Project Fundamental Research Report

- PROJECT DETAILS

File number	G0E5216N
Supervisor and co-supervisor(s)	Hugo Neels, Adrian Covaci and Ester Heath
Project start and end date	1.1.2016-31.12.2020
Title of the research project	Bisphenol A alternatives: transfer from food contact materials, fate and human exposure
Host Institution(s)	University of Antwerp (BE) and Jožef Stefan Institute (SLO)
FWO Expertpanel	Chemistry (W&T4)

- RESEARCH

1. Progress and (final or intermediate) results of the research project. If you deviate(d) from the approved application please describe and motivate.

1. Characterization of migration conditions for bisphenols from FCMs

To characterise the presence of nine bisphenols (BPA, BPAF, BPAP, BPB, BPC, BPE, BPF, BPS, and BPZ) and related compounds (4-cumylphenol and dihydroxybenzophenone) in honey and food simulant, an analytical method was developed and validated (LODs in sub ng/g). We analysed 36 honey samples from European and non-European countries and food simulant stored in selected corresponding containers. Honey samples contained BPA, BPAF, BPE, BPF, BPS, and BPZ in amounts up to 107, 53.5, 12.8, 31.6, 302, and 28.4 ng/g, respectively.

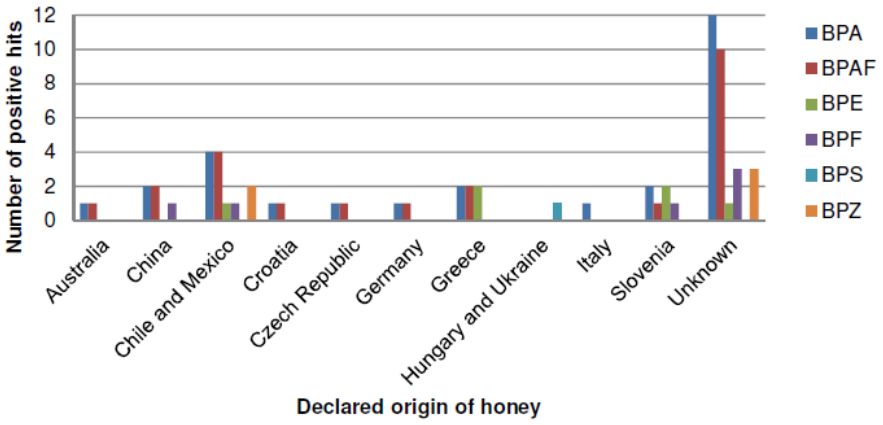

Fig. Under simulating conditions, BPA and BPAF were detected in food simulant up to 42.2 and 19.8 ng/mL, respectively. In certain cases, the detected bisphenols in honey probably derive from a source other than the final packaging (Česen et al., 2016).

In follow up, a GC-MS/MS method was developed and validated to establish the migration and stability of BPA and eleven other bisphenols in two food simulants (C: 20% ethanol, and B: 3% acetic acid) from beverage cans (n = 16) and reusable plastic and metal sports bottles (n = 51). Good method performance, based on ng/L LLOQs, acceptable recovery and uncertainty were achieved by following the Eurachem guidelines. Results showed that cans leached BPA (< 5865 ng/L), three BPF isomers (8.2–1286 ng/L) and

BPAP (1.6 ng/L), while bottles leached BPA (< 222 ng/L) and BPF, BPE, BPB and BPZ (1.1–4.6 ng/L). We found that simulant C was more aggressive than simulant B, and concentrations of bisphenols decreased with consecutive exposure to simulants.

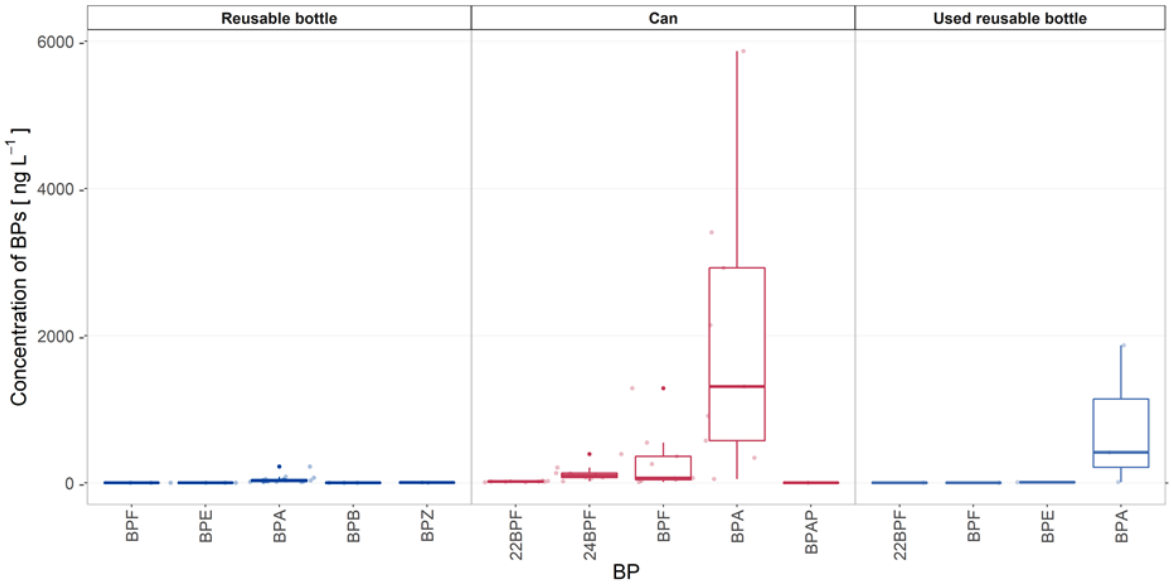

Fig. Box-plot showing concentrations of all detected BPs in both simulants (C and B) and all analysed containers (reusable sports bottle, cans and used reusable sports bottle).

The results show that BPA is remaining the primary bisphenol migrating from food contact materials (< SML) but at the same time confirm the migration of other bisphenols (Kovačič et al., 2020a).

2. *In vitro* biotransformation of bisphenols

The aims 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 pharmacokinetic studies. Metabolite identification was based on high resolution LC-QTOF-MS analysis combined with two complementary data analysis workflows: suspect and non-target 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 and environmental studies (Gys et al., 2018).

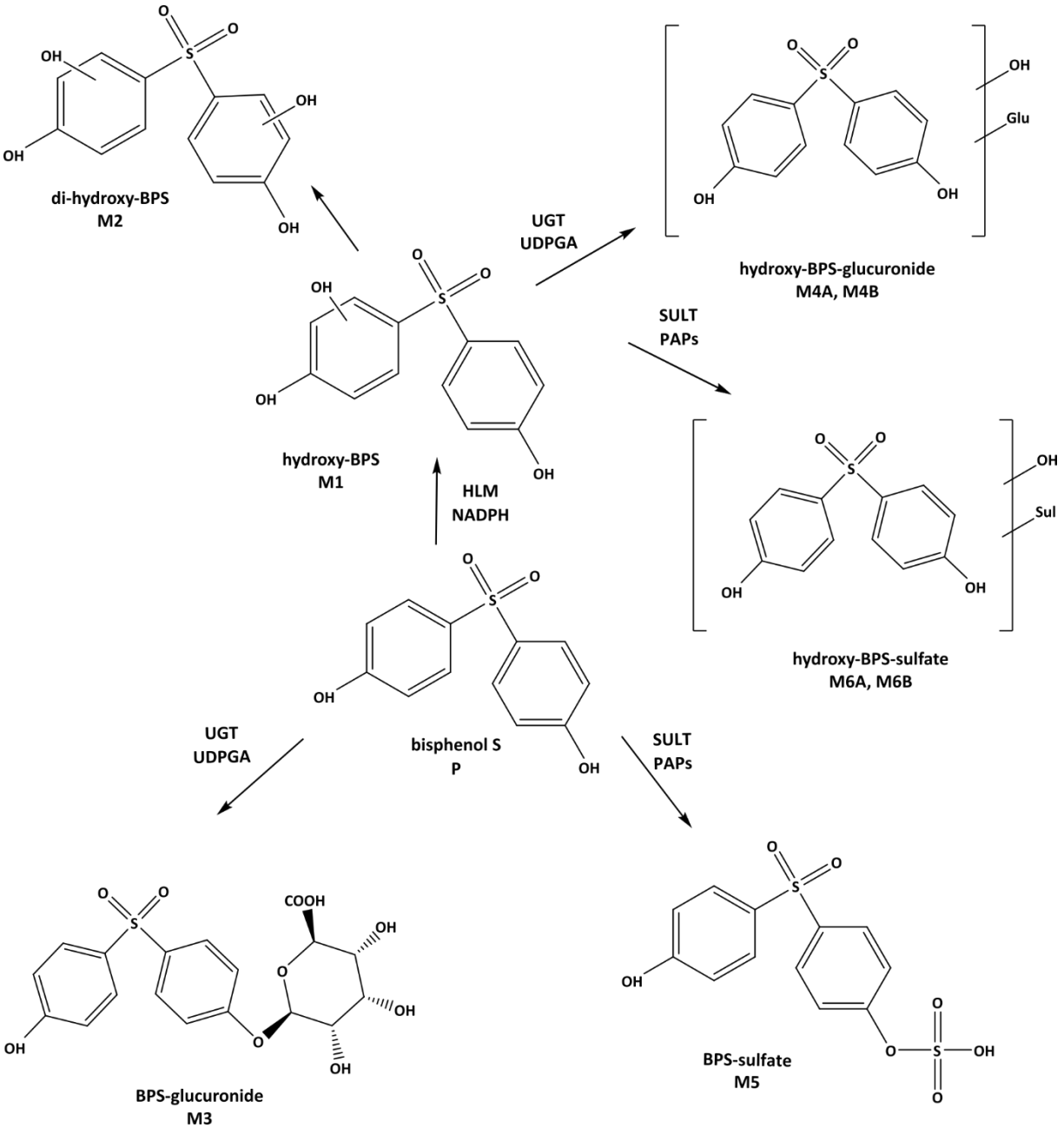

Fig. Proposed *in vitro* metabolic pathway for BPS. The arrows are marked with the enzymes and cofactors responsible for the reaction.

3. Removal/Transformation of bisphenols

Limited knowledge on the stability, removal, and transformation of BPA analogues in the aqueous environment and during wastewater treatment, exists. These data are of high importance because they can serve as basis for studying cycling and transformation of bisphenols in the environment and in FCMs.

In this context, we aimed to assess the stability and removal of eighteen common bisphenols by performing hydrolysis, adsorption, biological treatment and UV photolysis. Results showed that hydrolysis of bisphenols does not occur and confirmed their highest stability in methanol. We also found that biological treatment is efficient in removing the majority of bisphenols (> 85%).

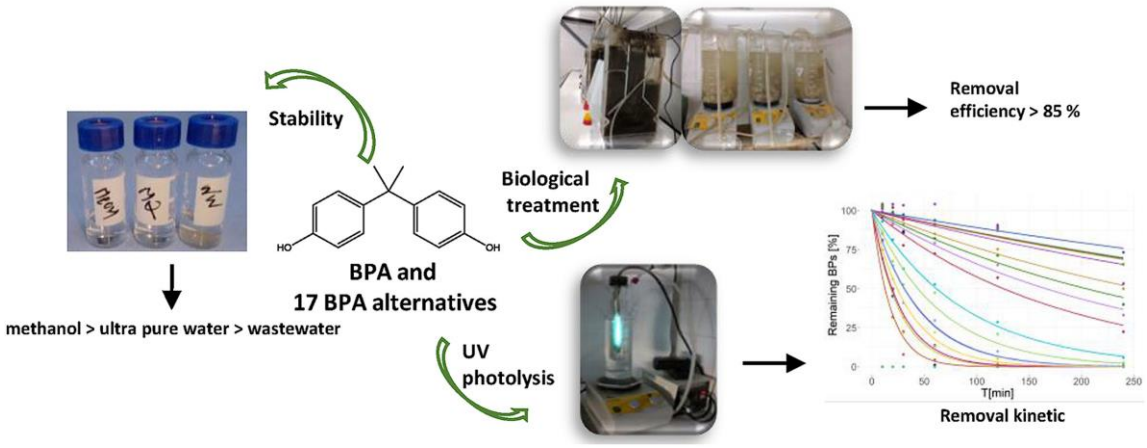

Fig. The removal of bisphenols was also successful by UV photolysis and followed pseudo-first-order kinetics (Kovačič et al., 2019a).

In continuation, we assessed photochemical degradation of three BPs commonly detected in the environment: BPF, BPS and BPZ. A comparison in degradation efficiency was made between 1) direct photolysis, 2) cyclodextrin-enhanced photolysis and 3) the photo-Fenton reaction in a bench top UV reactor.

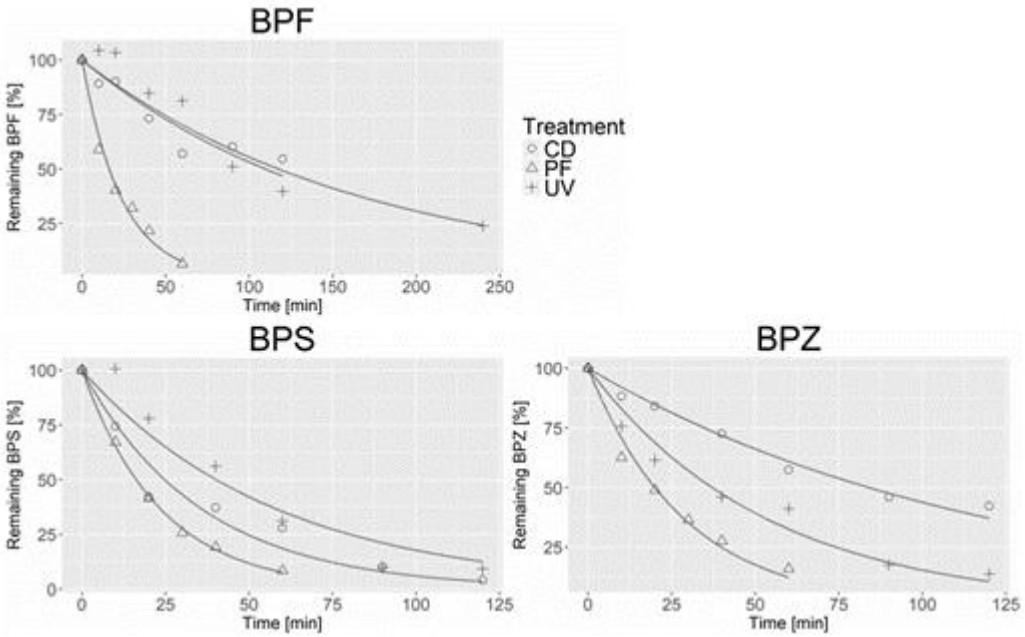

Fig. Kinetic profiles for BPF, BPS and BPZ using three different photodegradation treatments: exposure to UV irradiation (UV), exposure to UV with addition of ß-cyclodextrin (ß-CD) and exposure to UV with addition of H_2O_2 and Fe^{2+} (photo-Fenton reaction; PF).

Results showed that degradation followed pseudo-first order kinetics. The removal efficiency of BPs depended on the applied process, the photo-Fenton reaction resulting in the shortest half-lives. The study identified 11 novel transformation products and confirmed 8 previously reported TPs (Kovačič et al., 2019b).

In addition, the removal efficiency of twelve bisphenols from wastewater by hydrodynamic cavitation alone or in combination with UV irradiation was also investigated. The highest removal efficiencies of BPs were 15–63% on lab scale, while the pilot-scale experiments, performed at the real WWTP, showed the potential of hydrodynamic cavitation for a large-scale application as a pre-treatment technology (Kovačič et al., 2020b).

The occurrence and source identification of bisphenol compounds in wastewaters was also addressed. The investigated BPs were measured in WW within EU region for the first time. Most investigated BPs were detected > LOD in influents and effluents. BPZ is one of the mostly used BPA alternatives in Slovenia and BPC was quantified in WW for the first time.

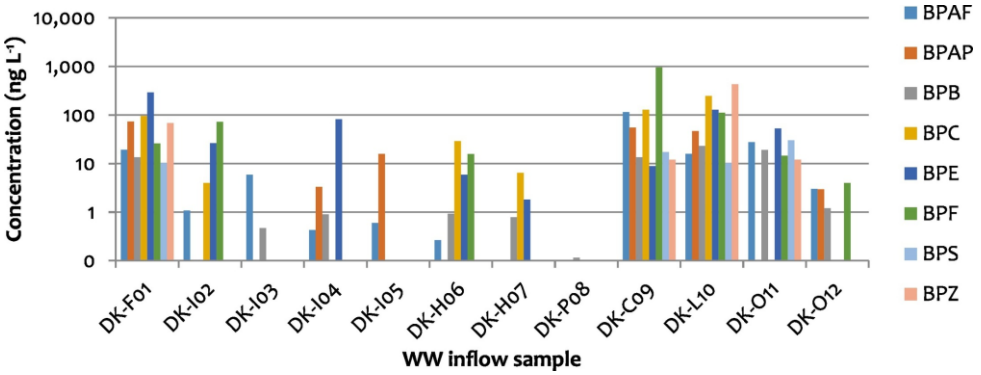

Fig. BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ concentrations in WW inflows collected in DK catchment.

As main point sources of BPs to WW food production/processing and textile cleaning facilities were identified (Česen et al., 2018a). Also, seasonal and spatial variations in the occurrence, mass loadings and removal of compounds of emerging concern including bisphenols in the aqueous environment were studied (Česen et al., 2018b, Česen et al., 2019).

Besides the investigation of removal efficiency, independent biodegradation experiments in batch reactors have been performed for studying the aerobic activated sludge kinetics and biotransformation of two most common BPA analogues: BPS and BPF. The degradation kinetics suggest that BPF and BPS do not tend to accumulate in biosolids or wastewater effluent.

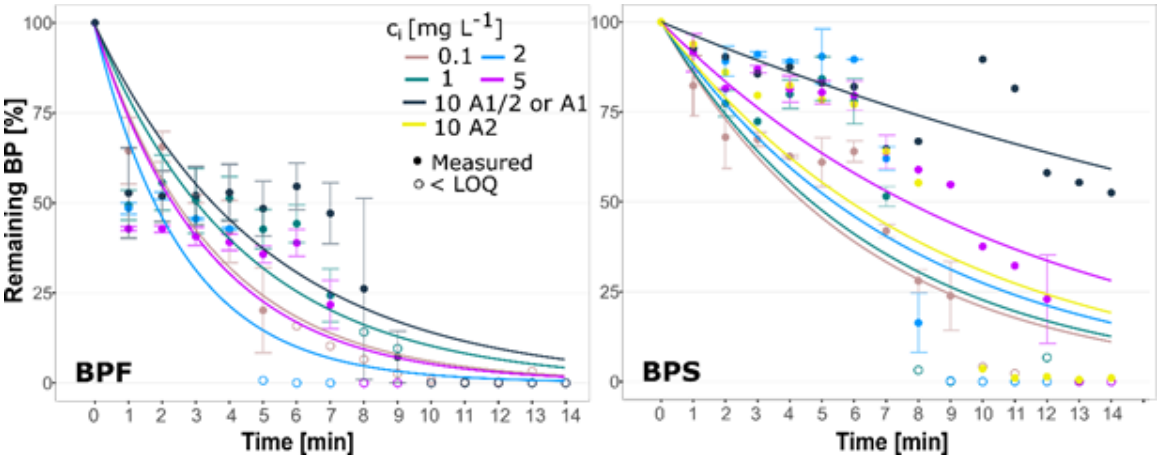

Fig. Aerobic degradation kinetics (single first-order) of BPF (left) and BPS (right) in AS. Vertical bars are standard deviations of parallels A1 and A2, except for BPS at 10 mg L^{-1}. Open circles are < LOQ.

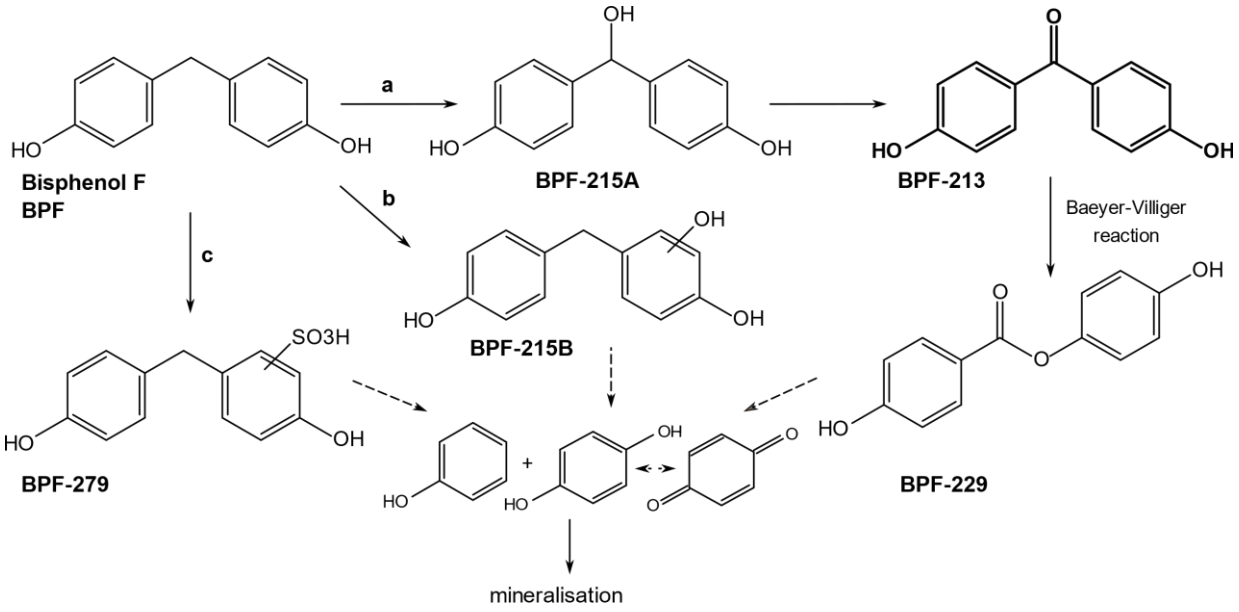

Fig. Proposed pathway for biodegradation of BPF in AS (a; aliphatic hydroxylation, b; aromatic hydroxylation and c; sulphation, dashed arrows; not identified).

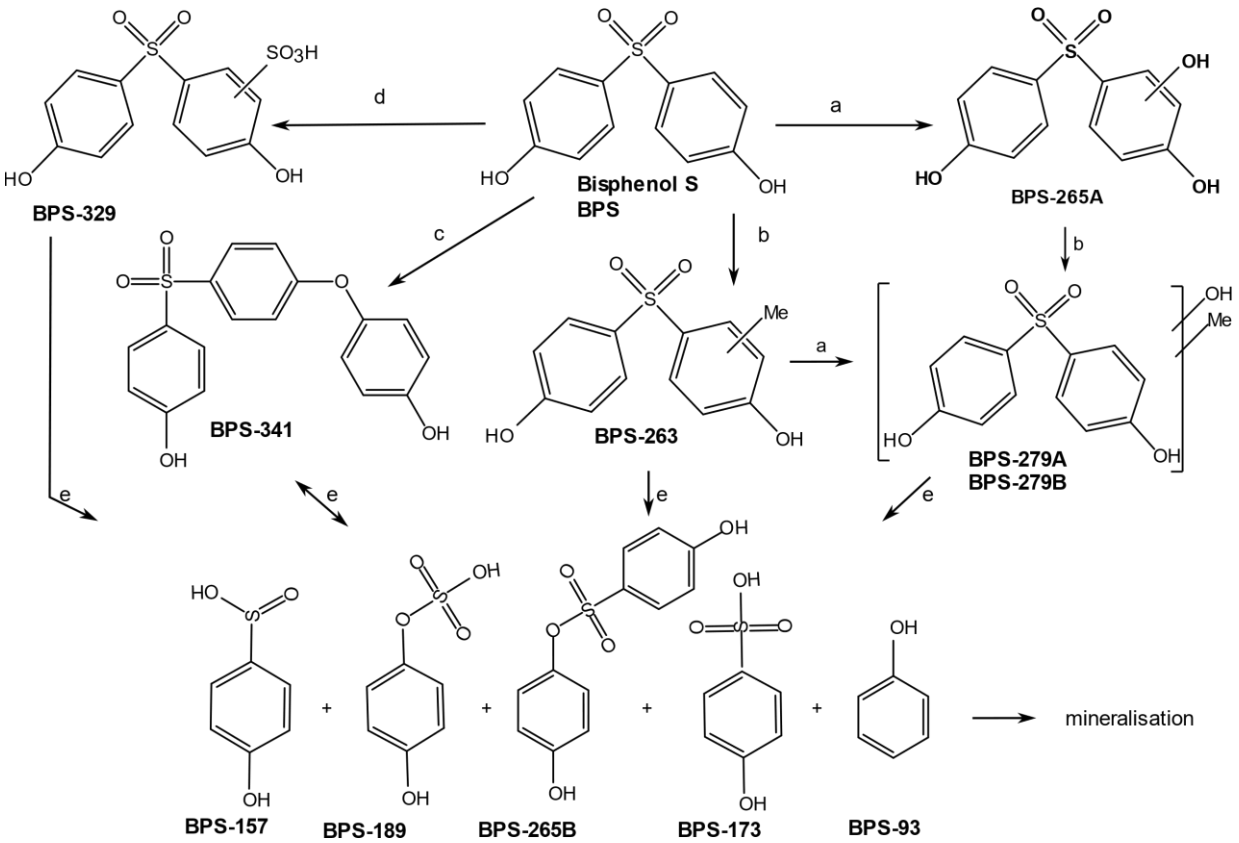

Fig. Proposed pathway for biodegradation of BPS in AS (a; hydroxylation, b; methylation, c; coupling of smaller BPS moieties, d; sulphation and e; cleavage of the S-C bond).

We identified one and ten novel biotransformation products and confirmed three and one previously reported biotransformation products of BPF and BPS, respectively. The results also reveal new biotransformation pathways, namely sulphation, methylation, cleavage of the S–C bond between the phenyl rings and the joining of smaller moieties. The findings of these studies will provide new insights, essential for understanding the fate of bisphenols during wastewater treatment and in the environment (Kovačič et al, 2020c).

The gained knowledge on non-target analysis in the identification of BP transformation during WW treatment was applied for studying BP transformation in FCMs by the assessment of chemical migration from FCM into food. Our initial findings were published (Kovačič et al., 2020a), where the presence of BPA, BPF, including its two isomers and the hydrolysis of diglycidyl ethers were suggested. Currently, we are optimizing the non-targeted analytical tools that could filter the relevant mass features from the number of false negatives, enabling the identification of unknown or unexpected leachable residues from FCM, including transformation products of bisphenols.

4. Toxicity of bisphenols

The estrogenic potential of different bisphenols (BPP, BPB, TBBPA, BPZ, BPPH, BPM, BPCII, BP26DM, BPAB, BPE, BP20H, BPC, BPAF, BPBP, BPFL) was determined by measuring their ability to induce MCF-7 breast cancer cell proliferation. Binding of estrogen to the estrogen receptor (ER) present on the surface of these cells led to activation of cell signaling pathways that promoted cell proliferation. The CyQuant DHA content assay was employed after optimization and validation using estrogen (17β-estradiol; E2), the ER antagonist ICI 182,780 and bisphenol. ICI 182,780 suppressed MCF-7 cell proliferation induced by BPA, BPS and BPF and confirmed to activate the estrogenic pathway. We found that all tested bisphenols, except BPFLinduce breast cancer cell proliferation with various potencies. The ability of BPB, BPZ and BPCII to induce cell proliferation was the highest among tested compounds (Petan et al., in preparation).

We also studied the toxicity of BPA, BPF and their mixture towards primary producers, the eukaryotic green alga *Pseudokircheneriella subcapitata* and the prokaryotic cyanobacterium *Synechococcus leopoliensis*. The results demonstrated that S. leopoliensis is more sensitive than P. subcapitata, whereas comparable to toxic potential of the two BPs. The toxicity of the binary mixture was predicted by different models and compared to experimental data. Additive effect was observed at low effect concentration range in *P. subcapitata*, whereas at high effect concentration range and in *S. leopoliensis* over the whole effect concentration range the effect was antagonistic. 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 represent environmental risk, whereas BPF does not (Eleršek et al, 2020).

5. Development and validation of multi-residue analytical method(s) for the simultaneous determination of bisphenols, their metabolites and transformation products in urine

At the Toxicological Centre, we developed an analytical method for measurement of 7 bisphenols in urine: BPA, BPAF, BPAP, BPB, BPF, BPS and BPZ. The method is based on deconjugation of bisphenol metabolites, solid phase extraction, derivatization and GC-MS/MS analysis and was successfully validated according to the guidelines for bioanalytical method validation of the European Medicines Agency. We assessed its performance and showed that the method reaches relevant and sufficiently low determination levels, between 0.03 µg/L and 0.25 µg/L.The performance was assessed for BPA, BPF, BPS and BPZ during successful participation in multiple rounds of the HBM4EU ICI scheme and the OSEQAS external quality assessment scheme (Gys et al., 2020a).

6. Assessment of human exposure

The levels of bisphenols were determined in urine of Slovenian children and adolescents, showing high detection rates for BPA and BPF. The urinary levels found in the investigated population are comparable to the levels found across the world. Adolescents showed higher urinary levels of BPF in comparison to children. BPA was significantly associated with high fat food items (Tratnik et al., 2019).

The internal exposure to seven bisphenols was assessed in urine samples (n = 396) from 7-year-old children from Japan, collected between 2012 and 2017. This allowed the evaluation of time trends in the concentrations of bisphenols. Information on demographic, indoor environment and dietary characteristics of participants were acquired through a self-administered questionnaire. All bisphenols were detected in the study population, with BPA, BPF and BPS showing detection frequencies > 50%. Concentrations of bisphenols measured in the Japanese children in this study were generally lower compared to studies worldwide. We found that BPA concentrations decreased significantly over the study time period (average 6.5% per year), whereas BPS rose with 2.8% per year. Levels of BPA and BPF were higher in autumn compared to winter.

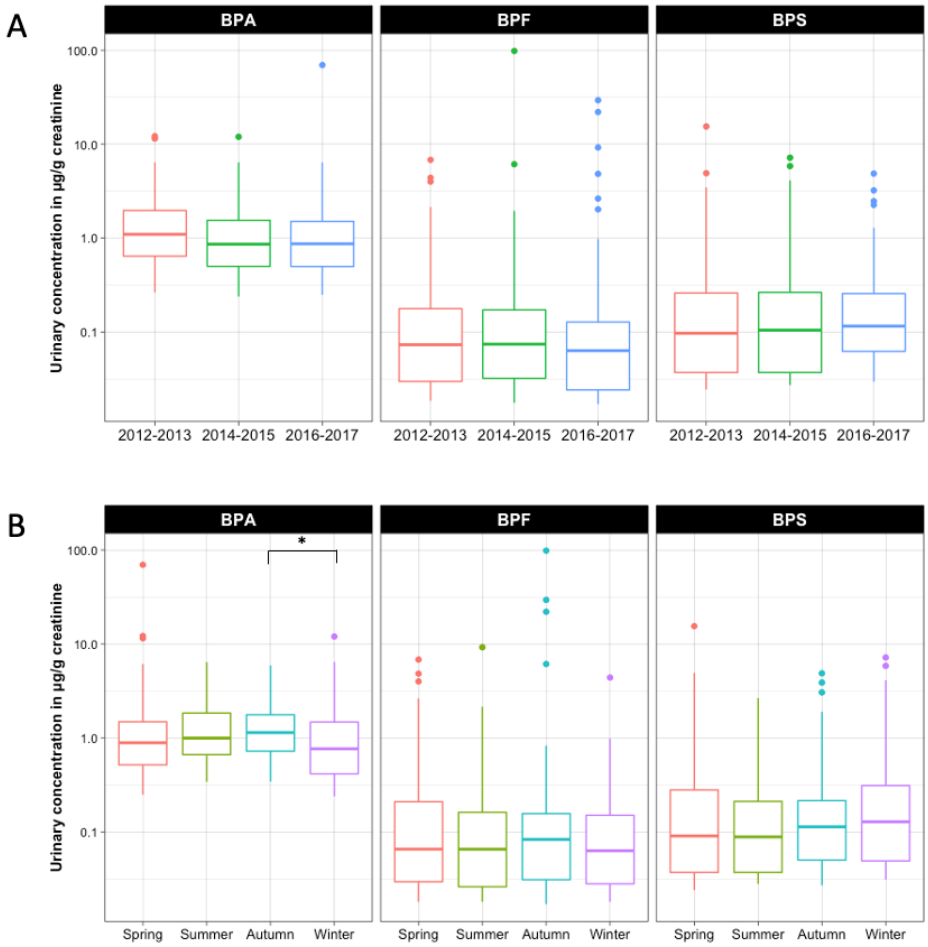

Fig. Boxplots for comparison of creatinine-corrected bisphenol concentrations with the year of urine collection (A) and the season of sampling (B). *: $p < 0.05$ for Kruskal-Wallis test with pairwise comparisons, Bonferroni-correction.

Higher urinary BPF levels were significantly associated with higher concentrations of the oxidative stress biomarker, 8-hydroxy-2'-deoxyguanosine. BPA and BPF levels were higher in children from families with lower household income. Bisphenol concentrations were significantly influenced by some other personal (e.g. household income), food intake (e.g. vegetables and cow milk) and indoor housing characteristics (e.g. flooring) (Gys et al., 2020a).

In the framework of the 4th cycle of the Flemish Environment and Health Study, BPA and 5 alternative bisphenols were measured in the urine of 423 Flemish adolescents. All included compounds were detected in the urine samples of the study population, with BPF, BPA and BPS showing high detection frequencies (83-97%), indicating extensive and continuous exposure. Despite still being the predominant bisphenol, showing highest levels, BPA concentrations in Flemish adolescents had decreased significantly compared to previous measurements during the second cycle of the Flemish Environment and Health Study in 2008. Levels of BPA, BPF and BPS were generally comparable to those reported in literature. Both active and passive smoking were associated with higher bisphenol levels. Some food consumption and product use variables showed significant associations with higher levels of BPA and BPF. Estimated daily intakes (EDI) of BPA, BPF and BPS were calculated based on measured internal exposure and were in the same range as or lower than reported values in literature. Even in a high-exposure scenario, the EDI of BPA stayed below the TDI that was established by the European Food Safety Authority (Gys et al., 2020b).

In order to evaluate the short-term variability of BPA and alternative bisphenols, we collected all spot urine samples from ten healthy adults for five consecutive days, and an additional 24 h pooled sample. We measured the concentrations of seven bisphenols (BPAF, BPF, BPA, BPB, BPZ, BPS and BPAP) in these samples. BPA, BPF and BPS were frequently found in spot samples (>80%), while bisphenol AP (BPAP) was detected in 43% of spot samples. Calculations of intra-class correlation coefficients showed that reproducibility of these four bisphenols was poor to fair (<0.01-0.200) but improved when concentrations were corrected for urine dilution using creatinine levels (0.128-0.401). In general, the within-participant variability of bisphenol levels was the largest contributor to the total variance (47-100%).

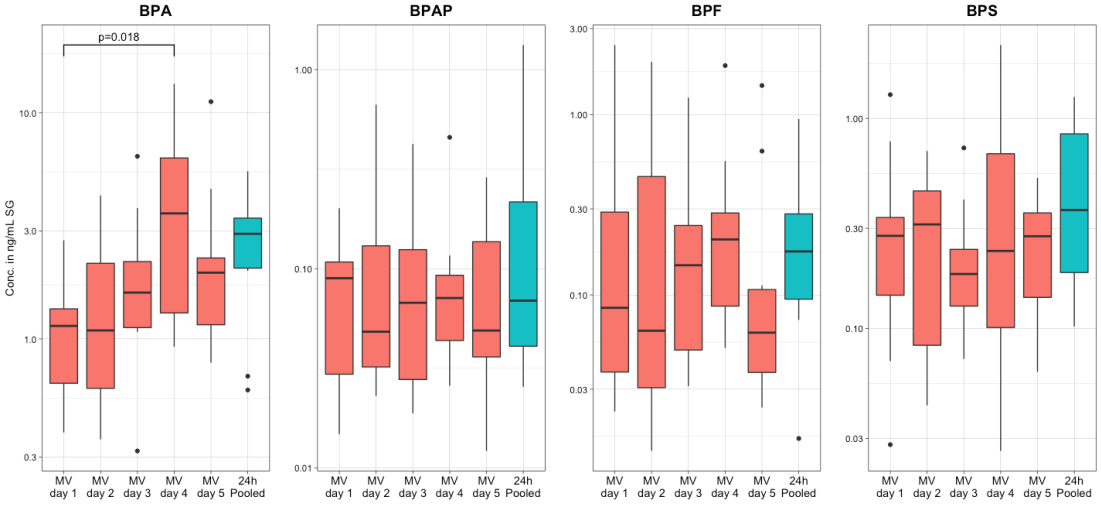

Fig. Boxplots of bisphenol concentrations in repeated morning void samples (MV, n = 50) and 24 h pooled urine samples (n = 10), corrected for specific gravity.

We compared repeated first morning voids to 24 h pooled urine and found no significantly different concentrations for BPA, BPF, BPS, or BPAP. Levels of BPA and BPF differed significantly depending on the sampling time throughout the day. The findings in this study suggest that collecting multiple samples per

participant over a few days, in predefined time windows throughout the day, could result in a more reliable estimation of internal exposure to bisphenols in future biomonitoring studies (Gys et al., 2020c).

7. Occurrence of Bisphenol developers in thermal paper

Thermal printing is a fast, widespread and inexpensive technology that uses a developer to produce a print on the paper, among many applications. A common developer is bisphenol A (BPA), used for this purpose in its free form. Consequently, the handling of thermal paper, as evaluated by the European Food Safety Authority, was reported to be the second largest source of external human exposure to this endocrine disruptor. Recently, reports have been made on the substitution of BPA by alternative developers, which are yet less studied. In this study, 311 receipts and other thermal paper products were collected from 14 countries in Europe, Asia, North America and Oceania and analysed using LC-QTOFMS.

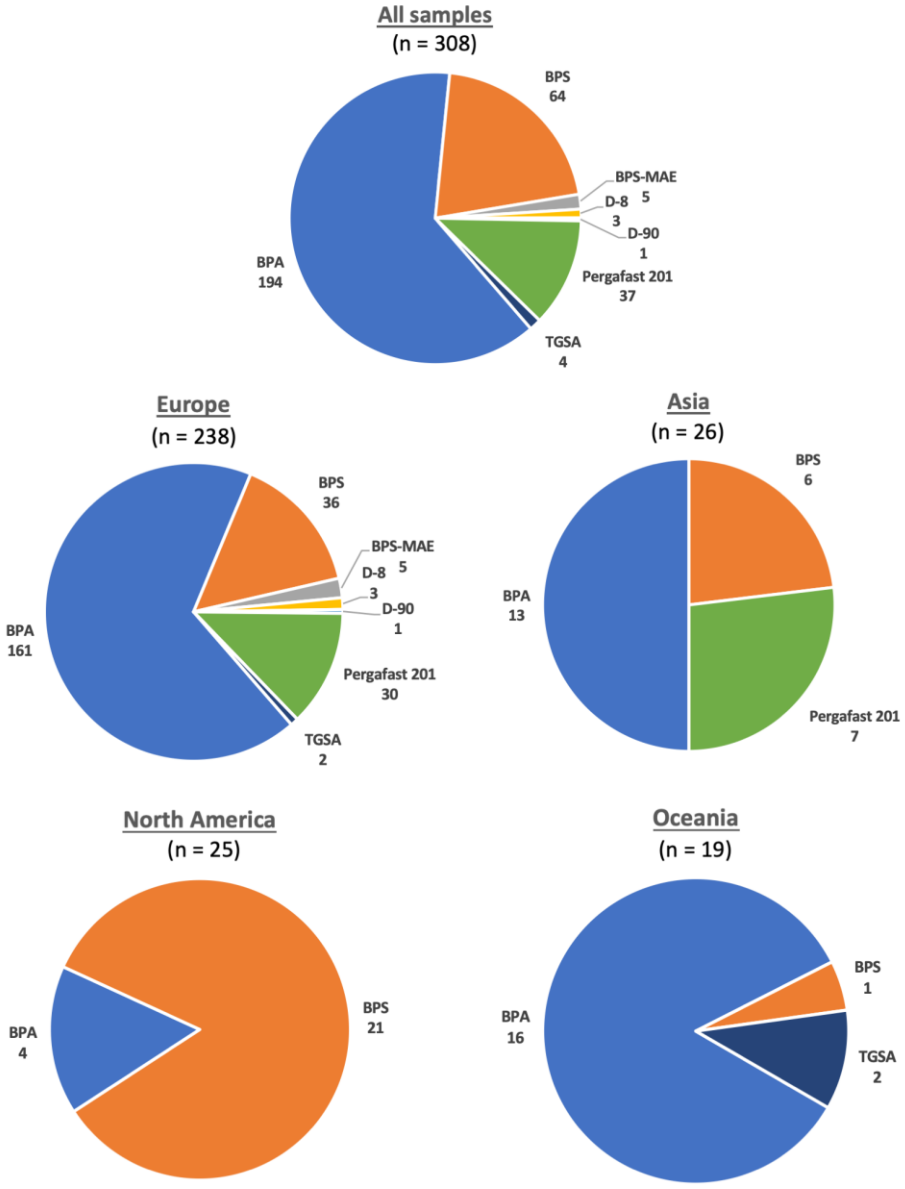

Fig. Overview of the main developer in the thermal paper samples with the absolute detection numbers, categorized by their continent of origin

BPA was the most frequently used main developer and was detected in 194 thermal paper samples, which represents a detection frequency of 63%. A statistically significant difference in the detection of BPA was shown between continents. BPA was followed by bisphenol S (BPS) which was detected in 64 samples as the main developer. Pergafast 201 was the third most abundant main developer and detected in 37 samples as the main developer. Less frequently used main developers included BPS-MAE, TGSA, d-8, and d-90, many of them being BPS derivatives. Two oligomers of d-90 (n = 1 and n = 2) were also identified. The sensitizer diphenyl sulphone (DPS) was identified using high-resolution mass spectrometry for the first time and detected in combination with other developers than BPS for the first time. Despite the lack of structural, nation-wide legislation prohibiting the use of BPA in thermal paper, it is clear that alternative developers are currently globally in use for the manufacturing of thermal paper.

2. List of publications based on the research project

GUIDELINES:

- ***Only mention publications realized since the beginning of the fellowship and directly relevant to the research project.*** *Other papers should not be listed and will not be considered by the panel.*
- *Provide* ***full bibliographical data*** *for each publication (journal, page numbers, impact factor in publication year, citations, etc.) and list them in chronological order.* ***Please indicate clearly for the publications resulting from the research supported by FWO the share of effort of each author funded by FWO. It is important to present correctly the real contribution and merit of the different researchers.***
- *Clearly indicate whether the publication is published, accepted for publication (indicate in or by which journal).* ***Papers that are merely 'in preparation' should not be listed and will not be considered by the panel.***
- *Use the following classification:*

Papers

a1.1. *Articles (a) included in Web of Science's (WoS) Science Citation Index Expanded, Social Science Citation Index and/or Arts and Humanities Citation Index, whose document type is labelled as "Article", "Review", "Letter", "Note" and/or "Proceedings Paper" or (b) in journals included in the Journal Citation Reports (JCR) of Web of Science.*

a1.2. *Peer-reviewed articles in journals with an approved by the "Gezaghebbend Panel van het Vlaams Academisch Bibliografisch Bestand in the Social Sciences and Humanities (VABB-SHW).*

a2. *Articles in scientific journals not included in A1.1. or A1.2.*

Books

b1. *Author or co-author of published books (limited to books published by a scientific publishing company; no syllabuses, no theses).*

b2. *Chapters in books (no conference proceedings).*

b3. *Books as editor (including editor of conference proceedings).*

Other publications

c1. *Papers in proceedings of scientific conferences, that do not belong to any of the previous categories (full articles, no abstracts).*

c2. *Doctoral dissertations*

c3. *Patents*

c4. *All other publications or items of scientific output which are relevant to the application and cannot be included in any of the previous categories.*

a1.1. Articles (a) included in Web of Science's (WoS) Science Citation Index Expanded

1. Česen, M, Lambropoulou, DA., Laimou-Geraniou, M, Kosjek, T, Blaznik, U, Heath, DJ, Heath, E. Determination of bisphenols and related compounds in honey and their migration from selected food

contact materials. *Journal of agricultural and food chemistry*, 2016, vol. 64, no. 46, str. 8866-8875, doi: 10.1021/acs.jafc.6b03924.

2. Česen, M, Lenarcic, K, Mislej, V, Levstek, M, Kovacic, A, Cimrmancic, B, Uranjek Ževart, Kosjek, T, Heahh, DJ, Sollner Dolenc, M, Heath, E. The occurrence and source identification of bisphenol compounds in wastewaters. *Science of the Total Environment*, 2018a, 616-617, 744-752, doi: 10.1016/j.scitotenv.2017.10.252
3. Česen, M., Heath, D., Krivec, M., Ko, J., Kosjek, T., Heath, E. 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* 2018b, 242. https://doi.org/10.1016/j.envpol.2018.06.052
4. Česen, M., Ahel, M., Terzić, S., Heath, D.J., Heath, E. The occurrence of contaminants of emerging concern in Slovenian and Croatian wastewaters and receiving Sava river. *Sci. Total Environ*. 2019, 650, 2446–2453. https://doi.org/10.1016/j.scitotenv.2018.09.238
5. Elersek T, Notersberg T, Kovačič A, Heath E, Filipič M. The effects of bisphenol A, F and their mixture on algal and cyanobacterial growth: from additivity to antagonism. *Environ Sci Pollut Res* (in press, 2020)
6. Gys, C, Kovacic, A, Huber, C, Lai, FY, Heath, E, Covaci, A. Suspect and untargeted screening of bisphenol S metabolites produced by in vitro human liver metabolism. *Toxicology Letters*, 2018, 295, 115-123, doi: 10.1016/j.toxlet.2018.05.034.
7. Gys, C., Bastiaensen, M., Ait Bamai, Y., Araki, A., Caballero-Casero, N., Kishi, R., Covaci, A. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. *Environmental Research*, in press. 2020a
8. Gys, C., Bastiaensen, M., Bruckers, L., Colles, A., Koppen, G., Govarts, E., Den Hond, E., Nelen, V., Schoeters, G., Covaci, A. Determinants of exposure levels of bisphenols in Flemish adolescents. *Environ Res*, in preparation, 2020b.
9. Gys, C., Bastiaensen, M., Malarvannan, G., Ait Bamai, Y., Araki, A., Covaci, A. Short-term temporal variability of bisphenols in spot, morning void and 24-hour urine samples. *Environmental Pollution*. Submitted, 2020c
10. 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
11. Kovačič, A, Gys, C, Kosjek, T, Covaci, A, Heath, E. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics. *Science of the Total Environment*, 2019b, 664, 595-604, https://doi.org/10.1016/j.scitotenv.2019.02.064
12. Kovačič, A., Gys, C., Gulin, M.R., Kosjek, T., Heath, D., Covaci, A., Heath, E., 2020a. The migration of bisphenols from beverage cans and reusable sports bottles. *Food Chem*. 127326. https://doi.org/10.1016/j.foodchem.2020.127326
13. Kovačič, A., David, Š., Zupanc, M., Gostiša, J., Bizjan, B., Kri, N., Sollner, M., Heath, E., The removal of bisphenols and other contaminants of emerging concern by hydrodynamic cavitation : From lab-scale to pilot-scale. *Sci Total Environ* 2020b, 743, 140724. https://doi.org/10.1016/j.scitotenv.2020.140724
14. Kovačič A, Gys C, Gulin MR, Gornik T, Kosjek T, Heath D, Covaci A, Heath E. Kinetics and Biotransformation products of Bisphenol F and S during Aerobic Degradation with Activated Sludge. *Journal of Hazardous Materials* (corrected manuscript submitted after major revision, 2020c).
15. Petan T., Kump A., Heath E., Križaj I. Estrogenicity testing of selected bisphenols. *Toxicology Letters.* In preparation.
16. Snoj Tratnik, J, Kosjek, T, Heath, E, Mazej, D, Čehic, S, Karakitsios SP, Sarigiannis, D, Horvat, M. Urinary bisphenol A in children, mothers and fathers from Slovenia : overall results and determinants of exposure. *Environ Res*, 2019, 168, 32-40, doi: 10.1016/j.envres.2018.09.004.

17. Vervliet, P.*, Gys, C.*, Caballero-Casero, N., Covaci, A. (2019). Current-use of developers in thermal paper from 14 countries using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Toxicology. 416: 54-61. (* contributed equally) https://doi.org/10.1016/j.tox.2019.02.003

3. Collaboration between the researchers c.q. research teams

The following research groups are involved in this project:
Group 1: Toxicological Centre, University of Antwerp, Belgium
Group 2: Group of Organic Analysis, Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia

The collaboration between the research groups is excellent. There were half-yearly meetings (mostly online) to present and discuss the progress of the research. The researchers were, also, in contact with each other on a daily/weekly basis.

Furthermore, there were various exchanges of researchers:

Ester Heath and Ana Kovačič visited the University of Antwerp in November 2016 to analyse samples generated in a biodegradation experiment on BPS, BPC2 and BPZ, using high resolution LC-QTOF-MS.

Ana Kovačič visited the University of Antwerp for two months in 2017 (November – December) and for two months in 2018 (November – December). The aim of the first visit was to participate in the study of *in vitro* metabolism of BPS and *in vivo* metabolism in serum and urine samples. The main aim of her second visit was to evaluate the migration of chemicals, focusing on bisphenols and their possible transformation products, from FCMs. Additionally, the biodegradation of selected bisphenols was monitored in samples generated in a benchtop wastewater treatment experiment previously carried out at Jožef Stefan Institute. During both visits, analyses were performed using high resolution LC-QTOF-MS.

Celine Gys visited the Jožef Stefan Institute for one month in March 2017 and for two months in 2018 (February – March). The aim of the first visit was to participate in the designing and performed of the photodegradation experiment of three selected bisphenols in a benchtop UV reactor. The aim of the second visit was to take part in a preliminary biodegradation study and estrogenic activity experiment.

Adrian Covaci visited Jožef Stefan Institute in March 2017 during the stay of Celine Gys. The aim of his visit was to discuss the progress of the photodegradation experiment and plan further collaboration.

Ester Heath visited the University of Antwerp in November 2017 and December 2018 to discuss the ongoing research work and plan further collaboration.

Milena Horvat, Nives Ogrinc, David Heath, Ester Heath, Dušan Žigon, Marjeta Česen, Tjaša Gornik and Ana Kovačič from the Jožef Stefan Institute visited the University of Antwerp to participate in the MassTwin Workshop Training: "Mass spectrometry in support of the environment, food, and health interaction and disease", which took place 18th until 20th April 2018. The purpose of the workshop was to present results in the form of oral and poster presentations, exchange knowledge and plan for future collaborations.

4. Other impact of the research

Fruitful collaboration between University of Antwerp and Jožef Stefan Institute scientists in this FWO-ARRS project led to another joint project (FWO-ARRS: Novel approaches for the estimation of the use of

psychoactive pharmaceuticals and illicit drugs by wastewater analysis, G060920N) that started in January 2020.

Also, an application for an ITN project (FoodTraNet: Advanced Research and Training Network in Food Quality, Safety, Security) was submitted in Jan., 2020, where JSI is coordinating and UAntwerp a partner organization. Results are expected in September, 2020.

Member of task force on advice 04-2019 "Toxicological concerns on bisphenol A alternatives in food contact materials" as external expert, commissioned by the scientific committee (SciCom) of the Belgian Federal Agency for safety of the food chain (FAVV).

This research will have an impact on the national and international debate on endocrine disruptors in humans and on decision making in the process on research priorities related to the current status of substances identified as endocrine disruptors (EDs), or under evaluation for endocrine disrupting properties within the EU

5. Science Communication

FWO encourages its researchers to disseminate the results of their research widely, and valorize them where possible. In this part you have to indicate which actions you have undertaken in the context of science communication and science in society.

Research carried out also within this FWO-ARRS project was included in series of conference presentations, taks at Universities and also radio/TV interviews. Some are listed below, full list is available at http://izumbib.izum.si/bibliografije/A20200714174119-12315.html.

The results of our project have been disseminated through different channels:
i) To peers: through presentations at international congresses and meetings and through publication in A1 journals.
(ii) To professionals and scientists through presentations at different occasions. in other fields, such as environmental scientists, human health safety, human exposure, etc.

Organization of the MASSTWIN Exploratory Workshop on mass spectrometry in support of the environment, food and health interaction and disease, held 18-20 April 2018, Antwerp, Belgium.

Lectures at Universities abroad

HEATH, Ester. *Case study : bisphenols in the environment, food and biological fluids : invited talk, Aristotle University of Thessaloniki, Department of Chemistry, 1st March 2018, Thessaloniki, Greece*.
HEATH, Ester. *Sources, cycling and fate of compounds of emerging concern : bisphenols as a case study : invited talk, University of Belgrade, Faculty of Chemistry, 15 December 2017, Belgrade, Serbia*.
HEATH, Ester. *Organic pollutants in the environment : occurrence, fate and cycling : invited talk, Aristotle University of Thessaloniki, Department of Chemistry, 27. Feb. 2018, Thessaloniki, Greece*.
HEATH, Ester. *Case study on cycling of compounds of emergig concern during wastewater treatment and in the environment : invited talk, Institute of Natural Sciences, Korea National University of Education, 3rd Nov. 2017, Chungbuk, Korea*.
HEATH, Ester. *Spojine, ki povzročajo motnje v endokrinem sistemu : vabljeno predavanje, Univerza v Ljubljani, Biotehniška fakulteta, 24. april 2016, Ljubljana, Slovenija*.

Invited lectures at conferences

HEATH, Ester, ČESEN, Marjeta, KOVAČIČ, Ana, KOSJEK, Tina, GYS, Celine, COVACI, Adrian, HEATH, David John. *Bisphenols in food and environment : presented at 16th International Conference on Chemistry and the Environment (ICCE), 18-22 June 2017, Oslo, Norway*.

HEATH, Ester. Compounds of emerging concern during wastewater treatment and in the environment : a case study on bisphenols : [keynote lecture]. V: DOĞAN, Nezahat (ur.). *Conference proceedings : er*. 2020, str. 105.

HEATH, Ester. Bisphenols in food and the environment : keynote lecture. V: *Abstracts & proceedings*, XX. Euroanalysis, September 1-5, 2019, Istanbul University, Istanbul, Tu. [S. l.: s. n.]. 2109. http://euroanalysis2019.com/ester-heath/.

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Adrian Covaci. 2nd International Conference on Environmental Pollution and Health. Hunting for human biomarkers for emerging contaminants Guangzhou, China, 14/06/2016

Adrian Covaci. 39th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2019) Human Biomonitoring and Exposomics of Legacy and Emerging Chemicals. Kyoto, Japan, 25-30/08/2019

Adrian Covaci. 1st Iberian Meeting in Separation Sciences and Mass Spectrometry. Trends in mass spectrometry for human biomonitoring and exposomics. Santiago de Compostella, Spain, 08-11/10/2019

Adrian Covaci. 9th International Symposium on Recent Advances in Food Analysis. Human biomonitoring of emerging chemicals: current trends and implications in the exposome, Prague, Czech Republic, 05-08/11/2019

Adrian Covaci. International Symposium for Exposure Science (ISES 2016). Emerging contaminants in the Flemish Environment and Health biomonitoring Surveys (FLEHS), Utrecht, the Netherlands, 10/10/2016

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HEATH, Ester. *isotopes in analytical chemisrty : presented at Training Workshop on Isotope Techniques in Ecologocal, Food and Environmetal Research, 29-30 January 2019, Ljubljana, Slovenia*.

HEATH, Ester, ČESEN, Marjeta, KOVAČIČ, Ana, KOSJEK, Tina, GYS, Celine, COVACI, Adrian, HEATH, David John. *Bisphenols in food and environment : presented at 16th International Conference on Chemistry and the Environment (ICCE), 18-22 June 2017, Oslo, Norway*.

HEATH, Ester. *Pharmaceutical residues : where do they end up? : presented at 6th EuCheMS Chemistry Conference, 14 September, Seville, Spain*.

Oral and poster presentations

Gys, C., Ait Bamai, Y., Araki, A., Caballero-Casero, N., Kishi, R., Covaci, A. Monitoring exposure levels to bisphenols in Japanese schoolchildren. 11th International Symposium on Biological Monitoring in Occupational and Environmental Health, Leuven, Belgium, 28-30 August 2019.

P. Vervliet, C. Gys, N. Caballero-Casero, A. Covaci, Current use of developers in thermal paper from 14 countries by LC-HRMS. Poster, 11th International Symposium on Biological Monitoring in Occupational and Environmental Health (ISBM-11), Leuven (Belgium), 28-30 August 2019.

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Gys, C., Kovačič, A., Huber, C., Oh, J., Ahn, YA., Kim, S., Lai, FY., Heath, E., Covaci, A. 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.

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Gys, C., Kovačič, A., Kosjek, T., Covaci, A., Heath, E. Photochemical degradation of bisphenols F, S and Z in aqueous solution: kinetics of degradation and identification of transformation products. International Conference on Chemistry and the Environment, Oslo, Norway, 18-22 June 2017.

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