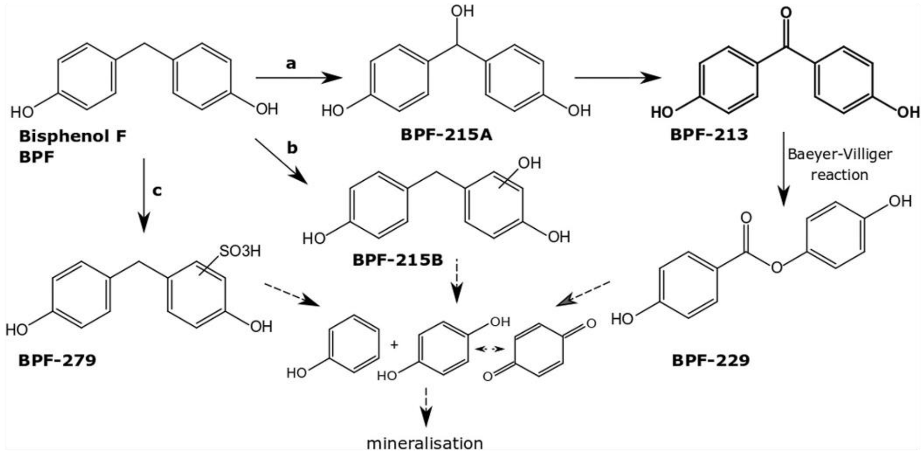


Fig. 3. Proposed pathway for biodegradation of BPF in AS (a: aliphatic hydroxylation, b: aromatic hydroxylation and c: sulphation, dashed arrows; not identified).

formed by a combination of hydroxylation and methylation. Although the positional isomerisation is assumed from their common product ions, neither the exact position of the hydroxyl- nor methyl groups could be determined (confidence level L3). Similar to BPF, BPS-329 ($[M\text{-}H]^-$, 328.9891) is probably a result of sulphate conjugation. In contrast, the spectra and RT 10.00 of BPS-341 ($[M\text{-}H]^-$, 341.0499) indicate the presence of a phenol group, which was also observed for BPF by Zdarta et al. (2018) during degradation with immobilised *Trametes versicolor laccase*.

Biodegradation pathway: Fig. 3 shows the biodegradation pathway of BPF, as proposed in this study. The pathway is the same as that suggested for *Sphingobium yanoikuyae,* where hydroxylation of the bridging carbon leads to complete mineralisation via the Baeyer-Villiger reaction (Inoue et al., 2008; Toyama et al., 2009). Recent studies (Choi and Lee, 2017; Ogata et al., 2013; Zühlke et al., 2016) have reported similar biodegradation pathways common to other bisphenols (Bisphenol A, C, E, B, P, AF, and Z), namely aromatic hydroxylation and meta- or ortho-cleavage of BPF and BPS in soil and sediments and phosphate

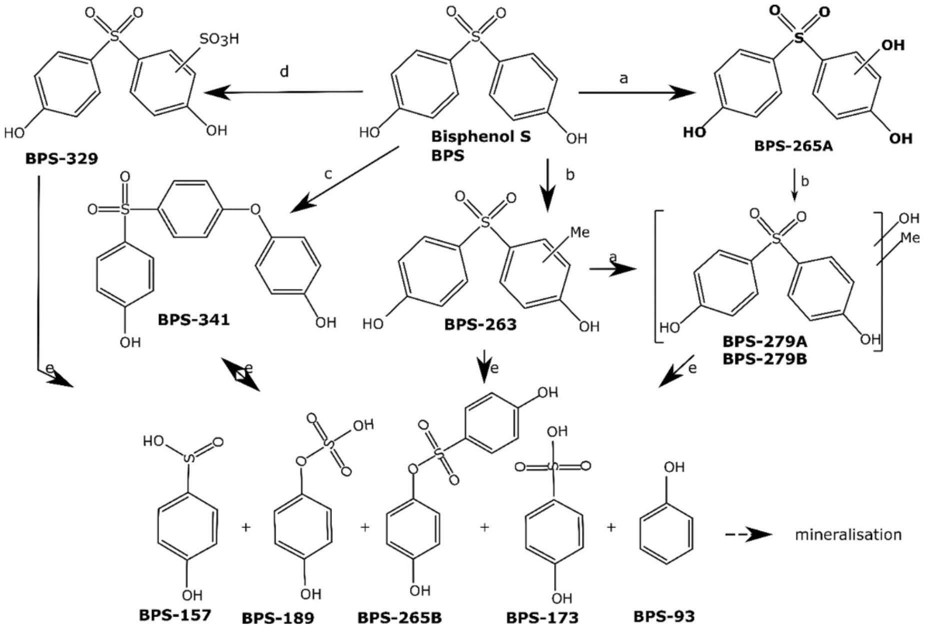


Fig. 4. Proposed pathway for biodegradation of BPS in AS (a: hydroxylation, b: methylation, c: the coupling of smaller BPS moieties, d: sulphation and e: cleavage of the S–C bond, dashed arrows; not identified).

conjugation of BPF by *Bacillus amyloliquefaciens* from AS. In this study, hydroxylation also forms part of the biodegradation pathways for BPS. In addition, the results show that degradation occurs by sulphation in the case of BPF and BPS and for BPS via methylation, cleavage of the S‐ C bond between the phenyl rings and the coupling of smaller moieties Figs. 3 and 4). The appearance and disappearance of BTPs imply the ability of certain bacteria in AS to slowly transform the parent compound by attaching smaller moieties until other bacteria, capable of removing the parent compounds and BTPs prevail (Fig. 2). We propose that the described biodegradation pathways for both compounds do not differ under the two applied conditions, but the detection of specific BTPs rather depends on the time of sampling. However, in the absence of any organic carbon source other than BPF or BPS (C), degradation is slower, especially in the case of BPS (Kosjek et al., 2013, 2018). Also, despite not having identified lower *m/z* BTPs of BPF, it is reasonable to assume that they are formed in both conditions but were lost due to rapid degradation and possible mineralisation. This assumption is supported by the relative quantities of the parent compounds and BTPs, i.e., BTP formation " parent compound degraded.

In summary, rapid biodegradation under aerobic conditions suggests that BPF and BPS ($c_i = 0.1$ mg L^{-1}, half-lives <2.5 and 4.3 days) will at environmental concentration most likely not accumulate in biosolids or wastewater effluent. The results reveal a new biodegradation pathway for BPF, based on sulphate-conjugated BTP. The slower degradation of BPS allowed for the identification of 11 BTPs, of which ten are novel and provide new evidence of additional biodegradation pathways. Importantly, new insights into the biodegradation of bisphenols and knowledge of novel biodegradation pathways will play an essential role in understanding the fate of bisphenols during biological water treatment and in the environment.

CRediT authorship contribution statement

Ana Kovačič – Conceptualization, Methodology, Investigation, Validation, Formal analysis, Data curation, Visualization, Writing - original draft. **Celine Gys** – Conceptualization, Methodology, Investigation, Writing - draft manuscript. **Tjaša Gornik:** Investigation. **Martin Rafael Gulin:** Software, Visualization. **Tina Kosjek:** Conceptualization, Supervision. **David Heath** - Data curation, Edite - original draft. **Adrian Covaci:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition. **Ester Heath:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition. All authors reviewed the manuscript and contributed to improving the quality of this paper.

Declaration of Competing Interest

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

Acknowledgments

Ana Kovačič acknowledges the Young researcher grant (Slovenian Research Agency). The study was performed with the financial assistance of the Slovenian Research Agency, namely Program Group P1–0143 projects L1–7544, L1–9191, N1–0047, J1–8147, J2–8162 and N1–0143. Celine Gys acknowledges a PhD fellowship from the Research Foundation Flanders (FWO): project G0E5216N.

Conflicts of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124079.

References

Beirnaert, C., 2018. tindeResting: Shiny app to rate the interestingness of metabolic time profiles. https://github.com/Beirnaert/tindeResting (accessed 26 March 2020).

Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53. https://doi.org/10.1016/j.aca.2015.12.034.

Česen, M., Ahel, M., Terzić, S., Heath, D.J., Heath, E., 2019. The occurrence of contaminantsof emerging concern in Slovenian and Croatian wastewaters and receiving Sava river. Sci. Total Environ. 650, 2446–2453. https://doi.org/10.1016/j.scitotenv.2018.09.238.

Česen, M., Heath, D., Krivec, M., Košmrlj, J., Kosjek, T., Heath, E., 2018. Seasonal and spatial variations in the occurrence , mass loadings and removal of compounds of emerging concern in the Slovene aqueous environment and environmental risk assessment. Environ. Pollut. 242, 143–154. https://doi.org/10.1016/j.envpol.2018.06.052.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity – a review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

Choi, Y.J., Lee, L.S., 2017. Aerobic soil biodegradation of bisphenol (BPA) alternatives bisphenol S and bisphenol AF compared to BPA. Environ. Sci. Technol. 51, 13698–13704. https://doi.org/10.1021/acs.est.7b03889.

Danzl, E., Sei, K., Soda, S., Ike, M., Fujita, M., 2009. Biodegradation of bisphenol A, bisphenol F and Bisphenol S in Seawater. Int. J. Environ. Res. Public Health. 6, 1472–1484. https://doi.org/10.3390/ijerph6041472.

Eftimov, T., Korošec, P., Potočnik, D., Ogrinc, N., Heath, D., Seljak, B.K., 2017. How to perform properly statistical analysis on food data? An e-learning tool: advanced statistics in natural sciences and technologies. In: Science within Food: Up-to-date Advances on Research and Educational Ideas, 2018, pp. 144–151.

Eio, E.J., Kawai, M., Tsuchiya, K., Yamamoto, S., Toda, T., 2014. Biodegradation of bisphenol A by bacterial consortia. Int. Biodeterior. Biodegrad. 96, 166–173. https://doi.org/10.1016/j.ibiod.2014.09.011.

George, K.W., Häggblom, M.M., 2008. Microbial O methylation of the flame retardant tetrabromobisphenol-A. Environ. Sci. Technol. 42, 5555–5561. https://doi.org/10.1021/es800038q.

Gornik, T., Kovacic, A., Heath, E., Hollender, J., Kosjek, T., 2020. Biotransformation study of antidepressant sertraline and its removal during biological wastewater treatment. Water Res., 115864 https://doi.org/10.1016/j.watres.2020.115864.

Gys, C., Kovačič, A., Huber, C., Lai, F.Y., Heath, E., Covaci, A., 2018a. Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism. Toxicol. Lett. 295, 115–123. https://doi.org/10.1016/j.toxlet.2018.05.034.

Huang, W.-C., Jia, X., Li, J., Li, M., 2019. Dynamics of microbial community in the bioreactor for bisphenol S removal. Sci. Total Environ. 662, 15–21. https://doi.org/10.1016/j.scitotenv.2019.01.173.

Inoue, D., Hara, S., Kashihara, M., Murai, Y., Danzl, E., Sei, K., Tsunoi, S., Fujita, M., Ike, M., 2008. Degradation of bis(4-hydroxyphenyl)methane (bisphenol F) by Sphingobium yanoikuyae strain FM-2 isolated from river water. Appl. Environ. Microbiol. 74, 352–358. https://doi.org/10.1128/AEM.01708-07.

Karthikraj, R., Kannan, K., 2017. Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere. 181, 216–223. https://doi.org/10.1016/j.chemosphere.2017.04.075.

Kosjek, T., Negreira, N., de Alda, M.L., Barceló, D., 2015a. Aerobic activated sludge transformation of methotrexate: Identification of biotransformation products. Chemosphere. 119, S42–S50. https://doi.org/10.1016/j.chemosphere.2014.04.081.

Kosjek, T., Negreira, N., Heath, E., López de Alda, M., Barceló, D., 2018. Aerobic activated sludge transformation of vincristine and identification of the transformation products. Sci. Total Environ. 610–611, 892–904. https://doi.org/10.1016/j.scitotenv.2017.08.061.

Kosjek, T., Perko, S., Žigon, D., Heath, E., 2013. Fluorouracil in the environment: analysis, occurrence, degradation and transformation. J. Chromatogr. A 1290, 62–72. https://doi.org/10.1016/j.chroma.2013.03.046.

Kovačič, A., Gys, C., Gulin, M.R., Kosjek, T., Heath, D., Covaci, A., Heath, E., 2020. The migration of bisphenols from beverage cans and reusable sports bottles. Food Chem., 127326 https://doi.org/10.1016/j.foodchem.2020.127326.

Kovačič, A., Gys, C., Kosjek, T., Covaci, A., Heath, E., 2019a. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: Identification of transformation products and degradation kinetics. Sci. Total Environ. 664, 595–604. https://doi.org/10.1016/j.scitotenv.2019.02.064.

Kovačič, A., Česen, M., Laimou-Geraniou, M., Lambropoulou, D., Kosjek, T., Heath, D., Heath, E., 2019b. Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions. Environ. Res. 179, 108738 https://doi.org/10.1016/J.ENVRES.2019.108738.

Liao, C., Liu, F., Moon, H.B., Yamashita, N., Yun, S., Kannan, K., 2012a. Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: Spatial and temporal distributions. Environ. Sci. Technol. 46, 11558–11565. https://doi.org/10.1021/es303191g.

Liao, C., Liu, F., Guo, Y., Moon, H.B., Nakata, H., Wu, Q., Kannan, K., 2012b. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. Environ. Sci. Technol. 46, 9138–9145. https://doi.org/10.1021/es302004w.

Liao, C., Liu, F., Alomirah, H., Loi, V.D., Mohd, M.A., Moon, H.B., Nakata, H., Kannan, K., 2012c. Bisphenol S in urine from the United States and seven Asian countries: Occurrence and human exposures. Environ. Sci. Technol. 46, 6860–6866. https://doi.org/10.1021/es301334j.

Lu, H., Weng, Z., Wei, H., Zhou, J., Wang, J., Liu, G., Guo, W., 2017. Simultaneous bisphenol F degradation, heterotrophic nitrification and aerobic denitrification by a bacterial consortium. J. Chem. Technol. Biotechnol. 92, 854–860. https://doi.org/10.1002/jctb.5069.

Magnusson, B., Örnemark, U., 2014. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, second ed. Eurachem https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.

Moreman, J., Lee, O., Trznadel, M., David, A., Kudoh, T., Tyler, C.R., 2017. Acute toxicity, teratogenic, and estrogenic effects of bisphenol A and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish embryo-larvae. Environ. Sci. Technol. 51, 12796–12805. https://doi.org/10.1021/acs.est.7b03283.

Mortelé, O., Vervliet, P., Gys, C., Degreef, M., Cuykx, M., Maudens, K., Covaci, A., van Nuijs, A.L.N., Lai, F.Y., 2018. In vitro Phase I and Phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. J. Pharm. Biomed. Anal. https://doi.org/10.1016/j.jpba.2018.02.032.

Neumegen, R.A., Fernández-Alba, A.R., Chisti, Y., 2005. Toxicities of triclosan, phenol, and copper sulfate in activated sludge. Environ. Toxicol. 20, 160–164. https://doi.org/10.1002/tox.20090.

Noszczyńska, M., Piotrowska-Seget, Z., 2018. Bisphenols: application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers. Chemosphere. 201, 214–223. https://doi.org/10.1016/j.chemosphere.2018.02.179.

Ogata, Y., Goda, S., Toyama, T., Sei, K., Ike, M., 2013. The 4-tert-butylphenol-utilizing bacterium sphingobium fuliginis OMI can degrade bisphenols via phenolic ring hydroxylation and meta-cleavage pathway. Environ. Sci. Technol. 47, 1017–1023. https://doi.org/10.1021/es303726h.

Perrin, C.L., 2017. Linear or nonlinear least-squares analysis of kinetic data? J. Chem. Educ. 94, 669–672. https://doi.org/10.1021/acs.jchemed.6b00629.

Pluskal, T., Castillo, S., Villar-Briones, A., Orešič, M., 2010. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry based molecular profile data. BMC Bioinform. 11 https://doi.org/10.1186/1471-2105-11-395.

Popova, T.P., Baykov, B.D., 2014. Decontamination of sewage sludge by treatment with formaldehyde in vitro. Int. J. Curr. Microbiol. Appl. Sci. 3, 982–989.

Python, Welcome to Python.org. https://www.python.org/ (accessed March 26, 2020).

RStudio Team, Open source & professional software for data science teams - RStudio. https://rstudio.com/ (accessed March 26, 2020).

Sakai, K., Yamanaka, H., Moriyoshi, K., Ohmoto, T., Ohe, T., 2007. Biodegradation of bisphenol A and related compounds by Sphingomonas sp. strain BP-7 isolated from seawater. Biosci. Biotechnol. Biochem. 71, 51–57. https://doi.org/10.1271/bbb.60351.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105.

Sun, Q., Wang, Y., Li, Y., Ashfaq, M., Dai, L., Xie, X., Yu, C.P., 2017. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ. Pollut. 225, 542–549. https://doi.org/10.1016/j.envpol.2017.03.018.

Takeo, M., Akizuki, J., Kawasaki, A., Negoro, S., 2020. Degradation potential of the nonylphenol monooxygenase of Sphingomonas sp. NP5 for bisphenols and their structural analogs. Microorganisms 8. https://doi.org/10.3390/microorganisms8020284.

Toyama, T., Sato, Y., Inoue, D., Sei, K., Chang, Y.C., Kikuchi, S., Ike, M., 2009a. Biodegradation of bisphenol A and bisphenol F in the rhizosphere sediment of Phragmites australis. J. Biosci. Bioeng. 108, 147–150. https://doi.org/10.1016/j.jbiosc.2009.03.011.

Vervliet, P., 2018 invitRo-master. doi:10.5281/zenodo.1252144.

Wang, H., Liu, Z. hua, Zhang, J., Huang, R. ping, Yin, H., Dang, Z., Wu, P. xiao, Liu, Y., 2019. Insights into removal mechanisms of bisphenol A and its analogues in municipal wastewater treatment plants. Sci. Total Environ. 692, 107–116. https://doi.org/10.1016/j.scitotenv.2019.07.134.

Wu, G., Geng, J., Shi, Y., Wang, L., Xu, K., Ren, H., 2020. Comparison of diclofenac transformation in enriched nitrifying sludge and heterotrophic sludge: transformation rate, pathway, and role exploration. Water Res. 184 https://doi.org/10.1016/j.watres.2020.116158.

Xue, J., Kannan, K., 2019. Mass flows and removal of eight bisphenol analogs, bisphenol A diglycidyl ether and its derivatives in two wastewater treatment plants in New York State, USA. Sci. Total Environ. 648, 442–449. https://doi.org/10.1016/j.scitotenv.2018.08.047.

Zdarta, J., Antecka, K., Frankowski, R., Zgoła-Grześkowiak, A., Ehrlich, H., Jesionowski, T., 2018. The effect of operational parameters on the biodegradation of bisphenols by Trametes versicolor laccase immobilized on Hippospongia communis spongin scaffolds. Sci. Total Environ. 615, 784–795. https://doi.org/10.1016/j.scitotenv.2017.09.213.

Zühlke, M.K., Schlüter, R., Henning, A.K., Lipka, M., Mikolasch, A., Schumann, P., Giersberg, M., Kunze, G., Schauer, F., 2016. A novel mechanism of conjugate formation of bisphenol A and its analogues by Bacillus amyloliquefaciens: detoxification and reduction of estrogenicity of bisphenols. Int. Biodeterior. Biodegrad. 109, 165–173. https://doi.org/10.1016/j.ibiod.2016.01.019.

Zühlke, M.K., Schlüter, R., Mikolasch, A., Henning, A.K., Giersberg, M., Lalk, M., Kunze, G., Schweder, T., Urich, T., Schauer, F., 2020. Biotransformation of bisphenol A analogues by the biphenyl-degrading bacterium Cupriavidus basilensis – a structure-biotransformation relationship. Appl. Microbiol. Biotechnol. 3569–3583. https://doi.org/10.1007/s00253-020-10406-4.

3.3 Migration from Food Contact Materials

3.3.1 The migration of bisphenols from beverage cans and reusable sports bottles

The paper "*The migration of bisphenols from beverage cans and reusable sports bottles*", by A. Kovačič, C. Gys, M. R. Gulin, T. Kosjek, D. Heath, A. Covaci and E. Heath was published in *Food Chemistry* in June 2020. I was fully responsible for the experimental work, method optimization, and validation, instrumental, and data analysis and writing the manuscript with assistance from M. R. Gulin in sample preparation, data analysis and visualization, and C. Gys who performed the LC-QTOF-MS analysis.

This study aimed to: (1) develop and validate an accurate and sensitive analytical method based on GC–MS/MS for the determination of BPA, 22BPF, 24BPF, BPF, BPE, BPC, BPB, BPZ, BPAP, BPC2, BPAF and BPS migrating from beverage cans and sports bottles; (2) test the influence of different materials, conditions and matrix on the migration of bisphenols from FCMs, and (3) assess the stability of bisphenol during migration studies. In this regard, a precise and accurate GC–MS/MS method with ng L^{-1} LOQ, acceptable recovery (78–107%) and estimated uncertainty (U>20%, except at LOQ) was developed following the Eurachem guidelines [208]. The migration and stability of selected bisphenols were established in two food simulants (C: 20% ethanol, and B: 3% acetic acid) from beverage cans and reusable metal and plastic sports bottles, following the standard EU protocol/migration tests for plastic material [217]. As such, a broad set of different beverage types and (extreme) migration conditions were covered. Among all monitored bisphenols, only BPC, BPC2, BPAF and BPS were <LOQs in all tested beverage cans and sports bottles. All cans leached BPA (<5865 ng L^{-1}), three BPF isomers (8.2–1286 ng L^{-1}) and BPAP (1.6 ng L^{-1}), while eight of the tested bottles leached BPA (<222 ng L^{-1}) and BPF, BPE, BPB and BPZ (1.1–4.6 ng L^{-1}). The highest concentrations of BPA and BPF originated from the linings of beverage cans and reusable steel bottles. Importantly, the highest estimate did not exceed t-TDI for BPA (4 μg kg bw^{-1} day^{-1}), neither for adults or children nor the SML (0.05 mg kg^{-1}) with any of the tested samples. However, the results suggest that cans present a significant source of bisphenols and BPA was determined in bottles even though being labelled as BPA free, while traces of other bisphenols were present in bottles that did not contain BPA. In addition, the non-target analysis also showed that the largest amount of compounds migrate from the beverage can linings and highlighted the existing challenges in improving data analysis workflows. Simulant C was more aggressive than simulant B, and concentrations of bisphenols decreased with consecutive exposure to simulants. From an analytical perspective, it is safe to store dried sample extracts at −20 °C for at least 8 weeks and derivatized samples at room temperature (21 °C) for up to 7 days.

This paper addresses three aims of the thesis, including (1) development, optimization and validation of GC-MS/MS for simultaneous determination of 12 bisphenol residues and LC-QTOF-MS for identification of their TPs and other compounds migrating from FCMs, (2) to examine bisphenols stability, and (3) to evaluate bisphenols migration from FCMs into food simulants. The findings of this paper are expected to bring about a better understanding of the occurrence and human exposure to bisphenols migrating from FCMs.

Food Chemistry 331 (2020) 127326

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

The migration of bisphenols from beverage cans and reusable sports bottles

Ana Kovačič[a,b], Celine Gys[c], Martin Rafael Gulin[a], Tina Kosjek[a,b], David Heath[a], Adrian Covaci[c], Ester Heath[a,b,*]

[a] *Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia*
[b] *International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia*
[c] *Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium*

ARTICLE INFO

Keywords:
Bisphenol
Migration
Food contact material
Stability
GC–MS/MS

ABSTRACT

A precise and accurate GC–MS/MS method with ng L^{-1} LLOQs, acceptable recovery (78–107%) and estimated uncertainty (U > 20%, except at LLOQ) was developed following the Eurachem guidelines. We established the migration and stability of twelve bisphenols in two food simulants (C: 20% ethanol, and B: 3% acetic acid) from beverage cans (n = 16) and reusable metal and plastic sports bottles (n = 51). Bisphenols were stable in dried (eight weeks, −20 °C) and derivatised extracts (seven days, 21 °C). Cans leached BPA (< 5865 ng L^{-1}), three BPF isomers (8.2–1286 ng L^{-1}) and BPAP (1.6 ng L^{-1}), while bottles leached BPA (< 222 ng L^{-1}) and BPF, BPE, BPB and BPZ (1.1–4.6 ng L^{-1}). Simulant C was more aggressive than simulant B, and concentrations of bisphenols decreased with consecutive exposure to simulants. Levels of BPA migrating from cans did not exceed the specific migration limits.

1. Introduction

Included among chemicals classified as endocrine disrupters is Bisphenol A (BPA), an industrial chemical mainly used as a monomer in the production of polycarbonate plastic and epoxy-resin (Kovačič, Česen, et al., 2019), both of which find broad application in food contact materials (FCMs) (Xiong et al., 2018). Food contact materials are defined as all materials and articles, which come in contact with food, for example, polycarbonate plastics in reusable food containers and drink bottles, and epoxy resins used as liners of cans and lids of glass jars and bottles for food and beverages, (Caballero-Casero, Lunar, & Rubio, 2016). These materials constitute a significant segment of the food packaging market which is becoming one of the largest global industrial sectors, amounting to around 839 billion USD in 2015 and set to reach 998 billion USD in 2020 (ALL4PACK, 2016; Sanchis, Yusà, & Coscollà, 2017).

Bisphenol A is a weak estrogen, which can generate reactive oxygen species (Pérez-Albaladejo, Fernandes, Lacorte, & Porte, 2017), presenting a risk for fetal development and reducing basal testosterone secretion in men at low concentrations (Rochester & Bolden, 2015a). Studies have linked exposure to BPA to numerous adverse health outcomes, like diabetes, obesity, and cardiovascular diseases (Rochester & Bolden, 2015a; Vilarinho, Sendón, van der Kellen, Vaz, & Silva, 2019). Due to uncertainties regarding these adverse health effects, the European Food and Safety Authority (EFSA) lowered the Tolerable Daily Intake (TDI) for BPA to a temporary TDI (*t*-TDI) of 4 µg/kg body weight/day (European Food Safety Authority CEF, 2015). More recently, the European Commission set a specific migration limit (SML) of 0.05 mg kg^{-1} for FCM (EU, 2018) and prohibited its use in baby bottles (EU, 2011). In the face of growing restrictions, alternative materials (e.g., polypropylene (PP), polyethersulphone (PES), polyamide (PA), Tritan™ and silicone) and compounds including other BPA like chemicals (collectively referred to as bisphenols, BPs) are entering the market (Onghena et al., 2016). Recent *in vitro* and *in vivo* studies demonstrate (Escrivá, Hanberg, Zilliacus, & Beronius, 2019; Gramec Skledar & Peterlin Mašič, 2016) that these compounds, including, for example, bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB) and bisphenol AF (BPAF) share a toxicity profile similar to BPA. Their examined hormonal activities are in the same order of magnitude as BPA with a similar estrogenic, anti-estrogenic, androgenic and, anti-androgenic action (Rochester & Bolden, 2015a; Usman, Ikhlas, & Ahmad, 2019).

Since the early 1990s, research has focused on the migration of BPA into various foods and food simulants (Fasano, Cirillo, Esposito, & Lacorte, 2015; Guart, Bono-Blay, Borrell, & Lacorte, 2014; Vilarinho et al., 2019), whereas there is little information concerning other BPs (Caballero-Casero et al., 2016; Česen et al., 2016; Vilarinho et al., 2019). So far, scientists have detected BPs in canned food (1–959 µg kg^{-1}) including vegetables, seafood, meat, milk products,

---

[*] Corresponding author at: Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
E-mail address: ester.heath@ijs.si (E. Heath).

https://doi.org/10.1016/j.foodchem.2020.127326
Received 13 January 2020; Received in revised form 26 April 2020; Accepted 10 June 2020

Table 1
A list of publications reporting concentrations of BPA and other BPs from various cans and plastic containers.

BPs studied	Material	Matrix	Target/Non-target	Instrument	Detected concentrations	Reference
BPA, BPC, BPAF, BPE, BPF, BPG, BPM, BPS, BPZ	plastic bottles	carbonated beverages	Target	GC–MS/MS	All: <LOQ to 13.43 ng mL $^{-1}$, BPS major	(Mandrah et al., 2017)
BPA, BPS, BPF, BPE, BPB	can	various beverages	Target	LC-MS/MS	BPA: 44–607 ng L^{-1}, BPF: 141, 218 ng L^{-1}	(Gallart-Ayala, Moyano, & Galceran, 2011)
BPA, BPS	plastic baby bottles	simulant	Target/Non-target	LC-MS/MS, GC–MS/MS	–	(Onghena et al., 2016, 2015)
BPA, BPF, BPS, BPB, BPAP, BPP, BPZ, BPAF	cans, paper, plastic, glass	various food	Target	LC-MSMS	All BPs: >LOQ − 9.0 µg kg $^{-1}$ BPA the most frequent, BPF the major (mustard products)	(Liao & Kannan, 2013, 2014)
BPA, BPB, BADGE, BFDGE	can	tuna (meat, oil, water)	Target	LC-FD	All (tuna meat): 6.3–187.0 ng g^{-1}	(Fattore et al., 2015)
BPA, BPB, BPF, BPE, BADGE, BFDGE	can	beer and simulant	Target	LC-FD	BPA, BADGE: 1.26–3.79 µg L^{-1}	(Xie et al., 2015)
BPA, BPF, BPAF, BPE, BPB, BPS, BPP	plastic baby package food	various food	Target	GC–MS/MS	BPA, BPP, BPE, BPB, BPS, BPF: 1–50 ng g $^{-1}$	(Driffield et al., 2018)
BPA, BPF, BPS, BPB, BPAP, BPP, BPZ, BPAF	plastic	simulant	Target	LC-MS/MS	–	(Hwang et al., 2018)
BPA, BPS, BPF, BPAF, BPB, BPZ, BPAP, BPM, BPP	baby teethers	simulant	Target/Suspect	GC–MS/MS	All BPs: 1.93–213 ng	(Asimakopoulos, Elangovan, & Kannan, 2016)
BPA, BPB	can	beverages, powder infant formula	Target	GC–MS	BPA: 0.03–4.70, BPB: 0.06–0.017 µg L^{-1}	(Cunha et al., 2011)
13BPs	cans, paper, plastic, glass	various beverages	Target	LC-MS/MS	Cans: BPA: <LOD − 1.26 µg L^{-1}, three isomers of BPF: 0.12–1 µg L^{-1}	(Regueiro & Wenzl, 2015)
11BPs	reusable plastic bottles	simulant	Target/Non-target	LC-MS/MS	–	(Tian et al., 2019)
BPA, BADGE derivatives	epoxy coatings	simulant	Target/Suspect	LC-DAD, LC-MS, UHPLC-HRMS	detected BPA, BADGE	(Paseiro-Cerrato et al., 2016)
BPA, BPF, BPZ, BPP	can	food and liquid	target	GC–MS	BPA, BPF, BPZ: >LOQ-14 ng L^{-1}	(Cacho et al., 2012)
21 BPs, BADGE derivatives	various	various milk products	Target	UHPLC-MS/MS	detected BPA, BPS, BPF, BPAF, BPAP, BPZ, TCBPA, BADGE derivatives	(Cheng et al., 2017)
18BPs	–	milk	Target	GC–MS/MS	BPA: <LOQ-1.16 µg L $^{-1}$, BPS: 0.23 µg L $^{-1}$	(Deceuninck et al., 2015)
9 BPs	–	milk	Target	HPLC-FLD	BPA: 13.74 µg kg^{-1}	(Xiong et al., 2018)
BPs and trace metals	can	vegetable foods	Target	HPLC-FLD	detected BPA, BADGE	(Noureddine El Moussawi et al., 2019)
BPA, BPB, BPF, BADGE, BFDGE	can	energy beverages	Target	UPLC-FL	detected BPA, BPF, BADGE, BFDGE	(Gallo et al., 2017)
BPA, BPB, BPF, BPE and BADGE derivatives	can	various food	Target	HPLC-FD	BPA, BPB, BPADGE derivatives: 7.1–959 µg kg^{-1}, BPF and BPE not present	(Alabi, Caballero-Casero, & Rubio, 2014)
BPA, BPF, BPS, BPB	cans, paper, plastic, glass	various food	Target	LC-MS/MS	BPA, BPS, BPF: 14.3, 1.6, 1.4 µg kg $^{-1}$	(Zhou et al., 2019)
9 BPs	plastic	honey and simulant	Target	GC–MS	BPA, BPAF, BPE, BPF, BPS (major), BPZ, BPAF: 12.8 – 302 ng g $^{-1}$	(Česen et al., 2016)

oils, eggs, cereal, honey and beverages (14–3790 ng L^{-1}) (Table 1). Monitoring BPs in unpacked milk has resulted in the detection of BPA (<LOQ – 13.74 µg L^{-1}) only (Xiong et al., 2018). However, the number of migration studies analysing BPA analogues from food simulants exposed to plastic containers or cans is scarcer. The studies by Xie et al. (Xie et al., 2015), using a HPLC-FD method, and by Paseiro-Cerrato et al. (Paseiro-Cerrato, Devries, & Begley, 2017) using a UHPLC-HRMS method combined with a suspect screening approach found only BPA and diglycidyl ether (BADGE) when performing migration tests on cans. The authors' focus was to verify the appropriateness of such tests rather than determining the investigated compounds at trace levels. Also, apart from BPA, BPs were not reported in migration studies of reusable baby bottles (Onghena et al., 2016), plastic bottles (Tian, Lin, & Bayen, 2019), nor in other FCMs made from different polymers (Česen et al., 2016; Hwang et al., 2018). The different aims of these studies, namely the verification of migration test adequacy, the evolution of non-target approaches, the evolution of short- and long-term migration testing for organic coating materials, justify the use of migration tests that do not follow EU testing guidelines (EU, 2011) or using methods with high limits of quantifications (LLOQs) despite BPs occurring in trace concentrations. For these reasons, there exists a real need for migration studies of BPs from cans and reusable sports bottles following EU migration tests using more accurate and sensitive analytical methods. Also, the stability of BPs during migration studies has been neglected and needs to be addressed.

This study aims to develop and validate an accurate and sensitive analytical method based on gas chromatography-tandem mass spectrometry (GC–MS/MS) for the determination of BPA, 22BPF, 24BPF, BPF, BPE, BPC, BPB, BPZ, BPAP, BPC2, BPAF and BPS migrating from beverage cans and sports bottles. In this context, the aim is to show the difference between the studied materials (metal with organic coating and different plastic materials), simulants (C: 20% ethanol and B: 3% acetic acid) that cover a broad set of different beverage types and (extreme) migration conditions. The work is also the first to highlight the stability of BPs during migration studies and their derivatives during analysis. The findings of this study are expected to bring about a better understanding of the occurrence and human exposure to BPs migrating from FCMs.

2. Materials and methods

2.1. Reagents, materials and standards

Analytical standards BPF (>98%), BPAF (>99%), 24BPF (>98%), 22BPF (>98%), BPE (>98%), BPA (>97%), BPC (>99%), BPC2 (>98%), BPZ (>98%), BPAP (>99%), BPS (>98%) were obtained from Sigma-Aldrich (St. Louis, USA), while BPB (Purity > 99.8%) was from Dr. Ehrenstoffer (Augsburg, Germany). Isotopically labelled BPF ($^{13}C_{12}$-BPF), BPS ($^{13}C_{12}$-BPS), and BPB ($^{13}C_{12}$-BPB) were purchased from CanSyn Chem. Corp. (Toronto, Canada), and deuterated BPA (BPA-d_{16}) purchased from Sigma Aldrich (USA). Compound specifications are given in Table SI-1. The derivatising agent *N*-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, Purity ≥ 99.0%) and anhydrous pyridine (Pyridine, Purity 99.8%) catalyst were purchased from Sigma-Aldrich (Schnelldorf, Switzerland and Steinheim, Germany). The solvents (HPLC grade) ethyl acetate (EtAc), ethanol (EtOH), methanol (MeOH), and water were obtained from J. T. Baker (Netherlands). Concentrated hydrochloric acid (37%, HCl) and formic acid (FA) used for solid-phase extraction (SPE) were purchased from Sigma-Aldrich (St. Louis, USA). Acetic acid (AA, Purity 99–100%) used for preparing the simulant was obtained from J. T. Baker (Germany). Ultrapure water was prepared using a Milli-Q water purification system (Millipore Merck Direct-Q^{TM}) to a specific resistance of > 18.0 MΩ cm $^{-1}$ at 25 °C.

2.2. Standard and quality control samples

Standard and internal standard stock solutions (concentration = 1.0 ± 0.1 mg mL^{-1}) were prepared by dissolving each compound in MeOH. A working standard solution (100 µg mL^{-1}) of 11 BPs and a solution of BPA (100 µg mL^{-1}) were then prepared in MeOH from the standard stock solutions (1.0 mg mL^{-1}). Similarly, an internal standard solution (1.0 µg mL^{-1}) in MeOH was prepared from a solution of deuterated (d_{16}BPA) and isotopically labelled ($^{13}C_{12}$-BPF, $^{13}C_{12}$BPS and $^{13}C_{12}$-BPB) compounds (1.0 mg mL^{-1}). Calibration standards were prepared by serial dilution from the working standard solution. Two calibration standards were prepared: 0.01 and 1.0 µg mL^{-1} for the BP mixture, and 0.1 and 1.0 µg mL^{-1} for BPA. Once prepared, all standards were stored in amber glass vials at 4 °C (maximum of 24 weeks). The same procedure was used to prepare stock and standard working solutions used for quality control (QC).

2.3. Preliminary experiment

Preliminary experiments focused on the migration of bisphenols, bisphenol diglycidyl ethers and their derivatives from FCMs including plastic bottles, reusable sports bottles, beverage cans, and baby teethers under standardised migration/leaching tests (EU 10/2011). The samples were incubated for 10 days at 60 °C or 3 times for 24 h at 70 °C using different aqueous simulants: 20% and 50% EtOH, 3% AA in ultrapure water, and 100% MeOH.

After extraction (described in section 2.6) and derivatisation, the samples were analysed using GC–MS/MS and the concentrations of 22BPF, BPAF, 24BPF, BPF, BPE, BPA, BPB, BPZ, BPS BPC2, BPC, BPM, BPPH, BPP, BPBP, BPAP, BP26DM and BPFL in the simulant were determined. Additionally, non-targeted analyses were performed on non-derivatised extracts using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in data-dependent acquisition mode. Elution was accomplished by combining two fractions. The first fraction was eluted with 3 × 0.6 mL 3% FA in EtAc while the second fraction was eluted with 3 × 0.6 mL dichloromethane. Data-analysis included two previously optimised parallel workflows: i) suspect screening using a database generated from the literature, and ii) non-targeted screening using the MZmine software combined with the R statistical software (Gys et al., 2018; Kovačič, Gys, Kosjek, Covaci, & Heath, 2019; Mortelé et al., 2018; R Development Core Team, 2011). The results generated from both workflows were used to design the final migration experiments and as the basis for future non-targeted studies of FCMs.

2.4. Sample collection

Samples included 1) aluminum beverage cans with internal coatings and lids designed for alcoholic (n = 4) and non-alcoholic beverages (n = 2) obtained from a single producer, and 2) reusable sports bottles (n = 11) made of various materials (PP, PE, Tritan™, PE-HD and steel), of different prices and brands. The bottles were purchased locally in sports shops and supermarkets in Slovenia between October 2018 and June 2019. Information on the type of coating used was not available, but mostly organic coatings are used to protect the integrity of the contents from the metal (Oldring and Nehring, 2007; Paseiro-Cerrato et al., 2017; Paseiro-Cerrato, Noonan, & Begley, 2016). None of the containers had been exposed to beverages before the experiment. Used sports bottles were also tested (n = 7). Detailed information about the beverage cans and bottles are presented in Table SI-2.

2.5. Migration testing

All plastic wrappings and labels were removed before testing, and the inner surface of each container rinsed with 10 mL of HPLC water and manually shaken for 30 s (Tian et al., 2019). Migration experiments followed the standard EU protocol (EU, 2011) for plastic materials. Unfortunately, there are no specific EU regulations for beverage cans, and only alternative testing migration conditions from the guidelines for rigid metal packaging coated with organic coatings (TSC23, 2017) are reported. Preliminary experiments, however, revealed no physical damage to the cans after testing. In the present study, two simulants were used based on Regulation EU No. 10/2011 (EU, 2011), which cover the majority of beverage types that come into contact with the selected containers. Simulant C (20% (v/v) ethanol in ultrapure water) is representative for clear non-alcoholic beverages or alcoholic beverages of an alcoholic strength lower than or equal to 6% v/v, and cloudy alcoholic beverages of an alcoholic strength of between 6% and 20% v/v, with a pH above 4.5. Simulant B (3% acetic acid (v/v) in ultrapure water) was used to represent beverages with a pH < 4.5.

Regulation EU No. 10/2011 states that "*For contact times above 30 days at room temperature and below the specimen shall be tested in an accelerated test at elevated temperature for a maximum of 10 days at 60 °C*". For this reason, each beverage can was filled with an equivalent volume of pre-heated simulant and incubated for 10 days at 60 °C (General; GEN). Current migration protocols for long-term storage of food products proved to be inappropriate for materials with organic coatings, and in each case, the migration test was extended to 30 days (Harsh; HAR) (Paseiro-Cerrato et al., 2017, 2016). The full cans were then sealed using their corresponding lids provided by the same manufacturer to avoid evaporation. Sports bottles, were tested using preheated simulant at 70 °C for 24 h in order to simulate worst-case conditions. Each bottle was tested using three consecutive volumes of fresh simulant (D1, D2 and D3). Used sports bottles were also tested using simulant B to simulate the most commonly stored beverages. For each migration test, two cans and two bottles from the same batch were analysed, and glass bottles were used to generate the controls and procedural blanks. Control samples were prepared in triplicate and analysed in the same batch as the samples. All glassware was cleaned and pyrolysed at 400 °C for 4 h to remove possible contamination (Česen et al., 2016).

2.6. Extraction

At the time of sampling, simulant (150 mL) was transferred to a glass bottle. In the case of simulant C, samples were further diluted with ultrapure water (300 mL) and acidified to pH 2 using HCl (37%). The internal standard mixture (25 µL; 1 µg mL^{-1}) was then added, and each sample was extracted using solid-phase extraction (SPE) and derivatised using a modified method as outlined in our previous studies (Kovačič, Česen, et al., 2019; Kovačič, Gys, et al., 2019). The influence of different sample volumes, pH and % of EtOH and AA used in the present study (data not shown) was also checked. It was found that loading 150 mL or 300 mL of the sample instead of 350 mL and the presence of 3% AA and 10% EtOH had no significant effect on extraction efficiency except for BPS, for which the use of 20% EtOH simulant resulted in < 50% SPE efficiency. Česen et al. (Česen et al., 2016) suggest rinsing the SPE cartridges with 15 mL MeOH, but since we found no significant contamination, the samples were loaded directly onto the cartridges. Samples for stability testing were prepared using the same procedure, except that the internal standard mixture was added before the derivatisation step.

2.7. GC–MS/MS analysis

Analyses were performed using a 7890B series gas chromatograph coupled to triple quadrupole mass analyser, GC–MS/MS, (7000 series, Agilent Technologies, United States). A list of the studied BPs is given in the supplementary material (Table SI-1). Separation was achieved using an Agilent Technologies DB-5 MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium (purity 6.0) operated under constant flow (1 mL min^{-1}) at an average velocity of 26 cm *sec*$^{-1}$ Chromatographic conditions were transferred and optimised from the method of Kovačič, Česen, et al. (2019). Samples were injected (2 µL) in the splitless mode at 270 °C (purge-off time, 2 min), and the oven temperature program was as follows: 120 °C (0 min), 30 °C min^{-1} until 220 °C (1 min) and 15 °C min^{-1} until 310 °C (0.7 min). Total runtime was 11.03 min. The MS transfer line was held at 280 °C. The mass spectrometer was operated in EI mode at 70 eV with the source temperature set at 250 °C. The collision gas was nitrogen at 1.5 mL min^{-1} (purity 6.0). Compounds were identified and quantified using multireaction monitoring (MRM) using two transitions; one for quantification (MRM 1) and one as a confirmation (MRM 2), for each analyte sequenced in twelve different time windows. The collision energy (CE) was optimised using the Analyze Experiments Assistant and the dwell time (DT) using the Dynamic MRM Assistant both provided by Agilent Technologies. The DT was such that 15 to 20 data points were collected per peak. The transition ions and optimised MS parameters are presented in Table SI-3. All data were acquired and processed using MassHunter software (Agilent Technologies).

2.8. Method validation

A full validation procedure was performed following the Eurachem guidelines (Magnusson & Örnemark, 2014) and the JRC guidelines on validation procedures for analytical methods in control of food contact materials (Bratinova, Raffael, 2009). The measurement uncertainty was estimated according to the Eurachem guide; Quantifying uncertainty in analytical measurement and Guide to the Expression of Uncertainty in Measurement (GUM) (EURACHEM/CITAC Guide CG 4, 2012; GUM, 2008). The method was validated for both simulant B and C. The optimum calibration curve was then selected using an internal standard, which covers the loss of analyte during sample preparation and analysis. The analytical method was validated using QC samples. Method validation parameters included: linearity, specificity, selectivity, accuracy, precision, the lowest limit of detection (LLOD) and LLOQ, reproducibility, and uncertainty. Controls (n = 3) and procedure blanks (n = 3) were systematically included in each batch of analyses to account for any contamination. The validation was performed at either four or three concentration levels for BPs and BPA: LLOQ = 1/3 and 10 ng L^{-1}, low (LQC) = 3 and 30 ng L^{-1}, medium (MQC) = 100 and 800 ng L^{-1} and high (HQC) = 0.6 and 2.5 µg L^{-1}, respectively. Based on the preliminary results and published data, a higher calibration range was used for BPA. Contamination due to BPs leaching from Parafilm® (used to seal cans) was also tested. A low and midpoint QC (LQC and MQC) sample and pure standard were included after every 20th sample, while solvent blanks were analysed after every 10th sample to evaluate potential carry-over.

2.9. Bisphenols stability in dried and derivatised extracts

The stability of the BPs in the stock solution, ultrapure water and wastewater were reported previously in Kovačič et al. (Kovačič, Česen, et al., 2019), while this study aim was to test their stability during migration experiments. Experimental temperatures and times were selected based on the following premises: storage of dried extracts at −20 °C is a common choice for long-term storage, and derivatised compounds are typically kept at room temperature (21 °C) before analysis. The studied compounds were only added to the dry extracts before being exposed to the test conditions to avoid extraction losses. Their stability was evaluated in terms of the average remaining percentage of the compound, calculated by dividing the determined concentration at a specific time-point by the initial concentration at time zero.

Exp. 1 Stability of BPs in dried extract during storage

To a series of glass vials containing dried extracts (prepared as described in section 2.5), a mixture of 12 BPs was added to give two final concentrations (LLOQ/LQC and MQC) of BPs and BPA. The samples were then reduced to dryness under a gentle stream of nitrogen (40 °C) and stored in the dark at − 20 °C for 0, 1, 7, 30, 60 and 180 days. At the time of sampling, the residues were spiked (25 µL) with the internal standards mixture (1 µg mL^{-1}), derivatised, and then analysed. Three replicate samples were prepared for each matrix, temperature setting, and sampling time. All controls were prepared in triplicate.

Exp. 2 Stability of BP derivatives in derivatised extract

The stability of the derivatised BPs was also verified. For this, sample extracts were prepared as described in *Exp. 1.* To each extract, target compounds and internal standards were added and immediately derivatised, stored at −20 °C and room temperature (21 °C) and analysed after 0, 1/3, 1, 2, 3 and 7 days. To provide reliable data, the stability of the derivatised internal standards: $^{13}C_{12}$-BPF, $^{13}C_{12}$-BPS, $^{13}C_{12}$-BPB and BPA-d_{16}, was also tested after 7 days. In this case, the instrumental response of the investigated compound was normalised to the response of underivatised diazepam at time 0, 1, 2, 3, 4, and 7 days.

2.10. Statistical analysis

All statistical analyses were carried out using the open-source software package R, version 3.5.2 (R Development Core Team, 2011). A comparison of two or more data samples was made to assess the influence of the matrix, concentration, temperature and storage time on the stability of target compounds in the dry and derivatised extracts. The Shapiro-Wilk test for testing normality and the Levene's test for checking homoscedasticity (significance level = 0.05) were used to select the appropriate statistical test (Eftimov et al., 2017). If two (paired) or more data samples (paired) satisfied the required conditions, parametric tests, namely a paired *t*-test or repeated measures ANOVA were performed. Otherwise, less powerful nonparametric tests such as a Wilcoxon signed-rank or the Friedman test were selected (Table SI-4). Statistical significance was evaluated at the 0.05 level.

3. Results and discussion

3.1. Preliminary experiment

The presence of BPA in all samples, up to 8 μg L^{-1} under harsh conditions and BPF in cans up to 1 μg L^{-1}, including its two isomers (ng L^{-1}), was confirmed using target and suspect screening in the preliminary experiment. None of the expected diglycidyl ethers were detected, suggesting that sample preparation needed to be optimised due to their high tendency for hydrolysis. Also, suspect screening did not reveal any compounds from the generated list. The identification of relevant mass features after data processing is difficult due to their large number generated from the untargeted analysis. However, despite this, a time trend for the migration of chemicals from reusable sports bottles (decrease in several mass features and the area of major features from day 1 to day 3) was detected. This preliminary experiment showed that the largest amount of compounds migrate from the beverage can linings (Figure SI-1) and highlights the existing challenges in improving data analysis workflows. The results also show that MeOH as a simulant was just as aggressive as simulants C and B. Based on these results and published data, the number of investigated BPs was reduced from 18 to 12, and the subsequent migration study of BPs from cans and reusable sports bottles into simulant C and B was investigated using targeted analysis.

3.2. Method validation

3.2.1. Lowest limits of detection (LLOD) and quantification (LLOQ), working range and linearity

The sensitivity of the method was evaluated in terms of LLOD and LLOQ, calculated (Table SI-4) by multiplying the standard deviation (SD) of the background (ten distinct matrix blanks) by a factor of 3 and 10, respectively (Magnusson & Örnemark, 2014). Using the LLOQ as the lowest calibration standards (see Accuracy and Precision) and at least five times the analyte signal of a blank sample, LLOQs were 1 and 3 ng L^{-1} for simulant C and B for the 11 BPs and 10 ng L^{-1} for BPA (Table SI–4).

The linearity of the method was obtained using spiked matrix-matched samples at different calibration points across the working range. The concentration of BPs and BPA in simulant C and B increased from < LLOQ to 1 μg L^{-1} and < LLOQ to 4 μg L^{-1}, respectively, whereas the concentration of each of the internal standards remained fixed (0.16 μg L^{-1}). Each calibrant was prepared in triplicate. The coefficient of determination (R^2) was > 0.99 for each BP when using a weighted (1/x or 1/y) linear calibration curve. The maximum residuals for all calibration levels were < 11.3%, and the RSD of the slopes were lower than 5.8%.

According to the JRC validation guidelines for Food Contact Materials (Bratinova, Raffael, 2009), the maximum relative standard deviation of the slope should not exceed 8%, and the residuals calculated for the lowest level (LLOQ) should be < 20%, and < 15% for all other levels to fulfil the linearity model. In addition, the adequateness of the linearity model was verified using the lack-of-fit (LOF) test. The resultant p-values, at a confidence level of 95%, were > 0.05 for all compounds. Table 2a summarises the results.

3.2.2. Recovery

Recoveries were estimated from the spiking experiments. The blank matrix samples of simulant C and B were spiked at three concentration levels (LLOQ or LQC, MQC and HQC) for the 11 BPs and BPA. The mean recoveries were 77.7–106.8%. The relative standard deviation ($RSD_{SPE\ recoveries}$) were < 16.8% at LLOQ/LQC and < 10.7% at higher MQC and HQC levels. The results are shown in Table 2a.

3.2.3. Accuracy and precision

Accuracy and precision were assessed by measuring five QC samples at four or three calibration levels LLOQ, LQC, MQC and HQC, as described above, within a single run and between different runs on three consecutive days. Also, instrument precision was obtained by repeated injections (n = 3) of one QC sample for each level. Twenty samples (for each batch) for each simulant including five samples of each QC level were analysed. The accuracy was expressed as a percentage of the nominal value, and precision expressed as the relative standard deviation (RSD, %) of the target BPs in simulant C and B, both in the intra-day (15 samples) and inter-day (10 samples) experiments. Accuracy, for the majority of BPs, was within required limits, i.e., 80.0–120.0% at LLOQ and 85.0–115.0% at higher QC levels. Precision was < 14.9%, except at the LLOQ (<19.9%). Instrument precision was 0.5–12.2%.

3.2.4. Reproducibility and uncertainty

Reproducibility was evaluated by determining the standard deviation in the measurement results (n = 5) using the same method but with a different operator (u_{ope}), time (u_{time}, six consecutive days) and equipment (u_{eqp}, stock standard solutions) at the LLOQ and varying only time (u_{time}, six consecutive days) at LQC, MQC and HQC. In all cases, reproducibility and method repeatability SD were the primary sources ($u_{ope,}$ u_{time}, u_{eqp}, u_{rep}) of measurement uncertainty. It also includes the effect of varying other parameters (e.g. standard preparation, equipment calibration, sample preparation and analysis, interpretation of results) that can affect the results. However, their contributions are negligible compared to method reproducibility and repeatability (EURACHEM/CITAC Guide CG 4, 2012). The combined standard uncertainties (u_c) were calculated using equation (1) (EURACHEM/CITAC Guide CG 4, 2012; GUM, 2008) to determine the type A uncertainty. Finally, the expanded uncertainties (U) were estimated by calculating the effective degrees of freedom ν_{df} using the Welch-Satterthwaite equation and a coverage factor (k), corresponding to a confidence level of 95%.

$$u_c = \sqrt{u_{ope}^2 + u_{time}^2 + u_{eqp}^2 + u_{rep}^2} \quad (1)$$

The expanded measurement uncertainties (U) for all of the compounds were 2–67% for simulant C and 4–46% for simulant B (Table 2a). Clearly, at LLOQ and LQC levels, U significantly increases (12–67% and 10–30%) in comparison to higher (MQC and HQC)

Table 2a
Linearity evaluation and recovery assay of the proposed methods with simulant C (20% EtOH) and B (3% AA) for 11BPs and BPA LLOQ/LQC = 3 and 30 $ng\,L^{-1}$, MQC = 100 and 800 $ng\,L^{-1}$, HQC = 0.6 and 2.5 $\mu g\,L^{-1}$: slope (k), slope standard deviation s_k, intercept, determination coefficient (R^2), weight, maximum residual, the lack of fit test (LOF, p values at a confidence level of 95%), lowest limit of quantification (LLOQ), recovery assays; SPE recoveries and relative standard deviation (RSD, n = 3), expanded uncertainty U [%].

BPs	k [$\mu g\,L^{-1}$]	Intercept	s_k [$\mu g\,L^{-1}$]	R2	Weight	Max residual [%]	LOF p-value	SPE recoveries [%]			RSD $_{SPE\ recoveries}$ [%]			Expanded uncertainty U [%]			
								LQC	MQC	HQC	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Simulant C																	
22BPF	3.38	−0.009	0.014	0.997	1/x	5.8	0.08	90.3	83.4	82.7	8.2	4.5	3.4	41	21	18	15
BPAF	1.54	0.004	0.021	0.998	1/x	3.1	0.15	106.8	85.5	84.4	13.6	4.4	3.1	26	/	14	15
24BPF	1.17	0.000	0.005	0.997	1/x	3.6	0.11	90.2	81.5	83.3	10.9	4.3	1.9	27	/	10	6
BPF	1.57	−0.003	0.007	0.998	1/x	4.9	0.11	97.1	84.9	83.4	6.7	2.6	3.0	43	14	12	7
BPE	2.56	−0.007	0.084	0.994	1/x	10.5	0.11	93.8	86.0	84.6	16.0	7.4	6.0	36	23	10	5
BPA	2.16	0.179	0.016	0.999	1/x	3.1	0.06	98.0	88.3	85.7	2.5	3.4	3.9	67	19	18	2
BPC	2.50	−0.009	0.020	0.997	1/x	3.8	0.11	94.7	80.4	77.7	15.1	4.2	5.2	24	15	11	10
BPB	1.33	−0.003	0.009	0.997	1/x	5.6	0.1	100.5	89.2	84.3	19.2	4.4	6.7	23	29	8	12
BPC2	0.69	−0.001	0.008	0.997	1/x	9.4	0.06	104.2	92.4	87.0	16.8	6.2	5.7	29	/	16	18
BPZ	3.03	−0.004	0.053	0.997	1/x	5.3	0.06	106.4	87.3	83.9	14.9	5.6	7.0	15	30	15	17
BPS	2.02	0.035	0.008	0.996	1/x	6.1	0.08	92.8	90.7	85.7	11.5	3.7	8.4	35	/	11	13
BPAP	1.61	−0.003	0.011	0.996	1/x	9.7	0.09	90.7	86.2	85.5	7.0	8.4	5.7	28	22	13	18
	Simulant B																
22BPF	3.519	−0.012	0.062	0.996	1/x	10.6	0.09	95.4	96.7	83.9	10.1	7.4	2.1	33	22	10	12
BPAF	0.919	0.000	0.004	0.997	1/y	6.3	0.15	88.3	93.7	84.4	16.0	4.9	3.1	37	12	11	8
24BPF	1.734	−0.004	0.041	0.996	1/x	9.4	0.09	89.0	104.9	83.3	12.4	14.9	1.9	12	/	9	10
BPF	1.418	−0.004	0.025	0.997	1/x	7	0.09	88.3	94.3	83.4	8.9	4.2	3.0	47	23	10	4
BPE	2.260	−0.007	0.054	0.996	1/x	8.4	0.09	95.8	93.7	84.6	4.9	3.2	6.0	31	23	4	10
BPA	1.749	0.580	0.017	0.997	1/x	4.3	0.06	85.0	88.6	85.7	5.4	1.9	3.9	20	15	12	16
BPC	1.183	−0.003	0.030	0.996	1/x	8.3	0.14	85.6	85.3	80.3	10.0	6.3	4.6	25	10	6	10
BPB	0.916	−0.002	0.011	0.997	1/x	5.3	0.08	94.6	94.8	86.1	14.3	6.9	5.9	34	23	9	13
BPC2	0.549	−0.001	0.017	0.993	1/x	9.1	0.09	87.4	98.3	87.0	12.5	10.7	5.7	29	/	7	8
BPZ	1.665	−0.003	0.051	0.995	1/y	8.2	0.08	94.8	95.0	85.1	8.7	6.8	6.2	42	19	12	14
BPS	1.977	0.002	0.020	0.996	1/x	8.1	0.06	94.4	99.7	85.7	11.1	3.5	8.4	27	/	11	14
BPAP	1.140	−0.002	0.064	0.996	1/x	11.3	0.08	87.3	93.7	85.5	6.9	9.9	5.7	31	16	14	16

calibration levels (U < 20%) for all compounds in simulant C and B. The highest estimated U (>40%) at LLOQ correspond to BPA, 22BPF, BPF and BPZ.

3.3. Stability study

The average concentration of BPs in the procedural blanks for each stability condition was < LLOQ. The RSDs of triplicates were < 15 and 10% for all BPs at LQC and MQC, respectively. The estimated U enabled confidence intervals to be set for the stability of BPs. Based on U (<15%) for the majority of BPs at MQC, the compounds were considered to be stable if their remaining percentages were between 85% and 115% (after a certain time compared to their concentration at time zero). A greater U at the LQC level meant that the interval tolerances were higher for the stability of individual BPs at this level (Table 2b).

Exp. 1 Stability of BPs in the dried extract

The general observation (Fig. 1a) is that all target compounds are stable at −20 °C for at least eight weeks in both dried simulant extracts (C and B) and at both concentration levels (LQC and MQC). After 24 weeks, eight BPs remained above or close to 85% (within their U range), proving their stability (Table SI-5). Bisphenol C, BPC2, BPZ and BPAP remained below the stability limit, especially at the MQC level, suggesting that a 24 week storage time is too long for these BPs. Statistical analysis also confirms that there are no significant differences ($p > 0.05$) measured at different concentration levels and between the simulants, but there is a significant difference ($p < 0.05$) over time (Table SI-8).

Exp. 2 Stability of BP derivatives in derivatised extract

The results (Table SI-6) confirm the stability (±17%) of all four internal standard derivatives under experimental conditions. The RSDs of their responses at both calibration levels, temperature and simulants were < 8.2%. The data allows us to calculate the ratio of the peak areas between the target BPs and relevant internal standards used in *Exp. 2*. The results (Table SI-8) show no significant difference ($p > 0.05$) in concentration overtime at each temperature, except at −20 °C at the MQC level, but there is a significant difference ($p < 0.05$) when stored at −20 and 21 °C (Fig. 1b), which was most evident at the MQC levels for both simulants. After 7 days at 21 °C, all BP derivatives remained above or close to 85%, except at the LQC concentration level (Table SI-7) where the deviations, although greater, are in line with U (Fig. 1b). A possible explanation for the lower remaining percentages when stored at −20 °C at the initial time vs later time points is that lower temperature can lead to lower derivatisation yields. In contrast, increased time reactivates the derivatisation process, which is confirmed by a p-value of < 0.05 when comparing Day 1 and Day 7 samples at −20 °C (Table SI-8). Generally, silylated derivatives are relatively unstable, and their long-term storage is not recommended (Villas-Bôas, Smart, Sivakumaran, & Lane, 2011). The results do show that derivatised samples can be stored for up to 7 days at room temperature (21 °C).

3.4. Migration study

3.4.1. Simulant analysis from beverage cans and reusable sports bottles

All investigated compounds were present at < LLOQ in both types of control samples from each sample batch. As expected, there was no leaching of BPs from Parafilm. Each compound was recorded as present only when measured values were above the LLOQ in both aliquots of one or both parallels, expressed as the average concentrations ($ng\,L^{-1}$) ± the standard deviation (aliquots or parallels). The results are given in Table 3 and shown graphically in Fig. 2a.

Beverage cans

Among targeted compounds, only BPA and BPF (including the two isomers) were > LLOQ. Bisphenol A ranged from 2144 to 5865 $ng\,L^{-1}$ in simulant C and from 51.6 to 1309 $ng\,L^{-1}$ in simulant B, whereas the dominant isomer of BPF (4,4′-BPF) varied from 257 to 1286 $ng\,L^{-1}$ and 13.0 to 64 $ng\,L^{-1}$. The levels of 22BPF (2,2′-BPF) and 24BPF (2,4′-BPF)

Table 2b

Accuracy evaluated as the percentage of the nominal value [%], and precision expressed as %RSD (n = 5) of the tested BPs (11BPs and BPA) in migration simulant C and B determined in the intra- and inter-day experiments at LLOQ = 1.0/3.0 and 10.0 ng L^{-1}, LQC = 3.0 and 30.0 ng L^{-1}, MQC = 100.0 and 800.0 ng L^{-1}, HQC = 0.60 and 2.50 µg L^{-1}.

Matrix		Simulant C				Simulant B			
BPs	QC level	Intra-day			Inter-day	Intra-day			Inter-day
		Accuracy [%](%RSD, n = 5)			Accuracy (%RSD, n = 10)	Accuracy [%](%RSD, n = 5)			Accuracy (%RSD, n = 10)
		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3	
22BPF	LLOQ	120.1(3.6)	116.3(3.9)	112.1(3.3)	118.4(9.7)	112.8(11.7)	109.0(5.1)	106.9(1.1)	116.0(14.1)
	LQC	99.9(8.7)	114.4(14.2)	94.9(12.4)	109.3(12.0)	100.8(7.5)	101.2(3.8)	85.9(0.8)	102.4(5.6)
	MQC	84.3(3.1)	94.8(7.6)	87.3(7.3)	89.3(8.7)	86.8(4.0)	83.4(4.0)	85(3.3)	79.5(13.9)
	HQC	100.5(2.3)	110.9(3.5)	86.0(2.1)	106.4(4.9)	98.0(3.3)	100.7(1.9)	87.0(1.6)	99.6(2.6)
BPAF	LLOQ	104.2(10.2)	113.5(9.5)	94.9(11.0)	109.9(9.8)	119.2(10.7)	83.4(7.2)	110.7(8.1)	105.2(18.8)
	LQC					95.0(7.3)	93.5 (5.0)	92.4(4.7)	94.5(7.2)
	MQC	86.1(5.0)	90.9(6.8)	87.3(8.39	87.5(6.9)	88.5(3.3)	83.4(2.7)	85.3(2.3)	79.4(14.5)
	HQC	103.0(2.9)	110.2(5.4)	86.0(5.5)	106.5(5.8)	99.7(3.0)	98.5(1.8)	92.8(1.8)	99.6(2.4)
24BPF	LLOQ	108.8(6.7)	115.2(12.9)	119.6(8.3)	115.2(5.4)	112.8(7.5)	114.0(11.6)	109.89(4.9)	112.0(11.5)
	MQC	97.2(14.3)	105.3(9.0)	91.3(5.3)	100.6(13.3)	92.987.6)	95.7(4.9)	100.9(3.6)	93.7(5.3)
	HQC	88.1(1.7)	85.3(5.9)	85.9(5.0)	86.8(4.7)	88.9(2.4)	82.8(3.6)	96.6(0.2)	85.7(8.5)
BPF	LLOQ	120.4(7.1)	81.0(19.9)	110.3(11.0)	107.9(9.3)	109.1(17.4)	109.2(8.9)	83.9(15.1)	114.3(12.7)
	LQC	90.9(6.6)	96.3(2.7)	95.2(5.0)	93.5(13.0)	96.3(14.0)	95.9(7.0)	95.3(6.2)	96.7(10.7)
	MQC	76.1(4.0)	83.2(4.3)	79.3(7.1)	82.9(7.7)	72.4(2.8)	77.7(4.4)	79.8(1.39)	75.3(9.0)
	HQC	78.2(3.0)	88.6(5.3)	90.2(5.6)	86.6(7.2)	83.1(1.5)	84.0(2.2)	85.2(6.3)	80.0(5.0)
BPE	LLOQ	114.1(4.1)	111.6(12.9)	117.7(1.0)	114.9(12.4)	114.2(13.8)	119.9(2.0)	115.0(2.9)	120.5(15.3)
	LQC	114.8(3.1)	113.2(5.8)	109.6(3.0)	114.9(12.2)	105.4(8.7)	108.0(4.1)	105.3(7.6)	109.3(5.5)
	MQC	99.1(4.4)	111.9(6.9)	113.6(2.6)	104.5(9.1)	109.8(5.7)	94.4(1.7)	111.1(1.2)	93.8(15.1)
	HQC	116.9(1.5)	128.2(0.4)	115.6(1.4)	119.9(6.1)	115.1(1.4)	114.4(0.5)	112.0(3.1)	116.4(1.5)
BPA	LLOQ	119.3(1.8)	89.7(7.4)	85.1(1.4)	118.6(11.8)	103.7(4.4)	104.2(5.8)	93.3(3.5)	105.6(5.6)
	LQC	98.0(14.2)	122.1(6.01)	85.1(2.1)	114.8(15.1)	119.3(2.8)	115.2(1.8)	89.7(7.4)	118.6(11.8)
	MQC	104.5(1.6)	104.4(6.9)	106.1(7.6)	104.5(0.8)	92.3(1.1)	101.7(1.2)	110.2(3.2)	101.7(1.2)
	HQC	111.0(1.0)	108.5(0.4)	106.5(6.2)	108.5(2.4)	98.9(0.2)	98.9(2.4)	108.8(5.1)	104.0(1.8)
BPC	LLOQ	118.0(11.1)	107.7(10.0)	114.6(5.0)	117.4(6.1)	116.8(2.9)	115.8(7.7)	100.5(10.2)	119.2(16.5)
	LQC	112.4(6.2)	113.7(6.6)	108.6(2.7)	112.0(6.7)	110.8(11.7)	106.1(7.5)	109.0(3.3)	111.4(5.8)
	MQC	94.6(2.2)	89.5(3.1)	109.4(6.4)	91.7(3.4)	104.8(5.8)	85.2(4.0)	106.4(1.5)	87.2(12.7)
	HQC	113.782.6)	111.7(4.8)	116.5(0.9)	112.1(3.7)	111.9(2.4)	100.3(5.2)	113.7(2.5)	105.5(7.4)
BPB	LLOQ	109.6(11.6)	111.5(9.7)	115.7(2.4)	108.9(9.7)	116.1(18.3)	117.5(8.6)	116.9(1.9)	119.2(13.4)
	LQC	105.3(10.5)	114.2(13.0)	95.5(11.1)	107.6(13.4)	109.8(5.7)	103.8(14.5)	114.2(3.9)	108.1(11.9)
	MQC	84.0(2.0)	85.8(5.1)	98.7(5.5)	84.3(3.8)	91.6(5.6)	86.3(3.3)	97.4(1.3)	87.3(4.9)
	HQC	98.8(4.0)	107.4(3.9)	105.6(4.0)	102.6(6.0)	99.5(2.2)	92.4(3.4)	111.5(1.6)	99.7(1.9)
BPC2	LLOQ	100.3(10.9)	92.1(12.9)	109.2(3.0)	97.0(13.5)	99.2(11.7)	104.8(7.5)	108.8(9.4)	100.0(11.8)
	MQC	89.8(5.9)	95.4(3.2)	111.1(4.6)	92.6(5.7)	89.6(5.4)	86.2(4.0)	108.4(1.2)	86.9(6.3)
	HQC	101.6(5.0)	111.1(4.6)	113.9(0.1)	105.2(7.0)	95.6(5.1)	102.3(5.2)	114.9(2.0)	101.0(4.5)
BPZ	LLOQ	100.9(11.6)	104.7(15.6)	106.7(3.7)	98.4(8.1)	117.8(11.9)	110(11.9)	112.5(4.5)	112.9(10.8)
	LQC	99.0(5.7)	111.1(12.9)	108.3(9.1)	105.3(11.4)	108.6(6.0)	85.8(9.1)	103.5(5.7)	103.2(11.6)
	MQC	88.5(6.0)	91.8(4.5)	113.8(3.3)	89.6(6.09)	99.9(3.9)	87(5.9)	114.9(1.5)	90.2(6.7)
	HQC	106.7(5.0)	111.4(3.7)	118.9()0.1	108.1(5.2)	104.0(2.6)	91.5(3.5)	113.4(4.3)	105.8(2.4)
BPS	LLOQ	109.5(15.5)	109.0(15.6)	92.1(2.9)	104.1(12.8)	107.5(8.7)	112(17.2)	113.7(3.7)	90.7(16.5)
	MQC	92.0(14.9)	94.8(8.4)	97.6(12.0)	95.5(12.6)	88.3(6.6)	95.2(8.4)	105.3(8.7)	92.7(9.3)
	HQC	93.4(3.4)	103.9(4.0)	101.3(3.2)	98.8(6.3)	95.0(2.5)	106.5(3.0)	101.1(7.5)	93.7(2.8)
BPAP	LLOQ	119.4(8.4)	115.6(6.8)	117.6(0.2)	118.6(6.6)	115.1()14.5	99.6(15.2)	111.9(7.2)	119.0(14.9)
	LQC	106.5(9.4)	111.3(3.5)	106.9(7.1)	111.6(12.8)	106.6(7.1)	92.6(7.5)	114.1(1.5)	98.1(9.3)
	MQC	89.5(4.4)	85.1(4.0)	103.8(5.9)	86.9(4.8)	97.5(8.7)	105.9(5.1)	99.5(2.3)	86.4(8.2)
	HQC	107.3(6.0)	107.5(5.9)	114.9(4.9)	106.7(5.9)	101.5(3.2)	93.9(4.0)	114.5(3.7)	100.7(3.2)

were from 8.8 to 391 ng L^{-1} in simulant C and from 8.2 to 99.8 ng L^{-1} in simulant B. These results agree with the literature findings (Table 1), except that measured concentrations are higher (Table 3) than those reported in beverages and food. For instance, BPAP was determined (1.7 ng L^{-1}) only in Can2 when exposed to simulant C. Unlike Cunha et al. (Cunha, Almeida, Mendes, & Fernandes, 2011), Cacho et al. (Cacho, Campillo, Viñas, & Hernández-Córdoba, 2012) and Fattore et al. (Fattore, Russo, Barbato, Grumetto, & Albrizio, 2015), we did not detect BPB.

From the data, it is evident that simulant C is more aggressive in comparison to simulant B (Fig. 2b) similar to the finding of Dreolin et al. (Dreolin, Aznar, Moret, & Nerin, 2019). Higher concentrations of BPs were observed after one month under HAR conditions, confirming a need for more specific regulation, similar to EU regulations for plastic material (EU, 2011), assigned to metal packaging with (organic) coatings. Increasing experimental time as suggested by Paseiro-Cerrato et al. (Paseiro-Cerrato et al., 2016) would more accurately reflect the long term storage, i.e., "*above 6 months at room temperature and below including heating up to 70 °C for up to 2 h, or heating up to 100 °C for up to 15 min*" as defined by EU, 2011.

The differences between the cans intended for different users (Can1 and Can2-6) or cans filled with different types of beverages (Can1-6) could be due to different formulations of the organic coating that are tailored to the intended contents (Driffield et al., 2018; Paseiro-Cerrato et al., 2017, 2016), or contamination during filling, transport, and storage. Can2, intended to hold craft beer, contained the highest amount of all detected BPs (Table 3).

Reusable sports bottles

Reusable sports bottles 1, 3 and 9 did not contain any BP residues (Fig. 3). The detected levels of BPA ranged from < LLOQ to 222 ng L^{-1} in simulant C and < LLOQ to 68 ng L^{-1} in simulant B, again suggesting simulant C is the more aggressive simulant (Fig. 2b). Migration tests

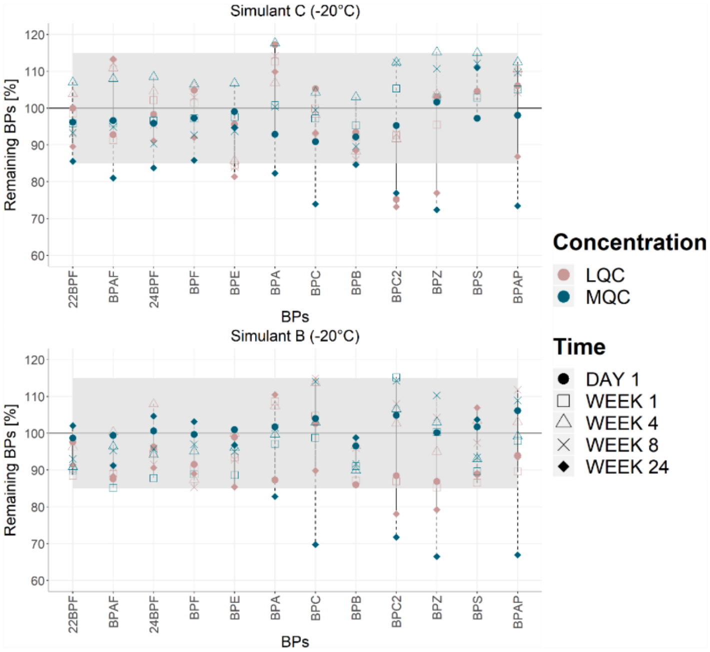


Fig. 1a. Stability expressed as remaining percentages of the 12 BPs in dried extracts of simulant C and B (initial concentration: LLOQ/LQC = 3 and 30 ng L^{-1}, MQC = 100 and 800 ng L^{-1}) stored at −20 °C for different lengths of time (up to 24 weeks).

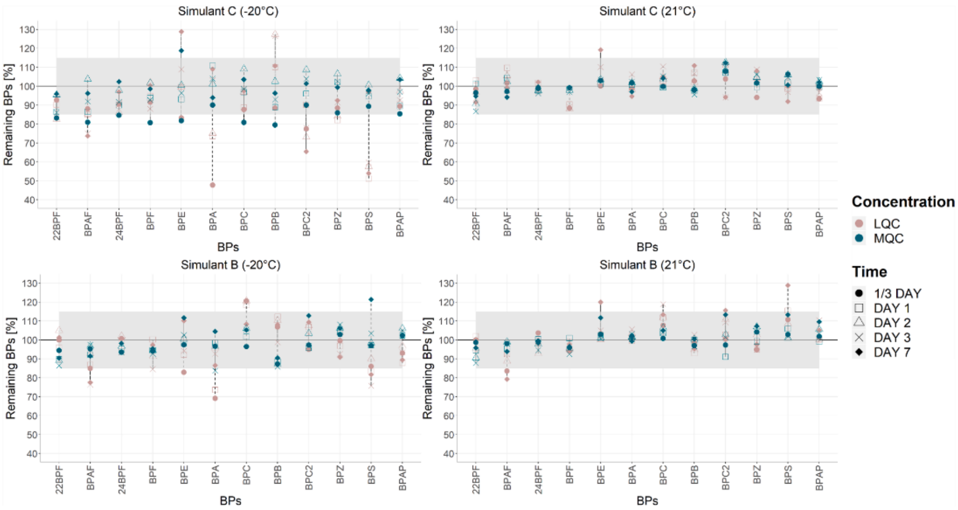


Fig. 1b. Stability expressed as remaining percentages of the 12 BP-derivatives in simulant C and B (initial concentration: LLOQ/LQC = 3 and 30 ng L^{-1}, MQC = 100 and 800 ng L^{-1}) stored at −20 °C and 21 °C for different lengths of time (up to 7 days).

Table 3

Results of migration test on simulant C and B from cans and reusable sports bottles expressed as average concentrations ± standard deviation (n = 2 or 4) in ng L^{-1}. Aliq.: when present in both aliquots of parallel a or b only (n = 2); Paral.: when present in both parallels (n = 4); Polyethylene (PE), Polypropylene (PP), high-density polyethylene (PD-PE), Polycarbonate (PC) plastic.

Simulant	Sample Type	Sample	Presence	Material type (#number)	Condition	Concentration ± sd [ng L^{-1}]												
						BPA	22BPF	24BPF	BPF	BPE	BPC	BPB	BPZ	BPAP	BPC2	BPAF	BPS	Σ BPs
Simulant C	Can	Can1	Paral.	organic	Gen	2144 ± 28	8.8 ± 1.2	131 ± 10	547 ± 84	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2831
		Can1	Paral.	coating	Har	2922 ± 132	18 ± 7	133 ± 4	361 ± 72	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	3433
		Can2	Paral.		Gen	5865 ± 137	14 ± 4	391 ± 34	1286 ± 213	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.7 ± 0.1	<LLOQ	<LLOQ	<LLOQ	7557
		Can3	Paral.		Gen	3402 ± 51	31 ± 11	208 ± 69	257 ± 11	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	3897
Simulant B		Can1	Paral.		Gen	52 ± 3	8.2 ± 0.3	21 ± 2	13 ± 1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	94
		Can1	Paral.		Har	1310 ± 113	23.8 ± 0.9	73 ± 8	64 ± 9	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1470
		Can4	Paral.		Gen	911 ± 151	23.5 ± 0.9	99.8 ± 0.2	63 ± 8	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1097
		Can5	Paral.		Gen	576 ± 73	14 ± 2	55 ± 11	42 ± 11	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	686
		Can6	Aliq. a		Gen	341 ± 4	18 ± 4	74 ± 25	29 ± 3	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	462
Simulant C	Reusable sports bottle	B2	Aliq. a	PE (4)	D1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.4 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.4
		B4	Paral.	steel	D1	51 ± 22	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	51
		B4	Aliq. b		D1	<LLOQ	<LLOQ	<LLOQ	1.1 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.1
		B4	Aliq. a		D3	14.1 ± 0.9	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	14
		B5	Aliq. b	PP (5)	D2	44.6 ± 0.9	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	45
		B5	Aliq. b		D3	82 ± 3	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	83
		B6	Paral.	PE (4)	D1	27.3 ± 0.7	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	27
		B6	Aliq. a		D2	10.6 ± 0.5	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	11
		B6	Aliq. b		D3	11.7 ± 0.2	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	12
		B7	Aliq. a	PE-HD (2)	D2	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	4.6 ± 0.8	<LLOQ	<LLOQ	<LLOQ	<LLOQ	4.6
		B8	Aliq. b	PP (5)	D1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.6 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.6
		B11	Paral.	stainless steel	D1	222 ± 87	<LLOQ	<LLOQ	1.7 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	224
		B11	Paral.		D2	31 ± 6	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	31
		B11	Paral.		D3	34 ± 12	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	34
Simulant B		B2	Aliq. b	PE (4)	D1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.0 ± 0.1	<LLOQ	1.1 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.0
		B2	Aliq. b		D2	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.6 ± 0.1	<LLOQ	1.7 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	3.3
		B2	Aliq. b		D3	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.7 ± 0.1	<LLOQ	2.2 ± 0.4	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	3.9
		B4	Paral.	stainless steel	D1	68 ± 18	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	68
		B4	Paral.		D2	18 ± 1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	18
		B4	Aliq. a		D3	14.6 ± 0.4	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	15
		B5	Aliq. a	PP (5)	D1	34 ± 5	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	34
		B10	Aliq. a	?	D2	20 ± 10	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	20
		B11	Paral.	stainless steel	D1	46 ± 17	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	46
		B11	Paral.		D2	19 ± 3	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	19
		B11	Paral.		D3	22 ± 2	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	22
	Used reusable sports bottle	B2	Aliq.	steel	Used	414 ± 3	1.3 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	415
		B4	Aliq.	PC (7)	Used	1867 ± 42	<LLOQ	<LLOQ	1.9 ± 0.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1869
		B6	Aliq.	PE (4)	Used	<LLOQ	<LLOQ	<LLOQ	<LLOQ	8.4 ± 0.7	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	8.4
		B7	Aliq.	PE (4)	Used	12 ± 1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	12

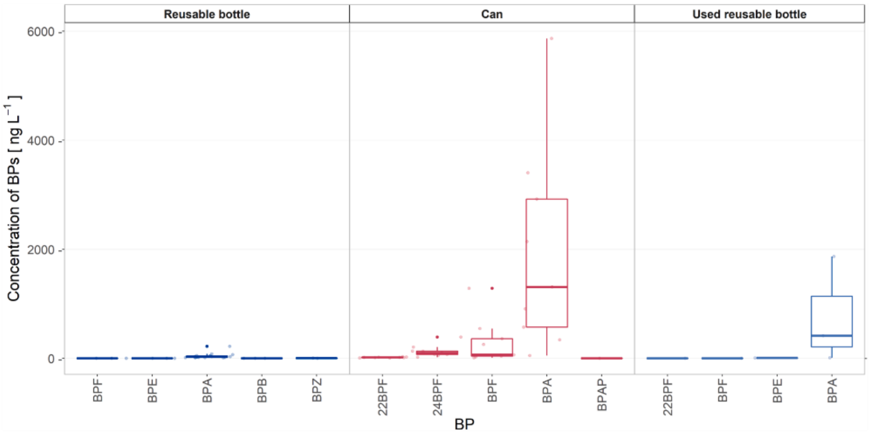


Fig. 2a. Box-plot showing concentrations of all detected BPs in both simulants (C and B) and all analysed containers (reusable sports bottle, cans and used reusable sports bottle).

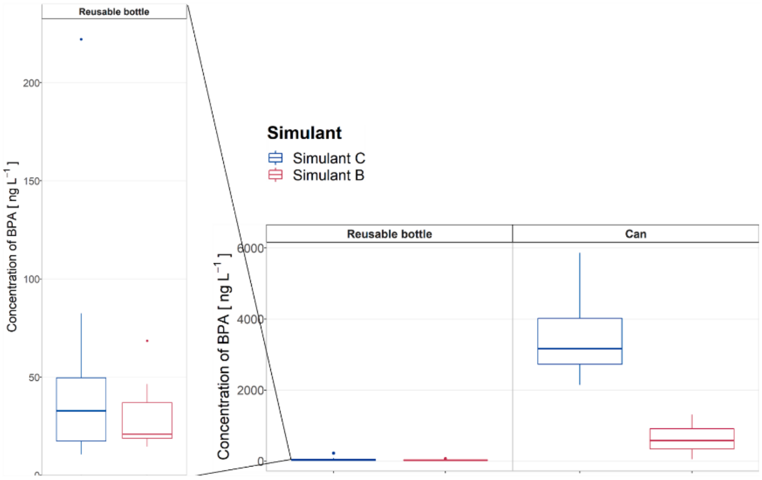


Fig. 2b. Box-plot showing differences in BPA concentration between cans and new reusable sports bottles and simulants C and B.

revealed a decrease in BPA concentration over time in case of simulant C and B (Figure SI-3), except for sample B5, where the trend is reversed. The bottles made from steel (B4 and B11) leached higher amounts of BPA (68 and 222 ng L^{-1}) after day 1 (Figure SI-3), and were the only ones to leach BPF (1.1 and 1.7 ng L^{-1}). Interestingly, B5 and B11, which were both labelled as BPA-free (Table SI-2) contained BPA (up to 44.6 and 222 ng L^{-1}). In contrast, B2 and B7 from PE material and B8 from PP material (Table 3) did not contain BPA, but the presence of BPE (1.6–2.0 ng L^{-1}), BPB (1.1–2.2 ng L^{-1}) and BPZ (2.6–4.6 ng L^{-1}) were observed following a similar time trend as for BPA. Unlike recent studies (Table 1), which identified BPS as a major contaminant detected in food and beverages from plastic (baby) bottles (García-Córcoles et al., 2018; Mandrah, Satyanarayana, & Roy, 2017), BPS was not present in any of the sports bottles tested. Unlike in this study, Tian et al. (Tian et al., 2019) and Hwang et al. (Hwang et al., 2018) did not detect any BPs in their simulant samples exposed to plastic bottles, but this is a likely result of their much higher LLOQ [μg L^{-1}]. In this study, used sports bottles were exposed to simulant B since it covers the majority of typical beverages kept in such bottles. The results revealed the presence of BPA and 22BPF in the case of steel sports bottles (Fig. 2a), and, as expected, the highest concentration of BPA (1867 ng L^{-1}) migrating from PC bottles, which also contained BPF (1.9 ng L^{-1}). Bisphenol E (8.4 ng L^{-1}) was present in case of PP bottles. The observed time trends imply that BPA concentrations would be lower from used bottles. Instead, 10 and 100 times higher concentrations were determined in the case of steel (B2), and PC (B4) used sports bottle. One possible explanation could be material damage or improvement in material quality over time; however to better understand the impact of material damage

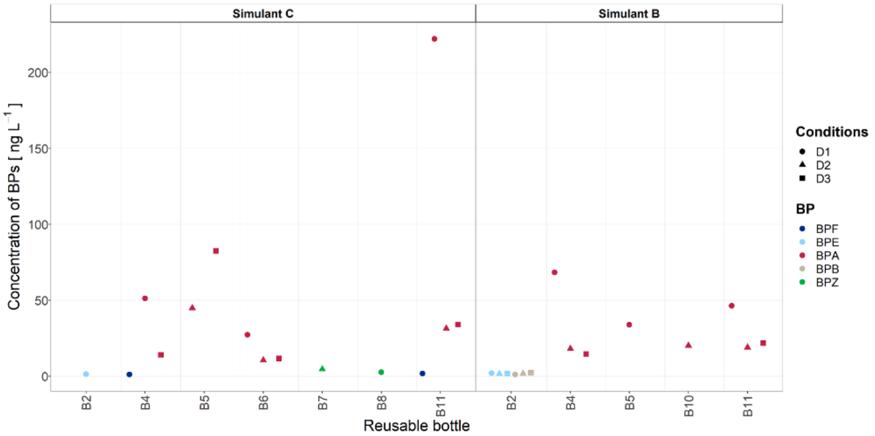


Fig. 3. The concentrations of all detected BPs from simulants C and B exposed to new reusable sports bottle over three consecutive days (D1, D2 and D3).

and to confirm the observed correlation, the analysis of a higher number of samples is required. Overall, a correlation exists between container material and the migration of BPs rather than in their price (Figure SI-4).

Beverage cans vs sports bottles

A total of 8 (BPA, 22BPF, 24BPF, BPF, BPE, BPB, BPZA and BPAP) out of the 12 investigated BPs were detected in the samples, 5 in beverage cans and 6 in reusable sports bottles, from which 24BPF and BPAP were found only in the simulant from beverage cans and BPE, BPB and BPZ from sports bottles (Fig. 2a). Table 3 suggests that BPA is still preferably used in the production of cans and sports bottles since it was the most frequently detected BP (100, 45 and 43% respectively) and present in the highest concentrations 5865, 222, and 1867 ng L^{-1} in cans, sports bottles, used sports bottles, respectively (Figure SI-2).

Bisphenol A and the three BPF isomers were determined in all samples obtained from cans at significantly higher concentrations in comparison to sports bottles - in one case up to two orders of magnitude higher (Can2). Although the level of BPA migrating from the cans did not exceed the SML, the results suggest that this type of container is a significant source of BPs (Fig. 2a). Ten out of the 18 reusable sports bottles did not contain BPA. Three out of 11 and 3 out of 7 new and used reusable sports bottles also did not contain any of the investigated BPs (Fig. 3), while a higher concentration of BPA leached from B4 and B11 (steel bottles) after day 1 and from B5 (made of PP) after day 3. The highest concentrations of BPA and BPF migrating from cans and steel bottles suggest that both contain an organic coating (Rochester & Bolden, 2015b). In the case of cans, the presence of BPF and low levels of its two isomers, suggest the use of an industrial mixture of BPA and BPF in the coating. Other BPs were determined only in case of plastic material at significantly lower concentrations, which does not imply their use as a replacement for BPA since the latter is present at significantly higher concentrations. Overall, these results together with the total BPs concentration per analysed container (Table 3) showed that consuming beverages from sports bottles (ΣBPs = < LLOQ – 68 ng L^{-1}) made from different non-polycarbonate polymers rather than steel (ΣBPs = 1.1–224 ng L^{-1}) pose a lower risk to human exposure to BPs compared to consuming beverages from cans (ΣBPs = 94–7557 ng L^{-1}).

3.5. *Exposure assessment*

The EFSA CEF Panel (EFSA Cef Panel, 2016) state that "The exposure for a food category can be calculated by combining the consumption level of the food category with the migration levels into the foods covered by the food category". For this reason, dietary exposure to BPs from beverage cans and reusable sports bottles was estimated for each sample at the 95th percentile (P95) of water consumption intake data, obtained from the EFSA Guidance using default assumptions (EFSA, 2010; EFSA, 2017). These assumptions included a default body weight (bw) for adults of 70 kg bw and 12 kg bw for children and a default value for daily total liquid intake in adults of 2 L and adequate water intake of 1.6 L for children (4–8 years) and used in this risk assessment. The risk to consumers from BPs was evaluated using the total determined concentration from each sample as the total concentration of BPA and comparing their exposure estimates with the established health-based guidance value for BPA. The assessment is also based on the assumption that BPs have similar toxicity as BPA (Gramec Skledar & Peterlin Mašič, 2016). According to results, estimated dietary exposure to BPs (Table 3 and Table SI-9) for adults is from 0.03 to 216 ng kg bw^{-1} day^{-1} and from 0.1 to 1008 ng kg bw^{-1} day^{-1} for children. The highest estimate for beverage cans (Can 2), either for adults or children, amounts to 5.4 or 25.4% of the *t*-TDI for BPA (4 µg kg bw^{-1} day^{-1}) (EFSA, 2015) and shows that the established value would not be exceeded with any of the tested samples. We underline that these values should be considered as a rough estimate of the risk.

4. Conclusions

A reliable GC–MS/MS method was developed and validated to analyse the migration and stability of BPA and eleven other BPs in food simulants. Good results, including LLOD and LLOQ, linearity, accuracy, precision, reproducibility and uncertainty, were achieved. From an analytical perspective, it is safe to store dried sample extracts at −20 °C for at least 8 weeks and derivatised samples at room temperature (21 °C) for up to 7 days. Bisphenol C, BPC2, BPAF and BPS were < LLOQs in all tested beverage cans and sports bottles. All cans leached BPA, BPF, 22BPF and 24BPF in amounts up to 5865, 1286, 391 and 99.8 ng L^{-1}. Eight of the tested bottles leached BPA, BPF, BPE, BPB and BPZ. The highest concentrations of BPA and BPF originated from the linings of beverages cans and reusable steel bottles. Importantly, BPA was determined in bottles that were labelled as being BPA free, while traces of BPs were present in bottles that did not contain BPA. Furthermore, the levels of BPA decreased with consecutive exposures of the sports bottles to simulant.

This work demonstrates that among BPs, BPA remains the primary bisphenol in FCMs but at the same time confirms the presence of other BPs in similar FCMs. It is also clear that simulant C is more aggressive than simulant B. Importantly, according to the BPA content of samples consuming beverages from cans is more concerning than consuming beverages from reusable sports bottles. Our findings provide insights into the problems surrounding the migration of chemicals from FCM and contribute to lowering the risk to consumer health.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by MASSTWIN (European Union's Horizon 2020 Research and Innovation Programme 692241) and ISO-FOOD project (ERA Chair for isotope techniques in food quality, safety, and traceability, under grant agreement no. 621329). Ana Kovačič acknowledges the Young researcher grant and the Slovenian Research Agency (Program Group P1-0143 and Projects L1-7544, L1-9191, N1-0047, J1-8147 and J2-8162). Celine Gys acknowledges a PhD fellowship from Research Foundation Flanders (project G0E5216N). The authors wish to thank Chemass d. o. o. (Ljubljana, Slovenia), an authorised distributor of Agilent Technologies, for allowing us to use their GC-MS/MS (7000 series, Agilent Technologies) instrument and local breweries for donating representative cans and their willingness to collaborate.

Credit authors statement

Ana Kovačič conceived and designed the study, conducted experiments, performed instrumental analysis, validate the method, analyzed and visualize the data and wrote the manuscript.

Celine Gys – a researcher collaborator, participated in planning the experiments and assisted in the non-target analysis.

Martin Rafael Gulin assisted in the experimental set-up, sample preparation, data analysis and visualization (preparation of the code, using R software).

Tina Kosjek – Co-supervisor of the first author, participated in developing the idea of the project.

David Heath assisted in the instrumental analysis and participated in the data interpretation and writing the manuscript.

Adrian Covaci, main supervisor of the second author, participate in planning the study and enable to perform the non-target analysis by hosting the researcher.

Ester Heath, main supervisor of the first author, participated from designing the experiment to reviewing the manuscript and conceptualised the research idea.

All authors reviewed the manuscript and contributed to improving the quality of this paper.

References

ALL4PACK, 2016. (n.d.). Packaging: market and challenges in 2016 – ALL4PACK Paris – Salon Emballage – Manutention Nov 2016. Retrieved December 6, 2019, from https://www.all4pack.com/Archives/Packaging-market-challenges-2016.

Bratinova, Raffael, S. (2009). EUR 24105 EN – Joint Research Centre – Institute for Health and Consumer Protection: Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. https://doi.org/10.2788/49046.

Caballero-Casero, N., Lunar, L., & Rubio, S. (2016). Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. *Analytica Chimica Acta, 908*, 22–53. https://doi.org/10.1016/j.aca.2015.12.034.

Cacho, J. I., Campillo, N., Viñas, P., & Hernández-Córdoba, M. (2012). Stir bar sorptive extraction coupled to gas chromatography-mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables. *Journal of Chromatography A, 1247*, 146–153. https://doi.org/10.1016/j.chroma.2012.05.064.

Česen, M., Lambropoulou, D., Laimou-Geraniou, M., Kosjek, T., Blaznik, U., Heath, D., & Heath, E. (2016). Determination of bisphenols and related compounds in honey and their migration from selected food contact materials. *Journal of Agricultural and Food Chemistry, 64*(46), 8866–8875. https://doi.org/10.1021/acs.jafc.6b03924.

Cunha, S. C., Almeida, C., Mendes, E., & Fernandes, J. O. (2011). Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 28*(4), 513–526. https://doi.org/10.1080/19440049.2010.542551.

Dreolin, N., Aznar, M., Moret, S., & Nerin, C. (2019). Development and validation of a LC–MS/MS method for the analysis of bisphenol a in polyethylene terephthalate. *Food Chemistry, 274*(February 2018), 246–253. https://doi.org/10.1016/j.foodchem.2018.08.109.

Driffield, M., Garcia-Lopez, M., Christy, J., Lloyd, A. S., Tarbin, J. A., Hough, P., ... Oldring, P. K. T. (2018). The determination of monomers and oligomers from polyester-based can coatings into foodstuffs over extended storage periods. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 35*(6), 1200–1213. https://doi.org/10.1080/19440049.2018.1440643.

EFSA (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal, 13*(1), 3978. https://doi.org/10.2903/j.efsa.2015.3978.

EFSA, (2010). Public consultation draft guidance on Default assumptions endorsed for public consultation scientific opinion 2 Guidance on Default assumptions used by the EFSA Scientific Panels and Committee, and EFSA Units in the absence of actual measured data 1 EFSA. Retrieved from http://www.efsa.europa.eu/en/efsajournal/doc/1459.pdf.

EFSA (2017). TECHNICAL REPORT Dietary Reference Values for nutrients Summary report. https://doi.org/10.2903/sp.efsa.2017.e15121.

EFSA CEF Panel. (2016). Recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials. EFSA Journal, 14(1), 1–28. Doi:10.2903/j.efsa.2016.4357.

Eftimov, T., Korošec, P., Potočnik, D., Ogrinc, N., Heath, D., & Seljak, B. K. (2017). How to perform properly statistical analysis on food data? An e-learning tool: Advanced Statistics in Natural Sciences and Technologies. *Science within Food Up-to-Date Advances on Research and Educational Ideas, 2018*, 144–151.

Escrivá, L., Hanberg, A., Zilliacus, J., & Beronius, A. (2019). Assessment of the endocrine disrupting properties of Bisphenol AF according to the EU criteria and ECHA/EFSA guidance. *EFSA Journal, 17*(July), https://doi.org/10.2903/j.efsa.2019.e170914.

EU. (2011). Commission Regulation (EU) No 10/2011. Official Journal of the European Union, 12, 1–89.

EU. (2018). Commission Regulation (EU) 2018/832 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materi. Official Journal of the European Union, 2001(May), 20–30. https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.

EURACHEM/CITAC Guide CG 4. (2012). EURACHEM/CITAC Guide CG 4; Quantifying uncertainty in analytical measurement, Third Edition.

Fasano, E., Cirillo, T., Esposito, F., & Lacorte, S. (2015). Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry. *LWT – Food Science and Technology, 64*(2), 1015–1021. https://doi.org/10.1016/j.lwt.2015.06.066.

Fattore, M., Russo, G., Barbato, F., Grumetto, L., & Albrizio, S. (2015). Monitoring of bisphenols in canned tuna from Italian markets. *Food and Chemical Toxicology, 83*, 68–75. https://doi.org/10.1016/j.fct.2015.05.010.

García-Córcoles, M. T., Cipa, M., Rodríguez-Gómez, R., Rivas, A., Olea-Serrano, F., Vílchez, J. L., & Zafra-Gómez, A. (2018). Determination of bisphenols with estrogenic activity in plastic packaged baby food samples using solid-liquid extraction and clean-up with dispersive sorbents followed by gas chromatography tandem mass spectrometry analysis. *Talanta, 178*(April 2017), 441–448. https://doi.org/10.1016/j.talanta.2017.09.067.

Gramec Skledar, D., & Peterlin Mašič, L. (2016). Bisphenol A and its analogs: Do their metabolites have endocrine activity? *Environmental Toxicology and Pharmacology, 47*, 182–199. https://doi.org/10.1016/j.etap.2016.09.014.

Guart, A., Bono-Blay, F., Borrell, A., & Lacorte, S. (2014). Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters. *Food Chemistry, 156*, 73–80. https://doi.org/10.1016/j.foodchem.2014.01.075.

GUM (2008). Evaluation of measurement data — Guide to the expression of uncertainty in measurement. International Organization for Standardization Geneva ISBN, 50(September), 134. https://doi.org/10.1373/clinchem.2003.030528.

Gys, C., Kova, A., Huber, C., Yin, F., Heath, E., & Covaci, A. (2018). Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism, 295(April), 115–123. https://doi.org/10.1016/j.toxlet.2018.05.034.

Hwang, J., Bae, I.-A., Lee, C., Lee, S., Choi, J. C., Park, S.-J., ... Kim, M. (2018). Simultaneous analysis and exposure assessment of migrated bisphenol analogues, phenol, and *p-tert*-butylphenol from food contact materials. *Food Additives & Contaminants: Part A, 1–9*. https://doi.org/10.1080/19440049.2018.1523571.

Oldring, K. T. P., & Nehring, U. (2007). Packing Materials – 7. Metal Packing for Foodstuffs. International Life Sciences Institute, 44. https://doi.org/D/2007/10.996/7.

Kovačič, A., Česen, M., Laimou-Geraniou, M., Lambropoulou, D., Kosjek, T., Heath, D., & Heath, E. (2019a). Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions. *Environmental Research, 179*, Article 108738. https://doi.org/10.1016/J.ENVRES.2019.108738.

Kovačič, A., Gys, C., Kosjek, T., Covaci, A., & Heath, E. (2019b9). Photochemical degradation of BPF, BPS and BPZ in aqueous solution: Identification of transformation products and degradation kinetics. *Science of the Total Environment, 664*, 595–604. https://doi.org/10.1016/j.scitotenv.2019.02.064.

Magnusson & Örnemark (2014). Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2nd ed. Retrieved from https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.

Mandrah, K., Satyanarayana, G. N. V., & Roy, S. K. (2017). A dispersive liquid-liquid microextraction based on solidification of floating organic droplet followed by injector port silylation coupled with gas chromatography–tandem mass spectrometry for the determination of nine bisphenols in bottled carbonated bev. *Journal of Chromatography A, 1528*, 10–17. https://doi.org/10.1016/j.chroma.2017.10.071.

Mortelé, O., Vervliet, P., Gys, C., Degreef, M., Cuykx, M., Maudens, K., ... Lai, F. Y. (2018). In vitro Phase I and Phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*. https://doi.org/10.1016/j.jpba.2018.02.032.

Onghena, M., Van Hoeck, E., Negreira, N., Quirynen, L., Van Loco, J., & Covaci, A. (2016). Evaluation of the migration of chemicals from baby bottles under standardised and duration testing conditions. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 33*(5), 893–904. https://doi.org/10.1080/19440049.2016.1171914.

Paseiro-Cerrato, R., Devries, J., & Begley, T. H. (2017). Evaluation of short-term and long-term migration testing from can coatings into food simulants: Epoxy and acrylic-phenolic coatings. *Journal of Agricultural and Food Chemistry, 65*(12), 2594–2602. https://doi.org/10.1021/acs.jafc.7b00081.

Paseiro-Cerrato, R., Noonan, G. O., & Begley, T. H. (2016). Evaluation of long-term migration testing from can coatings into food simulants: Polyester coatings. *Journal of Agricultural and Food Chemistry, 64*(11), 2377–2385. https://doi.org/10.1021/acs.jafc.5b05880.

Pérez-Albaladejo, E., Fernandes, D., Lacorte, S., & Porte, C. (2017). Comparative toxicity, oxidative stress and endocrine disruption potential of plasticizers in JEG-3 human placental cells. *Toxicology in Vitro, 38*, 41–48. https://doi.org/10.1016/j.tiv.2016.11.003.

R Development Core Team, R. (2011). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (vol. 1). https://doi.org/10.1007/978-3-540-74686-7.

Rochester, & Bolden (2015a). Bisphenol S and F: A systematic Review and Comparison of the Hormanal Activity of Bisphenol A Substitutes. Environmental Health Perspectives, 123(7), 643–650. https://doi.org/10.1289/ehp.1408989.

Rochester, J. R., & Bolden, A. L. (2015b). July 6). Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol a substitutes. *Environmental Health Perspectives. Public Health Services, US Dept of Health and Human Services*. https://doi.org/10.1289/ehp.1408989.

Sanchis, Y., Yusà, V., & Coscollà, C. (2017). Analytical strategies for organic food packaging contaminants. *Journal of Chromatography A, 1490*, 22–46. https://doi.org/10.1016/J.CHROMA.2017.01.076.

Tian, L., Lin, L., & Bayen, S. (2019). Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry. *Talanta, 193*(June 2018), 70–76. https://doi.org/10.1016/j.talanta.2018.09.070.

TSC23. (2017). TSC34 Migration Testing Guidelines for rigid metal packaging coated with organic coatings intended for direct food contact f or discussion with member states and JRC version 6. 2, (July), 1–12.

Usman, A., Ikhlas, S., & Ahmad, M. (2019). Occurrence, toxicity and endocrine disrupting potential of Bisphenol-B and Bisphenol-F: A mini-review. *Toxicology Letters*. https://doi.org/10.1016/j.toxlet.2019.05.018.

Vilarinho, F., Sendón, R., van der Kellen, A., Vaz, M. F., & Silva, A. S. (2019). Bisphenol A in food as a result of its migration from food packaging. *Trends in Food Science and Technology, 91*(June), 33–65. https://doi.org/10.1016/j.tifs.2019.06.012.

Villas-Bôas, S. G., Smart, K. F., Sivakumaran, S., & Lane, G. A. (2011). Alkylation or silylation for analysis of amino and non-amino organic acids by GC-MS? *Metabolites, 1*(1), 3–20. https://doi.org/10.3390/metabo1010003.

Xie, Y., Bao, Y., Wang, H., Cheng, Y., Qian, H., & Yao, W. (2015). Release of bisphenols from can coatings into canned beer in China market. *Journal of the Science of Food and Agriculture, 95*(4), 764–770. https://doi.org/10.1002/jsfa.6862.

Xiong, L., Yan, P., Chu, M., Gao, Y. Q., Li, W. H., & Yang, X. L. (2018). A rapid and simple HPLC–FLD screening method with QuEChERS as the sample treatment for the simultaneous monitoring of nine bisphenols in milk. *Food Chemistry, 244*, 371–377. https://doi.org/10.1016/j.foodchem.2017.10.030.

Alabi, A., Caballero-Casero, N., & Rubio, S. (2014). Quick and simple sample treatment for multiresidue analysis of bisphenols, bisphenol diglycidyl ethers and their derivatives in canned food prior to liquid chromatography and fluorescence detection. *Journal of Chromatography A, 1336*, 23–33. https://doi.org/10.1016/j.chroma.2014.02.008.

Asimakopoulos, A. G., Elangovan, M., & Kannan, K. (2016). Migration of parabens, bisphenols, benzophenone-type UV filters, triclosan, and triclocarban from teethers and its implications for infant exposure. *Environmental Science and Technology, 50*(24), 13539–13547. https://doi.org/10.1021/acs.est.6b04128.

Cheng, Y., Nie, X. M., Wu, H. Q., Hong, Y. H., Yang, B. C., Liu, T., ... Zhang, F. (2017). A high-throughput screening method of bisphenols, bisphenols digycidyl ethers and their derivatives in dairy products by ultra-high performance liquid chromatography-tandem mass spectrometry. *Analytica Chimica Acta, 950*, 98–107. https://doi.org/10.1016/j.aca.2016.11.006.

Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C.-Y., Legrand, A., Boscher, C., ... Le Bizec, B. (2015). Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 407*(9), 2485–2497. https://doi.org/10.1007/s00216-015-8469-9.

Gallo, P., Di Marco Pisciottano, I., Esposito, F., Fasano, E., Scognamiglio, G., Mita, G. D., & Cirillo, T. (2017). Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection. *Food Chemistry, 220*, 406–412. https://doi.org/10.1016/j.foodchem.2016.10.005.

Liao, C., & Kannan, K. (2013). Concentrations and profiles of bisphenol a and other bisphenol analogues in foodstuffs from the united states and their implications for human exposure. *Journal of Agricultural and Food Chemistry, 61*(19), 4655–4662. https://doi.org/10.1021/jf400445n.

Liao, C., & Kannan, K. (2014). A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 31*(2), 319–329. https://doi.org/10.1080/19440049.2013.868611.

Noureddine El Moussawi, S., Ouaini, R., Matta, J., Chébib, H., Cladière, M., & Camel, V. (2019). Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food. *Food Chemistry, 288*, 228–238. https://doi.org/10.1016/j.foodchem.2019.02.116.

Regueiro, J., & Wenzl, T. (2015). Determination of bisphenols in beverages by mixed-mode solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography A, 1422*, 230–238. https://doi.org/10.1016/j.chroma.2015.10.046.

Zhou, J., Chen, X.-H., Pan, S.-D., Wang, J.-L., Zheng, Y.-B., Xu, J.-J., ... Jin, M.-C. (2019). Contamination status of bisphenol A and its analogues (bisphenol S, F and B) in foodstuffs and the implications for dietary exposure on adult residents in Zhejiang Province. *Food Chemistry, 294*, 160–170. https://doi.org/10.1016/j.foodchem.2019.05.022.

3.4 Effects

3.4.1 The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism

The paper "*The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism*", by T. Eleršek, T. Notersberg, A. Kovačič, E. Heath and M. Filipič was published in *Environmental Science and Pollution Research* in September 2020. The study was performed in collaboration with the National Institute of Biology, Slovenia, and was led by Assist Prof Dr Eleršek under the supervision of Prof Dr Filipič. I carried out all the analytical work, including sample preparation, method optimization, instrumental analysis, and data evaluation as well as drafting the analytical part of the manuscript.

Although concern that BPA and other bisphenols in the environment can affect aquatic organisms has increased in recent years [85], [218], their ecotoxicological effect on algae and cyanobacteria, which as primary producers play an important role in aquatic ecosystems, remains largely unknown. In this study, the toxicity of BPA, BPF and their mixture towards eukaryotic green alga *Pseudokircheneriella subcapitata* and the prokaryotic cyanobacterium *Synechococcus leopoliensis* was addressed. The toxicity of the binary mixture was predicted using different models, including concentration addition (CA), independent action (IA), combination index, and the isobologram method over the whole effect concentration range (EC_5–EC_{90}). The comparison of the results obtained by these models with the experimental data enabled the appropriateness of using such models for prediction of mixture toxicity to be assessed. The results indicate the higher sensitivity of cyanobacteria compared to green algae to bisphenols. At the same time, the individual toxicity of BPA and BPF towards *P. subcapitata* and *S. leopoliensis* was comparable but higher towards *P. subcapitata* in the case of their binary mixture. Both models, CA and IA models underestimated mixture toxicity of bisphenols for P. *subcapitata* over the whole EC range. For S. *leopoliensis,* CA model predictions were close to the experimentally determined toxicity at EC_{50}–EC_{90} while with the IA model they were similar at EC_{10}–EC_{20}. The CI model and the isobologram method predicted the antagonistic effect of bisphenol mixture in both *S. leopoliensis* and *P. subcapitata.* The same effect was also revealed by the experimental data for *S. leopoliensis,* whereas in *P. subcapitata,* the additive effect was expressed. The environmental risk characterization based on the calculated ratio between reported concentrations of BPA and BPF in surface waters versus the predicted no-effect concentration calculated in this study showed that BPA represents an environmental risk, whereas BPF does not.

This work fulfils the aim of developing a simultaneous determination of bisphenol residues using GC-MS. In addition, it estimates the toxicity of bisphenols to aquatic species and contributes towards underestimating the fate and effects of bisphenol residues during WWT and in the environment. This study is the first to address the toxicity of individual bisphenol for cyanobacteria and toxicity of their mixtures for cyanobacteria and green algae, which is more likely to occur in the environment. Thus, this paper adds knowledge about the environmental risks of bisphenols and highlights new gaps in our understanding of their occurrence in the aquatic environment as well as ecotoxicological data for individual bisphenols and their mixtures.

Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-020-10329-7

RESEARCH ARTICLE

The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism

Tina Elersek[1] · Tilen Notersberg[1] · Ana Kovačič[2,3] · Ester Heath[2,3] · Metka Filipič[1]

Received: 1 February 2020 / Accepted: 30 July 2020

Abstract

Bisphenol A (BPA) is, due to its widespread use including the production of plastic materials, an ubiquitous pollutant in the aquatic environment. Due to evidence of adverse BPA effects on the environment and human health, its use has been restricted and replaced by analogues such as bisphenol F (BPF). This study examined the toxicity of BPA, BPF and their mixture towards primary producers, the eukaryotic green alga *Pseudokirchneriella subcapitata* and the prokaryotic cyanobacterium *Synechococcus leopoliensis.* The results demonstrated that *S. leopoliensis* is more sensitive than *P. subcapitata*, whereas toxic potential of the two BPs is comparable and represents comparable hazard for phytoplankton. The toxicity of the binary mixture was predicted by different models (concentration addition, independent action, combination index and the isobologram method) and compared to experimental data. Additive effect was observed in *P. subcapitata* over the whole effect concentration range (EC_5–EC_{90}), whereas in *S. leopoliensis*, no pronounced combined effect was observed. The environmental risk characterisation based on the comparison of reported concentrations of BPA and BPF in surface waters to the predicted no-effect concentration values obtained in this study showed that at certain industrial areas, BPA represents environmental risk, whereas BPF does not. However, BPF concentrations in aquatic environment are expected to increase in the future. To enable environmental risk assessment of BP analogues, more data on the toxicity to aquatic species, including combined effect, as well as data on their occurrence in the aquatic environment are needed.

Keywords Aquatic ecotoxicology · Plastic pollution · Toxic stress · Growth inhibition · Environmental risk assessment · Pollutant degradation

Introduction

Bisphenol A (BPA) is a commercial name for 4,4′-(propane-2,2-diyl)diphenol, which was first synthesised in 1891 (Staples et al. 1998). It is used as a raw material for the production of many products such as polycarbonates and epoxy resins, lining of food cans and metal lids (Eio et al. 2015). BPA is released to the environment from production sites, processing plants (Staples et al. 2000), wastewater effluent, landfill leachate (Corrales et al. 2015), plastic (Liao and Kannan 2013), microplastic and other materials where BPA is present, as a non-point source of environmental contamination (Crain et al. 2007). For the health of our aquatic environment, it is important to examine the impact of potentially toxic compounds on aquatic organisms, especially on primary producers, since they form the basis of an aquatic ecosystem's food web.

The concern for vertebrates was raised due to BPA estrogenic activity (Dodds and Lawson 1938) with a negative impact on the endocrine system of humans and animals (Eladak et al. 2015). Moreover, BPA is genotoxic in vitro and in vivo in rodents (Usman and Ahmad 2016). Studies also indicate a connection of exposure to BPA with many human diseases such as diabetes, obesity, cardiovascular, respiratory and kidney diseases, breast cancer and sexual disorders (Eladak et al. 2015). BPA is a well-known plastic-associated-compound

Responsible editor: Philippe Garrigues

✉ Tina Elersek
tina.elersek@nib.si

[1] Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

[2] Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

[3] Jožef Stefan, International Postgraduate School, 1000 Ljubljana, Slovenia

and is known to the wider public from the "BPA free" water bottles campaign. In 2011, the European Commission banned the use of BPA in baby bottles and sippy cups, and will prohibit its use in the production of thermal paper from 2020 onwards (EU 2016). Therefore, manufacturers have started to replace BPA with alternatives in similar applications, of which bisphenol F (BPF) is one of the most used BPA alternatives (Chen et al. 2016). BPF is a commercial name for 4,4′-methylenediphenol, and it is used as a monomer in the production of epoxy resins and polycarbonates for the lining of food containers, water pipes, roof coverings, roads, bridges, structural adhesives, coatings, lacquers, varnishes, adhesives and dental flosses (Rochester and Bolden 2015; Tisler et al. 2016). Due to its similar structure to BPA, BPF has the potential to exert similar ecological and health effects as BPA (Eladak et al. 2015; Liao and Kannan 2013; Tisler et al. 2016).

The BPA concentrations in the environment can reach up to 370 $\mu g\ L^{-1}$ in waste water treatment plant effluent (Corrales et al. 2015), 200 $\mu g\ kg^{-1}$ in river sediment (Fromme et al. 2002), 56 $\mu g\ L^{-1}$ in rivers (Corrales et al. 2015) and 17.2 $\mu g\ L^{-1}$ in leachate from plastic waste (Yamamoto et al. 2001). Although usual levels in river waters are $< 1\ \mu g\ L^{-1}$ (Staples et al. 2000; Fromme et al. 2002; Bhandari et al. 2014), the presence of BPA in the environment is global (Corrales et al. 2015). The levels in dust and air were reported to be up to 4.1 $\mu g\ g^{-1}$ (Liao et al. 2012) and 17.4 ng m^{-3} (Fu and Kawamura 2010), respectively. In food, concentrations of BPA can be up to 10 ng g^{-1} in products such as soups, eggs, sauces and syrups (Liao and Kannan 2013). BPA was also measured in energy drinks at levels up to 3.3 ng L^{-1} (Gallo et al. 2017). BPF has not been as thoroughly studied as BPA but compared to BPA, lower concentrations of BPF are reported. BPF concentrations were up to 0.3 $\mu g\ L^{-1}$ in surface waters (Yamazaki et al. 2015), 0.1 $\mu g\ g^{-1}$ in sediment (Fromme et al. 2002) and $< 0.5\ \mu g\ g^{-1}$ in dust powder (Liao et al. 2012). In food, the highest concentrations of BPF (4.63 ng g^{-1}) were measured in fish and seafood (Liao and Kannan 2013). In the environment, BPA is degraded abiotically with photo degradation as a result of UV radiation (Wang et al. 2007), and/or biotically with bacteria, algae and fungi (e.g. Ren et al. 2016; Ji et al. 2014). The biodegradability of different BPs in seawater and wastewaters was ranked as BPF $>>$ BPA $>$ BPS (Ike et al. 2006; Danzl et al. 2009).

Algae and cyanobacteria play a major role as primary producers at the bottom of food chain in aquatic ecosystems. Changes in their species composition can have wider implications for the biological community and ecosystem. Therefore, toxic stress to phytoplankton may affect the structure and functioning of the whole ecosystem (Ma 2005). The information on the effects of BPA and its analogues on phytoplankton species is limited to the studies in different algal species, which demonstrated some differences in their sensitivity towards BPA toxicity. Concern over BPA and alternatives in the environment affecting aquatic organisms has greatly increased over recent years (zebrafish e.g. Moreman et al. 2017; Le Fol et al. 2017; marine rotifers e.g. Park et al. 2018; algae e.g. Tisler et al. 2016; Li et al. 2009; Zhang et al. 2012; M'Rabet et al. 2018). Nevertheless, the ecotoxicological potential of BPA and alternatives on cyanobacterial primary producers, as an important part of aquatic algal community, remains largely unknown. To our best knowledge, the toxicity of BPA and its analogues has so far not been published for cyanobacteria. In general in algal group, diatoms from genera *Navicula*, *Stephanodiscus* and *Cyclotella* are more sensitive (EC_{50} 4–9 mg L^{-1}) (Liu et al. 2010; Li et al. 2009) than green algae from genera *Chlorella*, *Chlamydomonas* and *Desmodesmus* (EC_{50} 20–89 mg L^{-1}, Zhang et al. 2012; Tisler et al. 2016). Moreover, certain tolerant algal species have been reported to accumulate and degrade BPA (Hirooka et al. 2003, 2005; Ben Uoada et al. 2018).

Although algae and cyanobacteria have been shown to be comparatively sensitive to many chemicals (Real et al. 2003), for certain chemical differences in the sensitivity were demonstrated (Ma 2005; Brezovšek et al. 2014). In the aquatic community, such pollutants may cause changes of green algal and cyanobacterial group structure. Of particular concern is higher sensitivity of algae compared to cyanobacteria, which may result in a shift from dominance by green algae to dominance by cyanobacteria, and may even contribute to cyanobacterial blooms during the specific periods (Ma 2005). Therefore, for more reliable environmental risk assessment, studies comparing differential sensitivity of cyanobacteria and green algae are needed. Another aspect that has so far not been addressed is toxicity of mixtures of BPA and its analogues, which in the environment occur together.

In this study, we evaluated the toxicity of BPA, its analogue BPF and their binary mixture in the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*. To assess the interaction of BPA and BPF in combined exposure, the toxicity of the mixture has been predicted by several approaches: concentration addition (CA) and independent action (IA) models, determination of combination index (CI) and with isobologram method, and the calculated data have been compared to experimental data.

Materials and methods

Test substances

For the experiments, BPA (CAS 80-05-7, Merck), BPF (CAS 620-92-8, Sigma-Aldrich) and as a reference chemical DCP (3,5-dichlorophenol DCP, CAS 591-35-5, Sigma-Aldrich) were used. Concentrated stock solutions of BPA (300 mg L^{-1}) and BPF (30 mg L^{-1}) in OECD medium, and

DCP (1000 mg L^{-1}) in dimethilsulfoxide (DMSO) were prepared. DMSO was used at a final concentration of 0.04% v/v, which exceeds the OECD recommended value (0.01%), but as reported by Brezovšek et al. 2014, the concentration used does not affect the growth of the selected alga. The same final concentration of DMSO was added to the control cultures (solvent control). Stock solutions of BPA, BPF and DCP were stored in the refrigerator at − 4 °C, not longer than 6 months. BPA was tested at concentrations of 0.5, 1.5, 4.9, 15.6 and 50 mg L^{-1}; BPF at 0.3, 0.9, 2.9, 9.4 and 30 mg L^{-1} and DCP at 2.5, 5, 10, 20 and 40 mg L^{-1}. The highest test concentrations of BPA and BPF were chemically verified by gas chromatography-mass spectrometry (GC-MS). The following internal standards were used: deuterated BPA (BPA-d$_{16}$, CAS 96210-87-6, Sigma-Aldrich) and isotopically labelled BPF (^{13}C$_{12}$ BPF, > 95%, Toronto Research Chemicals CanSyn Chem. Corp.).

Stability testing of BPA and BPF during the assay

In order to assure reliable results and assess potential ability of *P. subcapitata* and *S. leopoliensis* to degrade BPA and BPF, the changes in the concentrations of investigated compounds were determined in culture medium in the presence and absence of the tested compounds (BPA 5 mg L^{-1}; BPF 3 mg L^{-1}) and in stock solutions (BPA 300 mg L^{-1}; BPF 30 mg L^{-1}). The concentrations in culture medium were determined at time 0 and after 72 h, using GC-MS.

Chemical analysis All samples were filtered through a RC membrane, 0.2 -μm filters, and diluted with OECD medium. Three aliquots ($V = 100$ μL) of the prepared sample were spiked ($V = 25$ μL) with mixed internal standards (^{13}C$_{12}$-BPF and ^{16}dBPA, $c = 1$ μg mL^{-1}) and dried under a gentle stream of N$_2$ at 40 °C. The samples were derivatised with 50 μL of BSTFA and 50 μL of pyridine for 16 h at 80 °C. Derivatised samples were transferred to glass vial inserts and analysed using an Agilent 7890B series gas chromatograph with a 5977A single-quadrupole mass spectrometer (Agilent, USA). Separation was achieved on a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA) with helium as the carrier gas (1 ml min^{-1}). One μL of each sample extract was injected in splitless mode at 250 °C. For optimal chromatographic separation, the following temperature program was used: an initial temperature of 120 °C was ramped at 20 °C/min to 200 °C and held for 2 min and then at 10 °C/min to 250 °C. Total GC-MS runtime was 11.0 min. The mass spectrometer was operated in EI mode at 70 eV. Selected compounds were determined using selective ion monitoring (SIM) mode using retention time matching (BPA 10.22 min, BPF 10.87 min, ^{16}dBPA 10.80 min, ^{13}C$_{12}$BPF 10.72 min) and by monitoring the following ions: *m/z* 344, 329 and 179 for BPF; *m/z* 372, 257 and 339 for BPA; *m/z* 356, 341 and 179 for 13C12 BPF; *m/z* 386, 368 and 217 for ^{16}dBPA. Data were processed using MassHunter Workstation Quantitative Analysis software (version B.07.00, Agilent Technologies).

BPs are ubiquitous in the laboratory environment due to their broad application (Caballero-Casero et al. 2016). Therefore, background contamination blanks were prepared to evaluate and minimise sources of contamination. Both BPA and BPF were present as contaminants in the blanks. To minimise contamination, all cleaned glasswares were heated to 400 °C for 4 h before the experiments. Procedural blanks were prepared for each experimental setup. Blank samples were analysed following the same instrumental analysis. Blank samples of ethyl acetate were analysed after every 10th sample injection to evaluate and prevent potential carry over between samples. All data were blank corrected.

Algal growth inhibition assay

To determine the toxicity of the BPA and BPF and their mixtures on algae and cyanobacteria, an algal growth inhibition test was performed according to OECD guideline 201 (OECD 2011) with minor modification in light regime. The cultures at exponential growth in a nutrient-enriched medium (OECD medium) were exposed to different concentrations of BPA, BPF or their binary mixtures and incubated in the light (16 h per day of light intensity at 80–120 μE/m^{2}s, lights Sylvania GRO-Lux F 18 W/GRO-T8) with constant shaking at 80 rpm (GFL 3017, Burgwedel, Germany) at 24 ± 2 °C (LTH, Slovenia). Two phytoplankton species were selected, the green alga *Pseudokirchneriella subcapitata* SAG 61.81 and the cyanobacterium *Synechococcus leopoliensis* SAG 1402-1, both obtained from SAG, Göttingen algae collection. The assays were performed in glass Erlenmeyer flasks. One replicate covered at least five different BP concentrations (arranged in a geometric series with a factor of 3.2) and a control sample. The culture volume was initially set to 20 mL with a cell density of 10^3–10^4 cells mL^{-1} for *P. subcapitata* and 10^4–10^5 cells mL^{-1} for *S. leopoliensis*. After 72 h, the observed response was growth rate inhibition in comparison to the control cultures. Each test was performed as 3 independent experiments; each tested concentration was set in triplicate. Flow cytometry (MacsQuant, Milteny) was chosen to detect cell growth for the following reasons: only a small sample volume is required (50 μL of sample per well of a microtiter plate); it can distinguish between live and dead cells and is also suitable for measuring cyanobacterial cells, which cannot be counted precisely using a counting chamber and a light microscope (Elersek 2012). Negative control (media) and solvent control were measured in the same microtiter plate. The performance of tests was validated according to OECD guideline 201 (OECD 2011); the growth rate was at least 0.92 per day; the cell count increased at least 16 times during 72 h; the coefficient of variability between individual test days was < 35%, and the coefficient of variability during the entire test was < 10%. The test system was confirmed using 3,5-dichlorophenol as a reference compound. The obtained EC_{50} 9.9 and 3.9 mg L^{-1} for *P. subcapitata* and *S. leopoliensis*, respectively, are in the range of the expected response.

The toxicities of the binary mixtures of BPA and BPF were tested at half the effective concentrations of each (EC5/2, EC10/2, EC20/2, EC50/2, EC90/2), as calculated from the dose-response curves of the single compounds (Table 2), and described previously (Cleuvers 2003; Brezovšek et al. 2014). This means that if the BPA and BPF effects follow the CA model, theoretically, the total effect of the mixture would be 5%, 10%, 20%, 50% and 90% growth inhibition, respectively. This is one of the possible approaches of mixture testing, where the focus is on testing different ratios in the mixture, based on the effects of individual compounds.

Statistical evaluations

Results expressed as growth inhibition were analysed using Prism 6 software (Graphpad Inc.). Data were analysed as measurements from each individual flask (pooled together) rather than means of replicates, to extract as much information as possible (OECD 2011). For the graphs showing percentage of inhibition vs. concentration of individual BPs, a nonlinear regression model "log(agonist) vs. response–find EC anything" function was applied (Eqs. (1)–(3)). Residual plots were also studied, and any outliers recognised by Prism were excluded from the statistical analyses. Statistical significance ($p < 0.05$) of an effect in comparison to the control was assessed using a non-parametric ANOVA (Kruskal-Wallis test) with the Dunnett's post-test at a 95% confidence interval.

$$\log EC_F = \log EC_{50} + (1/\text{slope}) \times \log(F\%/(100-F\%)) \tag{1}$$

$$F\% = (Y-\text{bottom})/(\text{top}-\text{bottom}) \times 100 \tag{2}$$

$$Y = \text{bottom} + (\text{top}-\text{bottom})/\left(1 + 10^{\left(\left(\log EC50 - X\right) \times \text{slope}\right)}\right) \tag{3}$$

Predicted toxicity of binary mixture of BPA and BPF

The predicted toxicity of the mixture was calculated as concentration addition (CA) (Loewe 1927; Loewe and Muischnek 1926) and independent action (IA) (Bliss 1939). Both models are frequently used for predicting mixture toxicity (Sumpter et al. 2006; Brezovšek et al. 2014; Elersek et al. 2016). Predicted toxicities were calculated based on the data obtained for individual compounds, and then the calculated values were compared to the measured, experimentally observed toxicities of the binary mixtures at each effect concentration.

Combination index (CI) for multiple drug effect interactions was introduced to give a quantitative definition of synergism (CI < 1), additive effect (CI = 1) and antagonism (CI > 1) using computerised simulations (Chou and Martin 2007; originally from Chou and Talalay 1984). The CI was modelled using CompuSyn software (ComboSyn, Inc.), which is based on the CI concept with CI algorithms and median-effect equation. The ratio of the fraction affected (fa) vs. the fraction unaffected (fu) is equal to the dose (D) vs. the median-effect dose (Dm) to the mth power, where Dm signifies potency and m signifies the sigmoidicity (shape) of the dose-effect curve. The isobologram is a curve that reveals whether a toxicant mixture displays additivity, synergism or antagonism (Chou 2006). The isobol equation is actually a special case of the CI equation and is often used to illustrate graphically the combined effect of binary mixtures (e.g. Altenburger et al. 1990).

Results and discussion

In aquatic environment, algae and cyanobacteria, as primary producers, represent an important target of toxic stress, and adverse effect to these organisms may severely affect the whole ecosystem function.

BPA and BPF stability

In general, the loss of tested substance can be a matter of volatilisation, sorption to test vessel or cells and/or uptake into algal cells. The results of our BPA and BPF stability study demonstrated that in the growth media (with no test organisms), no significant removal (decrease of concentration) of BPs was detected after 72 h (at maximum 9%, Table 1). In the presence of *P. subcapitata* during the 72-h exposure, 20% of BPA and 13% of BPF were removed from the medium, whereas in the presence of *S. leopoliensis*, the removal of BPA or BPF was only half of that observed in the presence of *P. subcapitata* (Table 1). These results indicate that *P. subcapitata* and *S. leopoliensis* do not exhibit significant BP removal capability.

Our results for BPA are comparable to the results of biotic degradation with green algae *Chlorella sorokiniana* (Eio et al. 2015), whereas green alga *Chlorella fusca* during 120-h exposure degraded up to 85% BPA (Hirooka et al. 2003, 2005). High BPA degradation ability has been reported also for extremophilic alga *Picocystis* sp. reaching 72% removal at 25 mg L^{-1} BPA (Ben Uoada et al. 2018). Concerning cyanobacterium *S. leopoliensis*, similar degradation of BPA has been reported for cyanobacterium from the genus *Anabaena* after 120 h (Hirooka et al. 2003).

According to OECD guideline recommendations (OECD 2011), the toxicological parameters (ECx, NOEC, LOEC)

Table 1 The stability (%) and measured concentrations (± standard deviation from 3 samples measured) of bisphenol A and F in the presence of algae and cyanobacteria (OECD medium, light, shaking, biotic factor for 72 h). Additionally, abiotic degradation was assessed during the experiment

	P. subcapitata	*S. leopoliensis*	Abiotic degradation
BPA	20%	11%	9%
Concentration at 0 h (mg L^{-1}) experiment	3.40 ± 0.19	3.38 ± 0.08	3.51 ± 0.35
Concentration after 72 h (mg L^{-1})	2.68 ± 0.57	3.01 ± 0.07	3.21 ± 0.06
BPF	13%	7%	0%
Concentration at 0 h (mg L^{-1}) experiment	2.67 ± 0.08	2.74 ± 0.19	2.69 ± 0.22
Concentration after 72 h (mg L^{-1})	2.32 ± 0.01	2.54 ± 0.02	2.82 ± 0.09

were calculated using nominal concentrations of the tested compounds. Additionally, stock solutions (saved in the refrigerator less than 6 months) from three experiments were also tested. Measured BPA stock concentrations matched the nominal value 93% (with standard deviation of 28%), and measured BPF stock matched the nominal value 89% (with standard deviation of 17%).

Toxicity of BPA and BPF

The results of the growth inhibition test of BPA and BPF in green alga *P. subcapitata* and cyanobacteria *S. leopoliensis* are shown in Fig. 1 and Table 2. In *P. subcapitata*, statistically significant toxic effects of BPA were detected at concentrations > 15.6 mg L^{-1} and BPF at concentrations > 9.4 mg L^{-1} (Fig. 1, left). In *S. leopoliensis*, statistically significant toxicity of BPA was observed at concentrations > 1.5 mg L^{-1}, and BPF at concentrations > 9.4 mg (Fig. 1, right). As can be seen on Fig. 1, growth inhibition of *P. subcapitata* after 72 h showed higher variability compared to *S. leopoliensis*. The reason might be in flow cytometer measurement, since the cells of *P. subcapitata* are more variable in the shape (curved croissant-like with variable dimensions) which could cause higher variations in cell count (e.g. during the cell division, the cells can be counted as 1 or 2). With *S. leopoliensis*, the morphology is more uniform (rod-like); thus, cell counts are less variable. However, we should perform more studies to confirm this speculation, but it was beyond the scope of the present study.

Algal and cyanobacterial growth inhibition test is in principle, a multigenerational test, and is considered as an acute and chronic toxicity test. Therefore, in the environmental risk assessment, EC_{50} values are used as a parameter of acute toxicity and EC_{10} and/or NOEC as parameters related to chronic toxicity (European Communities 2003). The obtained EC_{50}, EC_{10} and NOEC values are summarised in Table 2. The EC_{50} value for BPA in *P. subcapitata* (6.8 mg L^{-1}) was higher from previously reported EC_{50} value for this alga (2.7 mg L^{-1}) (Alexander et al. 1988). Lower EC_{50} values were obtained also in marine alga *Skeletonema costatum* (1 mg L^{-1}) (Alexander et al. 1988) and *Navicula incerta* (3.7 mg L^{-1}) (Liu et al. 2010), and comparable EC_{50} value (8.7 mg L^{-1}) to ours was reported for *Stephanodiscus hantzschii* (Li et al. 2009). On the other hand, much higher EC_{50} values (20–89 mg L^{-1}) were reported for *Chlorella vulgaris*, *Chlamydomonas mexicana* (Ji et al. 2014), *Chlorella pyrenoidosa*, *Scenedesmus obliquus* (Zhang et al. 2012) and *Desmodesmus subspicatus* (Tisler et al. 2016). The EC_{50} value for BPF in *P. subcapitata* (9.2 mg L^{-1}) is lower from that reported for *Desmodesmus subspicatus* (EC_{50} 22 mg L^{-1}), which is also the only published study on the toxicity of BPF in algae (Tisler et al. 2016). The differences in the algal growth inhibition by BPs in great deal depend on the test species and to certain extent to the differences in exposure conditions i.e. different exposure durations (72–120 h).

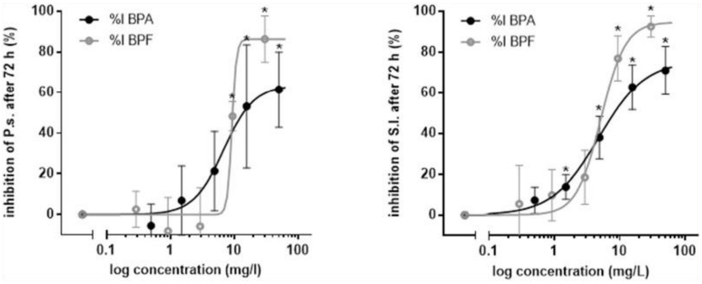


Fig. 1 Growth inhibition (%) of *P. subcapitata* (left) and *S. leopoliensis* (right) after 72 h. Bars denote 95% confidence interval. Asterisk denotes a statistical difference between the treated sample and the control based on ANOVA and the Dunnett's post test

Table 2 The effects of bisphenol A (BPA) and F (BPF) on *P. subcapitata* and *S. leopoliensis*. $EC_x = X\%$ effective concentration, *LOEC* lowest observed effect concentration, *NOEC* no observed effect concentration. At EC_x values, the 95% confidence intervals are reported in the parenthesis

	Effects of BPA and BPF-based non-lin. Fitting (EC_x in mg L^{-1})	
	BPA	BPF
Green alga *Pseudokirchneriella subcapitata*	$EC_5 = 1.4$ (0.3–5.6)	$EC_5 = 6.9$ (1.7–10.6)
	$EC_{10} = 2.1$ (0.7–5.8)	$EC_{10} = 7.4$ (2.7–10.4)
	$EC_{20} = 3.2$ (1.6–6.5)	$EC_{20} = 8.1$ (4.2–10.5)
	$EC_{50} = 6.8$ (3.4–13.8)	$EC_{50} = 9.2$ (7.1–13.8)
	$EC_{90} = 22.3$ (3.9–128.1)	$EC_{90} = 11.2$ (7.4–46.8)
	LOEC = 15.6	LOEC = 9.4
	NOEC = 4.9	NOEC = 2.9
Cyanobacterium *Synechococcus leopoliensis*	$EC_5 = 0.4$ (0.2–0.9)	$EC_5 = 1.5$ (0.9–2.3)
	$EC_{10} = 0.8$ (0.5–1.4)	$EC_{10} = 2.0$ (1.4–2.9)
	$EC_{20} = 1.5$ (1.1–2.2)	$EC_{20} = 2.8$ (1.1–3.8)
	$EC_{50} = 4.8$ (3.1–7.3)	$EC_{50} = 5.2$ (3.9–6.8)
	$EC_{90} = 28.6$ (10.4–79.0)	$EC_{90} = 13.2$ (7.5–23.2)
	LOEC = 1.5	LOEC = 9.4
	NOEC = 0.5	NOEC = 2.9

Nevertheless, the results of our study indicate that *P. subcapitata* belongs to more sensitive algal species.

Toxic potential of BPF was in alga and cyanobacteria comparable to that of BPA (Table 2). Also in *D. subspicatus*, BPA and BPF exerted comparable toxic potential (EC_{50}: 20 and 22 mg L^{-1}, respectively) (Tisler et al. 2016).

Regarding differences in the sensitivities towards the two BPs, *S. leopoliensis* was more sensitive than *P. subcapitata*, which is evident from EC*x* values and through the calculation of toxic units (Table 2). This may indicate higher sensitivity of cyanobacteria than green algae to BPs. However, as already discussed for BPA, lower and higher toxicities have been observed in other green alga species, whereas, to our best knowledge, in cyanobacteria, BPs have so far not been tested. Our results contribute to previously reported observations that toxicity levels cannot simply be extrapolated from one species to others or to natural assemblages (Ma 2005; Hagenbuch and Pinckney 2012).

Based on EC_{50} values, the EU Regulation (Regulation EC No 1907/2006) classifies chemicals as very toxic to aquatic organisms (EC_{50}, < 1 mg L^{-1}), toxic to aquatic organisms (EC_{50}, 1–10 mg L^{-1}) and harmful to aquatic organisms (EC_{50}, 10–100 mg L^{-1}). Based on our results, BPA and BPF are classified as toxic to primary producers in the aquatic environment.

Mixture effects and a comparison of different prediction models

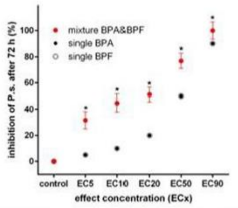
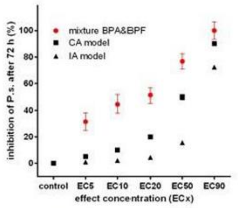
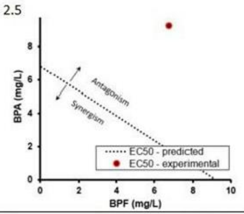
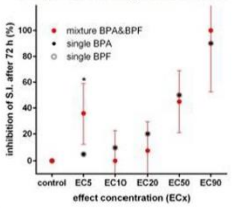
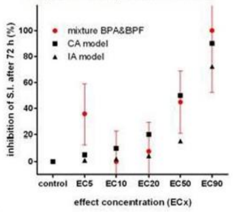
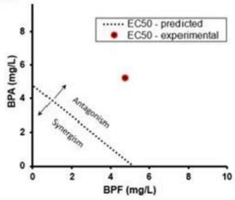
Results of a binary mixture of BPA/BPF showed different toxic responses and differences in the susceptibility of the two species. Like BPA and BPF, also their binary mixture exerted higher toxicity in *P. subcapitata* than in *S. leopoliensis* (Table 3). To assess the interaction of BPA and BPF in the mixture, the predicted effects were calculated by CA and IA models and by calculating the CI and compared to the experimental data (red dots in Table 3).

The CA model is based on the idea that chemicals have a similar mechanism of action, which means that it is more suitable for chemical combinations that are assumed to interact with common molecular target sites in the test organism (Backhaus et al. 2004) and would be the first logical choice in the case of BPA and BPF. When compared to experimental data, the CA model predictions underestimated mixture toxicity for *P. subcapitata* over the whole effect concentration range EC_5–EC_{90}, whereas for *S. leopoliensis*, CA model predictions were close to the experimentally determined toxicity at higher effect concentration range (EC_{50}–EC_{90}**)**.

Alternatively, IA model is a statistical concept based on independent random events (Bliss 1939, in Backhaus et al. 2004) and is based on the idea of dissimilar action of compounds in a mixture; thus, IA should be more suitable for chemical combinations that have different molecular target sites and modes of action (Backhaus et al. 2004). When compared to experimental data**,** the IA model (like CA model) underestimated mixture toxicity over the whole effect concentration range EC_5–EC_{90} for *P. subcapitata*, but for *S. leopoliensis* model, predictions were close to experimentally determined toxicity in the concentration range EC_{10}–EC_{20}. The critics of IA model that is based on probabilistic reasoning point out that in some cases, IA model is degraded to a simple calculation technique with no broader theoretical background (Hadrup et al. 2013).

The combination index (CI) indicated antagonism over the whole concentration range for *P. subcapitata* (CI > 2.1) and for *S. leopoliensis* (CI > 2.0), which is evident from the

Table 3 The effects of BPA and BPF mixture on *P. subcapitata* and *S. leopoliensis*—an overview of data analysing approaches and results. Graphs show effects on inhibition of proliferation after 72 h. Red colour is used for experimental data of BPA and BPF mixture. Isobolograms are modelled for EC_{50}. Error bars denote 95% confidence interval.

	effects of mixture BPA+BPF assessed by different approaches		
	experimental data	**independent action (IA), concentration addition (CA)**	**combination index (CI), isobologram method**
green alga ***Pseudokirchneriella subcapitata***	experimental data of BPA+BPF mixture exhibit higher toxicity* in comparison to single BPA and BPF (EC5-EC50)  	experimental data exhibit higher toxicity in comparison to IA model (only EC50-EC90) and CA model (only EC20-EC90)  	CI at EC5 = 5.1 CI at EC10 = 3.8 CI at EC20 = 2.9 CI at EC50 = 2.1 CI at EC90 = 2.5 according to this model the mixture effect should be antagonistic (EC5-EC90) 
cyanobacterium ***Synechococcus leopoliensis***	experimental data of BPA+BPF mixture exhibit similar toxicity (EC10-EC90) and higher toxicity * (at EC5 only) in comparison to single BPA and BPF  	experimental data exhibit similar toxicity in comparison to IA model (EC10-EC20), CA model (EC5-EC90) and (only at EC5) higher toxicity to both models  	CI at EC5 = 4.0 CI at EC10 = 2.9 CI at EC20 = 2.4 CI at EC50 = 2.0 CI at EC90 = 2.6 according to this model the mixture effect should be antagonistic (EC5 - EC90) 

*Asterisk denotes statistical difference in comparison to the control based on ANOVA testing and Dunnett's post test

isobologram (Table 3, right column). CI correctly predicted the antagonistic effect of the mixture in *S. leopoliensis* but not in *P. subcapitata* for which experimental data showed additive effect in the effect concentrations between EC_5 and EC_{50}.

Taken together for *P. subcapitata*, the data indicate additive effect at low-effect concentration range and antagonism at high-effect concentration range, while for *S. leopoliensis* the data indicate antagonism over the whole effect concentration range.

Environmental risk characterisation

As a result of high production, consumption and subsequent release into the environment, BPA became a ubiquitous contaminant in the environment (Corrales et al. 2015). The median concentrations in surface water range between 3 and 30 ng L^{-1} (Bhandari et al. 2014); however, the concentrations in surface water from dense industrial areas are considerably higher ranging up to 56 μg L^{-1} (Corrales et al. 2015; Petrie et al. 2015; Wilkinson et al. 2017). Despite an increasing use of BPF, the information on its occurrence in the aquatic environment is very limited. The maximal reported concentrations in samples of surface waters in Germany are 180 ng L^{-1} (Fromme et al. 2002) and around 300 ng L^{-1} in surface water samples in Japan and China (Yamazaki et al. 2015). An important component of the environmental risk assessment is hazard assessment. According to Technical Guidance Document on Risk Assessment (European Communities 2003), hazard assessment for aquatic environment is based on predicted no-effect concentration (PNEC) of the chemical for the most sensitive species that, if it does not exceed environmental concentration, ensures an overall protection of the environment. Here we characterised the environmental hazard for phytoplankton by comparing the measured environmental concentrations (MEC) of BPA or BPF to the predicted no-effect concentrations (PNEC) for the two species, which were derived from the EC_{50} values divided by the assessment factor of 1000 as recommended in the Technical Guidance Document on Risk Assessment (European Communities 2003). For BPA, PNEC values are 6.8 and 4.8 μg L^{-1} for

P. subcapitata and *S. leopoliensis*, respectively. These values are higher than the median BPA concentrations in surface waters (30 ng L^{-1}) (MEC/PNEC < 1), but lower than the measured BPA concentrations at the industrial areas (up to 28 μg L^{-1}) (MEC/PNEC > 1), indicating potential environmental risk for phytoplankton at such areas. The PNEC values for BPF are 9.2 and 5.2 μg L^{-1} for *P. subcapitata* and *S. leopoliensis*, respectively, and are higher than the BPF concentrations determined in surface waters (MEC/PNEC < 1), which indicates that BPF does not represent risk for phytoplankton. However, the available data on the environmental occurrence of BPF are very scarce and probably do not reflect the actual situation in the aquatic environment. However, BPA and BPF are endocrine disruptors; thus, aquatic invertebrates and vertebrates are expected to be more sensitive than phytoplankton. Wright-Walters et al. (2011) conducted an aquatic hazard assessment for BPA using a weight of evidence approach in which the PNEC value was derived using a non-parametric hazardous concentration for 5% of the species (HC_5) approach. They included 61 studies that yielded 94 no observed effect concentration (NOEC) values. The toxicity dataset suggested that mortality and inhibition of growth, development and reproduction are most likely to occur between the concentrations of 0.0483 and 2280 μg L^{-1}. They calculated a PNEC value for aquatic environment 0.06 μgL^{-1}, which is two orders of magnitude lower from PNEC values we obtained for phytoplankton.

Conclusions

The results of this study demonstrated that BPA and its analogue BPF exerted comparable toxicity in alga *P. subcapitata* and cyanobacteria *S. leopoliensis* and represented comparable hazard for phytoplankton. The prokaryotic cyanobacteria was more sensitive than the eukaryotic green alga to individual compounds. The toxicity of the binary mixture of BPA and BPF was predicted by CA and IA model and compared to the experimental data, which showed that for *P. subcapitata*, neither of the two models accurately predicted the actual effects of the mixture. In *P. subcapitata*, additive effect was observed over the whole effect concentration range, whereas in *S. leopoliensis*, the CA/IA model predictions were close to the experimentally determined toxicity. The environmental risk characterisation for phytoplankton based on comparison of reported concentrations of BPA and BPF in surface waters to the obtained PNEC values showed that at certain industrial areas, the concentrations of BPA exceeded PNEC values and thus BPA represents environmental risk. On the other hand, the data indicate that BPF does not represent risk for aquatic environment, but it should be noted that the exposure assessment to BPF is based on only few available data on its concentrations in the aquatic environment. Providing that BPF is increasingly used as a replacement for BPA, its concentrations in aquatic environment are very likely underestimated. Therefore, for a reliable environmental risk assessment, more information on the occurrence of BPF as well as other BP analogues in the aquatic environment as well as ecotoxicological data for individual analogues and their mixtures are needed.

Acknowledgements The authors thank to David Heath for corrections on language and grammar.

Funding This work was supported by the National Research Agency (grant numbers L1-7544 and N1-0047).

References

Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P (1988) Bisphenol A: acute aquatic toxicity. Environ Technol 70:19–26

Altenburger R, Bödeker W, Faust M, Grimme LH (1990) Evaluation of the isobologram method for the assessment of mixtures of chemicals: combination effect studies with pesticides in algal biotests. Ecotoxicol Environ Saf 20:98–114

Backhaus T, Sumpter J, Blank H (2004) On the ecotoxicology of pharmaceutical mixtures – chapter 16 from Kümmerer K., Pharmaceuticals in the environment. Springer, Berlin

Ben Uoada S, Ben Ali R, Leboulanger C, Ben Uoada H, Sayadi S (2018) Effect of Bisphenol A on the extremophilic microalgal strain *Picocystis* sp.(Chlorophyta) and its high BPA removal ability. Ecotoxicol Environ Saf 158:1–8

Bhandari KR, Deem LS, Holliday KD, Jandegian MC, Kassotis DC, Nagel CS, Tillitt ED, vom Saal SF, Rosenfeld SC (2014) Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviours in aquatic wildlife species. Gen Comp Endocrinol 214:195–219

Bliss CI (1939) The toxicity of poisons applied jointly. Ann Appl Biol 26: 585–615

Brezovšek P, Eleršek T, Filipič M (2014) Toxicities of four anti-neoplastic drugs and their binary mixtures tested on the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*. Water Res 52:168–177

Caballero-Casero N, Lunar L, Rubio S (2016) Analytical methods for the determination of mixture of bisphenols and derivatives in humans and environmental exposure sources and biological fluids. Anal Chim Acta 908:22–53

Chen D, Kannan K, Tan H, Zheng Z, Feng Y-L, Wu Y, Widelka M (2016) Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review. Environ Sci Technol 50:5438–5453

Chou TC (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 58:621–681

Chou TC, Martin N (2007) CompuSyn software for drug combinations and for general dose effect analysis, and user's guide. ComboSyn, Inc., Paramus, NJ [www.combosyn.com]. Accessed 2018

Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationship: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzym Regul 22:27–55

Cleuvers M (2003) Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett 142:185–194

Corrales J, Kristofco LA, Steele WB, et al. Global Assessment of Bisphenol A in the Environment: Review and Analysis of Its

Occurrence and Bioaccumulation. Dose-Response, An International Journal 13(3): 1559325815598308

Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, Guillette LJ Jr (2007) An ecological assessment of bisphenol-A: evidence from comparative biology. Reprod Toxicol 24:225–239

Danzl E, Sei K, Soda S, Ike M, Fujita M (2009) Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater. Int J Environ Res Public Health 6:1472–1484

Dodds EC, Lawson W (1938) Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene nucleus. Proc R Soc Lond B 125:222–232. https://doi.org/10.1098/rspb.1938.0023

Eio EJ, Kawai M, Niwa C, Ito M, Yamamoto S, Toda T (2015) Biodegradation of bisphenol A by an algal-bacterial system. Environ Sci Pollut Res 22:15145–15153

Eladak S, Grisin T, Moison D, Guerquin MJ, N'Tumba-Byn T, Pozzi-Gaudin S (2015) A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil Steril 103:11–21. https://doi.org/10.1016/j.fertnstert.2014.11.005

Elersek T (2012) The advantages of flow cytometry in comparison to fluorimetric measurement in algal toxicity test. Acta Biol Slov 55: 3–11

Elersek T, Milavec S, Korosec M, Brezovsek P, Negreira N, Zonja B, de Alda ML, Barcelo D, Heath E, Scancar J (2016) Toxicity of the mixture of selected antineoplastic drugs against aquatic primary producers. Environ Sci Pollut Res 23:14780–14790

EU (2016) /2235 - Commission Regulation of 12 December 2016 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bisphenol A

European Communities (2003) Technical guidance document on risk assessment (part II) in support of: commission directive 93/67/EEC on risk assessment for new notified substances, commission Regulation (EC) no 1488/94 on risk assessment for existing substances, directive 98/8/EC of the European Parliament and of the council concerning the placing of biocidal products on the market. Institute for Health and Consumer Protection, European Chemicals Bureau EUR 20418 EN/2

Fromme H, Küchler T, Otto T, Pilz K, Müller J, Wenzel A (2002) Occurrence of phthalates and bisphenol A and F in the environment. Water Res 36:1429–1438

Fu P, Kawamura K (2010) Ubiquity of bisphenol A in the atmosphere. Environ Pollut 158:3138–3143

Gallo P, Pisciottano ID, Esposito F, Fasano E, Scognamiglio G, Mita GD, Cirillo T (2017) Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection. Food Chem 220:406–412

Hadrup N, Taxvig C, Pedersen M, Nellemann C, Hass U, Vinnggaard AM (2013) Concentration addition, independent action and generalized concentration addition models for mixture effect prediction of sex hormone synthesis in vitro. PLoS One 8:e70490

Hagenbuch IM, Pinckney JL (2012) Toxic effect of the combined antibiotics ciprofloxacin, lincomycin, and tylosin on two species of marine diatoms. Water Res 46:5028–5036

Hirooka T, Akiyama Y, Tsuji N, Nakamura T, Nagase H, Hirata K, Miyamoto K (2003) Removal of hazardous phenols by microalgae under photoautotrophic conditions. J Biosci Bioeng 95:200–203

Hirooka T, Nagase H, Uchida K, Hiroshige Y, Ehara Y, Nishikawa J, Nishihara T, Miyamoto K, Hirata Z (2005) Biodegradation of bisphenol A and disappearance of its estrogenic activity by the green alga Chlorella fusca var. vacuolata. Environ Environ Toxicol Chem 24:1896–1901

Ike M, Chen MY, Danzl E, Sei K, Fujita M (2006) Biodegradation of a variety of bisphenols under aerobic and anaerobic conditions. Water Sci Technol 53:153–159

Ji MK, Kabra AN, Choi J, Hwang J, Kim JR, Abou-Shanab RAI, Oh Y, Jeon B (2014) Biodegradation of bisphenol A by the freshwater microalgae Chlamydomonas mexicana and Chlorella vulgaris. Ecol Eng 73:260–269

Le Fol V, Aït-Aïssa S, Sonavane M, Porcher JM, Balaguer P, Cravedi JP, Zalko D, Brion F (2017) In vitro and in vivo estrogenic activity of BPA, BPF and BPS in zebra fish-specific assays. Ecotoxicol Environ Saf 142:150–156

Li R, Chen GZ, Tam NF, Luan TG, Shi NPK, Cheung SG, Liu Y (2009) Toxicity of bisphenol A and its bioaccumulation and removal by a marine microalga Stephanodiscus hantzschii. Ecotoxicol Environ Saf 72:321–328

Liao C, Kannan K (2013) Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J Agric Food Chem 61: 4655–4662

Liao C, Liu F, Guo Y, Moon H, Nakata H, Wu Q, Kannan K (2012) Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. Environ Sci Technol 46:9138–9145

Liu Y, Guan Y, Gao Q, Tam NFY, Zhu W (2010) Cellular responses, biodegradation and bioaccumulation of endocrine disrupting chemicals in marine diatom Navicula incerta. Chemosphere 80: 592–599

Loewe S (1927) Die Mischarznei. Versuch einer allgemeinen pharmakologie der arzneikombinationen. Klin Wochenschr 6: 1077–1085

Loewe S, Muischnek H (1926) Über kombinationswirkungen. 1. mitteilung: Hilfsmittel der fragestellung. Nanyn-Schmiedebergs Arch Exp Pathol Pharmakol 114:313–326

Ma J (2005) Differential sensitivity of three cyanobacterial and five green algal species to organotins and pyrethroids pesticides. Sci Total Environ 341:109–117

Moreman J, Lee O, Trznadel M, David A, Kudoh T, Tyler CR (2017) Acute toxicity, teratogenic, and estrogenic effects of Bisphenol A and its alternative replacements Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish embryo-larvae. Environ Sci Technol 51:12796–12805

M'Rabet C, Pringault O, Zmerli-Triki H, Gharbia HB, Couet D, Yahia OK-D (2018) Impact of two plastic-derived chemicals, the Bisphenol A and the di-2-ethylhexyl phthalate, exposure on the marine toxic dinoflagellate *Alexandrium pacificum*. Mar Pollut Bull 126:241–249

OECD TG 201 (2011) OECD guidelines for the testing of chemicals, section 2: effects on biotic systems test no. 201: freshwater alga and cyanobacteria, Growth Inhibition Test OECD. OECD Publishing

Park JC, Lee M-C, Yoon D-S, Han J, Kim M, Hwang U-K, Jung J-H, Lee J-S (2018) Effects of bisphenol A and its analogs bisphenol F and S on life parameters, antioxidant system, and response of defensome in the marine rotifer *Brachionus koreanus*. Aquat Toxicol 199:21–29

Petrie B, Barden R, Kasprzyk-Hordern B (2015) A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res 72:3–27

Real M, Munoz I, Guasch H, Navarro E, Sabater S (2003) The effect of copper exposure on a simple aquatic food chain. Aquat.Toxicol 63: 283–291

Regulation EC No 1907 2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive

1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (Text with EEA relevance)

Ren L, Jia Y, Ruth N, Shi Y, Wang J, Qiao C, Yan Y (2016) Biotransformations of bisphenols mediated by a novel *Arthrobacter* sp. strain YC-RL1. Appl Microbiol Biotechnol 100: 1967–1976

Rochester JR, Bolden AL (2015) Bisphenol S and F: a systematic review and comparison of the hormonal Activity of Bisphenol A Substitutes. Environ Health Perspect 123(7):643-650

Staples CR, Dorn PB, Clecka GM, O'Block ST, Harris LR (1998) A review of the environmental fate, effect, and exposures of bisphenol A. Chemosphere 36:2149–2173

Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR, Branson DR (2000) Bisphenol A concentrations in receiving waters near US manufacturing and processing facilities. Chemosphere 40:521–525

Sumpter JP, Johnson AC, Williams RJ, Kortenkamp A, Scholze M (2006) Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. Environ Sci Technol 40: 5478–5489

Tisler T, Krel A, Gerželj U, Erjavec B, Dolenc MS, Pintar A (2016) Hazard identification and risk characterization of bisphenols A, F and AF to aquatic organisms. Environ Pollut 212:472–479

Usman A, Ahmad M (2016) From BPA to its analogues: Is it a safe journey?. Chemosphere 158:131–142

Wang B, Wu F, Li P, Deng N (2007) UV-light induced photodegradation of bisphenol A in water: kinetics and influencing factors. React Kinet Catal Lett 92:3–9

Wilkinson JL, Hooda PS, Swinden J, Barker J, Barton S (2017) Spatial distribution of organic contaminants in three rivers of Southern England bound to suspended particulate material and dissolved in water. Sci Total Environ 593-594:487–497

Wright-Walters M, Volz C, Talbott E, Davis D (2011) An updated weight of evidence approach to the aquatic hazard assessment of Bisphenol A and the derivation a new predicted no effect concentration (PNEC) using a non-parametric methodology. Sci Total Environ 409:676–685

Yamamoto T, Yasuhara A, Shiraisi H, Nakasugi O (2001) Bisphenol A in hazardous waste landfill leachates. Chemosphere 42:415–418

Yamazaki E, Yamashita N, Taniyasu S, Lam J, Lam PKS, MoonH-B, Jeong Y, Kannan P, Achyuthan H, Munuswamy N, Kannan K (2015) Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol Environ Saf 122:565–572

Zhang W, Xiong B, Sun WF, An S, Lin KF, Guo MJ, Cui XH (2012) Acute and chronic toxic effects of bisphenol A on *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Environ Toxicol 29:714–722

Chapter 4

Conclusions

This thesis contributes to our understanding of the occurrence and fate of bisphenol residues in the aqueous environment and during different water treatment technologies designed to limit their discharge into the environment. The novel contributions to the science are described within eight scientific papers published in SCI international journals and two related scientific publications. The results were also presented at international conferences, as oral (12) and poster (9) presentations (see Appendix).

An important part of this thesis was the development and validation of analytical methods for determining bisphenol residues, including sample preparation, extraction, and derivatization followed by GC-MS and GC-MS/MS. The outcome was the development of reliable and robust analytical methods with good linearity, sensitivity, low LOQ, high recovery and precision based on the Eurachem guidelines. These methods were used for successfully determining the stability of bisphenols in various aqueous matrices, including surface water, WW, drinking water, and food simulants. In addition, the removal efficiency of bisphenols during water treatment, and their toxicity towards algae and bacteria were determined. The analytical procedure, including sample preparation and extraction for identification and characterization of metabolites/TPs of bisphenols as well as their semi-quantification, was developed by using LC-QTOF-MS methods, with the ability to perform MS/MS fragmentation, having high resolution and mass accuracy. The optimized suspect and non-target screening approach enabled the identification of novel and confirmation of previously reported metabolites/TPs. These developed and optimized methods allowed the thesis hypotheses to be tested.

The following findings mean the first hypothesis H1: Residues of bisphenols are present in Slovenian WW, is accepted:

- In total, all of the target bisphenols were present in Slovenian WW influents and effluents.
- Food processing and textile cleaning company discharges are significant sources of bisphenols.
- Biological treatment is efficient at removing bisphenols ($\geq 96\%$, except for BPAP and BPB ($<43\%$)), but is not complete. Thus, the risk they pose to the environment should be estimated.

The second hypothesis is also accepted H2: The stability of bisphenols depends on storage temperature and time, concentration, matrix, and compound properties:

- The stability of bisphenols in various matrices are ranked as followed: MeOH > ultrapure water > WW.
- Bisphenols do not undergo hydrolysis in 48 h.
- The safe storage conditions and conditions during sample analysis were found to be for WW samples at −20 °C or 4 °C for up to four weeks, for dried food simulant

extracts at −20 °C for at least 8 weeks and derivatized samples at 21 °C for up to 7 days.
- The stability of bisphenols in WW samples depends on storage time.
- The stability of bisphenols in dried food simulant extracts depends on the storage time, while the main factor affecting the stability of derivatized samples is temperature.
- Concentration did not affect the stability of bisphenols.

The third hypothesis H3: Single treatment (biodegradation, photodegradation, or advance oxidation process) is not sufficient to completely mineralize bisphenols and TPs are formed during treatment, is accepted:

- Biological treatment, using either suspended or attached-growth biomass removed mostly > 85% of bisphenols.
- Adsorption to biomass was also recognized as an important removal mechanism, and the difference in bisphenol adsorption affinities reflects the differences in their K_{ow}.
- The degradation kinetics of BPF and BPS under biological treatment follows the first-order kinetics, and the rate depends on the tested compound and its initial concentration.
- The removal efficiencies between bisphenols in terms of UV photolysis differ but in nearly all cases, degradation followed pseudo-first-order kinetics.
- Photochemical processes, including direct photolysis using UV irradiation, cyclodextrin-enhanced photolysis and the photo-Fenton reaction, have shown to be an effective way of removing bisphenols from aqueous samples (> 90%) with the shortest half-lives in the case of photo-Fenton.
- Removal efficiencies for bisphenols observed in lab-scale HC set-ups and for CECs, including BPA, 44BPF and BPS were 15–63% and 15–90%, respectively. Temperature and K_{ow} affect HC removal efficiency. The results showed the potential of HC for large-scale application as a pre-treatment technology.
- When exposing BPS to human liver microsomes and cytosol fractions, two Phase I and four Phase II metabolites were formed through hydroxylation of the phenolic rings and conjugation with glucuronic acid or sulfate. Three metabolites were identified for the first time. The results provide insight into the fate and occurrence of possibly formed metabolites/TPs during water treatment and in the environment.
- The biological treatment with activated sludge yielded detectable bio-TPs of BPF and BPS. Of the 16 compounds identified, eleven BTPs were detected for the first time. New biotransformation pathways were postulated, namely sulfation in the case of BPF and additional methylation, cleavage of the S−C bond between the phenyl rings, and the joining of smaller moieties in the case of BPS. The obtained data confirmed that BTPs are formed during biological treatment.
- Nineteen TPs of BPF, BPS and BPZ during UV irradiation, UV/cyclodextrin and UV/Fenton reaction were identified, among which 11 are new to science. The formation of TPs depends on the applied process, from which the highest number of TPs was generated using the photo-Fenton process, confirming the formation of TPs during AOPs.

In addition, the toxicity tested models of BPA and BPF individually and as a binary mixture towards *S. leopoliensis* and *P. subcapitata* underestimated mixture toxicity of bisphenols. Bisphenol A showed to present a greater risk to the environment than BPF. Knowing that bisphenols are not entirely removed during WWT, they find their way into the aqueous environment. Therefore, their combined effects should be taken into account, and the risk they may pose to the environment should be estimated.

The last hypothesis H4: The migration of bisphenols from FCM depends on the material, food simulant, and migration conditions, is also accepted based on the following results:

- Bisphenols leach from cans and reusable plastic/steel sports bottles.
- Cans and steel bottles leached the highest concentrations of BPA and BPF, thus presenting a significant source of bisphenols.
- Migration does depend on the material, food simulant and the applied migration conditions.
- Importantly, even with the highest concentration of leaching bisphenols, both the t-TDI and SML for BPA were not exceeded.
- Our results suggest that consuming beverages from cans is more concerning than consuming beverages from reusable sports bottles and provide new insights into the migration of bisphenols from FCM, thereby lowering the risk to consumer health.

Chapter 5

General discussion and future perspective

The impact of BPA on both, human health and the environment is still not consensual. This thesis's overall goal was to gain more insight into the occurrence of bisphenols and their fate during WWT and in the environment. The uncertainties regarding human BPA exposure-associated health risks have led to the establishment of regulations, limiting its widespread applications in many countries and regions, e.g. American, Japan, the European Union and Canada [83]. Differences in opinion between EFSA and the FDA and national policymakers and scientists on the safe levels of BPA mean that BPA and bisphenols remain under constant review, which could lead to changes in regulation [2]. In the face of public concern and restrictions on its use, various BPA analogues are entering the market [29]. The problem is that there is insufficient data for assessing the presence, cycling and effects of residues of these compounds in the environment and identifying human exposure and health risks [60]. As I have stated in my thesis, this lack of data means there is no assurance that they are a safe replacement, especially since recent *in vitro* and *in vivo* studies show they have endocrine disrupting effects similar to BPA or even higher. It is also difficult to compare published studies, given the differences in experimental design, including sampling and applied analytical methods [3], [52], [57], [64], [198]. What is apparent is the need for analytical methods, capable of monitoring metabolites and TPs. This thesis and recent publications confirm the presence of bisphenols in the environmental matrices, wastewaters, food matrices and humans, suggesting their use in various applications and global contamination [16], [219].

This work's analytical achievements overcome one of the main issues in bisphenol research, i.e., the absence of validated and sensitive multi-residue analytical methods for determining bisphenols and their metabolites/TPs in various matrices and new GC-MS, GC-MS/MS, and LC-MS/MS-based methods have been developed and validated according to the Eurachem guidelines. Using these methods, we obtained improved LOQs [16], [57], [83] and identified TPs and metabolites. Despite this, there is still the absence of certified reference materials in suitable matrices, which are essential to establish comparability and accuracy of analytical results between different laboratories and over time. In the absence of certified reference materials, interlaboratory comparisons should be organized to check a laboratories' ability to deliver accurate testing results or determine whether a particular analytical method performs well and is fit for its purposes. I also developed new analytical protocols with assured stability of bisphenols in standard solutions and samples during storage and analysis, which has previously been neglected, but is an essential experimental design step.

My investigation of bisphenols in WWTP effluents confirms their presence in Slovenia and contributes to the corpus of knowledge on their occurrence in Europe. This thesis is the first European study addressing “point source” contamination of wastewater with bisphenols and their occurrence in WWTP influents and effluents. Importantly, this study can provide part of the necessary data supporting the decision whether or not to regulate bisphenols at the national or European level in wastewater and surface water, i.e., future versions of the Wastewater Directive and WFD. However, other environmental matrices, e.g., air, drinking and groundwater have received no or limited investigation [15], [220] and there is a need to establish their spatial (regionally and globally) and temporal distributions. The presence of bisphenols in the aqueous environment emphasizes the need for improved treatment technologies or combinations of existing treatments. Despite many investigations on BPA at both, real- and lab-scale, processes, limited information is available regarding removing BPA analogues in municipal WWTPs. The results also showed the potential of other advance processes, such as hydrodynamic cavitation for a large-scale application as a pre-treatment technology, apart from feasible, efficient and cost-effective biological treatment. However, this research area still needs to be further investigated. This thesis’s data fills the knowledge gap regarding efficiencies of different treatments for removing bisphenols from the aqueous environment and shows high removal efficiencies for biological, UV photolysis, and advanced oxidation processes, but not their complete mineralization. The methodology used in this thesis can be applied to other compounds of concern.

This thesis also addresses the ecotoxicological effects of BPA and BPF and their mixture towards green algae and cyanobacteria. The individual toxicity of bisphenols was lower than their mixture toxicity, and the comparison of predicted versus experimental data implied the potential for predictive models to underestimated mixture toxicity. This is important when considering the actual risk posed by bisphenol residues in the environment, including parent compounds’ cocktails. The presence of metabolites, TPs, their mixtures and their behaviour regarding environmental persistence and toxicity warrants further investigations.

Except for BPA, the metabolic pathways and pharmacokinetics are lacking for other bisphenols. Current studies report almost complete metabolization of BPA to its glucuronide conjugates with no endocrine disruptive activity [22], [94], [221]. Despite the structural similarity, it cannot be assumed that all bisphenols follow a similar detoxification mechanism [97]. The thesis did identify the formation of conjugated (glucuronide and sulphated) and oxidative metabolites of bisphenols using an *in vitro* assay with liver microsomes. The latter could still have endocrine activities in humans. The set of identified *in vitro* biotransformation products of BPS can potentially support a reliable assessment of BPS exposure and exposure to other bisphenols in future biomonitoring studies. In most pharmacokinetic studies, only conjugated metabolites, either directly or most commonly indirectly, were measured. The given information can significantly contribute knowledge about the metabolic pathways and metabolic biotransformations, which is essential for understanding and predicting their toxicity mechanisms. From this data, the TDI for BPA analogues can be established.

Human exposure to bisphenols occurs primarily through dietary sources such as ingested food, especially canned foods and beverages, which are the primary source of bisphenols [222], [223]. Food contamination with bisphenols may occur either through the unreacted monomer or through remobilized BPA from the final polymer used in FCMs [29], [224]. There is little information quantifying dietary sources of exposure to non-BPA analogues [16], [162], [225]. Especially migration studies following the EU guidelines had been lacking when addressing food simulants exposed to FCMs, e.g., plastic containers and cans. I applied standard EU migration tests to detect bisphenols leaching from cans and reusable sports bottles to address these gaps. The obtained results contribute to a critical understanding of dietary sources of exposure to bisphenols and help develop intervention strategies to reduce human and environmental exposure. Overall, the effects of bisphenols and their presence in different matrices together with warnings from EFSA and FDA [24] reveal uncertainty about non-dietary sources, strengthening the need to no longer consider them individually for both human and environmental exposure. Efforts should be made to develop a new TDI for bisphenols based on the co-exposure to different analogues and their toxic interactions from the mixture, including TPs, for an improved assessment of human exposure risks. To date, neither chemical analysis nor *in silico* methods (not adapted to mixtures) can identify the so-called "cocktail effect" in complex mixtures [226]. Although levels of bisphenols in the majority of recent studies, including this study, do not exceed the specific migration limits specified for BPA and only recently for BPS, changes in legislation are expected. Accordingly, our results could help better assess the provision of legislation and consequently reduce the human health risk.

In future work, understanding the occurrence and fate of bisphenols are critically needed. Regional and global environmental or biomonitoring should be extended to their mixture and especially their metabolites and TPs. In addition to these studies, alternative treatment technologies capable of mineralizing both, parent compounds and the metabolites/TPs, should be developed. The focus should be moved from the lab to pilot or ideally towards real scale investigations to obtain more reliable and realistic data. Importantly, further attempts to optimize analytical techniques and improve analysis, especially in non-target screening, for detecting and identifying bisphenols in combination with other CEC residues possibly entering the environment or humans are needed. Certified reference materials or at least interlaboratory comparisons are required to increase confidence in routine bisphenol analysis. In the case of TPs, this would require authentic compounds, either obtained commercially or in-house. Finally, future studies would be towards assessing the hazard posed by bisphenol residues and their possible interactions with other contaminants, including parent compounds, metabolites/TPs and their mixtures, to humans and the environment. *In vitro* bioassays together with analytical methods would be another promising strategy for assessing environmental and human health risks associated with bisphenols.

References

[1] R. M. Carvill Brian, Glasgow Katherine, “WO-2005005358-A1.pdf,” 2005.

[2] I. Rykowska, and W. Wasiak, “Properties , Threats , and Methods of Analysis of Bisphenol a and Its Derivatives,” *Acta Chromatogr.*, vol. 60–780, no. 16, p. 21, 2006.

[3] Y. Hu, Q. Zhu, X. Yan, C. Liao and G. Jiang, “Occurrence, fate and risk assessment of BPA and its substituents in wastewater treatment plant: A review,” *Environ. Res.*, vol. 178, no. July, 2019, doi: 10.1016/j.envres.2019.108732.

[4] Z. Theodor, “Mittheilungen aus dem chemischen Laboratorium der Universitat Marburg,” *Justus Leibigs Ann. Chemie*, vol. 343, p. pages 75-99, 1905.

[5] P. Allard, *Biomarkers in Toxicology, Chapter 27 - Bisphenol A*. 2014.

[6] X. Yu, J. Xue, H. Yao, Q. Wu, A. K. Venkatesan, R. U. Halden and K. Kannan, “Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the U . S . EPA targeted national sewage sludge survey,” *J. Hazard. Mater.*, vol. 299, pp. 733–739, 2015, doi: 10.1016/j.jhazmat.2015.07.012.

[7] J. Muncke, “Endocrine disrupting chemicals and other substances of concern in food contact materials: An updated review of exposure, effect and risk assessment,” *J. Steroid Biochem. Mol. Biol.*, vol. 127, no. 1–2, pp. 118–127, 2011, doi: 10.1016/j.jsbmb.2010.10.004.

[8] J. Michałowicz, “Bisphenol A - Sources, toxicity and biotransformation,” *Environ. Toxicol. Pharmacol.*, vol. 37, no. 2, pp. 738–758, 2014, doi: 10.1016/j.etap.2014.02.003.

[9] Dodds, “13, 1936,” *Nature*, vol. 137, p. 996, 1936.

[10] S. A. Vogel, “The politics of plastics: the making and unmaking of bisphenol a ‘safety’.,” *Am. J. Public Health*, vol. 99 Suppl 3, pp. 559–566, 2009, doi: 10.2105/ajph.2008.159228.

[11] A. Szymański, I. Rykowska and W. Wasiak, “New ketoimine sorbents in solid phase extraction for HPLC analysis of bisphenol A and other ‘endocrine disrupting’ residues in drinking water,” *Polish J. Environ. Stud.*, vol. 15, no. 3, pp. 479–483, 2006.

[12] G. Howard, “From BPA to BPZ: a toxic soup?,” *CHEM Trust*, no. March, p. 42, 2018, [Online]. Available: http://www.chemtrust.org.uk/efsa-corrects-its-risk-assessment-of-bi...

[13] I. Rykowska, and W. Wasiak, “Properties, threats, and methods of analysis of bisphenol A and its derivatives,” *Acta Chromatogr.*, no. 16, pp. 7–27, 2006.

[14] K. Pelch, J. A. Wignall, A. E. Goldstone, P. K. Ross, R. B. Blain, A. J. Shapiro, S. D. Holmgren, J. H. Hsieh, D. Svoboda, S. S. Auerbach, F. M. Parham, S. A. Masten,

V. Walker, A. Rooney and K. A. Thayer, “A scoping review of the health and toxicological activity of bisphenol A (BPA) structural analogues and functional alternatives,” *Toxicology*, vol. 424, no. February, p. 152235, 2019, doi: 10.1016/j.tox.2019.06.006.

[15] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y. L. Feng, Y. Wu and M. Widelka, “Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity - A Review,” *Environ. Sci. Technol.*, vol. 50, no. 11, pp. 5438–5453, 2016, doi: 10.1021/acs.est.5b05387.

[16] N. Caballero-Casero, L. Lunar and S. Rubio, “Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review,” *Anal. Chim. Acta*, vol. 908, pp. 22–53, 2016, doi: 10.1016/j.aca.2015.12.034.

[17] J. S. Siracusa, L. Yin, E. Measel, S. Liang and X. Yu, “Effects of bisphenol A and its analogs on reproductive health: A mini review,” *Reprod. Toxicol.*, vol. 79, no. June, pp. 96–123, 2018, doi: 10.1016/j.reprotox.2018.06.005.

[18] J. Liu, L. Zhang, G. Lu, R. Jiang, Z. Yan and Y. Li, “Ecotoxicology and Environmental Safety Occurrence , toxicity and ecological risk of Bisphenol A analogues in aquatic environment – A review,” *Ecotoxicol. Environ. Saf.*, vol. 208, p. 111481, 2021, doi: 10.1016/j.ecoenv.2020.111481.

[19] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.-L. Feng, Y. Wu and M. Widelka, “Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity—A Review,” *Environ. Sci. Technol.*, vol. 50, no. 11, pp. 5438–5453, Jun. 2016, doi: 10.1021/acs.est.5b05387.

[20] H. and F. safety Scientific commitee, *Opinion on The safety of the use of bisphenol A in medical devices*, no. February. 2015.

[21] G. Cao, J. Lu and G. Wang, “Photolysis kinetics and influencing factors of bisphenol S in aqueous solutions,” *J. Environ. Sci.*, vol. 24, no. 5, pp. 846–851, 2012, doi: 10.1016/S1001-0742(11)60809-7.

[22] D. Gramec Skledar, and L. Peterlin Mašič, “Bisphenol A and its analogs: Do their metabolites have endocrine activity?,” *Environ. Toxicol. Pharmacol.*, vol. 47, pp. 182–199, 2016, doi: 10.1016/j.etap.2016.09.014.

[23] Y. Yang, L. Lu, J. Zhang, Y. Yang, Y. Wu and B. Shao, “Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry,” *J. Chromatogr. A*, vol. 1328, pp. 26–34, 2014, doi: 10.1016/j.chroma.2013.12.074.

[24] H. Yang, Y. Huang, J. Liu, P. Tang, Q. Sun, X. Xiong, B. Tang, J. He and H. Li, “Binding modes of environmental endocrine disruptors to human serum albumin: Insights from STD-NMR, ITC, spectroscopic and molecular docking studies,” *Sci. Rep.*, vol. 7, no. 1, pp. 1–11, 2017, doi: 10.1038/s41598-017-11604-3.

[25] Y. Feng, Z. Jiao, J. Shi, M. Li, Q. Guo and B. Shao, “Effects of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells,” *Chemosphere*, vol. 147, pp. 9–19, Mar. 2016, doi: 10.1016/j.chemosphere.2015.12.081.

[26] T. Tišler, A. Krel, U. Gerželj, B. Erjavec, M. S. Dolenc and A. Pintar, “Hazard

identification and risk characterization of bisphenols A, F and AF to aquatic organisms," *Environ. Pollut.*, vol. 212, pp. 472–479, 2016, doi: 10.1016/j.envpol.2016.02.045.

[27] M. Ashfaq, Q. Sun, H. Zhang, Y. Li, Y. Wang, M. Li, M. Lv, X. Liao and C.-P. Yu, "Occurrence and fate of bisphenol A transformation products, bisphenol A monomethyl ether and bisphenol A dimethyl ether, in wastewater treatment plants and surface water," *J. Hazard. Mater.*, vol. 357, pp. 401–407, Sep. 2018, doi: 10.1016/J.JHAZMAT.2018.06.022.

[28] B. Petrie, L. Lopardo, K. Proctor, J. Youdan, R. Barden and B. Kasprzyk-Hordern, "Assessment of bisphenol-A in the urban water cycle," *Sci. Total Environ.*, vol. 650, pp. 900–907, Feb. 2019, doi: 10.1016/J.SCITOTENV.2018.09.011.

[29] G. Russo, F. Barbato, D. G. Mita and L. Grumetto, "Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe," *Food Chem. Toxicol.*, vol. 131, no. January, p. 110575, 2019, doi: 10.1016/j.fct.2019.110575.

[30] J. I. Cacho, N. Campillo, P. Viñas and M. Hernández-Córdoba, "Stir bar sorptive extraction coupled to gas chromatography-mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables," *J. Chromatogr. A*, vol. 1247, pp. 146–153, 2012, doi: 10.1016/j.chroma.2012.05.064.

[31] L.-H. Wu, X.-M. Zhang, F. Wang, C.-J. Gao, D. Chen, J. R. Palumbo, Y. Guo and E. Y. Zeng, "Occurrence of bisphenol S in the environment and implications for human exposure: A short review," *Sci. Total Environ.*, vol. 615, pp. 87–98, Feb. 2018, doi: 10.1016/J.SCITOTENV.2017.09.194.

[32] U.S. Environmental Protection Agency, "Bisphenol A Action Plan (CASRN 80-05-7) [CA Index Name: Phenol, 4,4'-(1-methylethylidene)bis-]," *Environ. Heal.*, p. 22, 2010, [Online]. Available: http://www.hhs.gov/safety/bpa/.%0Ahttps://www.epa.gov/sites/production/files/2015-09/documents/bpa_action_plan.pdf.

[33] USDA, "Draft Assessment of Bisphenol a for Use in," pp. 1–105, 2008, [Online]. Available: www.FDA.gov.

[34] Environment Canada, "Consultation Document Phenol, 4,4`-(1-Methylethylidene)bis- (Bisphenol A)," no. 80-05–07, p. 18, 2009.

[35] EU, "Commission Regulation (EU) No 10/2011," *Off. J. Eur. Union*, vol. 12, pp. 1–89, 2011.

[36] M. K. Björnsdotter, J. de Boer and A. Ballesteros-Gómez, "Bisphenol A and replacements in thermal paper: A review," *Chemosphere*, vol. 182, no. May, pp. 691–706, 2017, doi: 10.1016/j.chemosphere.2017.05.070.

[37] 2015 JORF, "France amended the law on the ban of BPA in food contact materials| TÜV SÜD," *Official Journal of the French Republic*, 2015. https://www.tuvsud.com/en/e-ssentials-newsletter/past-topics/france-amended-the-law-on-the-ban-of-bpa-in-food-contact-materials (accessed Jun. 08, 2020).

[38] EFSA, "Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs," *EFSA J.*, vol. 13, no. 1, p. 3978, Jan. 2015, doi: 10.2903/j.efsa.2015.3978.

[39] "Scientific Opinion on the risks to public health related to the presence of bisphenol

A (BPA) in foodstuffs," *EFSA J.*, vol. 13, no. 1, p. 3978, Jan. 2015, doi: 10.2903/j.efsa.2015.3978.

[40] EU, "Commission Regulation (EU) 2018/832 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materi," *Off. J. Eur. Union*, vol. 2001, no. May, pp. 20–30, 2018, doi: http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.

[41] European Commission, "Proposal for a Directive on the quality of water intended for human consumption," *Http://Ec.Europa.Eu/Environment/Water/Water-Drink/Review_En.Html*, vol. 0332, no. 2017, p. 02/03/2018, 2018, [Online]. Available: http://ec.europa.eu/environment/water/water-drink/review_en.html.

[42] M. Noszczyńska, and Z. Piotrowska-Seget, "Bisphenols: Application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers," *Chemosphere*, vol. 201, pp. 214–223, 2018, doi: 10.1016/j.chemosphere.2018.02.179.

[43] 2016 RS, "UREDBA o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo," 2014.

[44] C. Decision, "DECISION No 2455/2001/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 November 2001," *EU Water Fram. Dir.*, vol. 2001, no. May, pp. 20–30, 2000, doi: http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.

[45] O. F. T. H. E. Council, "Water environmental quality standards," *EU Water Fram. Dir.*, vol. 16, no. 1, pp. 11–14, 2008, [Online]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0105&from=EN.

[46] M. Kos Durjava, B. Kolar, F. Balk and W. Peijnenburg, "Water Framework Directive and Specific Pollutants in Surface Waters in Slovenia," *Acta hydrotechnica*, vol. 45, no. 2013, pp. 61–69, 2015.

[47] S. S. Andra, C. Austin, J. Yang, D. Patel and M. Arora, "Recent advances in simultaneous analysis of bisphenol A and its conjugates in human matrices: Exposure biomarker perspectives," *Sci. Total Environ.*, vol. 572, pp. 770–781, 2016, doi: 10.1016/j.scitotenv.2016.07.062.

[48] H. Zhang, Q. Quan, M. Zhang, N. Zhang, W. Zhang, M. Zhan, W. Xu, L. Lu, J. Fan and Q. Wang, "Occurrence of bisphenol A and its alternatives in paired urine and indoor dust from Chinese university students: Implications for human exposure," *Chemosphere*, vol. 247, p. 125987, 2020, doi: 10.1016/j.chemosphere.2020.125987.

[49] M. Petrović, S. Gonzalez and D. Barceló, "Analysis and removal of emerging contaminants in wastewater and drinking water," *TrAC - Trends Anal. Chem.*, vol. 22, no. 10, pp. 685–696, 2003, doi: 10.1016/S0165-9936(03)01105-1.

[50] A. Ballesteros-Gómez, F. J. Ruiz, S. Rubio and D. Pérez-Bendito, "Determination of bisphenols A and F and their diglycidyl ethers in wastewater and river water by coacervative extraction and liquid chromatography-fluorimetry," *Anal. Chim. Acta*, vol. 603, no. 1, pp. 51–59, 2007, doi: 10.1016/j.aca.2007.09.048.

[51] US EPA, “Bisphenol A Alternatives in Thermal Paper Hazard Evaluation of Bisphenol A (BPA) and Alternatives FINAL REPORT-Chapter 4,” no. August, 2015, [Online]. Available: https://www.epa.gov/sites/production/files/2015-09/documents/bpa_ch4.pdf.

[52] H. Jin, and L. Zhu, “Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China,” *Water Res.*, vol. 103, pp. 343–351, 2016, doi: 10.1016/j.watres.2016.07.059.

[53] R. Garg, and C. J. Smith, “Predicting the bioconcentration factor of highly hydrophobic organic chemicals,” *Food Chem. Toxicol.*, vol. 69, pp. 252–259, 2014, doi: 10.1016/j.fct.2014.03.035.

[54] J. Regueiro, and T. Wenzl, “Development and validation of a stable-isotope dilution liquid chromatography-tandem mass spectrometry method for the determination of bisphenols in ready-made meals,” *J. Chromatogr. A*, vol. 1414, no. 10, pp. 110–121, 2015, doi: 10.1016/j.chroma.2015.08.037.

[55] H. Wang, Z. hua Liu, Z. Tang, J. Zhang, H. Yin, Z. Dang, P. xiao Wu and Y. Liu, “Bisphenol analogues in Chinese bottled water: Quantification and potential risk analysis,” *Sci. Total Environ.*, vol. 713, p. 136583, 2020, doi: 10.1016/j.scitotenv.2020.136583.

[56] J. Q. Jiang, Z. Zhou and V. K. Sharma, “Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water - A review from global views,” *Microchem. J.*, vol. 110, pp. 292–300, 2013, doi: 10.1016/j.microc.2013.04.014.

[57] Q. Sun, Y. Wang, Y. Li, M. Ashfaq, L. Dai, X. Xie and C. P. Yu, “Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China,” *Environ. Pollut.*, vol. 225, pp. 542–549, 2017, doi: 10.1016/j.envpol.2017.03.018.

[58] H. Zhang, Y. Zhang, J. Li and M. Yang, “Occurrence and exposure assessment of bisphenol analogues in source water and drinking water in China,” 2018, doi: 10.1016/j.scitotenv.2018.11.053.

[59] L. H. Wu, X. M. Zhang, F. Wang, C. J. Gao, D. Chen, J. R. Palumbo, Y. Guo and E. Y. Zeng, “Occurrence of bisphenol S in the environment and implications for human exposure: A short review,” *Sci. Total Environ.*, vol. 615, pp. 87–98, 2018, doi: 10.1016/j.scitotenv.2017.09.194.

[60] J. R. Rochester, and A. L. Bolden, “Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol a substitutes,” *Environmental Health Perspectives*, vol. 123, no. 7. Public Health Services, US Dept of Health and Human Services, pp. 643–650, Jul. 06, 2015, doi: 10.1289/ehp.1408989.

[61] M. Liu, S. Jia, T. Dong, Y. Han, J. Xue, E. R. Wanjaya and M. Fang, “The occurrence of bisphenol plasticizers in paired dust and urine samples and its association with oxidative stress,” *Chemosphere*, vol. 216, pp. 472–478, 2019, doi: 10.1016/j.chemosphere.2018.10.090.

[62] W. Qiu, H. Zhan, J. Hu, T. Zhang, H. Xu, M. Wong, B. Xu and C. Zheng, “The occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a substitute of bisphenol A: A critical review of recent progress,” *Ecotoxicol. Environ. Saf.*, vol.

173, no. February, pp. 192–202, 2019, doi: 10.1016/j.ecoenv.2019.01.114.

[63] Q. Wang, M. Chen, G. Shan, P. Chen, S. Cui, S. Yi and L. Zhu, “Bioaccumulation and biomagnification of emerging bisphenol analogues in aquatic organisms from Taihu Lake, China,” *Sci. Total Environ.*, vol. 598, pp. 814–820, 2017, doi: 10.1016/j.scitotenv.2017.04.167.

[64] J. Xue, and K. Kannan, “Mass flows and removal of eight bisphenol analogs, bisphenol A diglycidyl ether and its derivatives in two wastewater treatment plants in New York State, USA,” *Sci. Total Environ.*, vol. 648, pp. 442–449, 2019, doi: 10.1016/j.scitotenv.2018.08.047.

[65] Č. Marjeta, K. Lenar, V. Mislej, M. Levstek, A. Kova, B. Cimrman, T. Kosjek, D. Heath, M. Sollner and E. Heath, “Science of the Total Environment The occurrence and source identi fi cation of bisphenol compounds in wastewaters,” vol. 617, pp. 744–752, 2018, doi: 10.1016/j.scitotenv.2017.10.252.

[66] C. Liao, F. Liu, Y. Guo, H. B. Moon, H. Nakata, Q. Wu and K. Kannan, “Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure.,” *Environ. Sci. Technol.*, vol. 46, no. 16, pp. 9138–9145, 2012, doi: 10.1021/es302004w.

[67] C. Liao, F. Liu, H. B. Moon, N. Yamashita, S. Yun and K. Kannan, “Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: Spatial and temporal distributions,” *Environ. Sci. Technol.*, vol. 46, no. 21, pp. 11558–11565, Nov. 2012, doi: 10.1021/es303191g.

[68] D. Lalwani, Y. Ruan, S. Taniyasu, E. Yamazaki, N. J. I. Kumar, P. K. S. Lam, X. Wang and N. Yamashita, “Nationwide distribution and potential risk of bisphenol analogues in Indian waters,” *Ecotoxicol. Environ. Saf.*, vol. 200, no. May, p. 110718, 2020, doi: 10.1016/j.ecoenv.2020.110718.

[69] Z. Yan, Y. Liu, K. Yan, S. Wu, Z. Han, R. Guo, M. Chen, Q. Yang, S. Zhang and J. Chen, “Bisphenol analogues in surface water and sediment from the shallow Chinese freshwater lakes: Occurrence, distribution, source apportionment, and ecological and human health risk,” *Chemosphere*, vol. 184, pp. 318–328, 2017, doi: 10.1016/j.chemosphere.2017.06.010.

[70] E. Yamazaki, N. Yamashita, S. Taniyasu, J. Lam, P. K. S. Lam, H. B. Moon, Y. Jeong, P. Kannan, H. Achyuthan, N. Munuswamy and K. Kannan, “Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India,” *Ecotoxicol. Environ. Saf.*, vol. 122, pp. 565–572, 2015, doi: 10.1016/j.ecoenv.2015.09.029.

[71] J. L. Zheng, D. X. Guan, J. Luo, H. Zhang, W. Davison, X. Y. Cui, L. H. Wang and L. Q. Ma, “Activated charcoal based diffusive gradients in thin films for in situ monitoring of bisphenols in waters,” *Anal. Chem.*, vol. 87, no. 1, pp. 801–807, 2015, doi: 10.1021/ac503814j.

[72] Y. Liu, S. Zhang, N. Song, R. Guo, M. Chen, D. Mai, Z. Yan, Z. Han and J. Chen, “Occurrence, distribution and sources of bisphenol analogues in a shallow Chinese freshwater lake (Taihu Lake): Implications for ecological and human health risk,” *Sci. Total Environ.*, vol. 599–600, pp. 1090–1098, 2017, doi: 10.1016/j.scitotenv.2017.05.069.

[73] C. Huang, L. H. Wu, G. Q. Liu, L. Shi and Y. Guo, "Occurrence and Ecological Risk Assessment of Eight Endocrine-Disrupting Chemicals in Urban River Water and Sediments of South China," *Arch. Environ. Contam. Toxicol.*, vol. 75, no. 2, pp. 224–235, 2018, doi: 10.1007/s00244-018-0527-9.

[74] M. Česen, D. Heath, M. Krivec, J. Ko, T. Kosjek and E. Heath, "Seasonal and spatial variations in the occurrence , mass loadings and removal of compounds of emerging concern in the Slovene aqueous environment and environmental risk assessment *," vol. 242, 2018, doi: 10.1016/j.envpol.2018.06.052.

[75] H. Jin, J. Zhu, Z. Chen, Y. Hong and Z. Cai, "Occurrence and Partitioning of Bisphenol Analogues in Adults' Blood from China," *Environ. Sci. Technol.*, vol. 52, no. 2, pp. 812–820, Jan. 2018, doi: 10.1021/acs.est.7b03958.

[76] W. Si, Y. Cai, J. Liu, J. Shen, Q. Chen, C. Chen and L. Ning, "Investigating the role of colloids on the distribution of bisphenol analogues in surface water from an ecological demonstration area, China," *Sci. Total Environ.*, vol. 673, pp. 699–707, 2019, doi: 10.1016/j.scitotenv.2019.04.142.

[77] M. Česen, M. Ahel, S. Terzić, D. J. Heath and E. Heath, "The occurrence of contaminants of emerging concern in Slovenian and Croatian wastewaters and receiving Sava river," *Sci. Total Environ.*, vol. 650, pp. 2446–2453, 2019, doi: 10.1016/j.scitotenv.2018.09.238.

[78] Z. Huang, J. L. Zhao, Y. Y. Yang, Y. W. Jia, Q. Q. Zhang, C. E. Chen, Y. S. Liu, B. Yang, L. Xie and G. G. Ying, "Occurrence, mass loads and risks of bisphenol analogues in the Pearl River Delta region, South China: Urban rainfall runoff as a potential source for receiving rivers," *Environ. Pollut.*, vol. 263, p. 114361, 2020, doi: 10.1016/j.envpol.2020.114361.

[79] H. Zhang, Y. Zhang, J. Li and M. Yang, "Occurrence and exposure assessment of bisphenol analogues in source water and drinking water in China," *Sci. Total Environ.*, vol. 655, pp. 607–613, Mar. 2019, doi: 10.1016/j.scitotenv.2018.11.053.

[80] S. Lee, C. Liao, G. J. Song, K. Ra, K. Kannan and H. B. Moon, "Emission of bisphenol analogues including bisphenol A and bisphenol F from wastewater treatment plants in Korea," *Chemosphere*, vol. 119, no. October, pp. 1000–1006, 2015, doi: 10.1016/j.chemosphere.2014.09.011.

[81] R. Karthikraj, and K. Kannan, "Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants," *Chemosphere*, vol. 181, pp. 216–223, 2017, doi: 10.1016/j.chemosphere.2017.04.075.

[82] M. Noszczyńska, and Z. Piotrowska-Seget, "Bisphenols: Application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers," *Chemosphere*, vol. 201. Elsevier Ltd, pp. 214–223, Jun. 01, 2018, doi: 10.1016/j.chemosphere.2018.02.179.

[83] H. Wang, Z. hua Liu, J. Zhang, R. ping Huang, H. Yin, Z. Dang, P. xiao Wu and Y. Liu, "Insights into removal mechanisms of bisphenol A and its analogues in municipal wastewater treatment plants," *Science of the Total Environment*, vol. 692. Elsevier B.V., pp. 107–116, Nov. 20, 2019, doi: 10.1016/j.scitotenv.2019.07.134.

[84] N. Cabaton, C. Dumont, I. Severin, E. Perdu, D. Zalko, M. Cherkaoui-Malki and M. C. Chagnon, "Genotoxic and endocrine activities of bis(hydroxyphenyl)methane

(bisphenol F) and its derivatives in the HepG2 cell line," *Toxicology*, vol. 255, no. 1–2, pp. 15–24, 2009, doi: 10.1016/j.tox.2008.09.024.

[85] J. Moreman, O. Lee, M. Trznadel, A. David, T. Kudoh and C. R. Tyler, "Acute Toxicity, Teratogenic, and Estrogenic Effects of Bisphenol A and Its Alternative Replacements Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish Embryo-Larvae," *Environ. Sci. Technol.*, vol. 51, no. 21, pp. 12796–12805, Nov. 2017, doi: 10.1021/acs.est.7b03283.

[86] C. Park, H. Song, J. Choi, S. Sim, H. Kojima, J. Park, M. Iida and Y. J. Lee, "The mixture effects of bisphenol derivatives on estrogen receptor and androgen receptor," *Environ. Pollut.*, vol. 260, p. 114036, 2020, doi: 10.1016/j.envpol.2020.114036.

[87] L. Y. Cao, X. M. Ren, C. H. Li, J. Zhang, W. P. Qin, Y. Yang, B. Wan and L. H. Guo, "Bisphenol AF and Bisphenol B Exert Higher Estrogenic Effects than Bisphenol A via G Protein-Coupled Estrogen Receptor Pathway," *Environ. Sci. Technol.*, vol. 51, no. 19, pp. 11423–11430, 2017, doi: 10.1021/acs.est.7b03336.

[88] A. K. Rosenmai, M. Dybdahl, M. Pedersen, B. M. A. van Vugt-Lussenburg, E. B. Wedebye, C. Taxvig and A. M. Vinggaard, "Are structural analogues to bisphenol a safe alternatives?," *Toxicol. Sci.*, vol. 139, no. 1, pp. 35–47, 2014, doi: 10.1093/toxsci/kfu030.

[89] F. Stossi, M. J. Bolt, F. J. Ashcroft, J. E. Lamerdin, J. S. Melnick, R. T. Powell, R. D. Dandekar, M. G. Mancini, C. L. Walker, J. K. Westwick and M. A. Mancini, "Defining estrogenic mechanisms of bisphenol A analogs through high throughput microscopy-based contextual assays," *Chem. Biol.*, vol. 21, no. 6, pp. 743–753, 2014, doi: 10.1016/j.chembiol.2014.03.013.

[90] A. Fic, B. Žegura, M. Sollner Dolenc, M. Filipič and L. Peterlin Mašič, "Mutagenicity and DNA damage of bisphenol a and its structural analogues in HepG2 cells," *Arh. Hig. Rada Toksikol.*, vol. 64, no. 2, pp. 189–200, 2013, doi: 10.2478/10004-1254-64-2013-2319.

[91] A. Fic, S. Jurković Mlakar, P. Juvan, V. Mlakar, J. Marc, M. Sollner Dolenc, K. Broberg and L. Peterlin Mašič, "Genome-wide gene expression profiling of low-dose, long-term exposure of human osteosarcoma cells to bisphenol A and its analogs bisphenols AF and S," *Toxicol. Vitr.*, vol. 29, no. 5, pp. 1060–1069, 2015, doi: 10.1016/j.tiv.2015.03.014.

[92] C. S. Rosenfeld, "Neuroendocrine Disruption in Animal Models Due to Exposure to Bisphenol A Analogues," *Physiol. Behav.*, vol. 176, no. 3, pp. 139–148, 2019, doi: 10.1016/j.yfrne.2017.08.001.Neuroendocrine.

[93] V. Mustieles, S. C. D'Cruz, S. Couderq, A. Rodríguez-Carrillo, J. B. Fini, T. Hofer, I. L. Steffensen, H. Dirven, R. Barouki, N. Olea, M. F. Fernández and A. David, "Bisphenol A and its analogues: A comprehensive review to identify and prioritize effect biomarkers for human biomonitoring," *Environ. Int.*, vol. 144, no. April, p. 105811, 2020, doi: 10.1016/j.envint.2020.105811.

[94] K. A. Thayer, D. R. Doerge, D. Hunt, S. H. Schurman, N. C. Twaddle, M. I. Churchwell, S. Garantziotis, G. E. Kissling, M. R. Easterling, J. R. Bucher and L. S. Birnbaum, "Pharmacokinetics of bisphenol A in humans following a single oral administration," *Environ. Int.*, vol. 83, pp. 107–115, 2015, doi:

10.1016/j.envint.2015.06.008.

[95] C. Karrer, T. Roiss, N. Von Goetz, D. G. Skledar and L. Peterlin, "Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA , BPS , BPF , and BPAF with New Experimental Metabolic Parameters : Comparing the Pharmacokinetic Behavior of BPA with Its Substitutes," vol. 126, no. July, pp. 1–17, 2018.

[96] O. Ousji, and L. Sleno, " In Vitro Metabolism of Five Bisphenol A Analogs Studied by LC-HRMS/MS ," *J. Am. Soc. Mass Spectrom.*, 2020, doi: 10.1021/jasms.0c00301.

[97] B. Peng, M. Liu, Y. Han, E. R. Wanjaya and M. Fang, "Competitive Biotransformation among Phenolic Xenobiotic Mixtures: Underestimated Risks for Toxicity Assessment," *Environ. Sci. Technol.*, vol. 53, no. 20, pp. 12081–12090, 2019, doi: 10.1021/acs.est.9b04968.

[98] L. Guo, Z. Li, P. Gao, H. Hu and M. Gibson, "Ecological risk assessment of bisphenol A in surface waters of China based on both traditional and reproductive endpoints," *Chemosphere*, vol. 139, pp. 133–137, 2015, doi: 10.1016/j.chemosphere.2015.06.001.

[99] M. D. Hernando, M. Mezcua, A. R. Fernández-Alba and D. Barceló, "Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments," *Talanta*, vol. 69, no. 2 SPEC. ISS., pp. 334–342, 2006, doi: 10.1016/j.talanta.2005.09.037.

[100] W. S. T. Aldenberg, "Confidence Limits for Hazardous Concentrations Based on Logistically Distributed NOEC Toxicity Data," *Ecotoxicol. Environ. Saf. Publ. Elsevier*, vol. 25, pp. 48–63, 1993.

[101] M. V. Bagal, and P. R. Gogate, "Wastewater treatment using hybrid treatment schemes based on cavitation and Fenton chemistry: A review," *Ultrasonics Sonochemistry*, vol. 21, no. 1. Elsevier B.V., pp. 1–14, 2014, doi: 10.1016/j.ultsonch.2013.07.009.

[102] A. Ullah, S. Hussain, A. Wasim and M. Jahanzaib, "Development of a decision support system for the selection of wastewater treatment technologies," *Sci. Total Environ.*, vol. 731, p. 139158, 2020, doi: 10.1016/j.scitotenv.2020.139158.

[103] E. Danzl, K. Sei, S. Soda, M. Ike and M. Fujita, "Biodegradation of Bisphenol A, Bisphenol F and Bisphenol S in Seawater," *Int. J. Environ. Res. Public Health*, vol. 6, no. 4, pp. 1472–1484, Apr. 2009, doi: 10.3390/ijerph6041472.

[104] J. Zdarta, K. Antecka, R. Frankowski, A. Zgoła-Grześkowiak, H. Ehrlich and T. Jesionowski, "The effect of operational parameters on the biodegradation of bisphenols by Trametes versicolor laccase immobilized on Hippospongia communis spongin scaffolds," *Sci. Total Environ.*, vol. 615, pp. 784–795, Feb. 2018, doi: 10.1016/j.scitotenv.2017.09.213.

[105] D. Kotoula, A. Iliopoulou, E. Irakleous-Palaiologou, G. Gatidou, M. Aloupi, P. Antonopoulou, M. S. Fountoulakis and A. S. Stasinakis, "Municipal wastewater treatment by combining in series microalgae Chlorella sorokiniana and macrophyte Lemna minor: Preliminary results," *J. Clean. Prod.*, vol. 271, p. 122704, 2020, doi: 10.1016/j.jclepro.2020.122704.

[106] P. Falås, P. Longrée, J. La Cour Jansen, H. Siegrist, J. Hollender and A. Joss,

"Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process," *Water Res.*, vol. 47, no. 13, pp. 4498–4506, 2013, doi: 10.1016/j.watres.2013.05.010.

[107] B. Erjavec, P. Hudoklin, K. Perc, T. Tišler, M. S. Dolenc and A. Pintar, "Glass fiber-supported TiO2 photocatalyst: Efficient mineralization and removal of toxicity/estrogenicity of bisphenol A and its analogs," *Appl. Catal. B Environ.*, vol. 183, pp. 149–158, 2016, doi: 10.1016/j.apcatb.2015.10.033.

[108] A. Gogoi, P. Mazumder, V. K. Tyagi, G. G. Tushara Chaminda, A. K. An and M. Kumar, "Occurrence and fate of emerging contaminants in water environment: A review," *Groundw. Sustain. Dev.*, vol. 6, no. September 2017, pp. 169–180, 2018, doi: 10.1016/j.gsd.2017.12.009.

[109] P. Guerra, M. Kim, S. Teslic, M. Alaee and S. A. Smyth, "Bisphenol-A removal in various wastewater treatment processes: Operational conditions, mass balance, and optimization," *J. Environ. Manage.*, vol. 152, pp. 192–200, 2015, doi: 10.1016/j.jenvman.2015.01.044.

[110] S. Jamal Khan, S. Ilyas, S. Javid, C. Visvanathan and V. Jegatheesan, "Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater," *Bioresour. Technol.*, vol. 102, no. 9, pp. 5331–5336, 2011, doi: 10.1016/j.biortech.2010.09.100.

[111] H. Wang, Z. hua Liu, J. Zhang, R. ping Huang, H. Yin, Z. Dang, P. xiao Wu and Y. Liu, "Insights into removal mechanisms of bisphenol A and its analogues in municipal wastewater treatment plants," *Sci. Total Environ.*, vol. 692, pp. 107–116, 2019, doi: 10.1016/j.scitotenv.2019.07.134.

[112] W.-C. Huang, X. Jia, J. Li and M. Li, "Dynamics of microbial community in the bioreactor for bisphenol S removal," *Sci. Total Environ.*, vol. 662, pp. 15–21, 2019, doi: 10.1016/j.scitotenv.2019.01.173.

[113] M. K. Zühlke, R. Schlüter, A. K. Henning, M. Lipka, A. Mikolasch, P. Schumann, M. Giersberg, G. Kunze and F. Schauer, "A novel mechanism of conjugate formation of bisphenol A and its analogues by Bacillus amyloliquefaciens: Detoxification and reduction of estrogenicity of bisphenols," *Int. Biodeterior. Biodegrad.*, vol. 109, pp. 165–173, 2016, doi: 10.1016/j.ibiod.2016.01.019.

[114] M. Takeo, J. Akizuki, A. Kawasaki and S. Negoro, "Degradation potential of the nonylphenol monooxygenase of Sphingomonas sp. NP5 for bisphenols and their structural analogs," *Microorganisms*, vol. 8, no. 2, 2020, doi: 10.3390/microorganisms8020284.

[115] C. Nicolucci, S. Rossi, C. Menale, T. Godjevargova, Y. Ivanov, M. Bianco, L. Mita, U. Bencivenga, • Damiano, G. Mita and N. Diano, "Biodegradation of bisphenols with immobilized laccase or tyrosinase on polyacrylonitrile beads," doi: 10.1007/s10532-010-9440-2.

[116] F. J. Benitez, J. L. Acero, F. J. Real, G. Roldan, F. J. Benitez, J. L. Acero, F. J. Real, G. Roldan and E. Rodriguez, "Toxic / Hazardous Substances and Environmental Engineering Modeling the photodegradation of emerging contaminants in waters by UV radiation and UV / H 2 O 2 system Modeling the photodegradation of emerging contaminants in waters by UV radiation and UV /

H," vol. 4529, no. March 2017, 2013, doi: 10.1080/10934529.2012.707864.

[117] A. Zhang, and Y. Li, "Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H2O2, and UV/H2O2oxidation processes: Effects of reaction conditions and sludge matrix," *Sci. Total Environ.*, vol. 493, pp. 307–323, 2014, doi: 10.1016/j.scitotenv.2014.05.149.

[118] M. Zupanc, T. Kosjek, M. Petkovšek, M. Dular, B. Kompare, B. Širok, Ž. Blažeka and E. Heath, "Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment," *Ultrason. Sonochem.*, vol. 20, no. 4, pp. 1104–1112, 2013, doi: 10.1016/j.ultsonch.2012.12.003.

[119] M. Gągol, A. Przyjazny and G. Boczkaj, "Wastewater treatment by means of advanced oxidation processes based on cavitation – A review," *Chem. Eng. J.*, vol. 338, no. September 2017, pp. 599–627, 2018, doi: 10.1016/j.cej.2018.01.049.

[120] Y. Wang, F. A. Roddick and L. Fan, "Direct and indirect photolysis of seven micropollutants in secondary effluent from a wastewater lagoon," *Chemosphere*, vol. 185, pp. 297–308, 2017, doi: 10.1016/j.chemosphere.2017.06.122.

[121] M. Gmurek, M. Olak-Kucharczyk and S. Ledakowicz, "Photochemical decomposition of endocrine disrupting compounds – A review," *Chem. Eng. J.*, vol. 310, pp. 437–456, 2017, doi: 10.1016/j.cej.2016.05.014.

[122] X. Wang, J. Ma, Z. Wang, R. Guo and X. Hu, "Aqueous phototransformation of bisphenol S: The competitive radical-attack pathway to p-hydroxybenzenesulfonic acid," *Water Sci. Technol.*, vol. 70, no. 3, pp. 540–547, 2014, doi: 10.2166/wst.2014.257.

[123] R. A. Torres, G. Sarantakos, E. Combet, C. Pétrier and C. Pulgarin, "Journal of Photochemistry and Photobiology A : Chemistry Sequential helio-photo-Fenton and sonication processes for the treatment of bisphenol A," vol. 199, pp. 197–203, 2008, doi: 10.1016/j.jphotochem.2008.05.016.

[124] S. Esplugas, J. Giménez, S. Contreras, E. Pascual and M. Rodríguez, "Comparison of different advanced oxidation processes for phenol degradation," *Water Res.*, vol. 36, no. 4, pp. 1034–1042, Feb. 2002, doi: 10.1016/S0043-1354(01)00301-3.

[125] N. Klamerth, S. Malato, A. Agu and A. Ferna, "Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in wastewater treatment plant effluents: A comparison," vol. 7, 2012, doi: 10.1016/j.watres.2012.11.008.

[126] H. Katsumata, S. Kawabe, S. Kaneco, T. Suzuki and K. Ohta, "Degradation of bisphenol A in water by the photo-Fenton reaction," vol. 162, pp. 297–305, 2004, doi: 10.1016/S1010-6030(03)00374-5.

[127] Y. Liu, X. Zhang and F. Wu, "Photodegradation of bisphenol AF in montmorillonite dispersions: Kinetics and mechanism study," *Appl. Clay Sci.*, vol. 49, no. 3, pp. 182–186, 2010, doi: 10.1016/j.clay.2010.05.002.

[128] G. Wang, L. Huang, R. Yu and N. Deng, "Photocatalytic degradation of 2,2-bis(4-hydroxy-3-methylphenyl) propane (BPP) based on molecular recognition interaction," *J. Chem. Technol. Biotechnol.*, 2008, doi: 10.1002/jctb.1837.

[129] I. P. Pozdnyakov, L. Guo, E. M. Glebov, F. Wu, V. F. Plyusnin, V. P. Grivin and N. Deng, "Aqueous photochemistry of bisphenol E in the presence of β-

cyclodextrin," *High Energy Chem.*, vol. 45, no. 3, pp. 214–221, 2011, doi: 10.1134/S001814391103012X.

[130] J. Xiao, G. Wang, X. Xue, F. Wu, H. Luan and N. Deng, "Enhanced Photodegradation Behavior of Bisphenol F in the Presence of -Cyclodextrin under UV Light," vol. 24, no. 6, pp. 812–820, 2007, doi: 10.1089/ees.2006.0107.

[131] G. Wang, P. Qi, X. Xue, F. Wu and N. Deng, "Photodegradation of bisphenol Z by UV irradiation in the presence of β-cyclodextrin," *Chemosphere*, vol. 67, no. 4, pp. 762–769, 2007, doi: 10.1016/j.chemosphere.2006.10.041.

[132] K. Chiang, T. M. Lim, L. Tsen and C. C. Lee, "Photocatalytic degradation and mineralization of bisphenol A by TiO 2 and platinized TiO2," *Appl. Catal. A Gen.*, vol. 261, no. 2, pp. 225–237, 2004, doi: 10.1016/j.apcata.2003.11.004.

[133] V. M. Daskalaki, Z. Frontistis, D. Mantzavinos and A. Katsaounis, "Solar light-induced degradation of bisphenol-A with TiO2 immobilized on Ti," *Catal. Today*, vol. 161, no. 1, pp. 110–114, 2011, doi: 10.1016/j.cattod.2010.09.018.

[134] N. Vela, M. Calín, M. J. Yáñez-Gascón, I. Garrido, G. Pérez-Lucas, J. Fenoll and S. Navarro, "Solar reclamation of wastewater effluent polluted with bisphenols, phthalates and parabens by photocatalytic treatment with TiO2/Na2S2O8 at pilot plant scale," *Chemosphere*, vol. 212, pp. 95–104, 2018, doi: 10.1016/j.chemosphere.2018.08.069.

[135] M. Petkovšek, and M. Dular, "Cavitation dynamics in water at elevated temperatures and in liquid nitrogen at an ultrasonic horn tip," *Ultrason. Sonochem.*, vol. 58, no. February, 2019, doi: 10.1016/j.ultsonch.2019.104652.

[136] S. M. Joshi, and P. R. Gogate, "Intensification of industrial wastewater treatment using hydrodynamic cavitation combined with advanced oxidation at operating capacity of 70 L," *Ultrason. Sonochem.*, vol. 52, no. December 2018, pp. 375–381, 2019, doi: 10.1016/j.ultsonch.2018.12.016.

[137] P. Braeutigam, M. Franke, R. J. Schneider, A. Lehmann, A. Stolle and B. Ondruschka, "Degradation of carbamazepine in environmentally relevant concentrations in water by Hydrodynamic-Acoustic-Cavitation (HAC)," *Water Res.*, vol. 46, no. 7, pp. 2469–2477, 2012, doi: 10.1016/j.watres.2012.02.013.

[138] J. Choi, M. Cui, Y. Lee, J. Kim, Y. Son and J. Khim, "Hydrodynamic cavitation and activated persulfate oxidation for degradation of bisphenol A: Kinetics and mechanism," *Chem. Eng. J.*, vol. 338, no. January, pp. 323–332, 2018, doi: 10.1016/j.cej.2018.01.018.

[139] M. Dietrich, M. Franke, M. Stelter and P. Braeutigam, "Degradation of endocrine disruptor bisphenol A by ultrasound-assisted electrochemical oxidation in water," *Ultrason. Sonochem.*, vol. 39, no. November 2016, pp. 741–749, 2017, doi: 10.1016/j.ultsonch.2017.05.038.

[140] M. Neamţu, and F. H. Frimmel, "Degradation of endocrine disrupting bisphenol A by 254 nm irradiation in different water matrices and effect on yeast cells," *Water Res.*, vol. 40, no. 20, pp. 3745–3750, 2006, doi: 10.1016/j.watres.2006.08.019.

[141] A. O. Kondrakov, A. N. Ignatev, F. H. Frimmel, S. Bräse, H. Horn and A. I. Revelsky, "Formation of genotoxic quinones during bisphenol A degradation by TiO2photocatalysis and UV photolysis: A comparative study," *Appl. Catal. B*

Environ., vol. 160–161, no. 1, pp. 106–114, 2014, doi: 10.1016/j.apcatb.2014.05.007.

[142] A. Zhang, and Y. Li, "Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H2O2, and UV/H2O2 oxidation processes: Effects of reaction conditions and sludge matrix," *Sci. Total Environ.*, vol. 493, pp. 307–323, 2014, doi: 10.1016/j.scitotenv.2014.05.149.

[143] V. Salomatova, I. Pozdnyakov, P. Sherin, V. Grivin and V. Plyusnin, "Photochemistry of bisphenol F in aqueous solutions: A mechanistic study," *J. Photochem. Photobiol. A Chem.*, vol. 305, pp. 45–50, 2015, doi: 10.1016/j.jphotochem.2015.03.008.

[144] X. Wang, J. Ma, Z. Wang, R. Guo and X. Hu, "Aqueous phototransformation of bisphenol S: the competitive radical-attack pathway to *p* -hydroxybenzenesulfonic acid," *Water Sci. Technol.*, vol. 70, no. 3, p. 540, Aug. 2014, doi: 10.2166/wst.2014.257.

[145] J. M. Pahigian, and Y. Zuo, "Occurrence, endocrine-related bioeffects and fate of bisphenol A chemical degradation intermediates and impurities: A review," *Chemosphere*, vol. 207, pp. 469–480, 2018, doi: 10.1016/j.chemosphere.2018.05.117.

[146] Y. Ogata, S. Goda, T. Toyama, K. Sei and M. Ike, "The 4-tert-butylphenol-utilizing bacterium sphingobium fuliginis OMI can degrade bisphenols via phenolic ring hydroxylation and meta-cleavage pathway," *Environ. Sci. Technol.*, vol. 47, no. 2, pp. 1017–1023, 2013, doi: 10.1021/es303726h.

[147] E. J. Eio, M. Kawai, K. Tsuchiya, S. Yamamoto and T. Toda, "Biodegradation of bisphenol A by bacterial consortia," *Int. Biodeterior. Biodegrad.*, vol. 96, pp. 166–173, Dec. 2014, doi: 10.1016/j.ibiod.2014.09.011.

[148] J. M. McCormick, T. Van Es, K. R. Cooper, L. A. White and M. M. Häggblom, "Microbially mediated O-methylation of bisphenol a results in metabolites with increased toxicity to the developing Zebrafish (Danio rerio) embryo," *Environ. Sci. Technol.*, vol. 45, no. 15, pp. 6567–6574, 2011, doi: 10.1021/es200588w.

[149] D. Inoue, S. Hara, M. Kashihara, Y. Murai, E. Danzl, K. Sei, S. Tsunoi, M. Fujita and M. Ike, "Degradation of Bis(4-Hydroxyphenyl)Methane (Bisphenol F) by Sphingobium yanoikuyae Strain FM-2 Isolated from River Water," *Appl. Environ. Microbiol.*, vol. 74, no. 2, pp. 352–358, 2008, doi: 10.1128/AEM.01708-07.

[150] V. Salomatova, I. Pozdnyakov, P. Sherin, V. Grivin and V. Plyusnin, "Photochemistry of bisphenol F in aqueous solutions: A mechanistic study," *J. Photochem. Photobiol. A Chem.*, vol. 305, pp. 45–50, Jun. 2015, doi: 10.1016/j.jphotochem.2015.03.008.

[151] J. Yao, M. Gao, X. Guo, F. Ai and Z. Wang, "Enhanced degradation performance of bisphenol M using peroxymonosulfate activated by zero-valent iron in aqueous solution: Kinetic study and product identification," *Chemosphere*, vol. 221, pp. 314–323, 2019, doi: 10.1016/j.chemosphere.2019.01.036.

[152] J. Li, S. Y. Pang, Y. Zhou, S. Sun, L. Wang, Z. Wang, Y. Gao, Y. Yang and J. Jiang, "Transformation of bisphenol AF and bisphenol S by manganese dioxide and effect of iodide," *Water Res.*, vol. 143, pp. 47–55, 2018, doi: 10.1016/j.watres.2018.06.029.

[153] 2016 ALL4PACK, "Packaging: market and challenges in 2016 - ALL4PACK Paris

- Salon Emballage - Manutention Nov 2016," *Packaging: market and challenges in 2016 - ALL4PACK Paris - Salon Emballage -*. https://www.all4pack.com/Archives/Packaging-market-challenges-2016 (accessed Dec. 06, 2019).

[154] Y. Sanchis, V. Yusà and C. Coscollà, "Analytical strategies for organic food packaging contaminants," *J. Chromatogr. A*, vol. 1490, pp. 22–46, 2017, doi: 10.1016/j.chroma.2017.01.076.

[155] Y. Sanchis, V. Yusà and C. Coscollà, "Analytical strategies for organic food packaging contaminants," *J. Chromatogr. A*, vol. 1490, pp. 22–46, Mar. 2017, doi: 10.1016/J.CHROMA.2017.01.076.

[156] A. Repossi, F. Farabegoli, T. Gazzotti, E. Zironi and G. Pagliuca, "Bisphenol a in edible part of seafood," *Ital. J. Food Saf.*, vol. 5, no. 2, pp. 98–105, 2016, doi: 10.4081/ijfs.2016.5666.

[157] J. Jing, C. Cao, S. Ma, Z. Li, G. Qu, B. Xie, W. Jin and Y. Zhao, "Enhanced defect oxygen of LaFeO3/GO hybrids in promoting persulfate activation for selective and efficient elimination of bisphenol A in food wastewater," *Chem. Eng. J.*, vol. 407, no. September 2020, p. 126890, 2021, doi: 10.1016/j.cej.2020.126890.

[158] H. Wang, S. Song, M. Shao, Y. Gao, C. Yang, Y. Li, W. Wang, Y. He and P. Li, "Determination of bisphenol analogues in food-contact plastics using diode array detector, charged aerosol detector and evaporative light-scattering detector," *Ecotoxicol. Environ. Saf.*, vol. 186, no. October, 2019, doi: 10.1016/j.ecoenv.2019.109778.

[159] L. Tian, L. Lin and S. Bayen, "Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry," *Talanta*, vol. 193, no. June 2018, pp. 70–76, Feb. 2019, doi: 10.1016/j.talanta.2018.09.070.

[160] E. Fasano, T. Cirillo, F. Esposito and S. Lacorte, "Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry," *LWT - Food Sci. Technol.*, vol. 64, no. 2, pp. 1015–1021, 2015, doi: 10.1016/j.lwt.2015.06.066.

[161] A. Guart, F. Bono-Blay, A. Borrell and S. Lacorte, "Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters," *Food Chem.*, vol. 156, pp. 73–80, 2014, doi: 10.1016/j.foodchem.2014.01.075.

[162] F. Vilarinho, R. Sendón, A. van der Kellen, M. F. Vaz and A. S. Silva, "Bisphenol A in food as a result of its migration from food packaging," *Trends Food Sci. Technol.*, vol. 91, no. June, pp. 33–65, 2019, doi: 10.1016/j.tifs.2019.06.012.

[163] Ş. Sungur, M. Koroğlu and A. Özkan, "Determination of bisphenol a migrating from canned food and beverages in markets," *Food Chem.*, vol. 142, pp. 87–91, 2014, doi: 10.1016/j.foodchem.2013.07.034.

[164] M. Fischnaller, R. Bakry and G. K. Bonn, "A simple method for the enrichment of bisphenols using boron nitride," *Food Chem.*, vol. 194, pp. 149–155, 2016, doi: 10.1016/j.foodchem.2015.07.117.

[165] S. C. Cunha, C. Almeida, E. Mendes and J. O. Fernandes, "Simultaneous

determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 28, no. 4, pp. 513–526, 2011, doi: 10.1080/19440049.2010.542551.

[166] H. Gallart-Ayala, E. Moyano and M. T. Galceran, "Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry," *Anal. Chim. Acta*, vol. 683, no. 2, pp. 227–233, 2011, doi: 10.1016/j.aca.2010.10.034.

[167] C. Liao, and K. Kannan, "A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 31, no. 2, pp. 319–329, 2014, doi: 10.1080/19440049.2013.868611.

[168] M. J. Gómez Ramos, A. Lozano and A. R. Fernández-Alba, "High-resolution mass spectrometry with data independent acquisition for the comprehensive non-targeted analysis of migrating chemicals coming from multilayer plastic packaging materials used for fruit purée and juice," *Talanta*, vol. 191, no. March 2018, pp. 180–192, 2019, doi: 10.1016/j.talanta.2018.08.023.

[169] R. Paseiro-Cerrato, J. Devries and T. H. Begley, "Evaluation of short-term and long-term migration testing from can coatings into food simulants: Epoxy and acrylic-phenolic coatings," *J. Agric. Food Chem.*, vol. 65, no. 12, pp. 2594–2602, 2017, doi: 10.1021/acs.jafc.7b00081.

[170] R. Paseiro-Cerrato, G. O. Noonan and T. H. Begley, "Evaluation of Long-Term Migration Testing from Can Coatings into Food Simulants: Polyester Coatings," *J. Agric. Food Chem.*, vol. 64, no. 11, pp. 2377–2385, 2016, doi: 10.1021/acs.jafc.5b05880.

[171] K. Mandrah, G. N. V. Satyanarayana and S. K. Roy, "A dispersive liquid-liquid microextraction based on solidification of floating organic droplet followed by injector port silylation coupled with gas chromatography–tandem mass spectrometry for the determination of nine bisphenols in bottled carbonated bev," *J. Chromatogr. A*, vol. 1528, pp. 10–17, 2017, doi: 10.1016/j.chroma.2017.10.071.

[172] J. Regueiro, and T. Wenzl, "Determination of bisphenols in beverages by mixed-mode solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry," *J. Chromatogr. A*, vol. 1422, pp. 230–238, 2015, doi: 10.1016/j.chroma.2015.10.046.

[173] J. Hwang, I.-A. Bae, C. Lee, S. Lee, J. C. Choi, S.-J. Park, J.-H. Hong, G. Lee and M. Kim, "Simultaneous analysis and exposure assessment of migrated bisphenol analogues, phenol, and *p-tert* -butylphenol from food contact materials," *Food Addit. Contam. Part A*, pp. 1–9, Oct. 2018, doi: 10.1080/19440049.2018.1523571.

[174] M. Onghena, E. Van Hoeck, N. Negreira, L. Quirynen, J. Van Loco and A. Covaci, "Evaluation of the migration of chemicals from baby bottles under standardised and duration testing conditions," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 33, no. 5, pp. 893–904, 2016, doi: 10.1080/19440049.2016.1171914.

[175] M. Onghena, E. Van Hoeck, J. Van Loco, M. Ibáñez, L. Cherta, T. Portolés, E. Pitarch, F. Hernandéz, F. Lemière and A. Covaci, "Identification of substances migrating from plastic baby bottles using a combination of low-resolution and high-resolution mass spectrometric analysers coupled to gas and liquid chromatography," *J. Mass Spectrom.*, vol. 50, no. 11, pp. 1234–1244, 2015, doi: 10.1002/jms.3644.

[176] A. G. Asimakopoulos, M. Elangovan and K. Kannan, "Migration of parabens, bisphenols, benzophenone-type UV filters, triclosan, and triclocarban from teethers and its implications for infant exposure," *Environ. Sci. Technol.*, vol. 50, no. 24, pp. 13539–13547, 2016, doi: 10.1021/acs.est.6b04128.

[177] Y. Xie, Y. Bao, H. Wang, Y. Cheng, H. Qian and W. Yao, "Release of bisphenols from can coatings into canned beer in China market," *J. Sci. Food Agric.*, vol. 95, no. 4, pp. 764–770, Mar. 2015, doi: 10.1002/jsfa.6862.

[178] P. Gallo, I. Di Marco Pisciottano, F. Esposito, E. Fasano, G. Scognamiglio, G. D. Mita and T. Cirillo, "Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection," *Food Chem.*, vol. 220, pp. 406–412, 2017, doi: 10.1016/j.foodchem.2016.10.005.

[179] Y. Cheng, X. mei Nie, H. qiu Wu, Y. he Hong, B. cheng Yang, T. Liu, D. Zhao, J. feng Wang, G. hong Yao and F. Zhang, "A high-throughput screening method of bisphenols, bisphenols digycidyl ethers and their derivatives in dairy products by ultra-high performance liquid chromatography-tandem mass spectrometry," *Anal. Chim. Acta*, vol. 950, pp. 98–107, 2017, doi: 10.1016/j.aca.2016.11.006.

[180] B. Vrana, I. J. Allan, R. Greenwood, G. A. Mills, E. Dominiak, K. Svensson, J. Knutsson and G. Morrison, "Passive sampling techniques for monitoring pollutants in water," *TrAC - Trends Anal. Chem.*, vol. 24, no. 10, pp. 845–868, 2005, doi: 10.1016/j.trac.2005.06.006.

[181] S. Xiao Mei, S. Deng Hui, W. Bing Shuang, L. Bei Bei and H. Fa Yuan, "Letter to the Editor Simultaneous Determination of Bisphenols and Alkylphenols in Water by Solid Phase Extraction and Ultra Performance Liquid Chromatography-tandem Mass Spectrometry *," *Biomed Env. Sci*, vol. 27, no. 6, pp. 471–474, 2014, doi: 10.3967/.

[182] Z. Yan, Y. Liu, K. Yan, S. Wu, Z. Han, R. Guo, M. Chen, Q. Yang, S. Zhang and J. Chen, "Bisphenol analogues in surface water and sediment from the shallow Chinese freshwater lakes: Occurrence, distribution, source apportionment, and ecological and human health risk," *Chemosphere*, vol. 184, pp. 318–328, 2017, doi: 10.1016/j.chemosphere.2017.06.010.

[183] S. R. Yazdinezhad, A. Ballesteros-Gómez, L. Lunar and S. Rubio, "Single-step extraction and cleanup of bisphenol A in soft drinks by hemimicellar magnetic solid phase extraction prior to liquid chromatography/tandem mass spectrometry," *Anal. Chim. Acta*, vol. 778, pp. 31–37, 2013, doi: 10.1016/j.aca.2013.03.025.

[184] X. Sun, J. Wang, Y. Li, J. Jin, B. Zhang, S. M. Shah, X. Wang and J. Chen, "Highly selective dummy molecularly imprinted polymer as a solid-phase extraction sorbent for five bisphenols in tap and river water," *J. Chromatogr. A*, vol. 1343, pp. 33–41, 2014, doi: 10.1016/j.chroma.2014.03.063.

[185] Affinisep, “AFFINIMIP ® SPE Bisphenols,” pp. 1–28.

[186] D. A. S. F. J. H. T. A. Nieman, *Principles of Instrumental Analysis*, vol. 5th Editio. 1998.

[187] P. He, and D. S. Aga, “Comparison of GC-MS/MS and LC-MS/MS for the analysis of hormones and pesticides in surface waters: Advantages and pitfalls,” *Anal. Methods*, vol. 11, no. 11, pp. 1436–1448, 2019, doi: 10.1039/c8ay02774a.

[188] J. J. Pitt, “Principles and Applications of Liquid ChromatographyMass Spectrometry in Clinical Biochemistry,” *Clin Biochem*, vol. 30, no. February, pp. 19–34, 2009.

[189] R. A. C. C. WILFRIED M. A. NIESSEN, *INTERPRETATION OF MS–MS MASS SPECTRA OF DRUGS AND PESTICIDES*. 2017.

[190] A. Ballesteros-Gómez, S. Rubio and D. Pérez-Bendito, “Analytical methods for the determination of bisphenol A in food,” *J. Chromatogr. A*, vol. 1216, no. 3, pp. 449–469, 2009, doi: 10.1016/j.chroma.2008.06.037.

[191] A. Agüera, M. J. Martínez Bueno and A. R. Fernández-Alba, “New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters,” *Environ. Sci. Pollut. Res.*, vol. 20, no. 6, pp. 3496–3515, 2013, doi: 10.1007/s11356-013-1586-0.

[192] M. Pourchet, L. Debrauwer, J. Klanova, E. J. Price, A. Covaci, N. Caballero-Casero, H. Oberacher, M. Lamoree, A. Damont, F. Fenaille, J. Vlaanderen, J. Meijer, M. Krauss, D. Sarigiannis, R. Barouki, B. Le Bizec and J. P. Antignac, “Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: From promises to challenges and harmonisation issues,” *Environ. Int.*, vol. 139, no. February 2020, p. 105545, 2020, doi: 10.1016/j.envint.2020.105545.

[193] V. Mayya, K. Rezaul, Y. S. Cong and D. Han, “Systematic comparison of a two-dimensional ion trap and a three-dimensional ion trap mass spectrometer in proteomics,” *Mol. Cell. Proteomics*, vol. 4, no. 2, pp. 214–223, 2005, doi: 10.1074/mcp.T400015-MCP200.

[194] T. De Vijlder, D. Valkenborg, F. Lemière, E. P. Romijn, K. Laukens and F. Cuyckens, “A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation,” *Mass Spectrom. Rev.*, vol. 37, no. 5, pp. 607–629, 2018, doi: 10.1002/mas.21551.

[195] X. F. Chen, H. T. Wu, G. G. Tan, Z. Y. Zhu and Y. F. Chai, “Liquid chromatography coupled with time-of-flight and ion trap mass spectrometry for qualitative analysis of herbal medicines,” *J. Pharm. Anal.*, vol. 1, no. 4, pp. 235–245, 2011, doi: 10.1016/j.jpha.2011.09.008.

[196] M. W. Senko, P. M. Remes, J. D. Canterbury, R. Mathur, Q. Song, S. M. Eliuk, C. Mullen, L. Earley, M. Hardman, J. D. Blethrow, H. Bui, A. Specht, O. Lange, E. Denisov, A. Makarov, S. Horning and V. Zabrouskov, “Novel parallelized quadrupole/linear ion trap/orbitrap tribrid mass spectrometer improving proteome coverage and peptide identification rates,” *Anal. Chem.*, vol. 85, no. 24, pp. 11710–11714, 2013, doi: 10.1021/ac403115c.

[197] L. L. Hohrenk, F. Itzel, N. Baetz, J. Tuerk, M. Vosough and T. C. Schmidt,

"Comparison of Software Tools for Liquid Chromatography-High-Resolution Mass Spectrometry Data Processing in Nontarget Screening of Environmental Samples," *Anal. Chem.*, vol. 92, no. 2, pp. 1898–1907, 2020, doi: 10.1021/acs.analchem.9b04095.

[198] D. Meng, D. ling Fan, W. Gu, Z. Wang, Y. jie Chen, H. zhong Bu and J. ning Liu, "Development of an integral strategy for non-target and target analysis of site-specific potential contaminants in surface water: A case study of Dianshan Lake, China," *Chemosphere*, vol. 243, p. 125367, 2020, doi: 10.1016/j.chemosphere.2019.125367.

[199] J. M. Weiss, E. Simon, G. J. Stroomberg, R. De Boer, J. De Boer, S. C. Van Der Linden, P. E. G. Leonards and M. H. Lamoree, "Identification strategy for unknown pollutants using high-resolution mass spectrometry: Androgen-disrupting compounds identified through effect-directed analysis," *Anal. Bioanal. Chem.*, vol. 400, no. 9, pp. 3141–3149, 2011, doi: 10.1007/s00216-011-4939-x.

[200] J. Hollender, E. L. Schymanski, H. P. Singer and P. L. Ferguson, "Nontarget Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go?," *Environ. Sci. Technol.*, vol. 51, no. 20, pp. 11505–11512, 2017, doi: 10.1021/acs.est.7b02184.

[201] M. J. Gómez Ramos, A. Lozano and A. R. Fernández-Alba, "High-resolution mass spectrometry with data independent acquisition for the comprehensive non-targeted analysis of migrating chemicals coming from multilayer plastic packaging materials used for fruit purée and juice," *Talanta*, vol. 191, pp. 180–192, Jan. 2019, doi: 10.1016/j.talanta.2018.08.023.

[202] J. Rusko, I. Perkons, J. D. Rasinger and V. Bartkevics, "Non-target and suspected-target screening for potentially hazardous chemicals in food contact materials: investigation of paper straws," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 37, no. 4, pp. 649–664, 2020, doi: 10.1080/19440049.2020.1711969.

[203] K. Dührkop, K. Scheubert and S. Böcker, "Molecular Formula Identification with SIRIUS," *Metabolites*, vol. 3, no. 2, pp. 506–516, 2013, doi: 10.3390/metabo3020506.

[204] E. L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H. P. Singer and J. Hollender, "Identifying small molecules via high resolution mass spectrometry: Communicating confidence," *Environmental Science and Technology*, vol. 48, no. 4. pp. 2097–2098, Feb. 18, 2014, doi: 10.1021/es5002105.

[205] C. Moschet, B. M. Lew, S. Hasenbein, T. Anumol and T. M. Young, "LC- and GC-QTOF-MS as Complementary Tools for a Comprehensive Micropollutant Analysis in Aquatic Systems," *Environ. Sci. Technol.*, vol. 51, no. 3, pp. 1553–1561, 2017, doi: 10.1021/acs.est.6b05352.

[206] M. Petrovic, and D. Barceló, "Liquid chromatography-mass spectrometry in the analysis of emerging environmental contaminants," *Anal. Bioanal. Chem.*, vol. 385, no. 3, pp. 422–424, 2006, doi: 10.1007/s00216-006-0450-1.

[207] EURACHEM/CITAC Guide CG 4, "EURACHEM/CITAC Guide CG 4; Quantifying uncertainty in analytical measurement, Third Edition," 2012.

[208] Magnusson & Örnemark, "Eurachem Guide: The Fitness for Purpose of Analytical

Methods – A Laboratory Guide to Method Validation and Related Topics," vol. 2nd ed, 2014, Accessed: Oct. 10, 2019. [Online]. Available: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.

[209] E. Trullols, I. Ruisánchez and F. X. Rius, "Validation of qualitative analytical methods," *TrAC - Trends Anal. Chem.*, vol. 23, no. 2, pp. 137–145, 2004, doi: 10.1016/S0165-9936(04)00201-8.

[210] EURACHEM, *Guide to Quality in Analytical Chemistry Eurachem/CITAC Guide Guide to Quality in Analytical Chemistry.* 2016.

[211] GUM, "Evaluation of measurement data — Guide to the expression of uncertainty in measurement," *Int. Organ. Stand. Geneva ISBN*, vol. 50, no. September, p. 134, 2008, doi: 10.1373/clinchem.2003.030528.

[212] EMEA, "Guideline on bioanalytical method validation," *Eur. Med. Agency, Comm. Med. Prod. Hum. Use*, vol. 44, no. July 2011, pp. 1–23, 2015, doi: EMEA/CHMP/EWP/192217/2009.

[213] P. Quevauviller, "Traceability of environmental chemical measurements," *TrAC - Trends Anal. Chem.*, vol. 23, no. 3, pp. 171–177, 2004, doi: 10.1016/S0165-9936(04)00314-0.

[214] A. A. for L. Accreditation, "G104 - Guide for Estimation of Measurement Uncertainty In Testing December 2014," *Am. Assoc. Lab. Accredit.*, no. December, pp. 1–31, 2014.

[215] S. Song, T. Ruan, T. Wang, R. Liu and G. Jiang, "Distribution and preliminary exposure assessment of bisphenol AF (BPAF) in various environmental matrices around a manufacturing plant in China," *Environ. Sci. Technol.*, vol. 46, no. 24, pp. 13136–13143, 2012, doi: 10.1021/es303960k.

[216] E. Yamazaki, N. Yamashita, S. Taniyasu, J. Lam, P. K. S. Lam, H.-B. Moon, Y. Jeong, P. Kannan, H. Achyuthan, N. Munuswamy and K. Kannan, "Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India," *Ecotoxicol. Environ. Saf.*, vol. 122, pp. 565–572, Dec. 2015, doi: 10.1016/J.ECOENV.2015.09.029.

[217] European Commission Regulation (EU), "European Commission Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A (BPA) in plastic infant feeding bottles. Text with EEA relevance.," no. June, pp. 3–4, doi: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R0321.

[218] V. Le Fol, S. Aït-Aïssa, M. Sonavane, J.-M. Porcher, P. Balaguer, J.-P. Cravedi, D. Zalko and F. Brion, "In vitro and in vivo estrogenic activity of BPA, BPF and BPS in zebrafish-specific assays.," *Ecotoxicol. Environ. Saf.*, vol. 142, pp. 150–156, Aug. 2017, doi: 10.1016/j.ecoenv.2017.04.009.

[219] C. J. Catenza, A. Farooq, N. S. Shubear and K. K. Donkor, "A targeted review on fate, occurrence, risk and health implications of bisphenol analogues," *Chemosphere*, vol. 268, p. 129273, 2021, doi: 10.1016/j.chemosphere.2020.129273.

[220] R. F. Lane, C. D. Adams, S. J. Randtke and R. E. Carter, "Bisphenol diglycidyl ethers and bisphenol A and their hydrolysis in drinking water," *Water Res.*, vol. 72,

pp. 331–339, 2015, doi: 10.1016/j.watres.2014.09.043.

[221] C. Gys, A. Kovačič, C. Huber, F. Y. Lai, E. Heath and A. Covaci, “Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism,” *Toxicol. Lett.*, vol. 295, pp. 115–123, Oct. 2018, doi: 10.1016/j.toxlet.2018.05.034.

[222] M. Fattore, G. Russo, F. Barbato, L. Grumetto and S. Albrizio, “Monitoring of bisphenols in canned tuna from Italian markets,” *Food Chem. Toxicol.*, vol. 83, pp. 68–75, 2015, doi: 10.1016/j.fct.2015.05.010.

[223] C. Liao, and K. Kannan, “Concentrations and profiles of bisphenol a and other bisphenol analogues in foodstuffs from the united states and their implications for human exposure,” *J. Agric. Food Chem.*, vol. 61, no. 19, pp. 4655–4662, 2013, doi: 10.1021/jf400445n.

[224] J. P. Buckley, H. Kim, E. Wong and C. M. Rebholz, “Ultra-processed food consumption and exposure to phthalates and bisphenols in the US National Health and Nutrition Examination Survey, 2013–2014,” *Environ. Int.*, vol. 131, no. August, p. 105057, 2019, doi: 10.1016/j.envint.2019.105057.

[225] M. Česen, D. Lambropoulou, M. Laimou-Geraniou, T. Kosjek, U. Blaznik, D. Heath and E. Heath, “Determination of Bisphenols and Related Compounds in Honey and Their Migration from Selected Food Contact Materials,” *J. Agric. Food Chem.*, vol. 64, no. 46, pp. 8866–8875, 2016, doi: 10.1021/acs.jafc.6b03924.

[226] I. Severin, E. Souton, L. Dahbi and M. C. Chagnon, “Use of bioassays to assess hazard of food contact material extracts: State of the art,” *Food Chem. Toxicol.*, vol. 105, pp. 429–447, 2017, doi: 10.1016/j.fct.2017.04.046.

Bibliography

Journal Articles published in peer-reviewed journals

Journal Articles Related to the Thesis

M. Česen, K. Lenarčič, V. Mislej, M. Levstek, A. Kovačič, B. Cimrmančič, N. Uranjek, T. Kosjek, D. Heath, M.S. Dolenc, E. Heath, The occurrence and source identification of bisphenol compounds in wastewaters, Sci. Total Environ. 616–617 (2018) 744–752. doi: 10.1016/j.scitotenv.2017.10.252.

C. Gys, A. Kovačič, C. Huber, F.Y. Lai, E. Heath, A. Covaci, Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism, Toxicol. Lett. 295 (2018) 115–123. doi: 10.1016/j.toxlet.2018.05.034.

A. Kovačič, C. Gys, T. Kosjek, A. Covaci, E. Heath, Photochemical degradation of BPF, BPS and BPZ in aqueous solution: Identification of transformation products and degradation kinetics, Sci. Total Environ. 664 (2019) 595–604. doi: 10.1016/J.SCITOTENV.2019.02.064.

A. Kovačič, M. Česen, M. Laimou-Geraniou, D. Lambropoulou, T. Kosjek, D. Heath, E. Heath, Stability, biological treatment and UV photolysis of 18 bisphenols under laboratory conditions, Environ. Res. 179 (2019) 108738. doi: 10.1016/J.ENVRES.2019.108738.

A. Kovačič, C. Gys, M.R. Gulin, T. Kosjek, D. Heath, A. Covaci, E. Heath. The migration of bisphenols from beverage cans and reusable sports bottles, Food Chem. (2020) 127326. doi: 10.1016/j.foodchem.2020.127326.

A. Kovačič, Š. David, M. Zupanc, J. Gostiša, B. Bizjan, N. Kri, M. Sollner, E. Heath. The removal of bisphenols and other contaminants of emerging concern by hydrodynamic cavitation: From lab-scale to pilot-scale. Sci. Total Environ. 743 (2020). doi: 10.1016/j.scitotenv.2020.140724.

T. Elersek, T. Notersberg, A. Kovačič, E. Heath, M. Filipič, The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism, Environ. Sci. Pollut. Res. (2020). doi: 10.1007/s11356-020-10329-7.

A. Kovačič, C. Gys, M.R. Gulin, T. Gornik, T. Kosjek, D. Heath, A. Covaci, E. Heath. Kinetics and biotransformation products of bisphenol F and S during aerobic degradation with activated sludge. Journal of hazardous materials, ISSN 0304-3894 (2020). doi: 10.1016/j.jhazmat.2020.124079.

Other Journal Articles

T. Gornik, A. Kovacic, E. Heath, J. Hollender, T. Kosjek, Biotransformation study of antidepressant sertraline and its removal during biological wastewater treatment, Water Res. (2020) 115864. doi: 10.1016/j.waters.2020.115864.

D. Škufca, A. Kovačič, M. Pflieger, E. Heath. Determination of 18 bisphenols in aqueous and biomass phase of high rate algal ponds: development and validation of analytical methods. Chemosphere, 129786 (2021). doi.org/10.1016/j.chemosphere.2021.129786

Submitted and in preparation paper

D. Škufca, A. Kovačič, F. Prosenc, M. Pflieger, D. Heath, E. Heath. Phycoremediation of municipal wastewater: removal of nutrients and contaminants of emerging concern. Under review at Sci. Total Environ (January 2021).

A. Kovačič, M.R. Gulin, C. Gys, Ž. Tkalec, T. Kosjek, D. Heath, A. Covaci, E. Heath. Non-target screening for intentionally and non intentionally added substances migrating from epoxy resins in cans: From food simulant to beer. To be submitted in Journal of hazardous materials (April 2021).

M.R. Gulin, A. Kovačič, C. Gys, D. Heath, A. Covaci, E. Heath. Development of the post-acquisition data processing and optimization of strategies for data reduction for the non-targeted screening for substances in food simulants, food and wastewater.
To be submitted in Analytical Chemistry (April 2021).

Conference Paper

D. Škufca, F. Prosenc, A. Kovačič, M. Pflieger, T. Kosjek, T. Griessler Bulc, E. Heath. Removal of bisphenols in laboratory-scale algal bioreactors. V: *Book of abstracts*, 2nd IWA Conference on Algal Technologies for Wastewater Treatment and Resource Recovery and 2nd IWA Specialist Group Conference in Wastewater Pond Technology, IWAlgae (July 2019).

M. Česen, K. Lenarčič, V. Mislej, M. Levstek, M. Stražar, M. Sollner Dolenc, A. Kovačič, C. Gys, D. Lambropoulou, M. Laimou-Geraniou, U. Blaznik, J. Snoj Tratnik, D. Mazej, M. Horvat, A. Covaci, D. J. Heath, T. Kosjek, E. Heath. BPA and its alternatives in environment, food and biological fluids. V: *Program book and abstracts*, 46th International Symposium on High Performance Liquid Phase Separations and Related Techniques, HPLC (November 2017).

A. Vehar, N. Hvala, M. Levstek, A. Kovačič, E. Heath, M. Stražar, A. Žgajnar, Gotvajn. Disinfection of wastewater with peracetic acid - toxicity and micropollutant removal. V: Dejanović, Igor (ed.), Vrsaljko, Domagoj (ed.), Žižek, Krunoslav (ed.). *Book of abstracts*, XIII. Croatian Society of Chemical Engineers (2020).

A. Vehar, N. Hvala, M. Levstek, A. Kovačič, E. Heath, M. Stražar, A. Žgajnar, Gotvajn. Disinfection of wastewater - tertiary treatment in municipal wastewater treatment plant with peracetic acid. V: Kljun, Jakob (ed.), Paljk, Tina (ed.). *Communicating in science:*

book of abstracts, Cutting Edge, Scientific conference for young researchers, Ljubliana (October 2019).

D. Škufca, F. Prosenc, A. Kovačič, M. Pflieger, T. Kosjek, T. Griessler Bulc, E. Heath Removal of bisphenols in lab-scale algal bioreactors. V: Topole, Martin (ed.), et al. *Book of abstracts*: S*cience of the future how to stay up-to-date with your research*: 11th Jožef Stefan International Postgraduate School Students' Conference (April 2019) and 13th Young Researchers' Day (May 2019) Planica, Slovenia.

D. Škufca, F. Prosenc, A. Kovačič, M. Pflieger, T. Kosjek, T. Griessler Bulc, E. Heath Bisphenols removal in algal wastewater treatment bioreactors: future prospects of product reuse. V: Heath, David John (ed.), Horvat, Milena (ed.), Ogrinc, Nives (ed.). *Programme and book of abstracts*, 1st ISO-FOOD International Symposium on Isotopic and Other Techniques in Food Safety and Quality (April 2019), Portorož, Slovenia, Ljubljana.

A. Kovačič, E. Heath, C. Gys, T. Kosjek, A. Covaci, D. Heath. Migration of bisphenol and their derivatives from food contact material by target and untargeted analyses. V: Heath, David John (ed.), Horvat, Milena (ed.), Ogrinc, Nives (ed.). *Programme and book of abstracts*, 1st ISO-FOOD International Symposium on Isotopic and Other Techniques in Food Safety and Quality (April 2019), Portorož, Slovenia.

A. Kovačič, M. Česen, C. Gys, T. Kosjek, A. Covaci, E. Heath. The occurrence and fate of BPA and its alternatives during water treatment. V: *MASSTWIN Workshop on Mass spectrometry in support of the environment, food, and health interaction and disease, 18th-20th, Antwerp Belgium*, Antwerpen (2018).

C. Gys, A. Kovačič, C. Huber, F.Y. Lai, E. Heath, A. Covaci. In vitro Phase I and Phase II metabolism of bisphenol S by liquid chomatography coupled to quadrupole time-of-flight mass spectrometry. V: *MASSTWIN Workshop on Mass spectrometry in support of the environment, food, and health interaction and disease, 18th-20th, Antwerp Belgium*, Antwerpen (2018).

C. Gys, A. Kovačič, C. Huber, J. Oh, YA. Ahn, S. Kim, F.Y. Lai, E. Heath, A. Covaci. Screening of *in vitro* and *in vivo* metabolites of bisphenol S by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *38th International Symposium on Halogenated Persistent Organic Pollutants and 10th International PCB Workshop – Dioxin,* Krakow, Poland (26-31 August 2018).

C. Gys, A. Kovačič, T. Kosjek, A. Covaci, E. Heath. Identification of photochemical degradation products of three bisphenols by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Journées Internationales de Toxicologie*, Liège, Belgium, 19-20 October 2017.

E. Heath, M. Česen, K. Lenarčič, V. Mislej, M. Levstek, M. Stražar, M. Sollner Dolenc, A. Kovačič, C. Gys, D. Lambropoulou, M. Laimou-Geraniou, U. Blaznik, J. Snoj Tratnik, M. Horvat, D. Mazej, D. J. Heath, T. Kosjek. Biosphenois in environment, food and biological fluids. V: *Xenowac II, Challenges and Solutions related to Xenobiotics and Antimicrobial Resistance in the Framework of Urban Wastewater Reuse: towards a blue circle society:* (October 2018) Limassol, Cyprus.

A. Kovačič, M. Česen, C. Gys, A. Covaci, T. Kosjek, E. Heath. V: *Xenowac II, Challenges and Solutions related to Xenobiotics and Antimicrobial Resistance in the Framework of Urban Wastewater Reuse: towards a blue circle society:* (October 2018) Limassol, Cyprus.

A. Kovačič, M. Česen, T. Kosjek, E. Heath. Study of the stability and fate of BPA and its alternatives during water treatment. V: Dežman, Miha (ed.), et al., 10th Jožef Stefan International Postgraduate School Students' Conference and 12th Young Researchers' Day 10th and 11th (May 2018), Piran, Slov. Ljubliana.

M. Česen, K. Lenarčič, V. Mislej, M. Levstek, M. Stražar, M. Sollner Dolenc, A. Kovačič, D. J. Heath, C. Gys, D. Lambropoulou, M. Laimou-Geraniou, U. Blaznik, A. Covaci, E. Heath. The occurrence and fate of bisphenols in the environment and food. V: *10th Micropol & Ecohazard Conference 2017,* (September, 2017) Vienna, Austria.

C. Gys, A. Kovačič, T. Kosjek, A. Covaci, E. Heath. Photochemical degradation of bisphenols -F, -S and - Z in aqueous solution: degradation kinetics and identification of transformation products. V: *Abstracts*, 16th International Conference on Chemistry and the Environment, ICCE 2017, (June 2017) Oslo.

T. Kosjek, M. Česen, K. Lenarčič, V. Mislej, M. Levstek, M. Stražar, M. Sollner Dolenc, A. Kovačič, D. J. Heath, J. Druškovič, H. Prosen, C. Gys, A. Covaci, E. Heath. The occurrence and fate of bisphenols in the environment and food. V: Sollner Dolenc, Marija (ed.), Peterlin-Mašič, Lucija (ed.). *Environmental pollutants and risk communication:* 3^{rd} *congress of Slovenian toxicology society (*2017), Ljubliana.

A. Kovačič, C. Gys, A. Covaci, T. Kosjek, E. Heath. Photodegradation of bisphenols F, S and Z in aqueous solution. V: Pavlin, Majda (ed.), et al., 9th Jožef Stefan International Postgraduate School Students' Conference and 11th Young researchers' Day, (April 2017) Ljubljana, Slovenia.

Invited lectures related to the Thesis

E. Heath, M. Česen, Kovačič, T. Kosjek, C. Gys, A. Covaci, D. J. Heath. Bisphenols in food and environment: presented at 16th International Conference on Chemistry and the Environment (ICCE), (June 2017) Oslo, Norway.

Biography

Education and research

- 2016 – Present: Jožef Stefan International Postgraduate School, Ljubljana: PhD student of Ecotechnology
- 2013 – 2016: University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia: Master's degree in Organic chemistry
- 2016 – Present: Demonstrator of laboratory work at The Faculty of Chemistry and Chemical Technology of the University of Ljubljana
- 2010 – 2013: University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia; Bachelor in Organic chemistry

Foreign work and study experience:

- 2018 (2 months): University of Antwerp, Antwerp, Belgium, MassTwin project
- 2017 (2 months): University of Antwerp, Antwerp, Belgium, MassTwin project
- 2014 (6 months): Erasmus exchange at the University of Padova/Department of Chemistry, Department of Material science, Department of Chemistry Engineering,
- 2014 (1 week): Erasmus Intensive Programme (Summer school) at the University of Crete
- 2012 (1 month): Practical training at the University of Trieste/Department of Life Sciences

Fellowships and scholarships:

- 2014: Erasmus Plus Internship scholarship
- 2006 – 2012: Zois scholarship

Research projects:

- Young researcher grant and the Slovenian Research Agency (ARRS):
  Program Group **P1-0143**: Cycling of substances in the environment, mass balances, modelling of environmental processes and risk assessment,
  Project **N1-0047**: Bisphenol A Substitutions: Transition from Food Contact Materials, Circulation and Human Exposure,
  Project **J2- 8162**: Closure of Material Pathways in Urban Wastewater Treatment with Green Technologies,

Project **L7-1848**: Photocatalytic water treatment - development of immobilized catalysts and compact reactor systems,

Project **L1-7544**: Improved treatment and monitoring of Water Framework Directive priority pollutants,

Project **N1-0143**: Novel approaches for the estimation of the use of psychoactive pharmaceuticals and illicit drugs by wastewater analysis.

- MASSTWIN (European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 692241)
- ISO-FOOD project (ERA Chair for isotope techniques in food quality, safety, and traceability, under grant agreement no. 621329)