3. CONTENTS

3.1. Introduction

Over the last decades, the incidence of gestational diabetes (GD) is increasing and currently one in seven births is affected by GD[1]. This is a significant public health concern, as GD is associated with dramatic adverse consequences, including preeclampsia; birth complications, cesarean delivery, as well as long-term risk of type 2 diabetes, obesity, and cardiovascular disease for both the mother and child[2]. Thus, it is critical to identify alternative root causes of GD[3]. Given the compelling evidences already available between exposure to phenolic compounds with T2D[4] and the extensive exposure of pregnant women to these chemicals, this study aims to evaluate the levels of these chemicals in pregnant women with and without GD.

3.2. Procedure

3.2.1. Sampling

This study was approved by the Ethics Committee of Centro Hospitalar do Baixo Vouga (in which Aveiro Hospital is included) and by the Portuguese National Data Protection Agency. Samples from 45 pregnant women were collected. From these women, morning spot urine samples were collected during the first consultation of the 1st trimester. An aliquot of each sample was transported to CMES in cool conditions and preserved at -20^{o}C until analysis.

3.2.2. Chemical analysis

At CMES, levels of triclosan, triclocarban, parabens (methyl, ethyl, propyl, butyl), benzophenones 2-OH-4-MeO-BP (BP-3); 2,4-diOH-BP (BP-1); 2,2'-diOH-4-MeO-BP (BP-8); 2,2',4,4'-tetraOH-BP (BP-2); 4-OH-BP) and bisphenols (BPA, BPB, BPC, BPF, BPS, BPZ, BPAF, TBBPA) were quantified in urine samples. The target compounds were analyzed following the protocol described by Kunisue et al (2010)[9] after some modifications and optimization by the team members (see LaMer 2017/18 report). Briefly, 50 µL of Internal Standards (ISs) Mixture was added to each sample after being hydrolised with β-glucuronidase/aryl-sulfatase for 16h at 37^{0}C. Afterwards, cold methanol, ultrapure water and ammonia solution (5% NH_4OH) were added. The samples were then loaded into a pre-conditioned OASIS MAX cartridge (MTBE, Methanol and ultrapure water). Prior to elution with formic acid:MTBE:methanol = 0.2: 3: 7 (v/v/v), the cartridges were washed with 5% NH_4OH, 5% NH_4OH in methanol, Milli-Q-water: methanol = 0.2: 6: 4 (v/v/v) and afterwards completely dried for 15 min. The eluted target compounds were evaporated to

dryness under nitrogen flux and re-dissolved with acetonitile and 50 µL of mixture of naproxen-$^{13}C,d_3$ and ketoprofen-$^{13}C,d_3$ (20 ng/mL each) was added. The samples were preserved in amber LC glass vials at 4^0C and before injection into the LC-MS/MS, Milli-Q water was added to a final volume of 1mL.

The internal standard (ISs) mixture contains 100 ng/mL of Triclosan-$^{13}C_6$, 20 ng/mL of Triclocarban-13C_6, 20 ng/mL of Methyl paraben-$^{13}C_6$ and 20 ng/mL of Butyl paraben-$^{13}C_6$).

The activity of β-glucuronidase/aryl-sulfatase was 290 units per mL of urine. The solution was prepared twice a week by adding 4.7 ml of 1.0 mol/L ammonium acetate; 5.3 mL of 1.0 mol/L acetic acid and 50 µL of β-glucuronidase/aryl-sulfatase solution (116,000 units/mL). The crude mixture of β-glucuronidase/sulfatase from Helix pomatia (Type HP-2, aqueous solution, 116,000 units/mL glucuronidase and 1020 units/mL sulfatase) was purchased from Sigma-Aldrich (St. Louis, MO, USA); (G7017).

3.4. Results

Of the 19 compounds analyzed, five were not detected in any sample being always bellow their respective detection limit (Triclocarban, Bisphenols AF, B, C and Z). Overall, it was possible to detect at least two EDCs in each sample, with an average of 6.4 compounds per sample. The most frequent compounds were Bisphenol-F and Methylparaben, detected in 97.8% of the samples, followed by Benzophenone-1 and Ethylparaben detected in 93.3 and 91.1% of the samples (Figure 1).

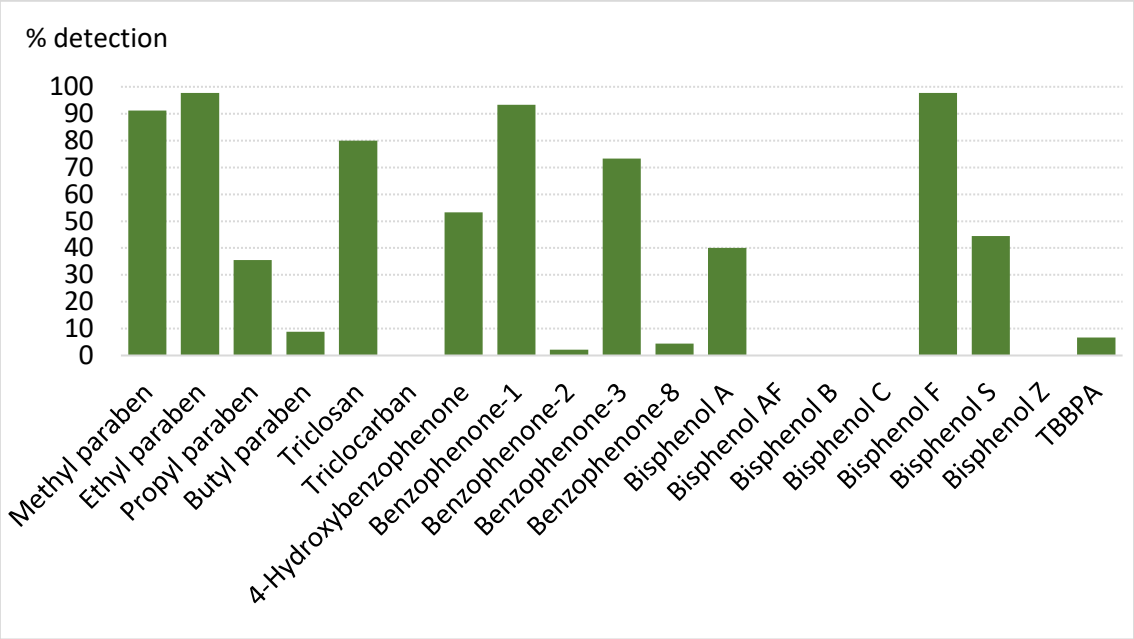

Figure 1. Percentage of detection of each EDC in all the urine samples analyzed (n=45).

Generally, the highest levels were associated with benzophenone-3 (12.9 ng/µg creatinine), methylparaben (3.4 ng/µg creatinine) and triclosan (1.1 ng/µg creatinine). Of the parabens, and as expected, methylparaben was the one with the highest concentrations (Figure 2).

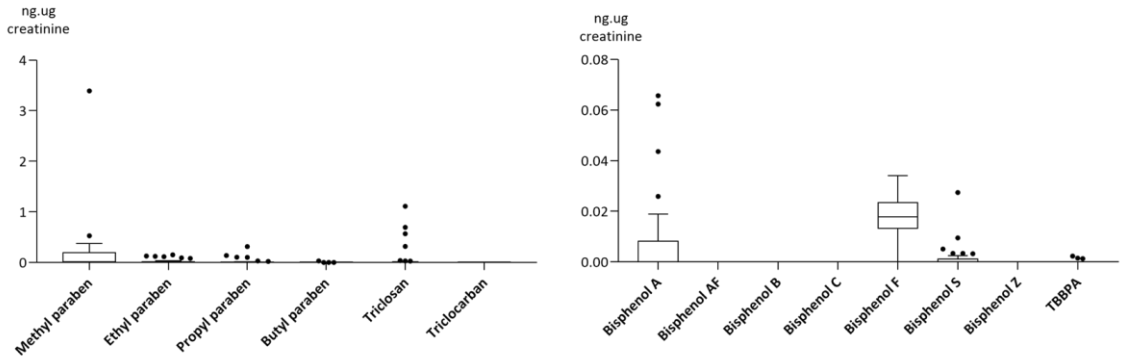

Figure 2. Boxplot summarizing the values of parabens and antimicrobilas (left panel) and bisphenols (right panel) detected in the analyzed urine samples.

As for the antimicrobials only triclosan was detected with values ranging from <MDL ($3.91x10^{-4}$ ng/µg creatinine) up to 1.1 ng/µg creatinine. In what concerns the benzophenon UV filters, benzophenone-1 was detected in 93.3% of the samples with values ranging between $3.91x10^{-4}$

ng/μg creatinine and 1.1 ng/μg creatinine and benzophenon-3 was detected in 73.3% of the samples with one sample registering values as high as 12.85 ng/μg creatinine.

Of the bisphenols, interestingly, BPA was not the most prevalent compound nor the one that registered the highest concentrations. In fact, it was detected in 40% of the samples with an average value of 0.0071 ng/μg creatinine whereas its substitute BPF was detected in 97.8% of the samples with an average value of 0.0186 ng/μg creatinine.

3.5. Future perspectives

Clinical data from all the patients is now being computed and analyzed. The obtained results from the chemical analysis will be analyzed by means of multivariate statistics and correlated with clinical data. Sampling continues in order to obtain a more robust data set.

3.6. Achievements

Oral Communications by Invitation

- Sousa ACA (2019) Environmental contaminants and endocrine disruptors: a global perspective on main sources, exposure pathways and health effects. *Ricardo Jorge Seminar,* National Institute of Health Dr. Ricardo Jorge, 24 October 2019, Lisbon, Portugal.
- Sousa ACA (2019) The interference of environmental contaminants on the thyroid gland. *VII Jornadas de Endocrinologia, Diabetes e Nutrição de Aveiro*, 23-24 May, Aveiro, Portugal.

Oral Communications

- Souto-Miranda S, Melo-Dias S, Valente C, Freite C, Sousa AM, Sousa ACA, Marques A (submitted) Patients' perspectives on exposure to environmental contaminants: a missing piece in pulmonary rehabilitation? 30th European Respiratory Society Annual Congress 2020, 5-9 September 2020, Vienna, Austria
- e Silva FA, Rocha B, Santo MJ, Neves MC, Sousa ACA, Freire MG (2019) New opportunities brought about by supported ionic liquids within the removal of anticancer drugs from aqueous solutions. BPP 2019, Biopartitioning & Purification Conference, 11-13 November 2019, Guarujá, SP, Brazil, p.29
- Sousa ACA, Souto-Miranda S, Marques A, Valente C, Barros R, Taborda-Barata L, Tanoue R, Kunisue T, Tanabe S, Pastorinho MR (2019) Endocrine Disrupting Chemicals in Patients with Chronic Obstructive Pulmonary Diseases. PTIM 2019, 3rd International Caparica

Conference on Pollutant Toxic Ions and Molecules, 4-7 November 2019, Caparica, Portugal, p. 97, ISBN 978-989-54470-3-9

Posters in Conferences/Workshops

- Sousa ACA, Assunção R, Coelho SD, Kunisue T, Tanabe S, Pastorinho MR (2020) Dietary exposure to Cadmium and the associated burden – results from a duplicate diet study in Portugal (2012). Glob 1st WG meeting & 2nd MC meeting Burden of Disease in Europe Taking stock and moving forward. National Food Institute, Technical University of Denmark, 18-19 February 2020, Copenhagen, Denmark, p. 21
- Sousa S, Barros R, Pastorinho MR, Sousa ACA (2019) Association between obesity and mercury: preliminary results from the elegance study. XIV Annual CICS-UBI Symposium 2019, 4-5 July 2019, Covilhã, Portugal, p. 71
- Sousa ACA, Souto-Miranda S, Marques A, Silva T, Henriques I, Barros R, Taborda-Barata L, Valente C, Tanoue R, Kunisue T, Tanabe S, Pastorinho MR (2019) Personal care products in matched human and Environmental samples collected under the framework of RESPIRA project. Jornadas CICECO, University of Aveiro, 11-12 June 2019, Aveiro, Portugal, p. 159

Peer-reviewed abstracts in international conferences

- Sousa ACA, Assunção R, Coelho SD, Kunisue T, Tanabe S, Pastorinho MR (2020) Dietary exposure to Cadmium and the associated burden – results from a duplicate diet study in Portugal (2012). Glob 1st WG meeting & 2nd MC meeting Burden of Disease in Europe Taking stock and moving forward. National Food Institute, Technical University of Denmark, 18-19 February 2020, Copenhagen, Denmark, p. 21, https://www.burden-eu.net/docs/m1wg-abstract-book.pdf

Book chapters

- Pastorinho MR, Sousa ACA (2020) Pets as sentinels of human exposure to neurotoxic metals. *In* Pastorinho MR, Sousa ACA (Eds) Pets as Sentinels, Forecasters and Promoters of Human Health. Springer International Publishing AG, Switzerland, ISBN: 978-3-030-30733-2, p.83-106; https://doi.org/10.1007/978-3-030-30734-9_5

Publications for science and technology dissemination

- Sousa ACA (2019) "The interference of environmental contaminants on the thyroid gland." Jornal das VII Jornadas de Endocrinologia, Diabetes e Nutrição de Aveiro, p. 9.

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(2) Kessous, R.; Shoham-Vardi, I.; Pariente, G.; Sherf, M.; Sheiner, E. Heart 2013, 99, 1118.

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