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Human exposure to hormone-active chemicals present in personal care products, and risk of onset and development of endometriosis in women of childbearing age

"Exposición humana a sustancias químicas con actividad hormonal presentes en productos de cuidado personal, y riesgo de aparición y desarrollo de endometriosis en mujeres en edad fértil"

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Francisco Manuel Peinado Rodríguez

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MEMORIA DE TESIS PRESENTADA POR EL DOCTORANDO

Francisco Manuel Peinado Rodríguez

PARA OPTAR AL TÍTULO DE DOCTOR POR LA UNIVERSIDAD DE GRANADA, DENTRO DEL PROGRAMA DE DOCTORADO EN MEDICINA CLÍNICA Y SALUD PÚBLICA

BAJO LA DIRECCIÓN DE LOS DOCTORES/AS:

D. Francisco Artacho Cordón

Profesor Titular

Departamento de Radiología y Medicina Física

Universidad de Granada

Dña. Mariana Fátima Fernández Cabrera

Catedrática

Departamento de Radiología y Medicina Física

Universidad de Granada

La vida y el tiempo son los mejores maestros. La vida nos enseña a aprovechar el tiempo y el tiempo nos enseña a valorar la vida

Anónimo

Life and time are the best teachers. Life teaches us to take advantage of time and time teaches us to value life

Anonymous

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RESUMEN

Introducción

La endometriosis es una enfermedad ginecológica crónica que afecta cada vez a un mayor número de mujeres en todo el mundo. A pesar del número creciente de artículos publicados en los últimos años, son muchas las lagunas del conocimiento existentes en torno a la etiología y fisiopatología de esta enfermedad. Además, las dificultades en su diagnóstico, principalmente quirúrgico, y en su tratamiento, enfocado a disminuir la sintomatología, obstaculizan aún más su abordaje. El conocimiento disponible sugiere que se trata de una enfermedad multifactorial, en la que interactúan de forma simultánea factores genéticos, inmunológicos, hormonales y ambientales. Junto con el incremento en la incidencia de esta enfermedad se ha producido, en las últimas décadas, un aumento paralelo de la presencia ambiental de sustancias químicas de síntesis con actividad hormonal. Muchas de estas sustancias químicas, disruptores endocrinos (EDCs), se utilizan en cosméticos y productos de cuidado personal (PCPs), haciendo que la población esté diariamente expuesta. Dado que la endometriosis está considerada como una enfermedad hormono-dependiente, se sospecha que la exposición humana a EDCs podría estar detrás del aumento en el riesgo de esta enfermedad.

Objetivo

El objetivo principal de esta tesis doctoral fue explorar la asociación entre la exposición a EDCs, específicamente a dos familias de estos compuestos, parabenos-PB y benzofenonas-BP, presentes en cosméticos y PCPs, y el riesgo de aparición y desarrollo de endometriosis en mujeres en edad fértil.

Para ello, se propusieron los siguientes objetivos específicos:

> Objetivo 1: Evaluar las concentraciones de PBs y BPs en sangre menstrual, una matriz en contacto íntimo con el endometrio, comparar las concentraciones de estos contaminantes en sangre menstrual y periférica, y explorar factores sociodemográficos y de estilo de vida relacionados.
>
> Objetivo 2: Investigar las asociaciones entre la exposición a PBs y BPs y el riesgo de endometriosis, así como con la frecuencia de uso de cosméticos y PCPs, y evaluar la influencia del estrés oxidativo en las asociaciones encontradas.

Objetivo 3: Describir el perfil de expresión en tejido endometriósico de diferentes genes relacionados con vías de señalización celular claves para el desarrollo y progresión de la endometriosis, y explorar su relación con las concentraciones de PBs y BPs en este tejido.

Objetivo 4: Evaluar la actividad biológica (estrogénica y anti-androgénica) de productos y formulaciones cosméticas de la empresa Inves Biofarm, y analizar la presencia de diferentes disruptores endocrinos.

Material y Métodos

Para dar respuesta al objetivo 1, se determinaron las concentraciones de PBs y BPs en muestras de sangre menstrual y periférica de mujeres voluntarias españolas sanas. Las concentraciones sanguíneas de PBs y BPs se determinaron mediante microextracción líquido-líquido dispersiva (DLLME) y cromatografía líquida de ultra alta resolución con espectrometría de masas en tándem (UHPLC-MS/MS). La información sociodemográfica y de estilo de vida se obtuvo mediante el uso de diferentes cuestionarios.

Para dar respuesta a los objetivos 2 y 3, se diseñó un estudio caso-control, recogiendo muestras de orina en ayunas de mujeres en edad fértil con diagnóstico confirmado de endometriosis mediante cirugía (casos), y de mujeres sin diagnóstico de endometriosis, sometidas a laparotomía o laparoscopia en el mismo hospital que los casos, pero con diagnóstico de enfermed no maligna (controles). Se recogieron también muestras de tejido endometriósico de los casos. Las concentraciones urinarias de PBs y BPs se determinaron mediante DLLME y UHPLC-MS/MS. Se evaluaron biomarcadores de estrés oxidativo en todas las muestras de orina mediante diferentes kits enzimáticos. Los perfiles de expresión de 36 genes involucrados en 9 vías de señalización celular, relacionadas con la endometriosis, se determinaron en muestras de tejido endometriosico mediante PCR en tiempo real. La información sociodemográfica, clínica y quirúrgica se obtuvo mediante el uso de diferentes cuestionarios.

Para dar respuesta al objetivo 4, se evaluaron las concentraciones de PBs y BPs y se analizó la actividad estrogénica y anti-androgénica de 6 productos cosméticos, además de 1 principio activo y 2 envases de plástico, proporcionados por la empresa privada Inves Biofarm.

Resultados

Artículo 1: Todas las muestras de sangre menstrual tenían niveles detectables de ≥3 de los compuestos seleccionados, y el 52.6% de las muestras contenían ≥6 compuestos. MeP, PrP y BP-3 fueron los compuestos detectados con mayor frecuencia (frecuencias de detección >90.0%). La edad, el uso de PCPs y el consumo de algunos alimentos (carne, pasta, queso o productos lácteos) se relacionaron con las concentraciones sanguíneas menstruales de algunos PBs/BPs. Las concentraciones de los EDCs medidos en sangre menstrual y periférica no se correlacionaron, o lo hicieron débilmente, siendo las concentraciones en sangre periférica más altas.

Artículo 2: La frecuencia de uso de ciertos cosméticos y PCPs se asoció significativamente con las concentraciones urinarias de PBs y BPs. Después de ajustar por posibles factores de confusión, el riesgo de endometriosis fue mayor cuando se compararon a las mujeres en el segundo *versus* primer tercil de MeP (OR=5.63; p<0.001), BP-1 (OR=5.12; p=0.011), BP-3 (OR=4.98; p=0.008) y ΣBPs (OR=3.34; p=0.032). No se encontraron asociaciones significativas cuando se compararon a las mujeres en el tercer *versus* primer tercil de exposición. El estrés oxidativo no modificaba las asociaciones encontradas entre exposición a PBs/BPs y riesgo de endometriosis.

Artículo 3: A pesar de que la inflamación podría desempeñar un papel central en la fisiopatología de la endometriosis y está considerada como un posible mecanismo de acción de los EDCs, una revisión sistemática de la literatura científica sobre exposición humana a PBs y BPs y biomarcadores inflamatorios mostró la escasez de estudios e información en este área de conocimiento y la necesidad de seguir investigando para comprender mejor los mecanismos de acción subyacentes a los PBs y BPs, y el papel fundamental que podría desempeñar la inflamación en la endometriosis.

Artículos 4 y 5: Más de la mitad de los genes seleccionados se encontraron expresados en >75% de las muestras de tejido endometriósico analizadas. La concentración de los congéneres de PBs y BPs analizados se asoció positivamente con la expresión de genes relacionados con vías celulares clave en el desarrollo de la endometriosis, concretamente genes de adhesión celular, migración/invasión/metástasis, inflamación, angiogénesis, proliferación celular-estimulación hormonal, ciclo celular, diferenciación

celular y de metabolismo lipídico. No se encontraron asociaciones entre la exposición a PBs y BPs y genes de la apoptosis.

Colaboración con la empresa Inves Biofarm: No se encontraron concentraciones detectables de PBs y BPs en ninguno de los envases de plástico analizados, ni tampoco en el principio activo proporcionado. Solo se encontraron concentraciones detectables de MeP, EtP y PrP en una muestra, y de BP-3 en cuatro de los productos cosméticos recibidos. Solamente algunos productos cosméticos y envases de plástico extraídos con cloroformo y acetonitrilo mostraron actividades estrogénica y antiandrogénica.

Conclusión

La exposición humana a sustancias químicas con actividad hormonal, presentes en algunos cosméticos y PCPs, podría aumentar el riesgo de endometriosis en mujeres en edad fértil. Esta exposición podría también estar relacionada con la expresión de genes involucrados en vías de señalización celular claves para el desarrollo de esta enfermedad. Es necesario, por tanto, eliminar o restringuir el uso de PBs o BPs en la composición de cosméticos y PCPs, lo que podría ayudar a disminuir la exposición humana a estos EDCs y, con ello, el riesgo de aparición y desarrollo de endometriosis.

Palabras clave: parabenos, benzofenonas, endometriosis, vías de señalización celular, cosméticos y productos de cuidado personal

ABSTRACT

Introduction

Endometriosis is a chronic gynecological disease that affects an increasing number of women worldwide. Despite the growing number of articles published in recent years, there are many gaps in knowledge about the etiology and pathophysiology of this disease. In addition, the difficulties in its diagnosis, mainly surgical, and in its treatment, focused on reducing the symptoms, further hinder its approach. Available knowledge suggests that it is a multifactorial disease, in which genetic, immunological, hormonal and environmental factors interact simultaneously. Along with the increase in the incidence of this disease, in recent decades there has been a parallel increase in the environmental presence of synthetic chemical substances with hormonal activity. Many of these chemicals, endocrine-disrupting chemicals (EDCs), are used in cosmetics and personal care products (PCPs), causing the population to be exposed on a daily basis. Since endometriosis is considered a hormone-dependent disease, it is suspected that human exposure to EDCs could be behind the increased risk of this disease.

Objective

The main objective of this doctoral thesis was to explore the association between exposure to EDCs, specifically to two families of these compounds, parabens-PB and benzophenones-BP, present in cosmetics and PCPs, and risk of onset and development of endometriosis in women of childbearing age.

For this goal, the following specific objectives were proposed:

> Objective 1: To evaluate the concentrations of PBs and BPs in menstrual blood, a matrix in intimate contact with the endometrium, compare the concentrations of these contaminants in menstrual and peripheral blood, and explore related sociodemographic and lifestyle factors.
>
> Objective 2: To investigate the associations between exposure to PBs and BPs and risk of endometriosis, as well as with the frequency of use of cosmetics and PCPs, and to evaluate the influence of oxidative stress on the associations found.
>
> Objective 3: To describe the expression profile in endometriotic tissue of different genes related to key cell signaling pathways for the development and

progression of endometriosis, and to explore their relationship with the concentrations of PBs and BPs in this tissue.

Objective 4: To evaluate the biological activity (estrogenic and anti-androgenic) of cosmetic products and formulations of the company Inves Biofarm, and to analyze the presence of different endocrine disruptors.

Material and Methods

To answer objective 1, PB and BP concentrations were determined in menstrual and peripheral blood samples from healthy Spanish volunteers. Blood concentrations of PBs and BPs were determined by dispersive liquid-liquid microextraction (DLLME) and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Sociodemographic and lifestyle information was obtained by using different questionnaires.

To answer objectives 2 and 3, a case-control study was designed, collecting fasting urine samples from women of childbearing age with a confirmed diagnosis of endometriosis by surgery (cases), and from women without a diagnosis of endometriosis, who underwent laparotomy or laparoscopy in the same hospital as the cases, but with a diagnosis of non-malignant disease (controls). Endometriotic tissue samples were also collected from the cases. Urinary concentrations of PBs and BPs were determined by DLLME and UHPLC-MS/MS. Oxidative stress biomarkers were evaluated in all urine samples using different enzyme kits. The expression profiles of 36 genes involved in 9 cell signaling pathways related to endometriosis were determined in endometriotic tissue samples by real-time PCR. Sociodemographic, clinical and surgical information was obtained using different questionnaires.

To answer objective 4, we evaluated the concentrations of PBs and BPs and analyzed the estrogenic and anti-androgenic activity of 6 cosmetic products, 1 cosmetic formulation and 2 plastic packages, provided by the private company Inves Biofarm.

Results

Article 1: All menstrual blood samples had detectable levels of ≥3 of the selected compounds, and 52.6% of the samples contained ≥6 compounds. MeP, PrP, and BP-3 were the most frequently detected compounds (detection frequencies >90.0%). Age, use

of PCPs and consumption of some foods (meat, pasta, cheese or dairy products) were related to menstrual blood concentrations of some PBs/BPs. Concentrations of EDCs measured in menstrual and peripheral blood did not correlate, or correlated weakly, with concentrations in peripheral blood being higher.

Article 2: The frequency of use of certain cosmetics and PCPs was significantly associated with urinary concentrations of PBs and BPs. After adjusting for possible confounders, the risk of endometriosis was higher when comparing women in the second *versus* first tertile of MeP (OR=5.63; p<0.001), BP-1 (OR= 5.12; p=0.011), BP-3 (OR=4.98; p=0.008), and ΣBPs (OR=3.34; p=0.032). No significant associations were found when comparing women in the third *versus* first tertile of exposure. Oxidative stress did not modify the associations found between exposure to PBs/BPs and risk of endometriosis.

Article 3: Although inflammation could play a central role in the pathophysiology of endometriosis and is considered as a possible mechanism of action of EDCs, a systematic review of the scientific literature on human exposure to PBs and BPs and inflammatory biomarkers showed the paucity of studies and information in this area of knowledge and the need for further research to get a better understanding on the underlying mechanisms of action of PBs and BPs, and the key role that inflammation could play in endometriosis.

Articles 4 and 5: More than half of the selected genes were found to be expressed in >75% of the endometriotic tissue samples analyzed. The concentration of PB and BP congeners analyzed was positively associated with the expression of genes related to key cellular pathways in the development of endometriosis, such as genes for cell adhesion, migration/invasion/metastasis, inflammation, angiogenesis, cell proliferation-hormone stimulation, cell cycle, cell differentiation and lipid metabolism. No associations were found between exposure to PBs and BPs and apoptosis genes.

Collaboration with the company Inves Biofarm: no detectable concentrations of PBs and BPs were found in any of the plastic packages tested, or in the cosmetic formulation provided. Detectable concentrations of MeP, EtP and PrP were only found in one sample, and BP-3 was detected in four of the received cosmetic products. The plastic packages showed estrogenic and anti-androgenic activity depending on the chosen

extraction methodology. Only some cosmetic products and plastic packages extracted with chloroform and acetonitrile showed estrogenic and antiandrogenic activities.

Conclusion

Human exposure to hormone-active chemicals, present in some cosmetics and PCPs, could increase the risk of endometriosis in women of childbearing age. This exposure could also be related to the expression of genes involved in key cell signaling pathways for the development of this disease. It is therefore necessary to eliminate or restrict the use of PBs or BPs in the composition of cosmetics and PCPs, which could help to decrease human exposure to these EDCs and, therefore, reduce the risk of onset and development of endometriosis.

Keywords: parabens, benzophenones, endometriosis, cell signaling pathways, cosmetics and personal care products

ABBREVIATION LIST

ALT: alanine transaminase
ANG: angiogenin
ANGPT1: angiopoietin 1
APOE: apolipoprotein E
ASRM: American society for reproductive medicine
AST: aspartate transaminase
BADGE: bisphenol A diglycidyl ether
BAX: BCL2-associated X Protein
BCL-2L1: BCL2 Like 1
BFDGE: bisphenol F diglycidyl ether
BisGMA: bisphenol A-glycidyl methacrylate
BMI: body mass index
BMI1: BMI1 Proto-Oncogene, polycomb ring finger
BP-1: benzophenone 1
BP-3: benzophenone 3
BP-6: benzophenone 6
BP-8: benzophenone 8
BPA: bisphenol A
BPF: biphenol S
BPs: benzophenones
BPS: biphenol S
BuP: butylparaben
BzP: benzylparaben
CCNB1: cyclin B1
CDFBS: charcoal/dextran treated fetal bovine serum
CDK1: cyclin dependent kinase 1
CDK1: cyclin dependent kinase 1
cDNA: complementary deoxyribonucleic acid
CI: confidence interval
CLDN7: claudin 7
CRP: C-reactive protein
Ct: cycle of quantification
CV: coefficient of variation
CYP19A1: cytochrome P450 family 19 subfamily A member 1
DDE: dichlorodiphenyldichloroethylene
DDT: dichlorodiphenyltrichloroethane
DLLME: dispersive liquid-liquid microextraction
DMEM: dulbecco's modified eagle medium
DUSP6: dual specificity phosphatase 6
E2: estradiol
EDCs: endocrine-disrupting chemicals
Eeq: estradiol equivalents
ELISA: enzyme-linked immunosorbent assay
EndEA: endometriosis y exposición ambiental
ERs: estrogen receptors
ERα: estrogen receptor alpha
ERβ: estrogen receptor β
ESI: electrospray ionization
EtP: ethylparaben
FBS: fetal bovine serum
FD: frequency of detection
FGF: fibroblast growth factor
FOXO3: forkhead box P3
FUT8: fucosyltransferase 8
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
GC-MS/MS: gas chromatography tandem mass spectrometry
GM: geometric mean
GM-CSF: granulocyte-macrophage colony-stimulating factor
GnRH: gonadotropin-releasing hormone
HCB: hexachlorobenzene
HOXA10: homeobox A10
HPLC: high performance liquid chromatography
HSD17β2: 17β hydroxysteroid dehydrogenase 2
ID-LC-MS/MS: isotope dilution-liquid chromatography-tandem mass spectrometry
IFN- γ: interferon γ
IL: interleukin
IL-1R: interleukin-1 receptor
IL-1ra: interleukin-1 receptor antagonist protein
IL1RL1: interleukin 1 receptor, type I
ILR2: interleukin 1 receptor, type II
IL-1α: interleukin 1α
IL-1β: interleukin-1β
IL-2: interleukin-2
IL-4: interleukin-4
IL-5: interleukin-5
IL-6: interleukin 6
IL6ST: interleukin 6 cytokine family signal transducer

IL-7: interleukin-7
IL-8: interleukin 8
IL-9: interleukin-9
IL-10: interleukin 10
IL-12: interleukin-12
IL-13: interleukin-13
IL-15: interleukin-15
IL-17A: interleukin-17A
IL-23: interleukin 23
IL-33: interleukin-33
IP-10: interferon-γ-induced protein 10
ITGB2: integrin beta-2
LC-MS/MS: liquid chromatography-mass spectrometry
LOD: limit of detection
LOQ: limit of quantification
MCP-1: monocyte chemoattractant protein 1
MCP-3: Monocyte Chemoattractant protein-3
MDK: midkine
MeP: methylparaben
MIP: macrophage inflammatory protein
MMP-1: matrix metalloproteinase-1
MMP-3: matrix metalloproteinase-3
MMP-7: matrix metalloproteinase-7
MMPs: matrix metalloproteinases
n.e.: not expressed
N.R.: not reported
ND: not detected
NK: natural killer
NR3C1: nuclear receptor subfamily 3 group C member 1
NSAIDs: non-steroidal anti-inflammatory drugs
OR: odds ratio
P25: percentile 25
P50: percentile 50
P75: percentile 75
PBDEs: polybrominated diphenyl ethers
PBs: parabens
PCBs: polychlorinated biphenyls
PCOS: polycystic ovary syndrome
PCPs: persistent organic pollutants
PCR: polymerase chain reaction
PDGBB: platelet-derived growth factor BB
PDGFRA: platelet derived growth factor receptor α
PFOA: perfluorooctanoic acid
PGR: progesterone receptor
PLC: phospholipase C
PLCG1: phospholipase C gamma 1
PLCG2: phospholipase C gamma 2
POPs: persistent organic pollutants
PPARs: peroxisome proliferator-activated receptors
PR: progesterone receptor
PRISMA: Preferred reporting items for systematic reviews and meta analyses
PrP: propylparaben
RANTES: regulated on activation, normal T expressed and secreted
RHOB: ras homolog gene, family, member B
RNA: ribonucleic acid
ROBINS-E: risk of bias in non-randomized studies of exposures
ROS: reactive oxygen species
RRM2: ribonucleotide reductase M2
RT-qPCR: reverse transcription-quantitative polymerase chain reaction
SALLE: salt-assisted liquid-liquid extraction
SD: standard deviation
SE: standard error
SEM: standard error of the mean
SNR: signal–to noise ratio
SOX2: SRY-box transcription factor 2
SPP1: secreted phosphoprotein 1
SPRY2: sprout homolog 2
STAR: steroidogenic acute regulatory protein
STROBE: reporting of observational studies in epidemiology
TAP: total antioxidant power
TBARS: 2-thiobarbituric acid reactive substances
TGF-β: transforming growth factor β
TIA: turbid-immunometric assay
TIMPs: tissue inhibitors of matrix metalloproteinases
TNFRSF1B: tumor necrosis factor receptor superfamily member 1B
TNF-α: tumor necrosis factor-α
UHPLC-MS/MS: ultra-high performance liquid chromatography with tandem mass spectrometry
URSA: unexplained recurrent spontaneous abortion

UV: ultraviolet
VEGF: vascular endothelial growth factor
VEGFA: vascular endothelial growth factor A
VEGFR-1: vascular endothelial growth factor receptor-1
WERF: world endometriosis research foundation
γ–GTP: γ glutamyl transpeptidase
γ-HCH: γ-hexachlorocyclohexane
4-OHBP: 4-hydroxybenzophenone
8-OHdG: 8-hydroxydeoxyguanosine

1. INTRODUCTION

1.1 DEFINITION OF ENDOMETRIOSIS

Endometriosis is a chronic gynecological disease commonly observed in women of childbearing age, characterized by the presence of functionally active endometrial tissue (glandular epithelium and stroma) outside the uterine cavity, mainly in the abdominal-pelvic cavity (peritoneal surface, ovaries and rectovaginal septum), and to a lesser extent in the diaphragm, pleura and pericardium (Giudice, 2010).

This ectopic tissue is sensitive to hormonal stimulation and has the ability to respond to cyclical changes in estrogen levels. It is, therefore, an estrogen-dependent disease in which the ectopic tissue can cyclically bleed and generate a local inflammatory reaction at the implantation site, giving rise to the formation of scar tissue, fibrosis and adhesions (Klemmt and Starzinski-Powitz, 2018). As a consequence, several symptoms could appear, such as chronic pelvic pain, dysmenorrhea, dyspareunia, menstrual irregularities and infertility (Giudice and Kao, 2004; Vercellini et al., 2014). Although it is considered a benign disease, hormonal stimulation could promote the development and progression of ectopic lesions, reaching a wide range of body locations, being able to spread in a similar way as neoplastic tissue does. In addition, it can also recur, mainly in deep forms. It is suspected that this disease could be associated with an increased risk of gynecologic cancer, adenomyosis, cardiovascular disease, fibromyalgia, chronic fatigue syndrome, and autoimmune disease (Benagiano et al., 2014; Kvaskoff et al., 2015; Matias-Guiu and Stewart, 2018). It is, therefore, a systemic disease and not predominantly pelvic (Taylor et al., 2021).

The diagnosis of endometriosis is challenging because of the overlap of symptoms with other diseases, both gynecological and non-gynecological, and also because of the frequent cases in which it presents asymptomatically. Surgical diagnosis is the "gold standard" for establishing an accurate diagnosis of the disease, and treatment is mainly symptomatic and not aimed at curing the disease (Dunselman et al., 2014).

Endometriosis is, definitely, a chronic, benign, estrogen-dependent, inflammatory, systemic and multifactorial gynecological disease, which can considerably reduce the quality of life of a large number of women worldwide. Despite growing concern and increasing research on this disease, the etiopathogenesis, pathophysiology, risk factors and its distribution in the population remain unclear, and endometriosis is currently still considered a challenging and controversial disease.

1.2 EPIDEMIOLOGY OF ENDOMETRIOSIS

It is complicated to accurately estimate both the incidence and prevalence of endometriosis due to the difficulty in diagnosis, asymptomatic cases and the use of surgery as the most reliable diagnostic tool (Dunselman et al., 2014; Giudice, 2010). Moreover, it is common to find cases of endometriosis in healthy populations, as well as in populations that are candidates for surgery or hospitalization for symptoms of pain and/or infertility. Estimates show that endometriosis could affect 10-15% of the female population of reproductive age, comprising more than 190 million women worldwide (Shafrir et al., 2018). This percentage may increase up to 50% in women diagnosed with infertility or with symptoms of pelvic pain (Shafrir et al., 2018). These estimates could, however, be biased as they were calculated for high-risk populations. In asymptomatic women, the prevalence ranges from 2 to 11% (Shafrir et al., 2018). More recent studies show a lower prevalence, between 1% and 5%, when considering low-risk population groups (Eisenberg et al., 2018). Considering all the extremely limited studies, it is estimated that the incidence of endometrosis could range between 5 and 10 cases per 10000 women, with an annual upward trend (Eisenberg et al., 2018); which makes this gynecological disease one of the most frequent. Therefore, endometrosis affects an increasing number of women of childbearing age, posing a problem for both their health and the healthcare system.

1.3 TOPOGRAPHICAL ANATOMY OF ENDOMETRIOSIS

The most frequent site of endometriosis is the pelvis, mainly the ovaries and nearby areas that are covered by peritoneum, such as the sac of Douglas, the broad ligaments, and the utero-sacral ligaments (Dmowski and Radwanska, 1984). Less frequently, it can be found in the fallopian tubes, cervix, vagina, vulva, rectum, sigma, ileocecal appendage, and round ligaments. However, endometriosis implants have been identified in extrapelvic sites, such as lymph nodes, liver, pancreas, bladder, abdominal wall, lungs, pleura, umbilicus, brain, and even in extremities (Jenkins et al., 1986; Vercellini et al., 2014). Furthermore, the distribution of bilateral organ lesions is asymmetric, probably due to anatomical differences in the right and left hemipelvis. An example can be found in the more frequent location of endometriotic implants in the left ovary and ureter, compared to those located on the right side.

1.4 HISTOPATHOLOGY AND CLASSIFICATION OF ENDOMETRIOSIS

Endometriotic tissue has a microscopic appearance similar to that of the endometrium of the uterine cavity, characterized by the presence of secretory-type epithelial tissue and stromal tissue. Endometrial glands are frequently irregular and their functional appearance is variable. Sometimes, they undergo cyclic changes similar to normal endometrium and other times they resemble the basal layer of the endometrium without undergoing secretory changes (Bergqvist et al., 1984). In contrast to the endometrium, endometriosis implants also often contain fibrous tissue, hemosiderin deposits, cysts, and pigmented histiocytes in varying amounts (Bonte et al., 2002; Nisolle et al., 1990). Interstitial bleeding is common in endometriosis. Chemical and mechanical irritation in the tissues causes proliferation of fibroblasts producing fibrosis around the lesion (Schweppe and Wynn, 1981).

Several endometriosis classification systems have been proposed, based on the anatomical location and the severity of the disease. From the macroscopic point of view, three forms of endometriosis can be distinguished (Kang et al., 2014):

Type I. Peritoneal endometriosis:

Endometriotic implants are located on the surface of the ovarian and pelvic peritoneum and often show a variable appearance and size. There are lesions characterized by numerous proliferative glands with a columnar or pseudostratified epithelium, called red, “flame” lesions (Nisolle and Donnez, 1997), which mainly affect the broad and uterosacral ligaments (Jansen and Russell, 1986), and are characterized by being hypervascularized active lesions. In addition, due to a reduction in vascularity, these lesions may undergo a scarification process and may become black and inactive lesions, as a consequence of the retention of blood pigments and the deposition of hemosiderin (Nisolle et al., 1990). In some cases, the inflammatory process and subsequent fibrosis totally devascularizes the endometriotic implant, causing white, opaque-looking lesions formed by inactive collagen plaques.

Type II. Ovarian endometriosis:

Endometriotic implants can appear as superficial lesions or as endometriomas and are usually present in one third of endometriosis cases. Superficial implants may have an atypical gross appearance with brownish lesions, red lesions, brownish deposits, and

adhesions on the ovarian surface. Endometriomas consist of pelvic masses composed of cystic structures that contain blood and menstrual waste. They tend to have thick walls and fibrotic adhesions, and are usually filled with a thick, chocolate-like liquid material ("chocolate cysts").

Type III. Deep infiltrating endometriosis:

Endometriotic implants are defined as subperitoneal nodules larger than 5 mm, which may affect the sac of Douglas, utero-sacral ligaments, recto-vaginal septum, ureters, bladder, and intestine. It has been estimated that more than 20% of patients with endometriosis present with this type and it is usually multifocal in 61% of cases, in addition to coexisting with other types of endometriosis (Dunselman et al., 2014). It is the most severe form of endometriosis, and can cause dysmenorrhea, dyspareunia, dyschezia, chronic pelvic pain and infertility.

Although numerous classifications for endometriosis have been proposed (Adamson, 2011; Buttram, 1987; Kistner et al., 1977), currently the most widely used is proposed by the American Society for Reproductive Medicine (ASRM) (ASRM, 1997). According to this classification, endometriosis is divided into four stages based on the presence of implants and/or adhesions to the peritoneum, ovaries or tubes, and assigning a score based on the size, depth and location of the implants (Figure 1).

- Stage I (minimum): assigned to patients who present a score less than or equal to 5 points. Superficial peritoneal endometriotic implants associated or not with small superficial ovarian involvement and limited loose adhesions are included in this group.
- Stage II (mild): includes patients with a score between 6 and 15 points. In these cases, in addition to the lesions described above, there may be deep peritoneal implants.
- Stage III (moderate): assigned to patients who score between 16 and 40 points. They may present with partial obliteration of the sac of Douglas and larger endometrioma, in addition to firm adhesions.
- Stage IV (severe): includes patients with a score greater than 40 points. In addition to the lesions already described, they may present complete obliteration of the sac of Douglas.

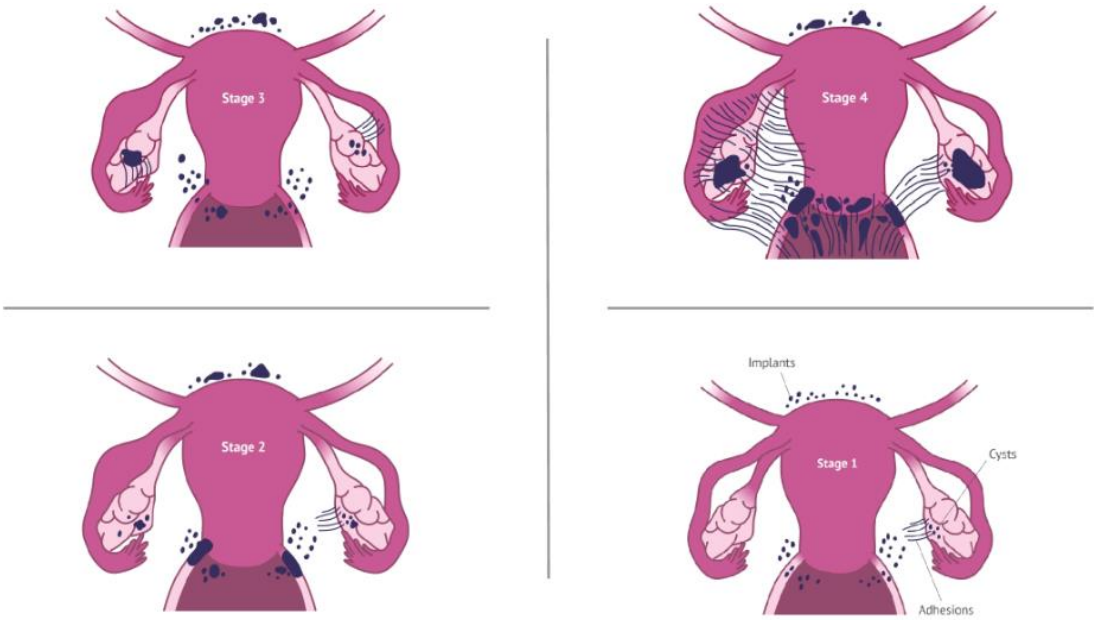


Figure 1. Clinical staging of endometriosis proposed by the American Society for Reproductive Medicine (ASRM, 1997).

However, this classification has some limitations, among which are the assignment of a score considering a subjective laparoscopic examination, an overestimation of the relevance of the endometrioma and of the obliteration of the sac of Douglas, assigning scores between 16 and 20 points, and 40 points, respectively. In contrast, deep nodules can only obtain a maximum of 6 points and lesions in the ureter or extra pelvic are not scored. In addition, there is no correlation between the different stages and fertility or pelvic pain, so it is not a good predictor of symptom severity, and the multifocal nature of deep endometriosis lesions, anatomical distribution, and difficulties surgical are not considered. Therefore, alternative classifications have emerged in recent years that attempt to address the aforementioned limitations. However, currently there is no ideal classification and each case must be individualized or a combination of several classifications must be used to integrate information and achieve a more complete vision of the disease.

1.5 SYMPTOMATOLOGY, DIAGNOSIS, TREATMENT AND PROGNOSIS

1.5.1 SYMPTOMATOLOGY

The symptoms of endometriosis are heterogeneous, variable, non-specific and there is no definite correlation with the stage of the disease. In addition, the symptoms may correlate with the activity and depth of the lesions, appear early in adolescence, and persist after the menopause in some cases.

The most frequent symptoms are menstrual irregularities, chronic pelvic pain, which is frequently exacerbated during menstruation (dysmenorrhea), subfertility or infertility, sexual intercourse (dyspareunia), defecation (dyschezia), or urination (dysuria) (Eskenazi and Warner, 1997; Giudice and Kao, 2004). When endometriotic lesions affect other anatomical structures, such as the thorax or lungs, other less frequent symptoms may appear, among which pneumothorax, catamenial hemoptysis, or neurological symptoms due to brain involvement stand out (Korom et al., 2004). Although it has been little studied, musculoskeletal alterations could also be produced (Álvarez-Salvago et al., 2020; Mabrouk et al., 2018).

Endometriosis may be also associated with other pathologies, such as pelvic inflammatory disease, cystitis syndrome, interstitial bladder or irritable bowel syndrome (Seaman et al., 2008), and has been related to an increased risk of gynecological cancer, adenomyosis, cardiovascular disease, fibromyalgia, chronic fatigue syndrome and autoimmune disease (Benagiano et al., 2014; Kvaskoff et al., 2015; Matias-Guiu and Stewart, 2018).

Finally, endometriosis can psychologically affect many cases, showing anxiety, depression and catastrophic thoughts. Quality of life can also be considerably reduced due to the variability and severity of the symptoms (Mundo-López et al., 2020).

1.5.2 DIAGNOSIS

Given the presence of asymptomatic cases, the non-specificity of some of the symptoms, and the need for surgical intervention to confirm the diagnosis of endometriosis, there may be a delay in the diagnosis of the disease of between 8 and 12 years from its onset and prolonged periods of time without treatment (Ghai et al., 2020; Kiesel and Sourouni, 2019).

There are several procedures for the diagnosis of endometriosis, such as a clinical examination, observing the patient's anamnesis to identify if the patient has presented or currently presents any of the characteristic symptoms of endometriosis, a biophysical examination, using different imaging tests, such as transvaginal ultrasound, transabdominal ultrasound, computerized axial tomography and nuclear magnetic resonance (Parasar et al., 2017), and a biochemical study, analyzing different serum, tissue and peritoneal fluid markers, which can be tumor and polypeptides, immunological, genetic or tissue (Bedaiwy and Falcone, 2004). Currently, the clinical and biochemical diagnosis of endometriosis has a low positive predictive value and has been questioned by numerous authors (Hornstein et al., 1992; Pittaway and Fayez, 1986). Thus, the "gold standard" for the diagnosis of endometriosis requires direct visualization of the lesions through laparoscopy together with their histopathological confirmation (Chapron et al., 2019; Falcone and Flyckt, 2018). However, because it is an invasive method and with associated costs (Dunselman et al., 2014), some experts mention that this disease can be suspected and that it should be diagnosed even in the absence of histological confirmation, when clinical, exploratory and/or imaging techniques provide well-founded suspicions of endometriosis.

1.5.3 TREATMENT

The treatment of endometriosis is mainly symptomatic and in most cases is not aimed at removing endometriotic lesions. In addition, it is sometimes ineffective and a reduction in symptoms is not achieved. Treatment should be individualized and consider the severity of symptoms, extent and location of disease, patient age, medical history, reproductive desires, previous treatments, surgical complications, and associated cost (Falcone and Flyckt, 2018).

There are different types of endometriosis treatments. Symptomatic medical treatment includes the administration of non-steroidal anti-inflammatory drugs (NSAIDs), oral contraceptives, or a combination of both. The hormonal medical treatment is based on the estrogen dependence of the implants and in the pregnancy, and aims to reduce pain by reducing the inflammatory response, interruption or suppression of ovarian cycle hormone production, inhibition of the action and oestradiol (E_2) synthesis and reduction or elimination of menstruation (Giudice, 2010). In this sense, oral contraceptives, such as danazol and genistrone are used. Gonadotropin-releasing hormone (GnRH) agonists

and aromatase inhibitors are also widely used (Bergqvist, 1995). Moreover, antiangiogenic treatments could prevent the development of endometriotic lesions, although several side effects have been evidenced (Hull et al., 2003; Nap et al., 2004).

These pharmacological treatments are symptomatic and not cytoreductive. Therefore, surgical treatment is considered the best therapeutic option in symptomatic cases (Vercellini et al., 2009). Surgery can be conservative, trying not to damage the reproductive system, or radical, which implies a bilateral oophorectomy, and can be extended to a hysterectomy with bilateral salpingo-oophorectomy (Koninckx and Martin, 1994). Due to the surgical complications that can occur, this type of treatment should only be considered when medical treatment is not effective (Jackson and Telner, 2006).

1.5.4 PROGNOSIS

Endometriosis is considered a benign disease, but it can also be progressive. The progression of the lesions over the years is variable, and may remain in the same location or progress or spread to other tissues. Previous studies have shown that 25% of endometriotic implants resolve spontaneously after 12 months, 50% worsen and the rest remain stable (Farquhar, 2007; Farquhar, 2000).

Currently available therapeutic measures offer limited benefit, and sometimes, medical-surgical treatment is not capable of eliminating symptoms (Becker et al., 2017). Medical therapy for endometriosis focuses on pain control, improving quality of life, preventing disease recurrence, preserving fertility, and reducing surgical intervention.

1.6 PATHOGENESIS AND PATHOPHYSIOLOGY OF ENDOMETRIOSIS

Despite the increasing number of studies on endometriosis, its etiology is still not well established. For several decades, different theories have been formulated to try to explain the origin of this disease, although none of them manages to explain all forms of endometriosis (Vitale et al., 2018):

1. Theory of retrograde menstruation

This theory, also called implantation theory, is currently the most widely accepted theory to explain the origin of endometriosis. It was proposed by Sampson in 1927 and states that fragments and cells of the menstrual endometrium are retrogradely

transported through the fallopian tubes towards the peritoneal cavity, producing implantation, growth and invasion in pelvic structures (Sampson, 1927a). Several factors must be met for the origin of this disease: occurrence of retrograde menstruation, presence of viable endometrial cells in retrograde menstrual reflux, and adhesive capacity of endometrial cells to be able to implant and proliferate in the pelvic cavity (Seli et al., 2003). This theory has been supported by several studies, showing a higher incidence of endometriosis in girls with obstructions of the genital tract, preventing the vaginal expulsion of menstrual flow and favoring retrograde menstruation (Jenkins et al., 1986).

2. Theory of coelomic metaplasia

This theory is based on embryological studies showing that the pelvic peritoneum, the germinal epithelium of the ovaries and Mullerian ducts could be derived from cells that line the coelomic cavity, and would explain the appearance of endometriosis in any location of the abdominal cavity and in the pleural cavity, as well as its diagnosis in the prepubertal stage or in women who have never menstruated, and rare cases of endometriosis in males (Tong et al., 2019).

3. Theory of induction

It is an extension of the coelomic metaplasia theory, and suggests that biochemical alterations and immunological factors may induce the development of endometriosis from totipotent stem cells present in connective tissue. This theory would only explain ovarian endometriosis, since the ovarian epithelium requires high concentrations of 17β estradiol, 10 times higher than those present in the peritoneal fluid of women with endometriosis and only present in the vicinity of the ovary (Laganà et al., 2018).

4. Mullerian embryonic remains theory

Residual cells from embryonic migration from the Müllerian ducts could develop endometriotic lesions as a consequence of estrogen stimulation during puberty or in response to estrogen mimicking molecules (Klemmt and Starzinski-Powitz, 2018).

5. Theory of lymphatic and vascular dissemination

Endometrial tissue implants that are located in extra-pelvic regions are a consequence of the migration of endometrial cells or tissue through the blood and lymphatic vessels (Klemmt and Starzinski-Powitz, 2018). This could be the cause of the appearance of endometriotic lesions in areas remote from the endometrium, such as the pleura, umbilical cord, retroperitoneal space, lower extremities, vagina, and cervix.

6. Theory of stem cells

This theory suggests that circulating stem cells derived from the bone marrow could differentiate into endometrial tissue at different locations (Sasson and Taylor, 2008). This theory would explain the development of endometriosis in patients without a uterus and in men with prostate cancer treated with high doses of estrogen.

Despite the different theories postulated over the years and supported by different data, there are relevant aspects that have yet to be clarified. In this sense, the frequent presence of a single endometrioma in the ovary, the different behavior of peritoneal lesions and the presence of adhesions in highly variable extensions are some of them. Furthermore, although the theory of retrograde menstruation is the most accepted, this type of menstruation is a universal physiological mechanism and a very common process, both in healthy women and with the disease, appearing in up to 90% of menstruating women. Therefore, since endometriosis only appears in 10-15% of women of childbearing age, its development must be associated with other factors, such as the amount of endometrial tissue that reaches the peritoneal cavity or the presence of a depressed immune system unable to remove ectopic endometrial tissue. In this sense, a "permissive" peritoneal environment is necessary for the establishment, growth, and progression of endometriotic lesions, promoting the development of pathophysiological mechanisms related to the origin and development of endometriosis (Klemmt and Starzinski-Powitz, 2018; Monnin et al., 2023) (Figure 2).

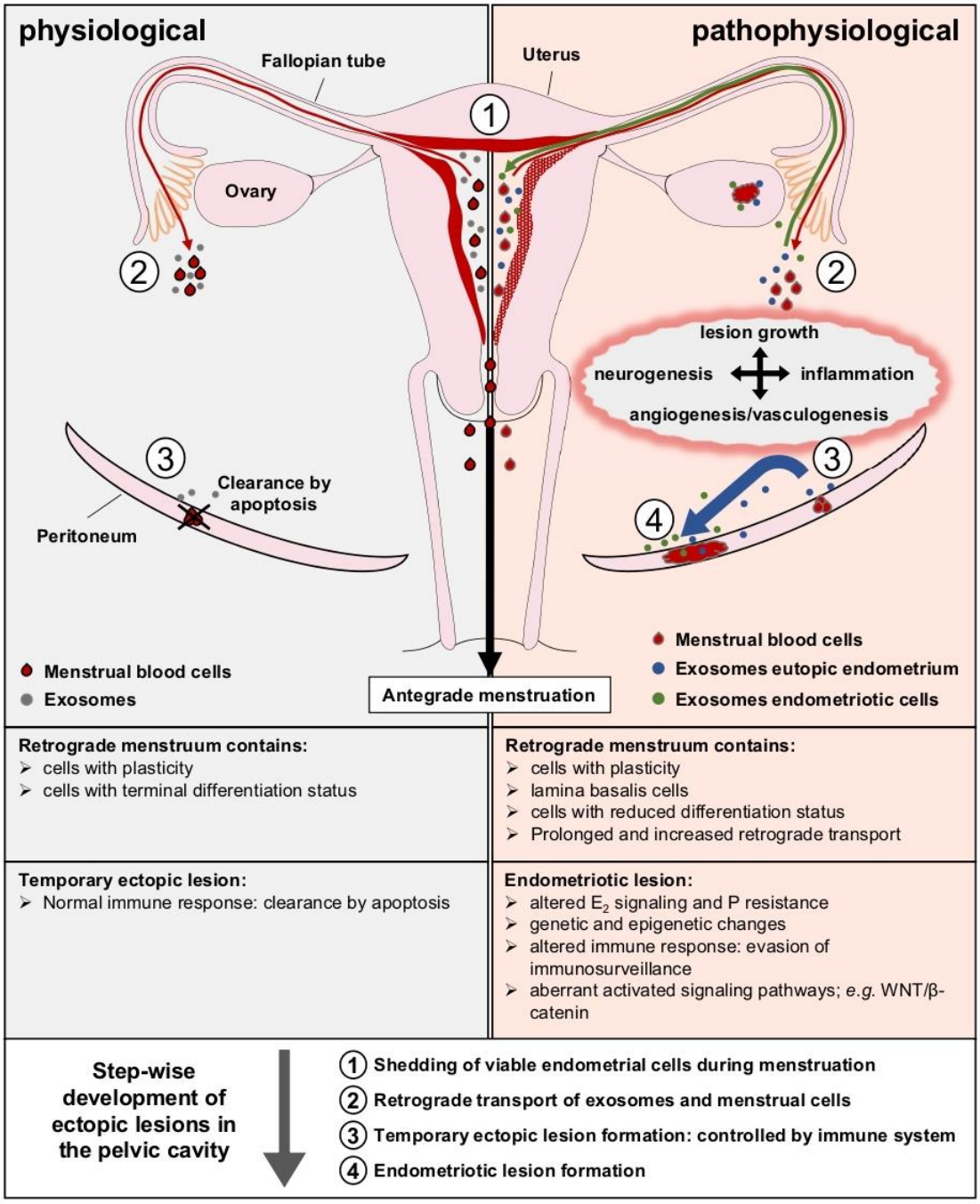


Figure 2. Schematic overview of endometriosis development (Klemmt and Starzinski-Powitz, 2018). Shed menstrual endometrium leaves the cavity mainly antegradely (black arrow) but is also flushed retrograde into the pelvic cavity (red arrow). Endometrial derived exosomes can also be transported retrograde into the pelvic cavity even in the absence of menstruation (grey and blue filled circles). Once in the pelvic cavity, menstrual cells can attach by gravity to the peritoneal surfaces and form temporary lesions. In healthy women, temporary ectopic lesions are removed by the immune sys- tem through apoptosis induction. In women developing endometriosis as a disease, these ectopic lesions evade the immunosurveillance and progress in response to *e.g.* locally present cytokines and/or growth factors (blue arrow). Exosomes derived from endometriotic cells (green filled circles) could act in an autocrine/paracrine manner but could also be transported back through the fallopian tubes into the uterine cavity (green arrow) and modulate signaling events in eutopic endometrium. Albeit the implantation theory is the most likely explanation for the pathophysiological lesion formation, the step-wise development of ectopic lesions could also be explained in part by other proposed theories.

For the origin and development of endometriosis, 5 critical molecular processes have been postulated: 1) Adhesion of endometrial cells to the peritoneal surface, 2) invasion, migration and metastasis of endometrial cells, 3) generation of a microenvironment inflammatory, 4) development of angiogenesis around the nascent endometriotic implant and 5) endometrial cell survival and proliferation (Seli et al., 2003).

1) Adhesion of endometrial cells to the peritoneal surface

Considering the theory of retrograde menstruation, endometrial cells from retrograde reflux must have the ability to adhere and grow on peritoneal surfaces for endometriosis to occur. The first studies that explored the adhesive capacity of endometrial cells were contradictory. On the one hand, it was observed that the intact mesothelium could constitute a defense barrier that prevents the adhesion of endometrial fragments to the peritoneal surface and that this adhesion would only occur in cases in which the mesothelium was damaged or absent (Groothuis et al., 1998; Groothuis et al., 1999; Koks et al., 1999). On the other hand, other studies observed an adhesive capacity of endometrial cells even when the endometrium was not damaged (Witz et al., 1999). As a consequence of these findings, further studies were directed and they observed that different cell adhesion molecules, such as integrins, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, could be involved in the cell adhesion process (Witz et al., 2000).

2) Invasion, migration and metastasis of endometrial cells

Once the endometrial cells are attached to the peritoneal mesothelium, the processes of invasion, migration, and metastasis of these cells to surrounding tissues begin. For this, the enzymes matrix metalloproteinases (MMPs) and their inhibitors [(tissue inhibitors of matrix metalloproteinases (TIMPs)] play a key role (Hulboy et al., 1997; Salamonsen and Woolley, 1996; Woessner, 1991). MMP family has the ability to degrade the extracellular matrix by degrading several of its components, such as collagen, gelatins, proteoglycans, laminin, gibronectin, and elastin (Hulboy et al., 1997; Matrisian, 1990; Rodgers et al., 1993; Salamonsen and Woolley, 1996; Woessner, 1991). MMPs and TIMPs are tightly regulated by steroid hormones and cytokines, and previous evidence has suggested that they may play a key role in the origin and development of endometriosis (Cox et al., 2001; Koks et al., 2000; Marbaix et al., 1992). In this sense, abnormal expression levels of MMPs and TIMPs have been reported. Matrix

metalloproteinase-1 (MMP-1), matrix metalloproteinase-3 (MMP-3) and matrix metalloproteinase-7 (MMP-7) have been expressed in endometriotic lesions and lower concentrations of TIMPs have been reported in peritoneal fluid from women with endometriosis (Sharpe-Timms et al., 1998). An *in vitro* study evaluated the role of MMPs in the establishment of ectopic lesions and found that suppression of their activity by progesterone or intraperitoneal TIMP injections suppressed the development of endometriotic implants (Bruner et al., 1997). Therefore, the aberrant expression of MMPs and TIMPs in the endometriotic environment could induce and facilitate the invasion, migration, and metastasis of endometriotic implants (Gottschalk et al., 2000; Matsuzaki et al., 2010).

3) Generation of an inflammatory microenvironment

The generation of an inflammatory microenvironment plays a central role in the origin and development of endometriosis, promoting both the processes of adhesion and invasion of endometrial cells, facilitating the initial contact of cells with the peritoneal surface, as well as the processes of angiogenesis and proliferation of endometriotic lesions (Augoulea et al., 2012). In addition, they play a key role in the regulation of chemotaxis, mitosis, angiogenesis, and cell differentiation (Augoulea et al., 2012). Several cytokines regulate the expression of MMPs. Previous evidence has reported that interleukin 1α (IL-1α) could regulate the expression of MMPs, being an important stimulator of MMP-3 (Osteen et al., 1999). It has been suggested that a chronic inflammatory environment could alter folliculogenesis, fertilization and embryo implantation, and lead to infertility (Lousse et al., 2012). In addition, a large number of inflammatory biomarkers, such as interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), interleukin-1 receptor (IL-1R) or transforming growth factor β1 (TGFβ1) have been reported in the peritoneal fluid and serum of patients with endometriosis (Jaiswal et al., 2020; Kalu et al., 2007; Tarokh et al., 2019).

4. Development of angiogenesis around the nascent endometriotic implant

Angiogenesis plays an important role in the pathogenesis and pathophysiology of endometriosis. The neovascularization of the surrounding tissues is necessary to the development and proliferation of endometriotic implants. This process involves the interaction of a large number of growth factors, such as angiogenin (ANG), angiopoietin 1 (ANGPT1) and VEGF, which is recognized as the main angiogenic

factor. Previous studies have shown increased levels of ANG, vascular endothelial growth factor A (VEGFA) and vascular endothelial growth factor receptor-1 (VEGFR-1) in the peritoneal fluid of women with endometriosis compared to healthy women (Cho et al., 2012).

5. Endometrial cell survival and proliferation

The molecular processes described above, an evasion of immune surveillance, and a permissive peritoneal microenvironment must be present for endometriotic lesions to survive and proliferate. In addition, inflammatory response and oxidative stress seem to play a key role in this process. Oxidative stress, defined as the imbalance between reactive oxygen species and antioxidant systems, could play a key role in the origin of the inflammatory microenvironment. High concentrations of oxidative stress biomarkers could damage the main cellular biomolecules (amino acids, lipids, and deoxyribonucleic acid) and produce alterations in the structure of cell membranes, protein activity, and gene expression (Donnez et al., 2016). Previous studies have shown significantly higher expression of oxidative stress biomarkers in women with endometriosis compared to healthy women (Amreen et al., 2019; Polak et al., 2013).

In addition to the above processes, alterations in other molecular processes, such as cell cycle, cell differentiation, apoptosis, and lipid metabolism, could also promote the origin and progression of the disease (Charrasse et al., 2000; Dmowski et al., 2001; Liu et al., 2021b; Van Langendonckt et al., 2010):

- The cell cycle needs to be fully regulated and previous evidence has suggested that alterations in this cycle could promote the proliferation and malignancy of endometriotic cells. Although there are few studies exploring the role of the cell cycle in endometriosis, it has been suggested that aberrant expression of several genes could cause cell cycle alterations in women with endometriosis. One example is found in the cyclin dependent kinase 1 (*CDK1*) gene, which controls the transition from the G2 phase to the M phase and could play an important role in the progression of endometriotic lesions (Chen et al., 2021).
- Although the theory of retrograde menstruation is the most widely accepted, endometriosis could also result from the differentiation of various cell types into endometrial tissue. Previous studies have shown an overexpression of the homeobox A10 gene (*HOXA10*), which plays an important role in the

differentiation of embryonic tissue from endometrial tissue, and abnormal expression has been reported in different types of endometriotic lesions (Browne and Taylor, 2006; Van Langendonckt et al., 2010). Another gene related to this process, the platelet derived growth factor receptor α gene (*PDGFRA*), which has been shown to be upregulated in ovarian endometriosis compared to eutopic endometrium (Matsuzaki et al., 2006).

- Apoptosis or programmed cell death consists of an efficient elimination of tissue cells without causing an inflammatory response (Kerr et al., 1972). This process is controlled by the expression of a number of regulatory genes, such as BCL2 Like 1 (*BCL-2L1*), which promotes cell survival by blocking apoptosis, and BCL2-associated X Protein (*BAX*), which antagonizes *BCL-2* activity. In previous studies, it has been observed that the increased survival capacity of the endometrial cells of patients with endometriosis would be regulated by the increase of *BCL-2* protein levels and the decrease of *BAX* levels (Meresman et al., 2000).
- Alterations in lipid metabolism could be related to the onset and progression of endometriosis (Lu et al., 2023). Elevated lipoprotein levels have been associated with an increased risk of disease (Crook et al., 1997; Jofre-Monseny et al., 2008). The apolipoprotein E gene (*APOE*) plays a key role in lipid metabolism and in cell proliferation and survival. Its expression has been upregulated in cases of endometriosis compared to women without endometriosis (Liu et al., 2021b). In addition, crucial enzymes in the phosphoinositide signal transduction system, such as phosphoinositide-specific phospholipase C (PLC), could be involved in inflammation (Lo Vasco et al., 2012), a central process for the development of endometriosis.

1.7 ENDOMETRIOSIS RISK FACTORS

Endometriosis is considered a multifactorial disease, originated as a consequence of molecular and cellular alterations, which have been previously described, and the interaction of numerous epidemiological, immunological, hormonal, genetic, epigenetic, and environmental factors.

- *Epidemiological factors:*

  There are anatomical factors that could increase the risk of endometriosis. In this sense, menstrual blood acquires a notable importance, which is a matrix in close contact with the endometrium, and the alterations that occur in it could promote the origin and development of endometriosis. Previous evidence has suggested higher levels of estradiol in the menstrual blood of women with endometriosis compared to the levels in this same matrix in healthy women (Takahashi et al., 1989). In addition, early age at menarche, late menopause, short menstrual cycles, and longer duration of menstrual bleeding (Vercellini et al., 2014) together with nulliparity, low birth weight (Hediger et al., 2005; Missmer et al., 2004) and low body mass index (BMI) (Kabir et al., 2015), are common features in patients with endometriosis. In addition, a family history of endometriosis and an unhealthy lifestyle (alcohol use, poor dietary habits, or little or no physical activity) may also increase the risk of endometriosis.

- *Immunological factors:*

  An impaired immune system unable to eliminate menstrual debris in ectopic locations could be one of the causes of the development of endometriosis (Burney and Giudice, 2012). Endometrosis has been associated with changes in cell-mediated and humoral component of innate and acquired immunity. It is suggested that immunological alterations could play a critical role in the development and progression of endometriotic lesions. Endometrial tissue, in patients with the disease, has been found to be more resistant to the action of natural killer (NK) cells than endometrial tissue from women without the disease (Kang et al., 2014), which could also be related to the high prevalence of autoimmune diseases in these patients (Porpora et al., 2020). In addition, decreased cytotoxic activity of peripheral blood lymphocytes against autologous endometrial cells has been reported in women with endometriosis. An increase in certain subpopulations of T lymphocytes, such as CD69+, has also been reported, although its impact on the pathophysiology of the disease is unknown (Guo et al., 2020). There could also be alterations in the complement system of women with endometriosis (Karadadas et al., 2020).

- *Hormonal factors:*

  Endometriosis is considered an estrogen-dependent disease due to the important role that the expression of hormone receptors and enzymes involved in estrogen metabolism play in its etiology and pathophysiology (Kitawaki et al., 2002). Significant differences have been observed in the expression levels between the endometrium of women without endometriosis, the eutopic endometrium of endometriosis cases, and the ectopic endometrium (Kitawaki et al., 2002). For example, an increased level of aromatase, an enzyme that converts androgens into estrogens, has been reported in the endometrium of patients. A high E_2 production due to aberrant aromatase activity, leads to a marked increase in the local bioavailable concentration of E_2, promoting the implant development (Bulun et al., 2015). Previous evidence has also shown resistance to progesterone in the endometrium of cases (Burney and Giudice, 2012), with endometriotic lesions showing decreased levels of progesterone receptors compared to eutopic endometrium. Progesterone exerts an antiestrogenic effect in the endometrium by inducing 17β hydroxysteroid dehydrogenase 2 (HSD17β2), catalyzing the conversion of estradiol to estrone, which is much less estrogenic.

- *Genetic and epigenetic factors:*

  Both in the origin and in the development of endometriosis, the deregulation of the expression of specific genes involved in the different key cell signaling pathways to the development of this disease seems to play an important role.

  Previous studies have shown the influence of genetic factors on the susceptibility to develop endometriosis, since family predisposition may increase the risk of disease. Women with first-degree relatives with endometriosis have a 7% higher risk, compared to 1% of women with no family history (Simpson et al., 1980). On the other hand, twin studies have estimated the heritability of endometriosis at 47% (Saha et al., 2015), probably following a multifactorial polygenic pattern of inheritance (Hansen and Eyster, 2010). Genetic predisposition increases when advanced stages of the disease are reached (Klemmt and Starzinski-Powitz, 2018).

  Epigenetic alterations have also been found in endometriosis. In this regard, the hypermethylation of gene promoters, such as the HOXA10 gene and the

progesterone receptor type B (Wu et al., 2005), which causes their silencing, is noteworthy. In addition, increased levels of estrogen receptor β (ERβ) and steroidogenic factor-1 have been described as a consequence of hypomethylation (Xue et al., 2007a; Xue et al., 2007b).

- *Environmental factors:*

  Some lifestyle factos may increase, or decrease, the risk of disease. Considering dietary habits, previous evidence has shown that a high intake of fresh vegetables and fruits could decrease the risk of endometriosis. On the contrary, a high consumption of red meat could increase it (Parazzini et al., 2013). It has also been suggested that physical activity could reduce the risk of disease, although a larger number of studies are required to confirm this (Garavaglia et al., 2014).

  Over the last decades, society has evolved and there has been an increase in hormone-active chemicals [so-called endocrine-disrupting chemicals (EDCs)]. Since endometriosis is an estrogen-dependent disease, there is growing concern about the effects that human exposure to EDCs could have on human health. Although there are currently few studies exploring EDC exposure and endometriosis risk, it is suspected that exposure to some EDCs could play a key role in the origin and development of disease (Dutta et al., 2023; Smarr et al., 2016).

1.8 ENDOCRINE-DISRUPTING CHEMICALS. DEFINITION & CHARACTERISTICS

The evolution and modernization of society in recent decades has been associated with an increase in the production and use of chemical compounds, including combustion products of fossil fuels, chemicals released into the environment as a consequence of industrial activity, pesticides used in agriculture, compounds derived from plastics, and substances released from cosmetics and personal care products (PCPs), among others. Over the years, there has been growing evidence reporting potential adverse effects from human exposure to these chemicals. This aspect has significantly increased public concern about the effect and inherent risks of human exposure to these chemicals. Currently, it is known that these chemical substances of synthetic origin can mimic or block hormonal behavior and alter the homeostasis of the endocrine system (Dekant and Colnot, 2013). In this sense, the World Health Organization defines an EDC as "any

exogenous substance or mixture of substances that alter one or more functions of the endocrine system and consequently cause adverse health effects in an intact organism, in its progeny, or in the (sub) population" (Bergman et al., 2014; WHO, 2002).

EDCs are highly diverse, with different origins, structures, and functions (Fernández and Olea, 2014). La Merrill et al. (2020) have reviewed the scientific evidence and summarized the main characteristics (*KCs, key characteristics*) that define an EDC. Researchers have established these characteristics in ten: **1**: binding or activation of one or more hormone receptors; **2**: antagonism of hormone receptors, inhibiting or blocking the effects of endogenous hormones and, therefore, acting as antagonists of these receptors; **3**: modulation of the expression of different hormone receptors; **4**: alteration of signal transduction, through membrane, intracellular and/or nuclear receptors, attenuating or potentiating the hormonal action; **5**: epigenetic modifications in cells by interfering with the action of hormones, hindering their ability to alter the expression and/or action of a hormone receptor or the transcription of hormone-responsive genes; **6**: alteration of the synthesis of hormones; **7**: modification of hormone transport; **8**: modulation of the distribution, circulation and concentration of hormones to (or in) target organs and tissues; **9**: alteration of the metabolism and/or elimination of the hormones and, consequently, modification of the concentration and activity of the hormones; and **10**: modification of the fate of cells that produce hormones or respond to them, affecting the structure and organization of tissues, by interfering with or stimulating cell proliferation, differentiation, migration, and/or death (La Merrill et al., 2020).

1.9 CLASSIFICATION OF ENDOCRINE-DISRUPTING CHEMICALS

Currently there are multiple classifications of EDCs considering their production, origin, chemical structure, activity, persistence in the environment, etc. The most widely used classification is based on resistance to physical, chemical and biological degradation, and on the degree of lipid solubility. Thus, two main groups are established: persistent and non-persistent EDCs.

- Persistent EDCs are lipophilic substances that resist environmental degradation, have long half-lives, are slowly metabolized and tend to bioaccumulate and biomagnify in the food chain (Arrebola et al., 2013; Fernandez et al., 2008). Therefore, concentrations of these compounds can appear in living organisms

even decades after they were banned. This group includes the so-called persistent organic pollutants (POPs), comprising organochlorine pesticides [dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB) and γ-hexachlorocyclohexane (γ-HCH)], polychlorinated biphenyls (PCBs), dioxins, furans, polybrominated diphenyl ethers (PBDEs), perfluorinated compounds, such as perfluorooctanoic acid (PFOA), and some heavy metals, such as mercury, lead or cadmium. Dietary intake should be the main route of these contaminants (Porta et al., 2008).

- Non-persistent EDCs are rapidly metabolized and excreted by the body, mainly through the urine (Frederiksen et al., 2013; Søeborg et al., 2014). However, a continuous and daily exposure to these compounds has been evidenced in biomonitoring studies, currently constituting an important threat to health. This group includes different families of compounds, such as phenols [bisphenols, parabens (PBs), benzophenones (BPs) and phthalates], among others. The main routes of exposure are dietary and dermal, mainly through cosmetics and PCPs, although other less common routes, such as the inhalation route, have also been found (IARC, 2013; Wang et al., 2013).

This doctoral thesis focuses mainly on two families of non-persistent EDCs, PBs and BPs, since these compounds are present in cosmetics and PCPs, products frequently used by women, and whose exposure could increase the risk of gynecological diseases in general and endometriosis in particular.

1.10 PARABENS AND BENZOPHENONES

PBs are alkyl or aryl homologs of the p-hydroxybenzoic acid, readily soluble in alcohol, ether and acetone. Physically they are small colorless crystal or crystalline powders at room temperature, odorless and tasteless, active over a wide pH range, and with good stability after mixing with the product (Fransway et al., 2019). They have been widely used as preservatives in cosmetics, pharmaceuticals, food and children's products (Soni et al., 2005) due to their chemical stability, low cost, broad spectrum antimicrobial activity and low risk of inducing allergic reactions. The compounds, methyl-, ethyl-, propyl- and butyl-paraben (MeP, EtP, PrP and BuP, respectively) are the most commonly used PB congeners (Nowak et al., 2018; Soni et al., 2005) (Figure 3). The

European Commission in its Regulation (EU) No. 1004/2014 on cosmetic products regulated the use of PBs, setting a maximum limit of 0.4% for single esters and 0.8% for mixed esters, as preservatives in these products (European-Comission, 2014; Gálvez-Ontiveros et al., 2021).

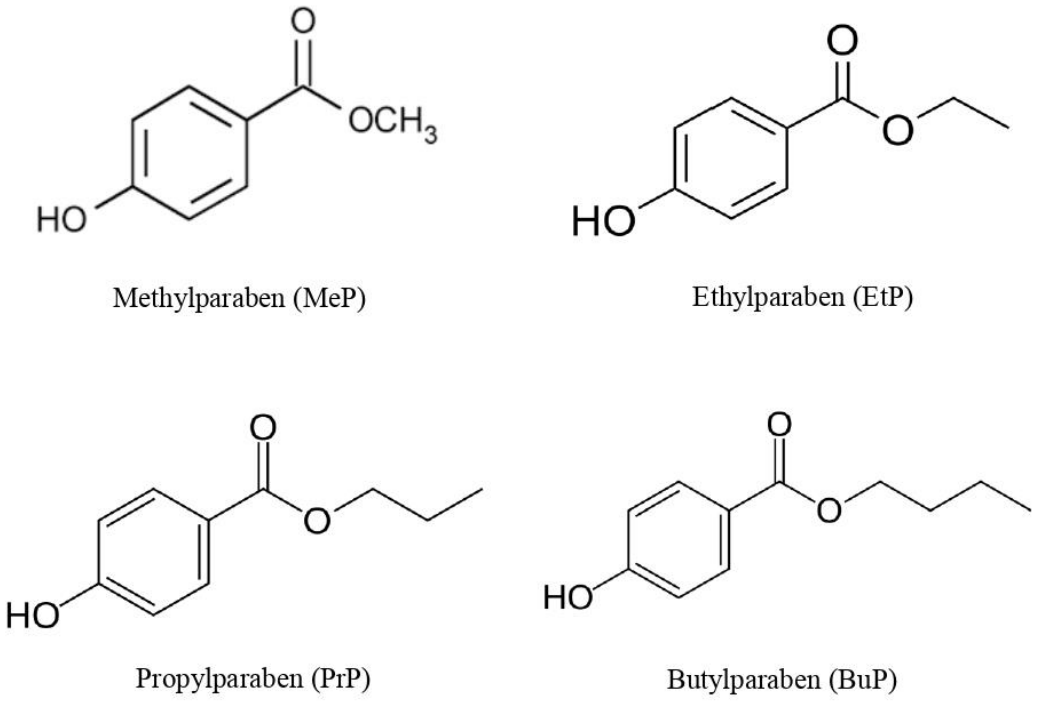


Figure 3. Chemical structure of paraben congeners.

BPs are aromatic ketones capable of absorbing ultraviolet light (λ: 280 – 400 nm) and dissipating it as heat. They are the most commonly used ultraviolet (UV) filters in industry, and are frequently included as components in cosmetic and PCPs formulations, food packaging, UV stabilizers, or as inks in printed food contact materials (Suzuki et al., 2005) and photoinitiators (Tsochatzis et al., 2020). They are also used in some plastic products and food packaging materials. Among the most common congeners are benzophenone 1 (BP-1), benzophenone 2 (BP-2), benzophenone 3 (BP-3), 4-hydroxybenzophenone (4-OHBP), benzophenone 6 (BP-6) and benzophenone 8 (BP-8) (Figure 4).

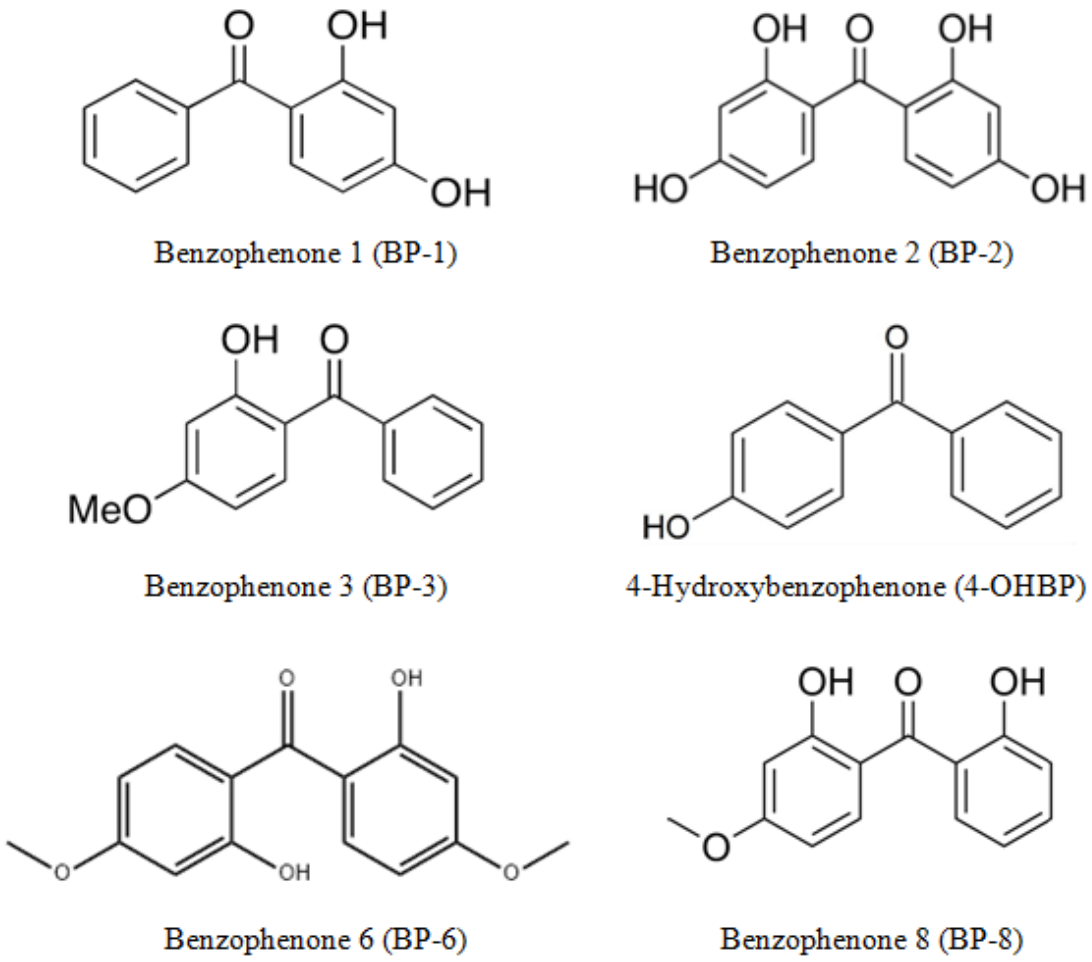


Figure 4. Chemical structure of benzophenone congeners.

Due to the favorable physico-chemical properties, PBs are the most prevalent ingredients after water in cosmetics and PCPs (Janjua et al., 2008). Therefore, the main route of exposure to PBs is dermal (Guo and Kannan, 2013); although to a lesser extent, the digestive route has also been considered a source of exposure to these EDCs (Nobile et al., 2020).

Similar to PBs, the dermal route is considered the main route of human exposure to BPs, although others are added such as inhalation, due to the presence of BPs in perfumes, cleaning products and in household dust (Surana et al., 2018; Wang et al., 2013), and digestive (IARC, 2013), due to the migration of BPs from food contact materials.

1.11 PARABENS AND BENZOPHENONES IN COSMETICS AND PERSONAL CARE PRODUCTS

PBs have gradually become the most abundant additives in cosmetics and pharmaceuticals (Andersen, 2008). In 1981, PBs were used in 13200 cosmetic products, increasing this number to over 22000 in the year 2006 (Andersen, 2008). Due to consumer demand for cosmetics, the amount of preservatives is expected to increase further in the future years (Nowak et al., 2021).

Previous literature has shown data on detection frequencies and concentrations of PBs in cosmetics and PCPs. One study reported detection rate of MeP, EtP and PrP of 98%, 32% and 38%, respectively, after studying the detection frequency of PBs in 215 cosmetic products (Rastogi et al., 1995). Considering more recent studies, the median detection frequencies for MeP, EtP, PrP, and BuP were 73%, 38%, 65%, and 25%, respectively, suggesting that PBs are ubiquitous in cosmetic products. The concentrations and detection frequencies of PBs could vary according to the type of PCP (rinse-off and leave-on products). In Spain, the PB concentrations of 22 shower gels were reported, finding that MeP was the most abundant PB followed by EtP and PrP (Esteve et al., 2016). The highest concentrations of MeP, EtP and PrP were detected in body or hand lotions and face creams (Guo et al., 2014). Another study analyzed 77 feminine hygiene products, showing that all of them contained at least one type of PB, and both MeP and EtP were found in >80% of these compounds, mainly in wipes, creams, bactericide solutions, deodorant sprays, and powders (Gao and Kannan, 2020). PB concentrations have also been detected in commercial dentifrices (Park et al., 2014), sanitary wipes, tickets, newspaper, food cartoons and paper currency (Liao and Kannan, 2014a).

Regarding BPs, previous studies have shown their presence in a wide variety of cosmetics and PCPs. In this sense, Rastogi et al. (1995) reported detected levels of up to 3 types of UV filters in 75 suscreen products from Europe and USA. BP-3 is the most studied congener, and concentrations of this compound have been found in shampoos, conditioners, fragrances, cosmetics, flavors, masks, powders, lip balms, and antiaging creams (Gonzalez et al., 2006; Schneider and Lim, 2019). A recent study showed detected levels of both BP-1 and BP-3 in 19.1% of the 283 products that were tested, mainly makeup products, which accounted for 45.2% of products with BP levels (Panico et al., 2019). Another study found detected levels of BP-3 in skin products and

lip products (16.9 and 23.8%, respectively) (Wahie et al., 2007). In the 2017 European Parliament Regulation, an acceptable dose of BP-3 was reduced from 10% to 6% in UV filters (European-Comission, 2017). In the USA, Japan, and South Korea, the BP-3 content is limited to 5%, due to suspected harmful effect (Food-Drug-Administration, 1998).

The non-persistent EDCs to which humans are exposed through the dermal route are metabolized by dermal esterases and their degree of dermal absorption will depend on the length of the ester chain and the formulation, decreasing with increasing length chain. On the other hand, lipid solubilizers could reduce percutaneous absorption, while penetration enhancers could increase it. Once the EDCs are absorbed, while the compounds ingested through the digestive tract are generally converted to more hydrophilic metabolites, those assimilated through the skin usually avoid first-pass metabolism, directly incorporating the original compound into the bloodstream, favoring its distribution in a wide variety of tissues and delaying its excretion through urine (Søeborg et al., 2014).

1.12 MECHANISMS OF ACTION OF ENDOCRINE-DISRUPTING CHEMICALS

Although the mechanisms of action through which EDCs can exert their effect are not yet fully elucidated, current knowledge indicates that they could act at different levels of complexity, interfering with a variety of hormone-signaling pathways and producing alterations in the homeostasis of the endocrine system. In this sense, EDCs can modify circulating hormone levels by acting on its synthesis, metabolism, or degradation. In addition, EDCs could reduce, increase or interfere with specific receptors for hormonal action and affect the response capacity of natural hormones (La Merrill et al., 2020). Considering the EDCs that interfere with the functioning of steroid hormones, it has been suggested that the observed effects could be a consequence of the activation or blockade of nuclear receptors. Furthermore, EDCs could also induce epigenetic modifications in hormone-responsive cells, altering the epigenetic action of endogenous hormones or directly altering epigenetic processes (La Merrill et al., 2020).

Both PBs and BPs have shown (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid activities (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Molina-Molina et al., 2008). For example, *in vitro* and *in vivo* experiments have revealed that PBs could interfere with the receptors for androgens,

estrogens, progesterone, glucocorticosteroids, and peroxisome proliferator-activated receptors (PPARs) (Nowak et al., 2018). Many BPs have also been involved in the disruption of the hypothalamic–pituitary–gonadal system (Schlumpf et al., 2004).

Complementary mechanisms of action have been postulated, such as inflammation and oxidative stress (Artacho-Cordón et al., 2019; Mustafa et al., 2015; Watkins et al., 2015). Although inflammation is part of the immune response and facilitates the defense, repair, turnover and adaptation of many tissues, chronic and systemic inflammation is detrimental and can alter normal physiological functions (Calder et al., 2017). In this regard, most studies considering bisphenol A (BPA) exposure have shown positive associations with inflammatory biomarker levels (Huang et al., 2017; Watkins et al., 2015; Yang et al., 2009), however few of these studies considered exposure to PBs and BPs. The available evidence exploring possible associations between EDCs and inflammatory biomarkers has shown limited and sometimes contradictory results, requiring a more studies in this regard.

Oxidative stress has also been related to exposure to EDCs (Artacho-Cordón et al., 2019; Thompson et al., 2015; Watkins et al., 2015), and increased levels of lipid peroxidation (Watkins et al., 2015). Decreased levels of antioxidant capacity (Artacho-Cordón et al., 2019) have been related to exposure to PBs and BPs. In this sense, it has been suggested that some EDCs could induce oxidative stress through estrogen receptor-α signaling pathways (Cho et al., 2018).

1.13 ADVERSE HEALTH EFFECTS

The adverse effects that exposure to EDCs can have on human health appear to be different based on age and gender. It is suspected that in men, exposure to EDCs could cause alterations in the development of the genitourinary system, including cryptorchidism, testicular cancer, and infertility (Ibarluzea et al., 2004; Olea and Fernandez, 2007). In women, exposure to EDCs has been linked to hormone-dependent cancers (either breast or ovarian) (Ibarluzea et al., 2004), uterine fibroids and endometriosis. In addition, in both men and women, exposure to EDCs could increase the risk of different chronic diseases, such as metabolic syndrome and its components (obesity, insulin resistance, hypertension, or dyslipidemia), neurobehavioral development disorders, and poor thyroid function (Arrebola et al., 2015; La Merrill et al., 2020; Pastor-Barriuso et al., 2016).

It is suspected that exposure to PBs and BPs could be associated with different gynecological diseases, including breast, vaginal, ovarian and endometrial cancers, uterine fibroids, gestational diabetes and other adverse pregnancy-related outcomes, impaired ovarian function, polycystic ovary syndrome, reduced fertility and endometriosis. Early studies suggesting that PCP use may increase the risk of breast cancer (Darbre, 2001), detected a wide variety of EDCs, including PBs, in these tumors, with high concentrations in the axillary region, suggesting that EDCs included in deodorants, body lotions, sprays, moisturizers and sunscreen products may be contributing to an increased risk of developing these diseases. Other studies with case-control design supported this hypothesis (Linhart et al., 2017). However, not all studies have found results along the same lines, e.g. Rylander et al. (2019) found no association between the use of skin care products and the risk of breast and endometrial cancer. It has also been observed that long-term exposure to ethinylestradiol-containing body care creams may increase the risk of abnormal genital bleeding and breast cancer (Komori et al., 2008). On the other hand, it has been observed that the use during pregnancy of lotions that included synthetic hormones in their composition was associated with very high concentrations in the hair of the offspring (Guarneri et al., 2008).

Despite these studies, it is difficult to establish the potential effect of the use of cosmetics and PCP, and their components, on human health. The difficulties are several, the wide variety of products and usage patterns, different formulations and individual susceptibility, among others (Darbre, 2001; Darbre and Harvey, 2008; Harvey and Darbre, 2004). However, EDC internal dose information could be a reflection of the magnitude and type of cosmetics and PCPs used. In this regard, urinary concentrations of PBs have been associated with an increased risk of breast cancer (Parada et al., 2019). Trace levels of PBs have also been found in endometrial tissue samples associated with an increased risk of endometrial carcinoma (Dogan et al., 2019). PrP levels were related to a decreased ovarian reserve (Smith et al., 2013) and PBs levels with early breast and pubic hair development, as well as earlier menarche (Harley et al., 2019). Urinary levels of BPs have also been associated with blood pressure during pregnancy (Liu et al., 2019a), and with thyroid hormone levels, growth factors, and reduced fetal growth (Krause et al., 2012). Exposure to BPs in experimental animals has been linked to feminized sexual behavior and increased uterine weight (Krause et al., 2012; Schlumpf et al., 2010). An *in vivo* study showed that exposure to BP-1 and BP-3

altered early events in ovarian cells, such as germ cell development and expression of crucial genes related to follicular assembly (Santamaria et al., 2019). Other studies reported BP-dependent induction of metastasis in an *in vivo* model for ovarian cancer (Shin et al., 2016).

Despite the wide variety of diseases that have been linked to human exposure to EDCs, the potential effect that exposure to PBs and BPs could have on the risk and development of endometriosis is still unknown. In this regard, previous systematic reviews have summarized the available literature on human exposure to persistent and non-persistent EDCs and endometriosis, reporting little or no studies on exposure to PBs and BPs and risk of endometriosis (Shirafkan et al., 2023; Wieczorek et al., 2022).

2. RATIONALE & HYPOTHESIS

Endometriosis is a chronic gynecological disease whose incidence has been progressively increasing in recent decades, affecting a large number of women of childbearing age worldwide. Despite the growing number of studies on this pathology, endometriosis is still considered an enigmatic, controversial, and challenging disease, due to still existing knowledge gaps on its etiology and pathophysiology, delayed in diagnosis and mainly symptomatological treatment. It is known to be a multifactorial disease, in which immunological, hormonal, genetic, epigenetic, and environmental factors could be involved. Since it is an estrogen-dependent disease, it is suspected that exposure to hormonally active environmental chemicals (EDCs), whose exposure has been increasing over the years due to the modernization of society and changes in lifestyle, could play an important role in the origin and development of this disease. The use of cosmetics and PCPs has also increased markedly over the years, and it is suspected that exposure to parabens and benzophenones, two families of EDCs present in a wide variety of cosmetics and PCPs, could be associated with different gynecological diseases, including endometriosis, although the mechanisms of action through which they exert their effect are still unknown.

An important part of this doctoral thesis has been carried out in collaboration with the private company, Inves Biofarm (Instituto de Investigación Biotecnológica, Farmacéutica y de Medicamentos Huérfanos S.L.; Spain), dedicated to the development of cosmetic and therapeutic products, both for mediators and for their own production. This collaboration has made it possible to investigate the presence and concentration of possible compounds with hormonal activity in a selection of cosmetic products, cosmetic formulations and plastic packaging, developed and used by Inves Biofarm, and to identify different families of EDCs in hormonally active products. As a result of this collaboration, the company will be able to market its products with the assurance of being EDC-free.

Hence, the main hypothesis of this doctoral thesis is that:

Exposure to hormonally active chemicals, present in cosmetics and PCPs, among other products, could increase the risk of endometriosis in women of childbearing age, and promote some of the key molecular processes in the pathophysiology of this disease.

3. OBJECTIVE

The general objective of this PhD thesis was to:

Explore the associations between exposure to two families of EDCs present in cosmetics and PCPs (PBs and BPs) and the risk of onset and development of endometriosis in women of childbearing age.

The specific objectives set out in this PhD thesis were to:

1. Assess concentrations of PBs and BPs in menstrual blood, a matrix in intimate contact with the endometrium, compare concentrations of these contaminants in menstrual and peripheral blood, and explore related sociodemographic and lifestyle factors.

2. Explore the associations between exposure to PBs and BPs and the risk of endometriosis, as well as, the frequency of use of cosmetics and PCPs, and to evaluate the influence of oxidative stress on the associations found.

3. Describe the expression profile, in endometriotic tissue, of different genes related to key cell signaling pathways for the development and progression of endometriosis, and to explore their relationship with the concentrations of PBs and BPs in this tissue.

4. Evaluate the biological activity (estrogenic and anti-androgenic) of cosmetic products and formulations of the company Inves Biofarm, and to analyze the presence of different endocrine disruptors.

4. MATERIAL & METHODS

4.1 OBJECTIVES 1, 2 AND 3

4.1.1 STUDY DESIGN AND POPULATION

Objective 1 was addressed using a cross-sectional design. A total of 57 healthy Spanish women were included between May 2015 and April 2016, recruited by invitation through advertisements at the University of Granada (Granada, Southern Spain) and in social networks of the Chair of Obstetrics and Gynecology, "Antonio Chamorro/Alejandro Otero". Inclusion criteria were established as age between 18 and 50 years, menstruation in the last 6 months and willingness to use menstrual cups. Pregnant women and/or women using hormonal contraceptives were excluded. All volunteers meeting the eligibility criteria were informed about the collection, storage, and delivery of menstrual blood samples.

Objectives 2 and 3 fall within the hospital-based case-control EndEA study (**End**ometriosis y **E**xposición **A**mbiental), which was designed to investigate from a holistic point of view the possible effect of exposure to EDCs on the risk of endometriosis, evaluating its role in hormonal balance, the generation of a possible inflammatory and oxidative microenvironment, and in the expression of endometriosis-promoting genes. The recruitment of the population was carried out from January 2018 to July 2019 in the two public hospitals 'San Cecilio' and 'Virgen de las Nieves' in the city of Granada. The inclusion criteria were: childbearing age (20-54 years), receipt of abdominal surgery (laparotomy or laparoscopy), and pathology report on the presence (cases) or absence (controls) of endometriosis. Further criteria for controls were: performance of laparotomy or laparoscopy for non-malignant disease in the same hospital as cases (e.g., acute appendicitis, biliary disease, hiatus hernia, ovarian torsion, corpus luteum and cystadenomas, among others), no findings of endometriosis during the surgery, and no history of endometriosis. Exclusion criteria for all participants were: morbid obesity (BMI >35), previous history of cancer (except non-melanoma skin cancer), pregnancy at recruitment, and inability to sign the informed consent. A study population of 124 women was included: 35 cases and 89 controls. Case women were staged according to the Revised American Fertility Society classification (Canis et al., 1997).

4.1.2 SAMPLE COLLECTION

Menstrual blood samples were collected in a menstrual cup at the participants' homes. Subsequently, the samples were stored in a polypropylene collection tube until it was full or menstruation ended. The samples were refrigerated for up to 24 hours and subsequently frozen at -20ºC. Finally, they were sent in a dry ice box to the laboratory, where they were weighed and stored at -20ºC. To make a comparison between the concentrations of PBs and BPs in peripheral and menstrual blood, both kinds of samples were collected from 12 volunteers from Granada who agreed to participate. Peripheral blood samples were collected by a hospital-trained technician, centrifuged at 2000 g (4 ºC) for 15 min to separate serum, and then stored at -20 ºC until extraction and chemical analysis.

Fasting early-morning urine samples were collected on the same day immediately before surgery. All samples were divided into 1 mL aliquots. Moreover, endometriotic tissue samples (3g) were collected by the surgeons during surgery and kept in QIAzol reagent (Qiagen, Hilden, Germany) to ensure RNA stability. Samples were stored in PB- and BP-free glass tubes, anonymously coded, and immediately stored at -80 ºC at the Biobank of the Public Andalusian Healthcare System.

All the participants included were previously informed and all signed a prior informed consent, which was approved by the Biomedical Research Ethics Committee of the province of Granada.

4.1.3 SAMPLE PREPARATION AND CHEMICAL ANALYSES

4.1.3.1 Extraction protocol

For the extraction of peripheral and menstrual blood and urine samples, a dispersive liquid–liquid microextraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS), using previously validated protocols were used (Jimenez-Diaz et al., 2016b; Vela-Soria et al., 2014). Urine and blood samples were thawed at room temperature. In the case of blood, each sample (1.0 mL) was spiked with 10 µL of surrogate standard solution (1 mg/L of BP-d^{10} and 2.5 mg/L of EtP-$^{13}C_6$) and enzymatically treated with 50 µL of an enzyme solution (β-glucuronidase/sulfatase), which was prepared dissolving 10 mg of β-glucuronidase/sulfatase (3.10^6 U/g solid) in 1.5 mL of 1 M ammonium acetate buffer

(pH 5.0). Subsequently, the samples were mixed and incubated at 37 ºC for 24 hours. Next, 1 mL of acetone was added and the mixture was vortexed for 30 seconds and centrifuged for 10 minutes at 2600g. Samples were diluted with 10 mL of 10% aqueous NaCl solution (w/v), and adjusted to pH 2.0 for DLLME. In the subsequent extraction phase, a mixture of acetone (dispersing solvent) and trichloromethane (extraction solvent) was injected by syringe, and samples were then shaken manually and centrifuged for 10 minutes. For blood samples, the volume of the mixture was 820 µL of acetone and 800 µL of trichloromethane, the shaking time was 20 seconds and the centrifugation speed was 2600 x g. In the urine samples, the volume of the mixture was 1.0 mL of acetone and 0.5 mL of trichloromethane, the manual shaking time was 30 seconds, and the centrifugation speed was 4000x g. Subsequently, the entire sedimented phase was transferred to a clean glass vial and evaporated under a nitrogen stream. Finally, regarding blood samples, the residue was dissolved with 100 µL of a mixture of methanol (0.1% ammonia)/water (0.1% ammonia) 60/40 (v/v), and vortexed for 30 seconds, and considering urine samples, the residue was dissolved with 100 µL of a mixture of acetonitrile (0.1% ammonia)/water (0.1% ammonia) 70/30 (v/v), and vortexed for 30 seconds. The extracts were then ready for analysis by UHPLC-MS/MS using ACQUITY UPLC™ H-Class equipment. A Xevo TQ-S tandem quadrupole mass spectrometer equipped with an orthogonal Zspray™ electrospray ionization source was employed for EDC detection. The compounds were separated using an Acquity UPLC©BEH C_{18} column. The total amounts (free and conjugated) of PBs and BPs were determined in both blood and urine, including 4 paraben congeners (methyl-, ethyl-, propyl- and butyl-paraben) in both matrices, three benzophenone congeners (BP-1, BP-3 and 4-OHBP) in urine samples and six of them in blood samples (BP-1, BP-2, BP-3, 4-OHBP, BP-6 and BP-8).

4.1.3.2 Calibration and quality control procedures

Blood samples and urine samples were assessed in three and eight batches, respectively, over a period of three weeks. Each batch included standards for calibration curved at beginning and end of each batch, 15-20 unknown samples, 2 blanks, and 2 quality-control samples (a sample of deionized water and a sample of menstrual blood or urine spiked with different concentrations of the target compounds). The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a signal–to noise ratio (SNR) of 3. The LOD obtained for menstrual blood samples were: 0.10

ng/mL for PrP, BuP, and BP-8; 0.20 ng/mL for MeP, EtP, BP-1, and 4-OHBP; and 0.30 ng/mL for BP-2, BP-3, and BP-6. LODs obtained for urine samples were: 0.10 ng/mL for MeP, EtP, PrP, and BuP, 0.05 ng/mL for BP-1, and 0.06 mg/mL for BP-3 and 4-OHBP.

4.1.4 BIOLOGICAL ASSESSMENT

4.1.4.1 Oxidative stress biomarkers

To assess the influence of oxidative stress on the associations between exposure to EDCs and the risk of endometriosis, biomarkers of oxidative lipid damage and total antioxidant power (TAP) were quantified in urine samples. Oxidative lipid damage was estimated by quantifying the levels of thiobarbituric acid reactive substances (TBARS), using the TBARS microplate colorimetric assay kit from Oxford Biomedical Research (Rochester Hills, MI, USA). The amount of TBARS was determined by comparing its absorbance of the urine samples with that of a known TBARS standard curve, carefully following the manufacturer's instructions. Each sample was measured in duplicate. In addition, to avoid interference from urine staining, the absorbance of untreated urine was analyzed in parallel.

First, a total of 200 µL of the sample (and 200 µL of the standard solution in the standard line tubes) were added to each tube. 200 µL of indicator solution was added to standard curve and samples, while 200 µL of the buffer solution was added to the blank urine samples. After incubation at room temperature for 45 minutes, 150 µL were transferred to the corresponding well to finally be read in a spectrophotometer, using 532 nm as the primary wavelength. The kit has a TBARS detection sensitivity range of 20 µM to 1 µM.

On the other hand, the urinary concentration of TAP was analyzed in a subset of participants with available urine sample (n=108), using a colorimetric microplate assay supplied by Oxford Biomedical Research (Rochester Hills, MI, USA). This assay measures the total capacity to reduce Cu^{++} to Cu^{+}, which selectively forms a stable 2:1 complex with the chromogenic reagent that has maximum absorption at 450 nm. A known concentration of uric acid was used to create a reference curve for comparison with readings in samples. Samples were diluted 1:40 in the provided dilution buffer, and 200 µL of diluted samples or standards from the standard curve were added to each

well. After performing a reference measurement, 50 µL of copper solution were added to each well and incubated for 3 minutes at room temperature. Finally, 50 µL of stop solution were added to each well and the plate was read in a spectrophotometer at a wavelength of 450 nm. The LOD was 0.50 mM.

4.1.4.2 RNA isolation & quantitative real-time polymerase chain reaction

For only 33 of the study women with endometriosis (n=35), sufficient endometriotic tissue sample was available to determine 36 genes, selected as being involved in 9 different cell signaling pathways related to endometriosis (cell adhesión, migration/invasión/metastasis, inflammation, angiogenesis, cell proliferation/hormone stimulation, cell cycle, apoptosis, cell differentiation and lipid metabolism) (Table 1). The criteria for the selection of these genes were based on previous evidence relating EDC exposure to gene expression of these genes, their participation in the different cell signaling pathways and/or their relationship with endometriosis (Chung et al., 2002; Roy et al., 2015).

First, total RNA was extracted from 30 mg of endometriotic tissue samples with the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Final RNA concentration and quality (260/280 ratio) were determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Secondly, total RNA (1000 ng) was transcribed into complementary deoxyribonucleic acid (cDNA) with the iScriptAdvanced cDNA Synthesis Kit for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (Bio-Rad Laboratories, Hercules, California, USA). Lastly, to measure gene expression, a real-time PCR was carried out using a CFX96 Real-time PCR detection system (Bio-Rad Laboratories, Hercules, California, USA) using SsoAdvanced SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, California, USA). Primers used for these studies were purchased from Bio-Rad Laboratories (Hercules, California, USA).

All values were normalized using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) RNA expression levels. Gene expression was analyzed by Bio-Rad CFX 96 Manager software to determine the cycle of quantification (Ct) and was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1. Genes and cell signaling pathways.

Cell signaling pathway	Gene symbol	Gene name
Cell adhesion	ITGB2	Integrin beta-2
	CLDN7	Claudin 7
Invasion, migration and metastasis	MMP1	Matrix Metallopeptidase 1
	MMP7	Matrix Metallopeptidase 7
	FUT8	Fucosyltransferase 8
	RRM2	Ribonucleotide Reductase M2
	MDK	Midkine
	RHOB	Ras Homolog Family Member B
	SPRY2	Sprout Homolog 2
Inflammation	IL1R2	Interleukin 1 Receptor, Type II
	IL1RL1	Interleukin 1 Receptor, Type I
	IL6ST	Interleukin 6 Cytokine Family Signal Transducer
	NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
	TNFRSF1B	Tumor Necrosis Factor Receptor Superfamily Member 1B
Angiogenesis	ANG	Angiogenin
	ANGPT1	Angiopoietin 1
	sVEGFR-1	Soluble Vascular Endothelial Growth Factor Receptor-1
	VEGFA	Vascular Endothelial Growth Factor A
Cell proliferation and hormonal stimulation	CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1
	DUSP6	Dual Specificity Phosphatase 6
	ERα	Estrogen Receptor 1
	PGR	Progesterone Receptor
	STAR	Steroidogenic Acute Regulatory Protein
Cell cycle	BMI1	BMI1 Proto-Oncogene, Polycomb Ring Finger
	CCNB1	Cyclin B1
	CDK1	Cyclin Dependent Kinase 1
Apoptosis	BAX	BCL2-associated X Protein
	BCL2L1	BCL2 Like 1
	FOXO3	Forkhead Box P3
	SPP1	Secreted Phosphoprotein 1
Cell diferentiation	HOXA10	Homeobox A10
	PDGFRA	Platelet Derived Growth Factor Receptor Alpha
	SOX2	SRY-Box Transcription Factor 2
Lipid metabolism	APOE	Apolipoprotein E
	PLCG1	Phospholipase C Gamma 1
	PLCG2	Phospholipase C Gamma 2

4.1.5 INFORMATION FROM THE PARTICIPANTS

The sociodemographic and lifestyle information required from the study population included in objective 1 was collected through an *ad hoc* questionnaire completed by each participant. The data obtained included sociodemographic characteristics, such as age, place of residence, education, occupation, height, and weight, and BMI calculation; characteristics related to dietary habits, including frequency of consumption of fish,

dairy products, cheese, cold meat, meat, fat, pulses, fresh and cooked vegetables, fruit, eggs, pasta, bread, canned food, and organic food; reproductive characteristics, including age at first menstruation, parity, interval since last pregnancy, and accumulated breastfeeding time; and frequency and use of cosmetics and PCPs, including hair dye and dermal oil/creams.

For the EndEA study, three questionnaires were used, adapting those designed by the 'World Endometriosis Research Foundation' (WERF): 1) epidemiological questionnaire, 2) clinical questionnaire; and 3) surgical questionnaire.

1) Epidemiological questionnaire, completed by the participants hours before surgery, that included the following sections: *sociodemographic information* (residence, studies, work, height, weight, weight change...), *dietary habits* (consumption of the main food groups: fish and shellfish, white and blue fish, dairy products, sausages, meat, butter, legumes, fruit, eggs, bread, vegetables, pasta, rice...), *use of personal care products* (frequency and extension of the use of sun creams, as well as frequency of use of shampoo, gel, deodorant, perfume, toothpaste, facial creams, body lotions, mask, makeup, eyeliner, lipstick...) and *physical activity* (weekly time dedicated to walking, running, cycling, dancing, tennis , swimming...).

2) Clinical questionnaire, also completed by the participants hours before surgery, that included the following sections: *menstrual and hormonal history* (age at menarche, characteristics of the menstrual cycle: regularity, abundance of bleeding, use of oral contraceptives or intensity and characteristics of premenstrual syndrome), *pregnancy and fertility* (number of pregnancies and children, use of fertility treatments, delivery characteristics, presence of gestational diabetes...), *pain* (presence and intensity of chronic pelvic pain, use of analgesic medication, intensity of pelvic pain chronic pain and dysmenorrhea, dyspareunia, pain interference in activities of daily living and bowel movements...), *medical history* (previous history of cancer, cardiovascular disease, chronic fatigue syndrome or multiple sclerosis, among others; previous intervention for endometriosis, family history of endometriosis, most relevant symptoms of endometriosis...), and *use of drugs*. In addition, an extra section was included in order to quantify pelvic dysfunction and quality of life in

women with and without endometriosis, adapting to Spanish the 'BENS Score' scale developed and validated by Riiskjær et al. (2017).

3) Surgical questionnaire, completed by the surgeons after the patients' surgery on the characteristics of the surgery (laparoscopy, abdominal laparotomy, or other), the reasons of the intervention, and the diagnosis after the intervention. In addition, in cases of endometriosis, information was collected on the presence of endometriomas, number, size and location of the lesions.

4.1.6 STATISTICAL ANALYSIS

Descriptive analysis of the sociodemographic, lifestyle and gynecological characteristics of the study population was conducted. Urinary and menstrual concentrations of individual BP and PB congeners, the sum of PBs (∑PBs) and BPs (∑BPs), oxidative stress biomarkers and endometriotic tissue gene expression levels were described using means, standard deviations, medians, 25^{th} – 75^{th} percentiles and minimum and maximum concentrations (quantitative variables), and frequencies (categorical variables). The Shapiro-Wilk test was applied to check the normality of variable distributions, and data found to be non-normally distributed underwent logarithmic transformation.

The Spearman test was performed for compounds detected in >75% of samples in order to evaluate correlations between pairs of the compounds detected in menstrual blood (intra-matrix correlation), and between the menstrual and peripheral blood concentrations of each compound (inter-matrix correlation). In addition, this statistical test was also performed to evaluate monotonic correlations between urinary concentrations of PBs/BPs and the expression of genes with detection/expression frequencies above 75%.

Bivariate analyses were also conducted between cases and controls using the chi-square and Student's (or Mann-Whitney) tests as appropriate. In this sense, the Mann-Whitney test was used to compare urinary concentrations of BPs, PBs, and oxidative stress biomarkers between cases and controls and between stage 1 cases and stage II/III/IV cases.

Multivariate linear regression analyses using a combination of backward and forward stepwise multiple linear regression were performed to explore the sociodemographic/lifestyle factors potentially related to menstrual blood concentrations of PBs and BPs, and to identify potential cosmetic and PCP-related predictors of urinary PB and BP concentrations. PBs and BPs for which <75% of samples were above the LOD were considered as dichotomous variables (<LOD/>LOD), and related factors were evaluated using bivariate and multivariate logistic regression models.

Unconditional logistic regression analyses were performed to determine the odds ratios (ORs) for endometriosis risk of urinary concentrations of BPs, PBs, and oxidative stress biomarkers. An additional model was performed, stratifying by tertiles concentrations of PBs, BPs and oxidative stress biomarkers. Regression analyses were sequentially adjusted for (1) urinary creatinine; (2) urinary creatinine, age, and BMI; and (3) urinary creatinine, age, BMI, residence, and parity. The relationship between PB or BP exposure and oxidative stress biomarkers was examined using linear regression models adjusted for urinary creatinine, age, BMI, residence, and parity, expressing the results as exp (β) with 95% confidence intervals.

Bivariate linear regression models were also developed to evaluate the relationship between PB/BP exposure and gene expression levels, expressing the results as β with 95% confidence intervals. In parallel, simple linear regression models were constructed after patient stratification (i) into lower or higher PB and BP exposure (below or above the median concentration of each congener); and (ii) into low, moderate, and high exposure, based on tertiles of PB and BP concentrations. Genes expressed in 25-75% of samples were considered as dichotomous variables (detected/not detected), and simple logistic regression models were used to evaluate their association with PBs and BPs, while no analyses were performed on genes expressed in <25% of samples. Moreover, the potential confounding effect of sociodemographic and reproductive characteristics was explored by introducing individual variables in the regression models.

Given the limited sample size, *post-hoc* analysis were conducted to estimate the statistical power (1-β), using G*Power 3.1.9.7 statistical software (Düsseldorf University, Germany). For the main analysis between BP and PB concentrations and the risk of endometriosis, it showed that the power was >0.99 for adjusted ORs ≥3.34, a total sample size of 124 women (35 cases and 59 controls), and an α-error of 0.05. For

the Spearman correlation analyzes exploring associations between exposure to PBs and BPs and gene expression levels, the power of the associations found ranged from 65% to 98% (ρ coefficients ranging from 0.48 to 0.65; n=22).

The significance level was set at p=0.05, although results with p-values between 0.10 and 0.05 were also cautiously discussed. SPSS Statistics 23.0 (IBM, Chicago, IL, USA) and R statistical computing environment v3.1 were used for data analyses.

4.1.7 DATA SOURCES AND SEARCH STRATEGY OF THE SYSTEMATIC REVIEW

The literature search was carried out in three databases: Medline (through the PubMed search engine), Web of Science, and Scopus. The search strategy included terms related to exposure to non-persistent EDCs, including PBs and BPs, and terms related to inflammation (Table 2). The question posed was: “Is there evidence for an association between human exposure to bisphenols, PBs and BPs, and inflammatory biomarkers?”. To help answer this question, the PECO (Participants, Exposure, Comparator, and Outcomes) statement was used (Morgan et al., 2018).

- Participants: Humans.

- Exposure: Bisphenols [bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol A-glycidyl methacrylate (BisGMA), bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE)], PBs [methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP) and butylparaben (BuP)] and BPs (BP1-12).

- Comparators: Not applicable.

- Outcomes: Inflammatory biomarkers (cytokines, intracellular adhesion molecules, humoral mediators, C-reactive protein, inflammatory milieu, phagocytic leukocytes, antibodies, complement proteins, receptor activator of nuclear factor-kappa B, prostaglandin-endoperoxide synthases).

Table 2. Search strategy in the Medline, Scopus and Web of Science databases.

Database	Pubmed (Medline)
Date	01/02/2023
Strategy	#1 AND #2
#1	("bisphenols" [Title/Abstract] OR "bisphenol A" [Title/Abstract] OR "bisphenol S" [Title/Abstract] OR "bisphenol F" [Title/Abstract] OR "bisphenol A-glycidyl methacrylate" [Mesh] OR "bisphenol A-glycidyl methacrylate" [Title/Abstract] OR "bisphenol A diglycidyl ether" [Title/Abstract] OR "bisphenol F diglycidyl ether" [Title/Abstract] OR "parabens" [Mesh] OR "parabens" [Title/Abstract] OR "methylparaben" [Title/Abstract] OR "ethylparaben" [Title/Abstract] OR "prophylparaben" [Title/Abstract] OR "buthylparaben" [Title/Abstract] OR "benzophenones" [Mesh] OR "benzophenones" [Title/Abstract] OR "benzophenone 1" [Title/Abstract] OR "2,4-dihydroxy-benzophenone" [Title/Abstract] OR "benzophenone 2" [Title/Abstract] OR "2,2',4,4'-tetrahydroxybenzophenone" [Title/Abstract] OR "benzophenone 3" [Title/Abstract] OR "oxybenzone" [Title/Abstract] OR "benzophenone 4" [Title/Abstract] OR "sulisobenzone" [Title/Abstract] OR "4-hydroxybenzophenone" [Title/Abstract] OR "benzophenone 5" [Title/Abstract] OR "benzophenone 6" [Title/Abstract] OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" [Title/Abstract] OR "benzophenone 7" [Title/Abstract] OR "5-chloro-2-hydroxybenzophenone" [Title/Abstract] OR "benzophenone 8" [Title/Abstract] OR "dioxybenzone" [Title/Abstract] OR "benzophenone 9" [Title/Abstract] OR "benzophenone 10" [Title/Abstract] OR "benzophenone 12" [Title/Abstract] OR "octabenzone" [Title/Abstract])
#2	("Inflammation Mediators" [Mesh] OR "inflammation mediators" [Title/Abstract] OR "Inflammation" [Mesh] OR "Inflammation" [Title/Abstract] OR "cytokines" [Mesh] OR "cytokines" [Title/Abstract] OR "intracellular adhesion molecules" [Title/Abstract] OR "humoral mediators" [Title/Abstract] OR "c reactive protein" [Title/Abstract] OR "inflammatory milieu" [Title/Abstract] OR "phagocytic leukocytes" [Title/Abstract] OR "antibodies" [Title/Abstract] OR "complement proteins" [Title/Abstract] OR "receptor Activator of Nuclear Factor-kappa B" [Mesh] OR "receptor Activator of Nuclear Factor-kappa B" [Title/Abstract] OR "prostaglandin-Endoperoxide Synthases" [Mesh] OR "prostaglandin-Endoperoxide Synthases" [Title/Abstract])
Database	Scopus
Date	01/02/2023
Strategy	#1 AND #2
#1	TITLE-ABS-KEY ("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#2	TITLE-ABS-KEY ("Inflammation Mediators" OR "Inflammation" OR "cytokines" OR "intracellular adhesion molecules" OR "humoral mediators" OR "c reactive protein" OR "inflammatory milieu" OR "phagocytic leukocytes" OR "antibodies" OR "complement proteins" OR "receptor Activator of Nuclear Factor-kappa B" OR "prostaglandin-Endoperoxide Synthases")
Database	Web of Science
Date	01/02/2023
Strategy	(#1 OR #2) AND (#3 OR #4)
#1	TI=("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#2	AB=("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#3	TI=("Inflammation Mediators" OR "Inflammation" OR "cytokines" OR "intracellular adhesion molecules" OR "humoral mediators" OR "c reactive protein" OR "inflammatory milieu" OR " phagocytic leukocytes" OR "antibodies" OR "complement proteins" OR "receptor Activator of Nuclear Factor-kappa B" OR " prostaglandin-Endoperoxide Synthases")
#4	AB=("Inflammation Mediators" OR "Inflammation" OR "cytokines" OR "intracellular adhesion molecules" OR "humoral mediators" OR "c reactive protein" OR "inflammatory milieu" OR " phagocytic leukocytes" OR "antibodies" OR "complement proteins" OR "receptor Activator of Nuclear Factor-kappa B" OR " prostaglandin-Endoperoxide Synthases")

4.1.8 STUDY SELECTION

The inclusion criteria of this systematic review were:

- Original scientific articles.
- Publication in English or Spanish.
- Report data on the associations between human exposure to bisphenols, PBs and BPs, and levels of inflammatory biomarkers.

The exclusion criteria were:

- Systematic and narrative reviews, case reports, conferences, meeting abstracts and editorials.
- *In vitro* and *in vivo* studies.

4.1.9 DATA EXTRACTION

A systematic review of peer-reviewed original research studies published up to February 2023 was conducted. Two experts proceeded to select and evaluate the articles and, in case of discrepancy, a third expert defined the result.

Table 3 shows all the information collected from each article, which included 1) general characteristics of the studies, 2) characteristics related to the exposure assessment, 3) characteristics related to the outcome assessment and 4) associations found between the concentrations of EDCs and levels of inflammatory biomarkers.

Table 3. Information collected from each article.

General characteristics of the studies
Country
Study design
Period of sample collection for exposure assessment
Period of sample collection for outcome assessment
Sample size
Reporting quality
Characteristics related to the exposure assessment
EDC family
Compounds
Matrix
Frequency of detetion (%)
Unit
Concentrations
Characteristics related to the outcome assessment
Inflammation parameter
Matrix
Unit
Concentrations
Association found between concentrations of EDCs and levels of inflammatory biomarkers
Exposure biomarker-inflammation biomarker
Satatistical test
Magnitude of the association
p-value

EDC: endocrine-disrupting chemical.

4.1.10 ASSESSMENT OF REPORTING QUALITY AND RISK OF BIAS

The quality of the studies included in this systematic review was assessed by considering the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist (von Elm et al., 2008). This list consists of six blocks and a total of 23 items:

- Title and abstract (2 items).
- Introduction (2 items).
- Method (9 items).
- Results (5 items).

- Discussion (4 items).
- Other information (1 items).

The reported quality of the articles was categorized according to Alvarenga et al. (2021) as:

- High (≥16 items)
- Medium (15-8 items)
- Low (<8 items).

Risk of bias was assessed using a modified version of the Risk of Bias in Non-randomized Studies of Exposures (ROBINS-E) tool (Higgins et al., 2022). This tool includes seven domains for the overall assessment of the risk of bias:

- Domain I: bias due to confounding.
- Domain II: bias in selecting participants in the study.
- Domain III: bias in exposure classification.
- Domain IV: bias due to departures from intended exposures.
- Domain V: bias due to missing data.
- Domain VI: bias in outcome measurement.
- Domain VII: bias in the selection of reported results.

The risk of bias in each study was classified as:

- Low
- Some concerns
- High
- Very high

4.2 OBJECTIVE 4

This doctoral thesis has been carried out in collaboration with the private company Inves Biofarm (Institute for Biotechnological, Pharmaceutical Research and Orphan Medicines S. L.), with which a research agreement was established that allowed us to apply for the predoctoral grant. The agreement included to achieve some of the

proposed objectives of the PhD thesis, among which were to determine the presence or absence of PBs (MeP, EtP, PrP and BuP) and BPs (BP-1, BP-3, 4-OHBP, BP-6 and BP-8) and the estrogenic and antiandrogenic activity in different cosmetic products, cosmetic formulations and plastic containers.

4.2.1 COMPANY DESCRIPTION

Inves Biofarm is a private company created in 2012 and located on the Health Technology Campus of Granada, which is dedicated to the development of both therapeutic and cosmetic products, both for mediators and for own production. Its laboratories are equipped with top brand equipment and machinery in the pharmaceutical industry that provide reliability and safety to the production process, as well as a more intelligent laser diffraction particle size analyzer, fluorimeter, ultraviolet and infrared spectrophotometers, HPLC, LC-MS/MS, climate bank for different atmospheric conditions, rotary evaporator, etc. The manufacturing plant is distributed in different areas dedicated to the processing of different injectable, liquid, semi-solid and solid pharmaceutical forms. This company allocates a high percentage of its profits to collaborating in the development of orphan drugs related to its experience in dermatology and different biotechnological-based growth factors, by including social responsibility among its objectives. It also uses products with proven benefits for dermatological diseases such as psoriasis or radiodermatitis. In fact, currently developed products for cosmetic purposes have been conceived based on previous therapeutic findings. Furthermore, these cosmetic products continue to be refined to (1) eliminate compounds with potential side effects and (2) incorporate new compounds that improve product efficacy. The cosmetic products currently being developed are for daily and domestic use, as well as cosmetic products for professional use in beauty salons.

4.2.2 SAMPLES PROVIDED BY INVES BIOFARM

Different samples, including 6 semi-solid samples (creams), one liquid sample (cosmetic formula), and two plastic containers were delivered by the company to carry out the chemical and biological analyses.

4.2.3 CHEMICAL ANALYSIS

4.2.3.1 Liquid and semi-solid samples

For the extraction of both liquid and semi-solid samples, a DLLME, a salt-assisted liquid-liquid extraction (SALLE) and UHPLC-MS/MS were used. A volume of milliQ water was added to 250 mg of each sample to reach a final volume of 1 mL. Each sample was spiked with 50 µL of surrogate standard solution. Subsequently, 50 µL of commercial formic acid and 6.5 mL of acetonitrile were added and the mixture was vortexed for 30 seconds. Next, 800 mg NaCl was added and immediately afterwards the samples were manually shaken for 60 seconds and centrifuged for 10 min at 4000 rpm (SALLE). The collected supernatant was dried under a stream of nitrogen at 45°C. Subsequently, 1 mL of acetonitrile was added to each sample. Next, samples were diluted with 10 mL of 10% aqueous NaCl solution (w/v), and adjusted to pH 2.0 for DLLME. 1.5 mL of trichloromethane was added and samples were then shaken manually and centrifuged for 10 minutes. Subsequently, the entire sedimented phase was transferred to a clean glass vial and evaporated under a nitrogen stream at 45ºC. Finally, the residue was dissolved with 100 µL of a mixture of milliQ water/acetonitrile 70/30 (v/v), vortexed for 60 seconds, and centrifuged at 10000 rpm for 45 minutes. The extracts were then ready for analysis by UHPLC-MS/MS. The LOD for both PBs and BPs was 0.20 ng/g.

4.2.3.2 Solid samples

The determination of PBs and BPs in plastic containers provided by Inves Biofarm was carried out using 4 different extraction methodologies, with different solvents and conditions (A-D). Thus, 50 mg of the containers were treated with 5 mL of:

A) Dichloromethane/Acetonitrile, in a 3/1 ratio.

B) Acetonitrile.

C) Acetonitrile/ milliQ water, in a 3/1 ratio.

D) MilliQ water.

All treated samples were incubated at 32 °C for 48 hours. Subsequently, 1 mL of the samples treated in condition A was dried completely under nitrogen stream and 1 mL of

acetonitrile was added. The mixture was vortexed for 60 seconds. 1 mL of the samples treated in conditions B, C and D was collected and, in this case, was not dried under a nitrogen stream. All samples were spiked with surrogate standard solution and were extracted by the DLLME technique. Briefly, the samples were diluted with 10 mL of 10% aqueous NaCl solution (w/v), and adjusted to pH 2.0. In the subsequent extraction phase, 1.5 mL of trichlomethane was injected and samples were then shaken manually for 60 seconds and centrifuged at 4000 rpm for 10 minutes. Next, the entire sedimented phase was transferred to a clean glass vial and evaporated under a nitrogen stream. Finally, the residue was dissolved with 100 µL of a mixture of milliQ water/acetonitrile 70/30 (v/v). The extracts were centrifuged at 4ºC for 30 minutes and were then ready for analysis by UHPLC-MS/MS. The LOD for both PBs and BPs was 0.20 ng/g.

4.2.4 BIOLOGICAL ANALYSIS

Estrogenicity (E-Screen) and anti-androgenicity (PALM) assays were carried out following previously described procedures (Molina-Molina et al., 2019; Molina-Molina et al., 2014).

4.2.4.1 E-SCREEN assay

This assay is based on the use of the MCF-7 breast cancer cell line. MCF-7 cells are considered estrogen-dependent and endogenously express ERα, respond to estradiol treatment by increasing their proliferative rate, synthesizing new proteins and transcribing specific genes. The assay compares the number of cells or cell proliferation obtained after 6 days of culture. The cells are grown in a culture medium supplemented with human serum/bovine serum devoid of estrogens, in the presence and/or absence of E_2, as well as chemical compounds of suspected estrogenic activity. MCF-7 cells at confluence are trypsinized, and aliquots of this suspension are seeded into 96-well plates at initial concentrations of 4000-5000 cells per well, in maintenance medium, Dulbecco's Modified Eagle Medium (DMEM) (with phenol red), supplemented with 10% Charcoal/Dextran treated Fetal Bovine Serum (CDFBS). Once the cells are attached to the plate (usually 24-48 hours), the maintenance medium is removed and experimental medium DMEM (without phenol red) supplemented with 10% CDFBS is added. Estradiol-17β and the compounds to be tested are also added to the culture medium at the required concentrations/dilutions. A dose–response curve (0:1–1000 pM) for E_2, a negative control of cells treated solely with hormone-free medium (test culture

medium), and a solvent control (0.1% ethanol in test culture medium) were included in each experiment. The assay ends at 144 hours (6 days) of culture (exponential phase) after aspiration of the medium and fixation of the cells for the application of the sulforhodamine-B technique. Finally, cell growth for each experimental group is referred to the growth obtained for the negative control (absence of hormone) and with respect to estradiol (positive control). Since the proliferative effect obtained in the E-Screen assay only provides information relative to a given sample/dilution in the assay, in order to quantify the estrogenic activity with respect to the total amount of original sample, estradiol equivalents (Eeq) are calculated by extrapolating the values of the proliferative effects obtained in an estradiol dose-response curve. The extracts were tested in triplicate and at dilutions from 1:1 to 1:10. The Eeq obtained are corrected by the dilution factor and expressed as Eeq per gram of compound (Eeq/g).

4.2.4.2 PALM assay

This assay is based on the use of the cell line (PALM), derived from the human prostate cancer cell line PC3, co-transfected with an androgen response element, the human androgen receptor gene and the luciferase gene. In this PhD thesis we used the stock of PALM cells provided by Dr. P. Balaguer (INSERM U-896, Montpellier, France). The maintenance medium for this cell line was Ham's F12 supplemented with 10% FBS, 1% penicillin/streptomycin, 1 mg/ml gentamicin and 1 µg/ml pyromycin. For this cell line the experimental medium used was Ham's F12 supplemented with 6% bovine serum, devoid of estrogens.

Cells were seeded at a density of 5 x 10^4 cells per well, in opaque white 96-well plates, in 100 µl of experimental medium. After 8 hours, the compounds to be tested were added to each well and dissolved in experimental medium (50 µl). Serial dilutions of the synthetic human androgen receptor agonist methyltrienolone-R1881 (1-10000 pM) were included in each plate, performing quadruplicate assays for each dilution, together with the test culture medium alone, and the test samples as positive and negative controls, respectively. The cells were incubated for 40 hours at 37°C. At the end of incubation, the medium was removed and replaced with fresh medium containing a concentration of 0.3 mM luciferin. Finally, the luciferase activity for each experimental group was expressed as percentage of luciferase activity. For the study of the antagonistic hormonal effect (anti-androgenic activity), the compound was added simultaneously to

the culture medium together with R1881 at the concentration (0.3 nM) that produces a luciferase activity of 80%. Data were expressed as procymidone equivalents (Proceq) per gram of sample (Proceq/g).

5. RESULTS

OBJECTIVE 1

ARTICLE 1: Iribarne-Durán LM, Domingo-Piñar S, **Peinado FM**, Vela-Soria F, Jiménez-Díaz I, Barranco E, Olea N, Freire C, Artacho-Cordón F, Ocón-Hernández O. **Menstrual blood concentrations of parabens and benzophenones and related factors in a sample of Spanish women: An exploratory study.** Environmental Research 2020, 183:109228. DOI: 10.1016/j.envres.2020.109228

OBJECTIVE 2

ARTICLE 2: Peinado FM, Ocón-Hernández O, Iribarne-Durán LM, Vela-Soria F, Ubiña A, Padilla C, Mora JC, Cardona J, León J, Fernández MF, Olea N, Artacho-Cordón F. **Cosmetic and personal care product use, urinary levels of parabens and benzophenones, and risk of endometriosis: results from the EndEA study.** Environmental Research 2021, 196:110342. DOI: 10.1016/j.envres.2020.110342

OBJECTIVE 3

ARTICLE 3: Peinado FM, Iribarne-Durán LM, Artacho-Cordón F. **Human exposure to bisphenols, parabens, and benzophenones, and its relationship with the inflammatory response: A Systematic Review.** International Journal of Molecular Sciences 2023, 24(8). DOI: 10.3390/ijms24087325

ARTICLE 4: Peinado FM, Olivas-Martínez A, Lendínez I, Iribarne-Durán LM, León J, Fernández MF, Sotelo R, Vela-Soria F, Olea N, Freire C, Ocón-Hernández O, Artacho-Cordón F. **Expression profiles of genes related to development and progression of endometriosis and their association with paraben and benzophenone exposure.** Submitted to Ecotoxicology and Environmental Safety.

ARTICLE 5: Peinado FM, Olivas-Martínez A, Iribarne-Durán LM, Ubiña A, León J, Vela-Soria F, Fernández-Parra J, Fernández MF, Olea N, Freire C, Ocón-Hernández O, Artacho-Cordón F. **Cell cycle, apoptosis, cell differentiation, and lipid metabolism gene expression in endometriotic tissue and exposure to parabens and benzophenones.** Science of the Total Environment 2023, 879:163014. DOI: 10.1016/j.scitotenv.2023.163014

OBJECTIVE 4

Collaboration with the private company Inves Biofarm.

ARTICLE 1

Iribarne-Durán LM, Domingo-Piñar S, **Peinado FM**, Vela-Soria F, Jiménez-Díaz I, Barranco E, Olea N, Freire C, Artacho-Cordón F, Ocón-Hernández O. **Menstrual blood concentrations of parabens and benzophenones and related factors in a sample of Spanish women: An exploratory study.** Environmental Research 2020, 183:109228.

Category: Public, Environmental & Occupational Health

Ranking: 16/203

Impact factor: 6.50; D1

DOI: 10.1016/j.envres.2020.109228

Menstrual blood concentrations of parabens and benzophenones and related factors in a sample of Spanish women: an exploratory study

Iribarne-Durán LM[1,2#], Domingo-Piñar S[3#], Peinado FM[1,2], Vela-Soria F[1*], Jiménez-Díaz I[1], Barranco E[4], Olea N[1,2,5], Freire C[1,5], Artacho-Cordón F[1,2,5*], Ocón-Hernández O[1,6].

[1]Instituto de Investigación Biosanitaria (ibs. GRANADA), Granada, Spain

[2]University of Granada, Radiology and Physical Medicine Department, Granada, Spain.

[3]Obstetrics and Gynecology Service. Hospital of Guadix, Granada, Spain

[4]University of Granada, Department of Obstetrics and Gynecology, Granada, Spain

[5]CIBER de Epidemiología y Salud Pública (CIBERESP), Spain

[6]Obstetrics and Gynecology Service. San Cecilio University Hospital, Granada, Spain

[#]These authors equally contributed to this work

***Corresponding authors:**

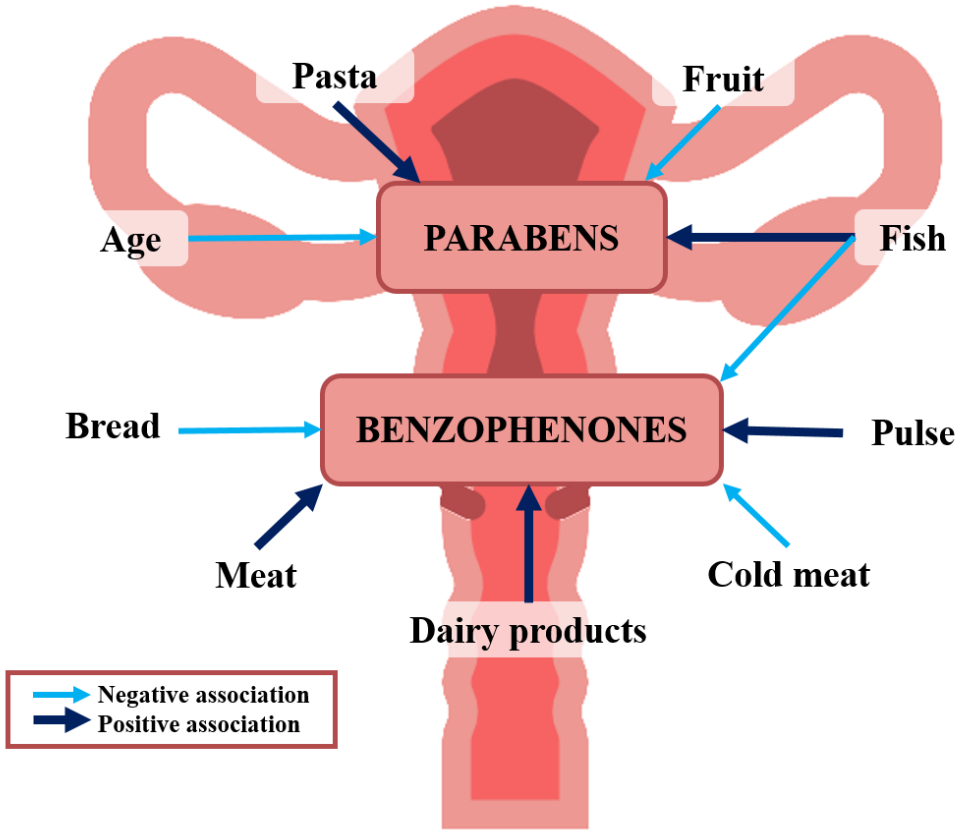
Francisco Artacho-Cordón. University of Granada (Radiology and Physical Medicine Department), Granada, Spain. E-mail: fartacho@ugr.es

Fernando Vela-Soria. Instituto de Investigación Biosanitaria (ibs. GRANADA), Granada, Spain. E-mail: fervs@ugr.es

HIGHLIGHTS

- All menstrual blood samples had detectable levels of ≥3 compounds
- MeP, PrP, and BP-3 were detected in more than 90.0% of menstrual blood samples
- The use of PCPs and dietary habits were related to PB and BP menstrual blood levels

GRAPHICAL ABSTRACT



ABSTRACT

Aim: To evaluate concentrations of parabens (PBs) and benzophenones (BPs) in menstrual blood and explore related sociodemographic/lifestyle factors, and to compare between menstrual and peripheral blood concentrations in a subset of samples.

Material and methods: Concentrations of 4 PBs [methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butyl-paraben (BuP)] and 6 BPs [BP-1, BP-2, BP-3, BP-6, BP-8 and 4-OHBP] were determined in menstrual blood from 57 women and in both menstrual and peripheral blood samples from 12 women, all healthy Spanish women of reproductive age. Socio-demographic characteristics and lifestyle habits [diet and use of cosmetics and personal care products (PCPs)] were gathered using an online questionnaire. Spearman correlation analysis was performed to examine the relationship between menstrual and peripheral blood concentrations, while multivariable linear regression was used to identify potential explanatory variables for menstrual PB and BP concentrations.

Results: Globally, all menstrual blood samples had detectable levels of ≥3 compounds, and 52.6% of the samples contained ≥6 compounds. MeP, PrP, and BP-3 were the most frequently detected compounds (detection frequencies >90.0%), with median concentrations of 1.41, 0.63, and 1.70 ng/mL of menstrual blood, respectively. Age, the use of PCPs, and consumption of some food items (meat, pasta, cheese, or dairy products) were related to the menstrual blood concentrations of some PBs/BPs. Serum: menstrual blood ratios of PBs/BPs ranged from 1.7 to 3.6, with no inter-matrix correlations.

Conclusions: This study reveals, to our knowledge for the first time, the widespread presence of several PBs and BPs in intimate contact with gynecological tissues, although their concentrations in menstrual blood were not correlated with those in peripheral blood from the same women. These results shed light on the information provided by the menstrual blood as a potential matrix for characterizing exposure to PBs and BPs, whose consequences for women's reproductive health need to be addressed.

Keywords: benzophenones, menstrual blood, parabens, women

1. INTRODUCTION

Little attention has been paid in recent decades to the composition of menstrual blood. It is acknowledged to be a complex biological fluid composed of blood from endometrium spiral arteries, vaginal secretions, and endometrial cells from the uterine wall (van der Molen et al., 2014; Yang et al., 2012). A mixture of steroidal hormones (e.g., estradiol, prolactin, and progesterone) is also diluted in this matrix and plays a crucial role in the homeostasis of the endometrium, an endocrine active organ. It has been reported that greater concentrations of these hormones are present in menstrual blood than in peripheral blood (Zhou et al., 1989). This difference may be explained not only by a tissue-specific production rate but also by the filtration of peripheral blood at the blood-uterine lumen barrier (McRae, 1988).

Human exposure to endocrine-disrupting chemicals (EDCs) has been related to various negative effects on female reproductive health, including impaired fertility, birth defects in offspring, endometriosis, and breast cancer (Kunisue et al., 2012; Minguez-Alarcon and Gaskins, 2017; Parada et al., 2019; Smarr et al., 2017). There have been increasing public concerns in recent years about the use of consumer products containing EDCs, including personal care products (PCPs). EDCs contained in PCPs belong to very different chemical groups, including parabens (PBs), benzophenones (BPs), bisphenols, phthalates, alkylphenols, oxycinnamates, camphenes, glycol ethers, and silicones. The PB family includes methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butylparaben (BuP) congeners, which are constitutively present in PCPs as preservatives. In addition to cosmetic and pharmaceutical industries (Darbre and Harvey, 2008), PBs are also widely used in the food industry as food preservatives (Liao et al., 2013b). The BP family includes benzophenone 1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), benzophenone-6 (BP-6), benzophenone-8 (BP-8), and 4-hydroxybenzophenone (4-OHBP), among others. BPs are the most common ultraviolet filters used in industry, and are frequently included as a component in PCP formulas, food packaging (Ding et al., 2018) and cloths (Freire et al., 2019). Parabens have been found to exert weak estrogenic activity (Karpuzoglu et al., 2013; Lange et al., 2014) and to stimulate the proliferation of breast cancer cells *in vitro* (Pan et al., 2016). Likewise, a wide range of *in vitro* and *in vivo* studies has revealed that some UV filters also exhibit estrogenic and anti-androgenic activities (Krause et al., 2012).

Humans are widely exposed to PBs and BPs in their daily lives *via* ingestion, inhalation, or dermal routes (Søeborg et al., 2014). It is suspected that PBs and BPs from most PCPs access internal body compartments through inhalation or dermal pathways, while exposure to PBs and BPs from foodstuff packaging occurs *via* ingestion (Nicolopoulou-Stamati et al., 2015; Søeborg et al., 2014). In humans, PBs and BPs circulate in the bloodstream until they are metabolized and then excreted in urine within the first 24-72 h (Asimakopoulos et al., 2014). During this time, these hormonally-active compounds might exert biological effects on susceptible tissues such as the mammary gland (Gopalakrishnan et al., 2017), fat compartment (Quiros-Alcala et al., 2018), or liver (Kizhedath et al., 2019). Tissue-specific concentrations of EDCs appear to be more relevant for the evaluation of local EDC-driven biological effects (Artacho-Cordón et al., 2019), especially in sensitive tissues with some degree of blood-tissue barrier.

With this background, the primary objectives of this study were to determine PB and BP concentrations in menstrual blood, a non-invasive biological matrix in intimate contact with the endometrium, and to explore related sociodemographic/lifestyle factors. A secondary objective was to compare concentrations of PBs and BPs between peripheral and menstrual blood in a subset of samples.

2. MATERIAL AND METHODS

2.1. Study population and sample collection

This study was conducted between May 2015 and April 2016 and included 57 healthy Spanish women. Volunteers were invited to participate through announcements at the University of Granada (Spain) and in social networks of the Chair of Obstetrics and Gynecology 'Antonio Chamorro/Alejandro Otero'. Inclusion criteria were: age between 18-50 years, menstruation in the previous 6 months, and willingness to use menstrual cups. Exclusion criteria were pregnancy and the receipt of hormonal contraceptives. Volunteers who met these eligibility criteria received: detailed instructions on the collection, storage, and delivery of menstrual blood samples; an informed consent form; five PB/BP-free polypropylene collection tubes; and a menstrual cup, unless already used by the participant. All samples were collected at the home of participants, who were asked to collect menstrual blood from the menstrual cup in a polypropylene collection tube until it was filled or menstruation ended, keeping the sample refrigerated for up to 24 hours and then frozen at -20 ºC. At the end of the collection period,

participants placed the tube in a dried ice box provided for its delivery to the laboratory, where it was weighed and stored at -20 ºC.

For the comparison between peripheral and menstrual blood concentrations of PBs and BPs, 12 volunteers from Granada were invited to provide a blood sample at the same time as their menstrual blood was collected. Peripheral blood samples were drawn by a trained technician at the hospital, centrifuged at 2000 g (4 ºC) for 15 min to separate the serum, and then stored at -20 ºC until extraction and chemical analyses.

All volunteers signed the informed consent form to participate in the study, which was approved by the Research Ethics Committee of Granada.

2.2. Sample preparation and chemical analyses

2.2.1. Extraction protocol

The extraction protocol design was previously validated (Jimenez-Diaz et al., 2016b). In summary, menstrual blood samples were thawed completely at room temperature, and all samples from each participant were pooled before the extraction procedure. Each pooled sample (1.0 mL) was spiked with 10 µL of surrogate standard solution (1 mg/L of BP-d^{10} and 2.5 mg/L of EtP-$^{13}C_6$) and 50 µL of enzyme solution (β-glucuronidase/sulfatase). The enzyme solution was prepared daily by dissolving 10 mg of β-glucuronidase/sulfatase (3.10^6 U/g solid) in 1.5 mL of 1 M ammonium acetate buffer (pH 5.0). After mixing, the sample was incubated at 37 ºC for 24 hours. Next, 1 mL of acetone was added, and the mixture was vortexed for 30 seconds and centrifuged for 10 minutes at 2600g. The supernatant was transferred to the test tube, diluted to 10 mL with 10% NaCl aqueous solutions (w/v), and adjusted to pH 2.0 for dispersive liquid-liquid microextraction (DLLME). After using a syringe to rapidly inject 800 µL of trichloromethane (extraction solvent) and 820 µL of acetone (dispersant solvent) into the aqueous sample, the mixture was manually shaken for 20 seconds and then centrifuged at 2600 g for 10 minutes. The entire sedimented phase was transferred to a clean glass vial and evaporated under a nitrogen stream. The residue was dissolved with 100 µL of a mixture of methanol (0.1% ammonia)/water (0.1% ammonia) 60/40 (v/v), and vortexed for 30 seconds. Finally, 5 µL was injected into the ultra-high-performance liquid chromatography (UHPLC) system.

2.2.2. UHPLC-MS/MS conditions

Four parabens and six benzophenones were determined by UHPLC-MS/MS analyses using an ACQUITY UPLC™ H-Class (Waters, Manchester, UK). A Xevo TQS tandem quadrupole mass spectrometer (Waters) equipped with an orthogonal Z-spray™ electrospray ionization (ESI) source was used for PB and BP detection. Chromatographic separation of the target compounds was performed using an Acquity UPLC©BEH C_{18} column.

2.2.3. Calibration and quality control

The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a signal–to noise ratio (SNR) of 3. LODs obtained were: 0.1 ng/mL for PrP, BuP, and BP-8; 0.2 ng/mL for MeP, EtP, BP-1, and 4-OHBP; and 0.3 ng/mL for BP-2, BP-3, and BP-6.

Menstrual blood samples were extracted in three batches over a period of three weeks. Each batch included standards for calibration curves (injected at beginning and end of each batch), 15-20 unknown samples, 2 blanks, and 2 quality-control samples. These quality-control samples included 1 field blank (deionized water) and 1 blank sample of menstrual blood spiked with the target compounds at a final concentration of 2.5 ng/mL. Recoveries for all target compounds in the spiked quality control samples ranged from 88 to 106%, with a coefficient of variation (CV) of 12%.

2.3. Independent variables

Independent variables were gathered using an *ad-hoc* questionnaire completed online by each participant and included age, place of residence, education, occupation, height, and weight. The body mass index (BMI) was calculated as weight/height squared (kg/m^2), and the place of residence was classified as urban (>150.000 inhabitants) or semi-urban/rural (<150.000 inhabitants). The questionnaire also included a dietary section on the consumption frequency of the main food groups (fish, dairy products, cheese, cold meat, meat, fat, pulses, fresh and cooked vegetables, fruit, eggs, pasta, bread, canned food, and organic food). Data were also gathered on age at first menstruation, parity, interval since last pregnancy, and accumulated breastfeeding time. Finally, a short section contained items on the participants' utilization of cosmetics and PCPs, including hair dye and dermal oil/creams.

2.4. Statistical analysis

In a descriptive analysis, PB and BP concentrations (ng/mL) were expressed as means with standard deviation (SD), medians, 25th and 75th percentiles, and minimum and maximum concentrations. PB and BP concentrations below the LOD were assigned a value of LOD/√2. The Spearman test was performed for compounds detected in >75% of samples in order to evaluate correlations between pairs of the compounds detected in menstrual blood (intra-matrix correlation) (N=57) and between the menstrual and peripheral blood concentrations of each compound (inter-matrix correlation) (N=12).

Given the non-normal distribution of PB and BP concentrations, these were log-transformed to minimize the influence of extreme values, and β coefficients were exponentially transformed (exp β). Sociodemographic/lifestyle factors potentially related to menstrual blood concentrations of PBs and BPs were explored by bivariate and multivariate linear regression analyses using a combination of backward and forward stepwise multiple linear regression. PBs and BPs for which <75% of samples were above the LOD were considered as dichotomous variables (<LOD/>LOD), and related factors were evaluated using bivariate and multivariate logistic regression models (EtP, BuP, BP-1, 4-OHBP and BP-6). The significance level was set at p=0.05, although results with p-values between 0.10 and 0.05 were also cautiously discussed. All tests were two-tailed and R-statistical computing environment 3.0 (http://www.r-project.org/) and SPSS Statistic v23.0 (IBM, Chicago, IL) were used for data analyses.

3. RESULTS

3.1. Characteristics of study participants

Table 1 summarizes the general characteristics of the study population. The mean (± SD) age of the 57 participants was 36.4±6.0 years. There was a higher population of normal weight (77.2%) than overweight/obese individuals, and more than one-third of the population (35.1%) reported weight loss during the previous year. A total of 28 (49.1%), 38 (66.7%), and 36 (63.2%) participants lived in a semi-urban/rural area, were non-manual workers, and had university education, respectively. Finally, 22 out of 57 participants (38.6%) were smokers at their enrolment.

Menarche had started before the age of 12 years old in 30 (52.6%) participants; 54.4% of the women were nulliparous; and the mean (SD) accumulated breastfeeding duration for the primiparous/multiparous women was 14.5 (±24.6) months.

More than 80% of the women declared the consumption of cold meat and pasta at least once a week, and more than half of them the consumption of cheese, fresh vegetables, cooked vegetables, and fruit at least twice weekly (56.1%, 64.9%, 75.2%, and 82.5%, respectively). Finally, 39 (68.4%) participants declared the consumption of at least one organic item per week

The weekly use of dermal oil and cream was declared by 48 (84.2%) of participants, the use of hair dye by 20 (35.1%), and the weekly use of cosmetics by 34 (59.6%).

Table 1. Characteristics of the study population (N=57).

	N (%)		N (%)
Sociodemographic characteristics		***Frequency of dietary intake***	
Age *(years)*	36.4±6.0*	**Frequency of fish consumption** = *At least 2 times/week*	5 (8.8)
BMI = *Overweight/Obese (>25 Kg/m^2)*	13 (22.8)	**Frequency of dairy products consumption** *Everyday*	23 (40.4)
Residence = *Urban*	29 (50.9)	**Frequency of cheese consumption** = *More than 2 times/week*	32 (56.1)
Time living in residence *(years)*	18.8±13.7*	**Frequency of cold meat consumption** = *At least 1 time/week*	47 (82.5)
Greenhouse proximity = *Yes*	6 (10.5)	**Frequency of meat consumption** = *At least 2 times/week*	45 (78.9)
Agriculture proximity = *Yes*	28 (49.1)	**Frequency of fat consumption** = *More than 3 times/week*	9 (15.8)
Industry proximity = *Yes*	27 (47.4)	**Frequency of pulse consumption** = *At least 2 times/week*	26 (45.6)
Currently working = *Yes*	43 (75.4)	**Frequency of fresh vegetable consumption** = *More than 2 times/week*	37 (64.9)
Currently studing = *Yes*	13 (22.8)	**Frequency of cooked vegetable consumption** = *More than 2 times/week*	43 (75.2)
Currently working and/or studying = *Yes*	10 (17.5)	**Frequency of fruit consumption** = *More than 2 times/week*	47 (82.5)
Type of work = *Manual worker*	19 (33.3)	**Frequency of egg consumption** = *More than 2 times/week*	14 (24.6)
Academic degree = *University*	36 (63.2)	**Frequency of pasta consumption** = *At least 1 time/week*	46 (80.7)
Current smoker = *Yes*	22 (38.6)	**Frequency of bread consumption** = *Everyday*	40 (70.2)
Perceived weight loss = *Yes*	20 (35.1)	**Consumption of canned food** = *Yes*	35 (61.4)
Dental filling = *Yes*	51 (89.5)	*Frequency of cans (cans/week)*	1.1±1.5*
		Consumption of organic food = *Yes*	39 (68.4)
Reproductive characteristics		*Proportion of organic food (%)*	0.3±0.3*
Age at first menstruation = *After 12 years old*	30 (52.6)		
Parity =*Yes*	26 (45.6)	***Personal care products use***	
Time since last pregnancy *(months)*	21,3±15.7*	**Use of hair dye** = *Yes*	20 (35.1)
Cumulative breasfeeding time *(months)*	14.5±24.6*	*Frequency of hair dye use (times/year)*	2.0±3.5*
		Weekly use of dermal oil and cream = *Yes*	48 (84.2)
		Weekly use of cosmetics = *Yes*	34 (59.6)

**Mean±Standard deviation; BMI=body mass index.*

3.2. PBs and BPs in menstrual blood

Menstrual blood samples from all 57 women were positive for ≥3 of the studied EDCs, and samples from 30 (52.6%) of the women were positive for ≥ 6 PBs and/or BPs (Figure 1). Detection frequencies and concentrations of PBs and BPs are exhibited in Table 2. The frequency of PB detection ranged from 56.2% (BuP) to 98.2% (MeP), and the frequency of BP detection from 0.0% (BP-2 and BP-8) to 98.2% (BP-3). MeP, PrP, and BP-3 had the highest median concentrations (1.41 ng/mL, 0.63 ng/mL, and 1.70 ng/mL, respectively). A high variability was observed in PB and BP concentrations, with some samples containing MeP and EtP concentrations up to 16-fold higher than the mean. Results for the xenobiotics detected in >75% of menstrual blood samples (MeP, PrP, and BP-3) were not correlated (rho of 0.05-0.15, p-value >0.05).

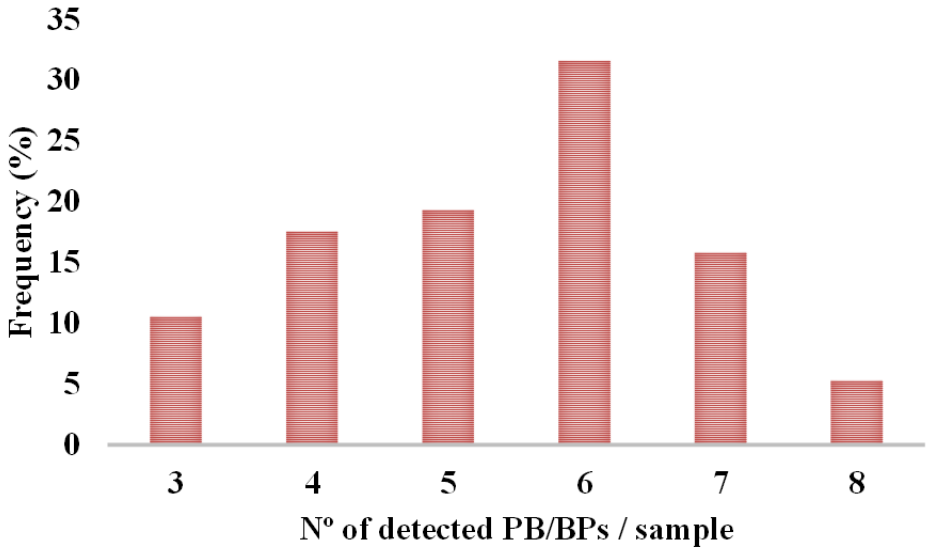


Figure 1. Frequency of detection of PBs and BPs in menstrual blood from the study population.

Table 2. Concentrations of parabens and benzophenones in menstrual blood (ng/mL) (N=57).

		N(%) >LOD	Mean	SD	Percentiles			Mín.	Max.
					25	50	75		
PBs	**MeP**	56 (98.2)	3.19	6.52	0.79	1.41	2.74	<0.20	45.54
	EtP	34 (59.6)	0.89	2.26	<0.20	0.40	0.70	<0.20	16.00
	PrP	53 (93.0)	1.15	1.72	0.25	0.63	1.17	<0.10	9.04
	BuP	32 (56.2)	0.45	0.56	<0.10	0.37	0.70	<0.10	3.05
	ΣPBs	-	5.68	9.41	2.10	3.17	4.87	0.80	65.50
BzPs	**BP-1**	23 (40.3)	0.46	0.68	<0.20	<0.20	0.40	<0.20	3.03
	BP-3	56 (98.2)	1.93	1.59	0.85	1.70	2.48	<0.30	9.70
	BP-6	31 (54.4)	0.59	0.58	<0.30	0.60	0.87	<0.30	3.19
	4-OHBP	23 (40.3)	0.30	0.28	<0.20	<0.20	0.48	<0.20	1.02
	ΣBPs	-	3.48	1.87	2.14	3.23	4.14	1.10	11.00

MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PBs: parabens; BP-1: benzophenone 1; BP-3: benzophenone 3; BP-6: benzophenone 6; 4-OHBP: 4-hydroxybenzophenone; BPs: benzophenones; LOD: limit of detection; LOQ: limit of quantification; SD: standard deviation.

3.3. Factors associated with PB and BP concentrations in menstrual blood

Sociodemographic and lifestyle factors associated with menstrual blood PB and BP concentrations are shown in Tables 3 and 4. Bivariate associations between potential predictors of exposure and the concentrations of PBs and BPs are summarized in Supplementary tables 1 and 2.

According to the multivariate models, age was negatively associated with detectable EtP concentrations, which were more frequently detected in younger volunteers. In bivariate analyses, detectable levels of 4-OHBP and EtP were inversely related to age at first menstruation; however, no variable related to reproductive history was significantly associated with the menstrual blood concentration of any PB or BP in the multivariate analysis.

A number of positive associations were found between the consumption frequency of selected food items and the menstrual blood concentration of PBs and BPs. A higher frequency of meat consumption was associated with a higher BP-3 concentration and a higher consumption frequency of pasta and pulses with detectable EtP and BP-1 concentrations, respectively, while the consumption of dairy products was related to detectable levels of BP-6. In contrast, the consumption of cold meat and fruit was inversely associated with detectable EtP and BP-1 concentrations, respectively, bread consumption was inversely associated with detectable menstrual blood levels of BP-1, and a non-significant trend was observed for an inverse association between fat

consumption and higher menstrual blood PrP concentrations (p = 0.063), and between bread consumption and detectable levels of 4-OHBP (p = 0.074). Interestingly, fish consumption was inversely associated with detectable BP-6 concentrations but positively with MeP concentrations. Non-significant inverse relationships were also observed between the consumption of organic food and concentrations of MeP and detectable levels of 4-OHBP (p < 0.080).

Regarding PCPs, bivariate analysis showed a non-significant association between BP-3 and the weekly use of dermal oil and cream that persisted in the multivariate models (p-value = 0.064). Likewise, the weekly use of dermal oil and cream was also related to detectable levels of BP-6 and the weekly use of cosmetics to detectable BuP concentrations in bivariate analysis. In the same way, non-significant relationships were found between the weekly use of cosmetic and higher MeP concentrations in the bivariate analysis (p = 0.076) and between the frequency of hair dye use and higher BP-3 concentrations in bivariate analysis (p = 0.060).

Table 3. Factors associated with menstrual blood levels of MeP, PrP and BP-3 (N=57). Multivariate linear regresion analyses

	MeP (R^2=0.114)				PrP (R^2=0.063)				BP-3 (R^2=0.201)			
	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
Frequency of fish consumption (= At least 2 times/week[a])	***1.75***	***1.00***	***3.05***	***0.049***	-	-	-	-	-	-	-	-
Frequency of meat consumption (= More than 2 times/week[b])	-	-	-	-	-	-	-	-	***1.79***	***1.23***	***2.59***	***0.003***
Frequency of fat consumption (= At least 3 times/week[c])	-	-	-	-	0.47	0.21	1.04	0.063	-	-	-	-
Consumption of organic food (= Yes[d])	0.58	0.32	1.04	0.066	-	-	-	-	-	-	-	-
Weekly use of dermal oil and cream (= Yes[d])	-	-	-	-	-	-	-	-	1.61	0.97	2.67	0.064

CI: confidence intervals, [a] Reference category= <2 times/week; [b] Reference category= ≤2 times/week; [c] Reference category= <3

Table 4. Factors associated with menstrual blood levels of EtP, BuP, BP-1, BP-6, and 4-OH-BP (N=57). Multivariate logistic regression analyses.

	EtP (R^2=0.346)				BuP (R^2=0.304)			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Age *(years)*	***0.86***	***0.77***	***0.97***	***0.017***	-	-	-	-
Frequency of fish consumption = *At least 1 time/week* [a]	-	-	-	-	-	-	-	-
Frequency of dairy products consumption = *At least 2 times/week* [b]	-	-	-	-	-	-	-	-
Frequency of cold meat consumption = *At least 1 time/week* [a]	-	-	-	-	-	-	-	-
Frequency of pulse consumption = *More than 2 times/week* [c]	-	-	-	-	-	-	-	-
Frequency of fruit consumption = *Everyday* [d]	***0.25***	***0.07***	***0.90***	***0.033***	-	-	-	-
Frequency of pasta consumption = *At least 3 time/week* [e]	***9.95***	***1.76***	***56.28***	***0.009***	-	-	-	-
Frequency of bread consumption = *Everyday* [d]	-	-	-	-	-	-	-	-
Frequency of bread consumption = *More than 1 time/day* [f]	-	-	-	-	-	-	-	-
Consumption of organic food = *Yes* [g]	-	-	-	-	-	-	-	-
Weekly use of cosmetics = *Yes* [g]	-	-	-	-	***0.11***	***0.03***	***0.37***	***<0.001***

	BP-6 (R^2=0.223)				4-OHBP (R^2=0.157)			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Age *(years)*	-	-	-	-	-	-	-	-
Frequency of fish consumption = *At least 1 time/week* [a]	***0.17***	***0.04***	***0.70***	***0.014***	-	-	-	-
Frequency of dairy products consumption = *At least 2 times/week* [b]	***5.36***	***1.34***	***21.45***	***0.018***	-	-	-	-
Frequency of cold meat consumption = *At least 1 time/week* [a]	-	-	-	-	-	-	-	-
Frequency of pulse consumption = *More than 2 times/week* [c]	-	-	-	-	-	-	-	-
Frequency of fruit consumption = *Everyday* [d]	-	-	-	-	-	-	-	-
Frequency of pasta consumption = *At least 3 time/week* [e]	-	-	-	-	-	-	-	-
Frequency of bread consumption = *Everyday* [d]	-	-	-	-	-	-	-	-
Frequency of bread consumption = *More than 1 time/day* [f]	-	-	-	-	0.34	0.11	1.11	0.074
Consumption of organic food = *Yes* [g]	-	-	-	-	0.31	0.08	1.14	0.078
Weekly use of cosmetics = *Yes* [g]	-	-	-	-	-	-	-	-

	BP-1 (R^2=0.491)			
	OR	**95% CI**		**p-value**
Age *(years)*	-	-	-	-
Frequency of fish consumption = *At least 1 time/week* [a]	-	-	-	-
Frequency of dairy products consumption = *At least 2 times/week* [b]	-	-	-	-
Frequency of cold meat consumption = *At least 1 time/week* [a]	***0.07***	***0.01***	***0.68***	***0.022***
Frequency of pulse consumption = *More than 2 times/week* [c]	***7.12***	***1.15***	***44.30***	***0.035***
Frequency of fruit consumption = *Everyday* [d]	-	-	-	-
Frequency of pasta consumption = *At least 3 time/week* [e]	-	-	-	-
Frequency of bread consumption = *Everyday* [d]	***0.03***	***0.00***	***0.29***	***0.002***
Frequency of bread consumption = *More than 1 time/day* [f]	-	-	-	-
Consumption of organic food = *Yes* [g]	-	-	-	-
Weekly use of cosmetics = *Yes* [g]	-	-	-	-

CI: confidence intervals, [a] *Reference category= <1 times/week;* [b] *Reference category= <2 times/week;* [c] *Reference category= ≤2 times/week;* [d] *Reference category= <1 time/day;* [e] *Reference category=<3 times/week;* [f] *Reference category=≤1 time/day;* [g] *Reference category=No.*

3.4. Comparison of PB and BP concentrations between peripheral and menstrual blood

PB and BP serum: menstrual blood ratios and the inter-matrix Spearman correlations for MeP, PrP, and BP-3 (detected in > 75% of the 12 samples) are exhibited in Table 5. Geometric means of serum:menstrual blood ratios ranged from 1.7 to 3.6, while inter-matrix Spearman correlation coefficients did not reach statistical significance (p-values > 0.090).

Table 5. Relative concentration and Spearman correlations of parabens and benzophenones between serum and menstrual blood (N=12).

	MeP	PrP	BP-3
Serum:menstrual blood ratio*	3.6 (1.3-9.4)	1.7 (1.0-2.5)	1.8 (1.0-2.7)
Rho coefficient	0.44	0.51	0.20
P-value	0.155	0.090	0.536

** Values expressed as geometric mean (25^{th}-75^{th} percentiles).*

4. DISCUSSION

To the best of our knowledge, this preliminary study represents the first exploration of concentrations of EDCs, specifically PBs and BPs, in intimate contact with the endometrium. Numerous PBs and BPs were present in the menstrual blood samples and therefore in the uterine microenvironment, and certain sociodemographic and lifestyle factors were associated with the menstrual blood concentrations of these EDCs. Finally, menstrual blood concentrations of PBs and BPs were not correlated or were only weakly correlated with concentrations in serum from peripheral blood, which were higher. These findings suggest that menstrual blood might offer additional information to that obtained in urine or serum, widely used for exposure assessment, and may be a useful matrix for investigating the potential effects of EDCs on gynecological disorders in women of reproductive age, such as endometriosis.

Only a few studies have compared the composition of menstrual and peripheral blood, finding clearly distinct immunologic profiles (van der Molen et al., 2014; Yang et al., 2012) and differences in the concentrations of sexual hormones such as estradiol, progesterone, or prolactin (Zhou et al., 1989). Our results were consistent with these reports, finding lower concentrations of some PB/BPs in menstrual blood than in serum, with an apparent lack of inter-matrix correlation. Hence our findings support the proposal that the blood-uterine barrier may act as a physical and/or metabolic barrier that can hamper the passage of certain substances (McRae, 1988) and induce the glucuronidation of EDCs, facilitating their excretion (Matsumoto et al., 2007). The present results indicate that the uterine lumen is in contact with lower concentrations of xenoestrogenic compounds such as PBs or BPs than those present in the bloodstream. A similar trend was also detected in other tissues with some degree of blood-tissue barrier. Thus, concentrations of PBs were recently reported in the human brain (van der Meer et al., 2017), indicating that some EDCs can cross the blood-brain barrier, although

concentrations were lower in the brain than in the blood, suggesting that they may be influenced by the permeability of the blood-brain barrier.

We found detectable levels of all studied PBs and BPs with the exception of BP-2 and BP-8, which were below the limit of detection in all menstrual blood samples, possibly reflecting their limited industrial use (Mikkelsen et al., 2015). The PB and BP concentrations found in our menstrual blood samples were also lower than reported in serum samples by previous studies of exposure to PB/BPs (Artacho-Cordon et al., 2017; Hines et al., 2015; Vela-Soria et al., 2013). In contrast, MeP, PrP, and BP-3 concentrations were higher those recently described in lipid-rich tissue (Artacho-Cordon et al., 2018). Our results are in agreement with previous reports of lower PB and BP concentrations in the brain than in the blood, indicating that blood-tissue barriers may play an important role in the distribution of xenobiotics among different body compartments (van der Meer et al., 2017).

Exploratory analysis revealed certain factors associated with menstrual blood concentrations of PBs and BPs, mainly related to lifestyle habits. Thus, in addition to the inverse relationship of age with EtP concentrations, as previously reported (Engel et al., 2014; Larsson et al., 2014), the use of cosmetics, dermal oil/creams and hair dyes were related to increased menstrual blood concentrations of certain PBs and BPs, in line with previous studies that found an association between the frequency of PCP use and higher urinary concentrations of MeP (Berger et al., 2019) and BPs (Ko et al., 2016). These results are also consistent with reports of the generalized presence of PBs and BPs in a wide variety of PCPs (Guo and Kannan, 2013; Liao and Kannan, 2014b). Finally, the consumption frequency of some food items was related to the menstrual blood concentrations of some PBs and BPs, suggesting that food additives and packaging may make a relevant contribution to human exposure to these compounds. Little information is available on the role of diet in exposure to PBs and BPs, and contradictory findings have been published. Thus, Larsson et al. (2014) found no association between dietary habits and PB exposure, whereas other studies found that urinary concentrations of BPs and PBs were related to the consumption of meat and non-fresh vegetables in young girls (Mervish et al., 2014). The consumption of bread and fats was also reported to be inversely associated with PBs in Norwegian adults, while their consumption of beverages was related to higher urinary concentrations of BuP (Husøy et al., 2019). In addition, a previous study by our group found a

relationship between the consumption of fat, fish, cheese, bread, or fruit and adipose tissue concentrations of some PBs and BPs in adults (Artacho-Cordon et al., 2018). According to the present results, the intake of meat, pasta, pulses, or dairy products may be associated with greater exposure to PBs and BPs, while the higher consumption of other food items such as fruit or bread may be inversely related to the concentrations of certain PBs and BPs. In this regard, the widespread presence of PBs has been reported in various foodstuffs examined in the USA and China (Liao et al., 2013a; Liao et al., 2013b). However, no study has distinguished between chemicals deriving from the food itself and those leaching from the plastic materials used to contain it. Interestingly, lower MeP and 4-OHBP concentrations were observed in menstrual blood samples from women who reported consuming some organic food in comparison to those who did not. No research has been published on the influence of organic food consumption on the internal burden of PBs and BPs; however, organic food is more likely to be free of pesticides and to be sold in packaging with lesser BP-leaching potential.

The presence of PBs and BPs in the uterine microenvironment might have adverse effects on the physiology of gynecological tract tissues such as the endometrium or ovary, as evidenced in previous *in vitro* and *in vivo* studies. For instance, an *in vitro* study revealed that the exposure of ovarian cells to BP-3 (5.8 nM) perturbed early events in germ cell development, decreasing total oocytes, the number of nests per ovary, and early primary follicles, and disrupting the expression of crucial genes related to follicular assembly (Santamaria et al., 2019). Another study observed upregulation of the epithelial-mesenchymal transition pathway in BG-1 ovarian cells exposed to BP-1, inducing ovarian cancer metastasis (Shin et al., 2016). In a mouse model of endometriosis, Jones et al. (Jones et al., 2018) recently showed that bisphenols can disrupt ovarian gene expression and steroidogenic pathways, reducing progesterone concentrations and increasing atretic oocytes. Hence, the presence of PBs and BPs in the uterine cavity might potentially lead to various gynecological disorders in humans, including endometriosis, polycystic ovarian syndrome, impaired fertility, or birth defects in the offspring (Kunisue et al., 2012; Minguez-Alarcon and Gaskins, 2017; Parada et al., 2019; Smarr et al., 2017).

The sample size was limited, reducing the statistical power of the study to identify a more comprehensive group of determinants of PB and BP exposure. One explanation is the difficulty of collecting menstrual blood samples from the general population, which

requires volunteers to use a menstrual cup and keep frozen samples at home for 5 days. In addition, the comparison of peripheral and menstrual blood samples was conducted in a small number of samples. Further studies are needed to verify these preliminary findings in wider samples of women. In addition, although volunteers were enrolled from different regions of Spain, they cannot be considered representative of the general population of Spanish women of reproductive age, because the need for participants to use menstrual cups may have introduced a potential selection bias in terms of lifestyle habits. Another potential study limitation was the 5-day collection period during menstruation, which sometimes lasted longer; therefore, not all of the blood produced by menstruation was always collected, and we cannot confirm that collected and uncollected blood had the same composition. Finally, the questionnaire section on PCPs could have been longer, allowing the detection of associations between PB/BP concentrations and more PCPs, and PCPs for intimate body parts were not included. Strengths of our study include the use of a non-invasive matrix to measure PB and BP exposure, facilitating the collection of samples with no or little inconvenience for the participant. Moreover, this biological matrix may offer a more appropriate approach for the *in-situ* assessment of environmental chemicals with endocrine-disrupting properties in the gynecological tract. This is of crucial relevance, given the suspicion that EDCs might be involved in the disruption of gynecological tissue physiology and in the development of various female disorders, as discussed above.

5. CONCLUSIONS

To the best of our knowledge, this preliminary study is among the first to contribute evidence on the presence of PBs and BPs in menstrual blood and therefore in the uterine microenvironment. We consider these results to be of special public health interest, given the consequences of hormone-mimicking chemicals for the physiology of estrogen-sensitive tissues, such as the endometrium, and for the reproductive health of women.

CONFLICT OF INTEREST

The authors declare no conflicts of interest to disclose.

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ARTICLE 2

Peinado FM, Ocón-Hernández O, Iribarne-Durán LM, Vela-Soria F, Ubiña A, Padilla C, Mora JC, Cardona J, León J, Fernández MF, Olea N, Artacho-Cordón F. **Cosmetic and personal care product use, urinary levels of parabens and benzophenones, and risk of endometriosis: results from the EndEA study.** Environmental Research 2021, 196:110342.

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Cosmetic and personal care product use, urinary levels of parabens and benzophenones, and risk of endometriosis: results from the EndEA study

Peinado FM[1], Ocón-Hernández O[1,2], Iribarne-Durán LM[1], Vela-Soria F[1], Ubiña A[3], Padilla C[1,2], Mora JC[4], Cardona J[1,2], León J[1,5,6], Fernández MF[1,7,8], Olea N[1,7,8,9], Artacho-Cordón F[1,7,8*].

[1] Biohealth Research Institute in Granada (ibs.GRANADA), E-18012 Granada, Spain

[2] Gynecology and Obstetrics Unit, 'San Cecilio' University Hospital, E-18016 Granada, Spain;

[3] General Surgery, San Cecilio University Hospital, E-18016 Granada, Spain

[4] Gynecology and Obstetrics Unit, 'Virgen de las Nieves' University Hospital, E-18014 Granada, Spain

[5] Digestive Medicine Unit, 'San Cecilio' University Hospital, E-18012 Granada, Spain

[6] CIBER Hepatic and Digestive Diseases (CIBEREHD), E-28029 Madrid, Spain

[7] CIBER Epidemiology and Public Health (CIBERESP), E-28029 Madrid, Spain

[8] Radiology and Physical Medicine Department, University of Granada, E-18016 Granada, Spain

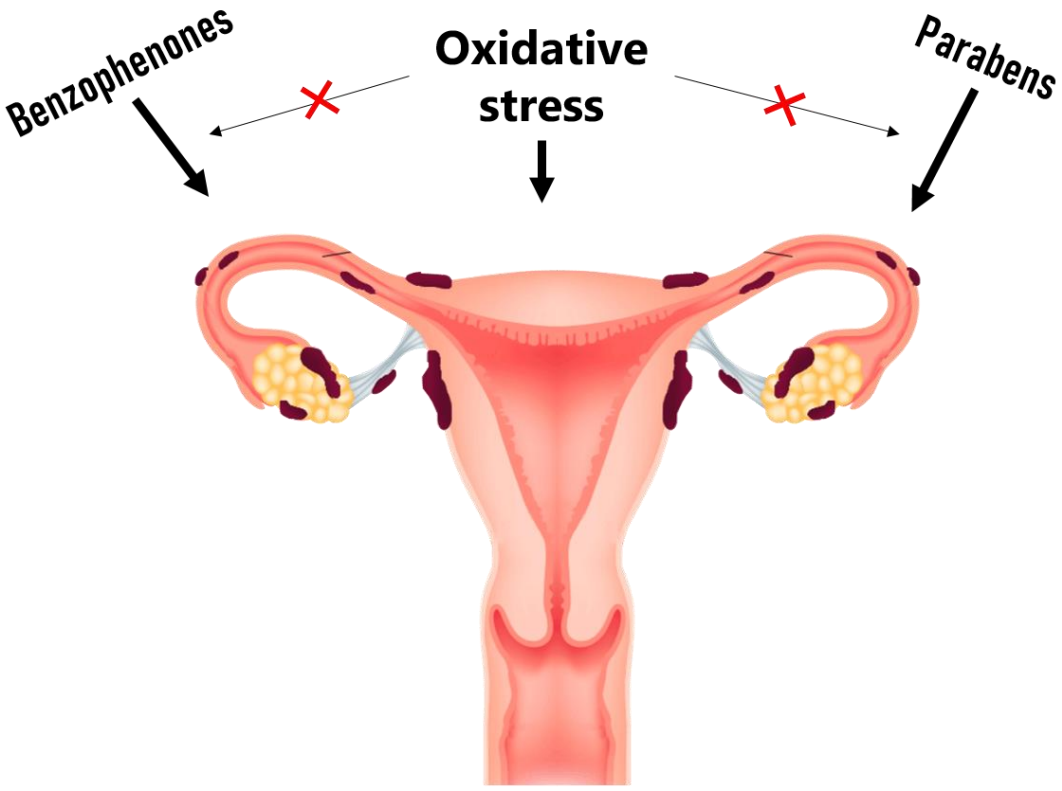
[9] Nuclear Medicine Unit, 'San Cecilio' University Hospital, E-18016 Granada, Spain

***Corresponding author:** Francisco Artacho-Cordón. University of Granada, Department of Radiology and Physical Medicine. 18071 Granada, Spain. Phone: +34 958 249983; E-mail: fartacho@ugr.es ORCID (0000-0003-3850-6173).

HIGHLIGHTS

- MeP, BP-1, BP-3 and ΣBPs are related to higher risk for endometriosis
- TAP and TBARS are related to lower and higher risk for endometriosis, respectively
- Oxidative stress did not modify the associations between exposure and the disease

GRAPHICAL ABSTRACT



ABSTRACT

Aim: To explore the relationship of urinary concentrations of different congeners of benzophenones and parabens with the utilization of cosmetics and personal care products (PCPs) and their impact on the risk of endometriosis, and to evaluate the influence of oxidative stress on associations found.

Methods: This case-control study comprised a subsample of 124 women (35 cases; 89 controls). Endometriosis was confirmed (cases) or ruled out (controls) by laparoscopy, with visual inspection of the pelvis and biopsy of suspected lesions (histological diagnosis). Urinary concentrations of benzophenone-1 (BP-1), benzophenone-3 (BP-3), 4-hydroxibenzophenone (4-OHBP), methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butyl- paraben (BuP), and biomarkers of oxidative stress [lipid peroxidation (TBARS) and total antioxidant power (TAP)] were quantified. Information was gathered on the frequency of use of cosmetics and PCPs. Associations between the frequency of cosmetics/PCP use, urinary concentrations of benzophenones and parabens, oxidative stress, and endometriosis risk were explored in logistic and linear multivariable regression analyses.

Results: The frequency of utilization of certain cosmetics and PCPs was significantly associated with urinary concentrations of benzophenones and parabens. After adjustment for potential confounders, the risk of endometriosis was increased in women in the second *versus* first tertiles of MeP (OR=5.63; p-value<0.001), BP-1 (OR=5.12; p-value=0.011), BP-3 (OR=4.98; p-value=0.008), and ΣBPs (OR=3.34; p-value=0.032). A close-to-significant relationship was observed between TBARS concentrations and increased endometriosis risk (OR=1.60, p-value 0.070) and an inverse association between TAP concentrations and this risk (OR=0.15; p-value=0.048). Oxidative stress results did not modify associations observed between benzophenone/paraben exposure and endometriosis risk.

Conclusions: Our findings indicate that the frequency of cosmetics and PCP utilization is a strong predictor of exposure to certain benzophenone and paraben congeners. These compounds may increase the risk of endometriosis in an oxidative stress-independent manner. Further studies are warranted to corroborate these findings.

Keywords: benzophenones, endocrine disruption, endometriosis, oxidative stress, parabens

1. INTRODUCTION

Endometriosis is a chronic gynecological disease that affects a growing number of women of childbearing age, being present in more than 176 million women worldwide according to recent estimates (Zondervan et al., 2018). A definitive diagnosis can only be confirmed surgically, and the disease is frequently asymptomatic; nevertheless, the suspected prevalence is 10-15% of women of childbearing age (Buck Louis et al., 2011; Eisenberg et al., 2018). It is a complex disease characterized by the presence of functionally active endometrial-like tissue (endometrial glands and stromal cells) outside the uterine cavity (Vercellini et al., 2014). The most common symptoms are menstrual irregularities, infertility, and chronic pelvic pain, which is frequently exacerbated during menstruation (dysmenorrhea), sexual intercourse (dyspareunia), defecation (dyschezia), or urination (dysuria) (Eskenazi and Warner, 1997; Giudice and Kao, 2004). It has also been reported that women with endometriosis are at higher risk of gynecological cancer, adenomyosis, cardiovascular disease, fibromyalgia, chronic fatigue syndrome, and autoimmune disease (Benagiano et al., 2014; Kvaskoff et al., 2015; Matias-Guiu and Stewart, 2018; Ruderman and Pavone, 2017).

The etiology of endometriosis has yet to be fully elucidated; however, it is known to be multifactorial (Ferguson et al., 1969; Gargett, 2004; Koninckx et al., 1999; Levander and Normann, 1955; Sampson, 1927b; Starzinski-Powitz et al., 2001; Starzinski-Powitz et al., 2003), and hormonal, genetic, lifestyle, and environmental risk factors have been implicated in recent decades (Vercellini et al., 2014). For instance, given the known sensitivity of this endometrial-like tissue to estrogens, it has been proposed that daily exposure to so-called endocrine-disrupting chemicals (EDCs), which can alter the homeostasis of the endocrine system and are associated with multiple adverse effects (Sifakis et al., 2017), may play a key role in the onset and progression of endometriosis (Smarr et al., 2016). Cosmetics and personal care products (PCPs) have been found to release numerous EDCs (Gao and Kannan, 2020), and the number, variety, and consumption of these products have markedly increased over the last few decades. The highest consumption of cosmetics and perfumery is in the USA (78.6 billion euros), followed by China (52 billion euros), Japan (32 billion euros), and Brazil (28 billion

euros) (Stanpa, 2018). Most of the EDCs released by cosmetics and PCPs belong to the benzophenone (BP) and paraben (PB) families. BPs include BP-1, BP-3, and 4-hydroxybenzophenone (4-OHBP), among others, and are widely used in sunscreens and cosmetics as UV-filters and in the manufacture of plastics and food-packaging materials (Kim and Choi, 2014). PBs, which include methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP) congeners, are alkyl esters of p-hydroxybenzoic acid with antimicrobial properties and are frequently used in cosmetics, PCPs, pharmaceuticals (Darbre and Harvey, 2008), and beverage/food packaging (Ye et al., 2006). *In vivo* and *in vitro* studies have demonstrated that BPs and PBs both have estrogenic and antiandrogenic activities (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Molina-Molina et al., 2008; Oishi, 2002; Schlumpf et al., 2001; Suzuki et al., 2005). There is also emerging evidence that oxidative stress might act as a mechanism of action for these EDCs (Thompson et al., 2015), with suggestions that BPs and PBs might contribute to systemic and local redox disturbances (Artacho-Cordón et al., 2019; Watkins et al., 2015). It has been reported that these disturbances play a determinant role in endometriosis (Gupta et al., 2006; Lambrinoudaki et al., 2009) and may be involved in its development (Scutiero et al., 2017), progression (Ito et al., 2017; Scutiero et al., 2017) and severity (Amreen et al., 2019).

Although females are considered to make greater use of PCPs than men (Biesterbos et al., 2013), scant epidemiological evidence is available on the relationship between PCP-released chemicals and endometriosis. To our best knowledge, only one study has investigated this relationship, finding that concentrations of certain BP congeners might be related to increased endometriosis risk (Kunisue et al., 2012), and no study has addressed the role of PBs in endometriosis. As part of a wider project on the contribution of environmental factors to endometriosis and their potential mechanisms of action, the present study was designed to explore exposure to BPs and PBs from the use of cosmetic and PCPs and its association with the risk of endometriosis in women of childbearing age. A further objective was to evaluate the influence of oxidative stress on associations found between BP/PB exposure and endometriosis.

2. MATERIAL Y METHODS

2.1. Study population and sample collection

The study population (N=124) is part of the hospital-based case–control EndEA study (**End**ometriosis y **E**xposición **A**mbiental), which has been described elsewhere (Peinado et al., 2020b). Study participants were 35 cases and 89 controls, paired by frequency, recruited from January 2018 through July 2019 in two public hospitals ('San Cecilio' and 'Virgen de las Nieves') in Granada, Southern Spain.

Inclusion criteria were: childbearing age (20-54 years), receipt of abdominal surgery (laparotomy or laparoscopy), and pathology report on the presence (cases) or absence (controls) of endometriosis. Further criteria for controls were: performance of laparotomy or laparoscopy in the same hospital as cases for non-malignant disease (e.g. acute appendicitis, biliary disease, hiatus hernia, ovarian torsion, corpus luteum and cystadenomas, among others), no findings of endometriosis during the surgery, and no history of endometriosis. Exclusion criteria for all participants were: morbid obesity (BMI> 35), previous history of cancer (except non-melanoma skin cancer), pregnancy at recruitment, and inability to sign the informed consent. Clinicians involved in the research told each patient about the objectives of this research and invited them to sign their informed consent to participation in the study, which was approved by the Research Ethics Committee of Granada (0464-N-18).

All participants underwent clinical and anthropometrical examination, recording their height and weight for calculation of their body mass index (BMI) and gathering data on: sociodemographic, lifestyle, clinical, and surgical variables, including age (years), residence (urban or suburban), educational level (university degree or less), occupational status (working outside the home or not), current smoking (yes or no), parity (nulliparous, primiparous, or multiparous) and average intensity of menstrual bleeding (mild, moderate, or severe). The women were asked to fill out a questionnaire about their patterns of use of different cosmetics and PCPs (lipstick; facial cream; hair lacquer, cream, and foam; face mask; body lotion; hand cream; makeup; facial tonic; eye pencil; eyeshadow; facial milk; nail polish; shampoo; gel; deodorant; conditioner; cologne/perfume; toothpaste; mouthwash; and sunscreen). The frequency of use was classified in seven categories (>4 times/day, 2-3 times/day, 1 time/day, 5-6 times/week,

3-4 times/week, 1-2 times/week, and rarely/never). The frequency of activities related to the use of cosmetics and PCPs (pedicure, manicure, massage, facial treatment, acrylic nail application, or hair dying) was classified in five categories (weekly, more than once a month, monthly, less than once a month, or never). The questionnaires were completed by 99 of the 124 participants: 25 cases and 74 controls.

Clinicians involved in this research completed a surgical questionnaire on primary and secondary diagnoses and the classification of endometriosis according to Revised American Fertility Society categories (Canis et al., 1997). Fasting early-morning urine samples were collected before the surgery in 10 mL PB- and BP-free glass tubes and immediately stored at -80 ºC until their analysis at the Biobank of the Public Andalusian Healthcare System. Banking of biological samples and completion of questionnaires followed the standardized procedures of the EHPect project (http://endometriosisfoundation.org/ephect/).

2.2. Sample preparation and chemical analysis

Dispersive liquid–liquid microextraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) were used to determine urinary concentrations of three BPs (BP-1, BP-3, and 4-OHBP) and four PBs (MeP, EtP, PrP, and BuP), as previously described (Vela-Soria et al., 2014).

Urine samples were thawed at room temperature and centrifuged at 2600 x g for 10 min, taking 1.0 mL for analysis. First, the total amounts (free and conjugated) of the aforementioned benzophenones and parabens were determined in the urine samples by enzymatic treatment with 50 µL of an enzyme solution of β-glucuronidase/sulfatase, previously prepared by dissolving 10 mg of β-glucuronidase/sulfatase ($3 \cdot 10^6$ U g solid^{-1}) in 1.5 mL of 1 M ammonium acetate/acetic acid buffer solution (pH 5.0). The enzymatically treated samples were incubated at 37 ºC for 24 h and then transferred to 15 mL conical-bottom glass tubes for the addition of 20 µL of standard replacement solution (5 mg / L of EP–$^{13}C_6$, 2 mg/L of BPA-D_{16}, and 2 mg/L of BP-d_{10}) and dilution with 10 mL of 10% aqueous NaCl solution (pH 2.0, adjusted with 0.5 M HCl). In the subsequent extraction phase, 1.5 mL of a mixture of 1 mL of acetone (dispersing solvent) and 0.5 mL of trichloromethane (extraction solvent) was injected by syringe, and samples were then shaken manually for 30 seconds and centrifuged at

4000x g for 10 minutes. Next, the organic phase was carefully collected from the bottom of the glass tube using a 1 mL pipette and placed in 2 mL glass vials. All of the extracted fluid was then evaporated under a nitrogen stream, and the residue was dissolved with 100 µL of an acetonitrile/water mixture (0.1% ammonia, 70:30 (v/v)) and vortexed for 30 seconds. The extract was then ready for analysis by UHPLC-MS/MS using ACQUITY UPLC™ H-Class equipment. A Xevo TQ-S tandem quadrupole mass spectrometer equipped with an orthogonal Zspray ™ electrospray ionization source was employed for EDC detection. The compounds were separated using a gradient mobile phase consisting of 0.1% (v/v) ammoniac aqueous solution (solvent A) and 0.1% (v/v) ammonia in methanol (solvent B). The injection volume was 2 µL. The column temperature was maintained at 40 ◦C. Total run time was 10.0 min.

Regarding quality control, urinary samples were analyzed in eight batches over a period of three weeks. Each batch included standards for calibration curves (injected at beginning and end of each batch), 15–20 unknown samples, 2 blanks, 2 urine pool controls, and 2 urine pool controls with a mixture of tested chemicals at low and high concentration levels. The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a signal-to-noise ratio ≥3. The LODs obtained were 0.05 ng/mL for BP-1, 0.06 mg/mL for BP-3 and 4- OH-BP, and 0.10 ng/mL for MeP, EtP, PrP, and BuP.

2.3. Measurement of oxidative stress biomarkers

Oxidative lipid damage was evaluated in all urine samples by quantifying the concentration of thiobarbituric acid reactive substances (TBARS) using the Colorimetric TBARS Microplate Assay Kit from Oxford Biomedical Research (Rochester Hills, MI, USA). This enzyme-linked immunosorbent assay (ELISA) kit is based on the reaction of 2-thiobarbituric acid with malondialdehyde (compound resulting from decomposition of polyunsaturated fatty acid lipid peroxides) at 25 ºC to yield a chromophore with maximum absorbance at 532 nm. In addition, the urinary concentration of total antioxidant power (TAP) was analyzed in a subset of participants with available urine sample (n=108) using a colorimetric microplate assay supplied by Oxford Biomedical Research (Rochester Hills, MI, USA). This assay measures the total capacity to reduce Cu^{++} to Cu^{+}, which selectively forms a stable 2:1 complex with the chromogenic reagent that has maximum absorption at 450 nm. A known concentration

of uric acid was used to create a reference curve for comparison with readings in samples. The LOD was 1.0 μM for TBARS and 0.5 mM for TAP.

2.4. Statistical Analysis

Descriptive analysis was conducted of the sociodemographic, lifestyle, and gynecological characteristics of cases and controls. Continuous variables were expressed as means±standard deviation and categorical variables as percentages. Urinary concentrations of individual BP and PB congeners, the sum of BPs (∑BPs) and PBs (∑PBs), and oxidative stress biomarkers were expressed as means±standard deviation and percentiles (25, 50 and 75). Urinary concentrations of BPs, PBs, and oxidative stress biomarkers below the LOD were assigned a value of LOD/2. Urinary concentrations of BPs, PBs, and oxidative stress biomarkers were log-transformed to minimize the influence of extreme values.

Bivariate analyses were conducted between cases and controls using the chi-square and Student's (or Mann-Whitney) tests as appropriate. The Mann-Whitney test was used to compare urinary concentrations of BPs, PBs, and oxidative stress biomarkers between cases and controls and between stage 1 cases and stage II/III/IV cases. Bivariate and multivariate linear regression analyses with backward stepwise selection were applied to identify potential cosmetic and PCP-related predictors of log-transformed BP and PB concentrations in the subset of women with available urine sample and information on cosmetic and PCP use (N=99). Unconditional logistic regression analyses were performed to determine the odds ratios (ORs) for endometriosis risk of urinary concentrations of BPs, PBs, and oxidative stress biomarkers in the whole study population (N = 124). In additional models, concentrations of BPs, PBs, and oxidative stress biomarkers were entered in tertiles. Given the limited sample size, regression analyses were sequentially adjusted for (1) urinary creatinine; (2) urinary creatinine, age, and BMI; and (3) urinary creatinine, age, BMI, residence, and parity. Given the similarity in results obtained, only those from fully adjusted models are discussed. Results are summarized as ORs with corresponding 95% confidence intervals. Because the independent variables (BPs, PBs and oxidative stress biomarkers) were log-transformed, OR estimates reflect the odds of endometriosis risk for each 1 log unit of the concentration of the corresponding biomarker. The relationship between BP or PB exposure and oxidative stress biomarkers was examined using linear regression models

adjusted for urinary creatinine, age, BMI, residence, and parity, expressing the results as exp (β) with 95% confidence intervals. SPSS Statistics 23.0 (IBM, Chicago, IL, USA) and R statistical computing environment v3.1 (http://www.r-project.org/) were used for data analyses. Post-hoc analysis was conducted to estimate the statistical power (1-β), using G*Power 3.1.9.7 statistical software (Düsseldorf University, Germany). For the main analysis between BP and PB concentrations and the risk of endometriosis, it showed that the power was >0.99 for adjusted ORs ≥3.34, a total sample size of 124 women (35 cases and 59 controls), and an α-error of 0.05.

3. RESULTS

3.1. Characteristics of study population

No difference was found between cases and controls in age, BMI, residence, educational level, parity, smoking habits, or menstrual bleeding intensity (p-values >0.050). Among the 35 cases, 24 (68.6%) had ovarian/peritoneal endometriosis and 11 (31.4%) had endometriosis lesions in deep localizations; 14 (40.0%) were diagnosed with stage I endometriosis (Table 1). Supplementary tables S1 and S2 show the type of job and frequency of use of each cosmetic and PCP item, respectively, of the 99 participants who completed the questionnaires.

Table 1. Characteristics of endometriosis cases and controls.

	Cases (n=35)		Controls (n=89)		
	n	**%**	**n**	**%**	**p-value**
Age *(years)**	38.3 ± 9.3		35.8 ± 10.4		0.251
Weight *(kg)**	68.5 ± 13.8		66.4 ± 13.6		0.334
Height *(m)**	1.6 ± 0.1		1.6 ± 0.1		0.897
Body mass index *(kg/m^2)**	25.8 ± 5.4		25.0 ± 4.8		0.671
Normal weight (BMI < 25)	20	57.1	21	57.3	0.182
Overweight (BMI 25-30)	6	17.1	26	29.2	
Obese (BMI > 30)	9	25.7	12	13.5	
Residence					0.156
Rural	14	40.0	24	27.0	
Urban /sub-urban	21	60.0	65	73.0	
Parity					0.626
Nulliparous	16	45.7	37	41.6	
Primiparous/Multiparous	19	54.3	52	58.4	
Educational level					0.105
Less than university degree	19	54.3	62	69.7	
University degree	16	45.7	27	30.3	
Working outside home					0.806
Yes	20	57.1	53	59.6	
No	15	42.9	36	40.4	
Current smoker					0.354
Yes	7	20.0	25	28.1	
No	28	80.0	64	71.9	
Amount of menstrual bleeding					0.366
Mild	14	40.0	28	31.5	
Moderate/severe	21	60.0	61	68.5	
Urinary creatinine *(ng/mL)**	143.1 ± 71.6		115.5 ± 61.3		0.052
Endometriosis stage					-
I	14	41.2	-	-	
II	9	26.5	-	-	
III	7	20.6	-	-	
IV	4	11.8	-	-	
Endometrioma location					-
Deep infiltrating endometriosis	10	29.4	-	-	
Ovarian/peritoneal endometriosis	24	70.6	-	-	

**Mean ± standard deviation.*

3.2. Urinary concentrations of PBs and BPs and habits related to the use of cosmetics and PCPs

Table 2 exhibits the detection frequencies and concentrations of BP-1, BP-3, 4-OHBP, ΣBPs, MeP, EtP, PrP, BuP, and ΣPBs. All of these were detected in >89.0% of urine samples with the exception of BuP, detected in 65.3% of samples. In comparison to controls, higher median urinary concentrations of MeP (46.15 *vs* 13.4 ng/mL), EtP (3.83 *vs* 2.75 ng/mL), PrP (2.54 *vs* 1.04 ng/mL), ΣPBs (68.31 *vs* 36.31 ng/mL), BP-3 (3.22 *vs* 2.99 ng/mL), and ΣBPs (7.66 *vs* 5.85 ng/mL) were observed in cases, although these differences did not reach statistical significance. Likewise, BP-1 (1.45 *vs* 1.76 ng/mL), EtP (2.41 *vs* 7.66 ng/mL), and PrP (1.32 *vs* 2.69 ng/mL) observed in women with stages III/IV *vs* stages I/II endometriosis were not statistically significant (Supplementary Table S3). In addition, no significant differences were observed among the women with ovarian, peritoneal, and deep infiltrating endometriosis (data not shown in tables).

Table 2. Urinary concentrations of benzophenones, parabens and oxidative stress biomarkers (ng/mL)

		Total (N=124)	Cases (N=35)					Controls (N=89)					p-value
		N(%) >LOD	Mean	St. Dev	Percentiles			Mean	St. Dev	Percentiles			
					25	50	75			25	50	75	
BzPs	**BP-1**	114 (91.9)	3.37	4.99	0.93	1.45	4.04	22.73	107.95	0.45	1.71	9.10	0.788
	BP-3	122 (98.4)	10.84	32.61	1.64	3.22	5.77	35.07	115.36	1.06	2.99	12.33	0.830
	4-OH-BP	119 (96.0)	0.99	1.01	0.36	0.66	1.19	3.26	19.61	0.30	0.80	1.51	0.686
	ΣBPs	-	15.21	34.41	3.37	7.66	10.91	61.05	198.35	2.46	5.85	24.39	0.786
PBs	**MeP**	124 (100.0)	210.98	512.79	11.93	46.15	161.78	148.91	272.94	3.94	13.40	153.19	0.130
	EtP	117 (94.4)	35.18	79.27	0.76	3.83	17.10	37.12	118.82	0.89	2.75	9.65	0.760
	PrP	111 (89.5)	14.35	30.22	0.33	2.54	8.77	8.59	18.87	0.22	1.04	6.04	0.386
	BuP	81 (65.3)	1.15	3.42	0.05	0.14	0.52	1.40	2.25	0.05	0.39	1.66	0.057
	ΣPBs	-	261.66	554.33	16.66	68.31	236.09	196.03	354.58	11.37	36.31	201.68	0.347
Ox. stres	**TAP***	107 (99.1)	1.76	0.42	1.42	1.98	2.09	1.80	0.43	1.56	2.00	2.08	0.682
	TBARS	101 (81.5)	4.36	3.32	1.80	4.18	6.98	3.67	3.72	1.22	2.18	4.95	0.113

*St. Dev: standard deviation; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BzP-1: benzophenone-1; BzP-3: benzophenone-3; 4-OH-BzP: 4-hydroxibenzophenone; BzP: benzophenone; TAP: total antioxidant power; TBARS: thiobarbituric acid reactive substance;*measured in 108 urine samples.*

Table 3a shows the determinants of urinary BP concentrations related to the use of cosmetics and PCPs and Table 3b those of urinary PB concentrations related to this use. Multivariate models revealed a positive association between urinary concentrations of certain BPs and a more frequent use of face masks and creams and hair dye treatments and between urinary concentrations of certain PBs and greater use of lipstick, facial creams, and hair lacquers and foam and more frequent hair dye and pedicure treatments.

Table 3a. Factors associated with urine levels of BP-1, BP-3, 4-OHBP and ΣBP. Multivariate linear regresion analyses (N=99).

	BP-1 (R^2=0.163)				BP-3 (R^2=0.133)			
	exp(β)	**95% CI**		**p-value**	**exp(β)**	**95% CI**		**p-value**
Frequency of use of mask = *At least 1 time/week* [a]	***3.74***	***0.48***	***2.15***	***0.002***	***3.02***	***0.28***	***1.93***	***0.009***
Frecuency of hair dye = *Less than 1 time/month* [b]	***2.28***	***0.04***	***1.60***	***0.039***	1.93	-0.11	1.42	0.094
Use of use of facial cream = *More than 1 time/day* [c]	-	-	-	-	-	-	-	-
	4-OHBP (R^2=0.079)				**ΣBP (R^2=0.088)**			
	exp(β)	**95% CI**		**p-value**	**exp(β)**	**95% CI**		**p-value**
Frequency of use of mask = *At least 1 time/week* [a]	-	-	-	-	***2.17***	***0.06***	***1.49***	***0.034***
Frecuency of hair dye = *Less than 1 time/month* [b]	-	-	-	-	-	-	-	-
Use of use of facial cream = *More than 1 time/day* [c]	***2.11***	***0.08***	***1.41***	***0.028***	-	-	-	-

CI: confidence intervals, [a] *Reference category= < 1 time/week;* [b] *Reference category= Never;* [c] *Reference category= ≤ 1 time/day.*

Table 3b. Factors associated with urine levels of MeP, EtP, PrP, BuP and ΣPB. Multivariate linear regresion analyses N=99.

	MeP (R^2=0.154)				EtP (R^2=0.207)			
	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
Frequency of use of lipstick								
= *More than 1 time/day* [a]	3.21	-0.07	2.40	0.063	***5.95***	***0.58***	***2.99***	***0.004***
Frecuency of pedicure = *Monthly* [b]	2.80	-0.08	2.14	0.069	-	-	-	-
= *More than 1 time/month* [c]	-	-	-	-	-	-	-	-
Frecuency of hair dye = *Yes* [d]	2.12	-0.10	1.60	0.083	-	-	-	-
= *Monthly* [b]	-	-	-	-	***2.50***	***0.02***	***1.81***	***0.045***
Frequency of use of facial cream								
= *Everyday* [e]	***2.51***	***0.04***	***1.80***	***0.040***	-	-	-	-
Frequency of use of lacquer, cream and foam								
= *Yes* [d]	-	-	-	-	***2.35***	***0.01***	***1.70***	***0.048***
= *Everyday* [e]	-	-	-	-	-	-	-	-

	PrP (R^2=0.179)				BuP (R^2=0.032)			
	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
Frequency of use of lipstick								
= *More than 1 time/day* [a]	-	-	-	-	-	-	-	-
Frecuency of pedicure = *Monthly* [b]	-	-	-	-	-	-	-	-
= *More than 1 time/month* [c]	-	-	-	-	-	-	-	-
Frecuency of hair dye = *Yes* [d]	***3.60***	***0.40***	***2.16***	***0.005***	2.03	-0.09	1.51	0.083
= *Monthly* [b]	-	-	-	-	-	-	-	-
Frequency of use of facial cream								
= *Everyday* [e]	2.28	-0.06	1.71	0.066	-	-	-	-
Frequency of use of lacquer, cream and foam								
= *Yes* [d]	-	-	-	-	-	-	-	-
= *Everyday* [e]	***3.91***	***0.24***	***2.49***	***0.018***	-	-	-	-

	ΣPB (R^2=0.117)			
	exp(β)	95% CI		p-value
Frequency of use of lipstick				
= *More than 1 time/day* [a]	***3.39***	***0.11***	***2.33***	***0.031***
Frecuency of pedicure = *Monthly* [b]	-	-	-	-
= *More than 1 time/month* [c]	***3.87***	***0.09***	***2.62***	***0.036***
Frecuency of hair dye = *Yes* [d]	-	-	-	-
= *Monthly* [b]	-	-	-	-
Frequency of use of facial cream				
= *Everyday* [e]	1.96	-0.13	1.47	0.098
Frequency of use of lacquer, cream and foam				
= *Yes* [d]	-	-	-	-
= *Everyday* [e]	-	-	-	-

CI: confidence intervals, [a] *Reference category= ≤ 1 time/day;* [b] *Reference category= < 1 time/month;* [c] *Reference category= ≤ 1 time/month;* [d] *Reference category= Never;* [e] *Reference category= <1 time/day.*

3.3. Urinary concentrations of BPs and PBs, oxidative stress and risk of endometriosis

Table 4 exhibits the associations found between urinary concentrations of BPs or PBs and risk of endometriosis. No significant association was observed when exposure was treated as a continuous variable; however, a significantly higher endometriosis risk was found for the second *versus* first tercile of exposure to MeP (OR = 5.63;

p-value = 0.005), BP-1 (OR = 5.12; p-value = 0.011), BP-3 (OR = 4.98; p-value = 0.008), and ΣBPs (OR = 3.34; p-value = 0.032).

Table 4. Relationship between urinary benzophenones and parabens and endometriosis. Logistic regression analyses.

	OR	95% CI		aOR*	95% CI			OR	95% CI		aOR*	95% CI	
Benzophenones							**Parabens**						
BP-1	0.99	0.81	1.20	0.99	0.79	1.25	**MeP**	1.15	0.95	1.40	1.07	0.86	1.33
<LOD-0,90	1.00			1.00			*<LOD-9,21*	1.00			1.00		
>0,90-3,60	**3.37**	**1.25**	**9.10**	**5.12**	**1.46**	**17.99**	*>9,21-86,06*	**4.57**	**1.58**	**13.22**	**5.63**	**1.70**	**18.70**
>3,60	1.16	0.40	3.38	1.72	0.49	6.06	*>86,06*	2.21	0.73	6.71	1.62	0.47	5.58
BP-3	0.93	0.76	1.15	0.96	0.76	1.23	**EtP**	1.03	0.85	1.24	0.99	0.80	1.22
<LOD-1,48	1.00			1.00			*<LOD-1,28*	1.00			1.00		
>1,48-5,77	**3.56**	**1.33**	**9.56**	**4.98**	**1.52**	**16.31**	*>1,28-7,38*	1.49	0.56	3.95	1.37	0.48	3.94
>5,77	1.03	0.35	3.08	1.46	0.42	5.09	*>7,38*	1.28	0.48	3.42	0.98	0.33	2.91
4-OHBP	0.98	0.72	1.34	0.91	0.63	1.32	**PrP**	1.08	0.90	1.30	0.97	0.78	1.21
>LOD-0,46	1.00			1.00			*<LOD-0,49*	1.00			1.00		
>0,46-1,03	1.61	0.61	4.20	1.43	0.49	4.18	*>0,49-3,75*	1.28	0.48	3.42	1.05	0.36	3.09
>1,03	1.18	0.43	3.18	0.88	0.28	2.75	*>3,75*	1.49	0.56	3.95	0.98	0.32	3.01
ΣBPs	0.93	0.73	1.18	0.95	0.73	1.25	**BuP**	0.80	0.62	1.02	0.77	0.58	1.03
<LOD-1,24	1.00			1.00			*<LOD*	1.00			1.00		
>1,24-2,53	**3.22**	**1.23**	**8.45**	**3.34**	**1.11**	**10.05**	*>0,05-0,68*	0.64	0.25	1.63	0.39	0.12	1.20
>2,53	0.73	0.24	2.20	0.77	0.23	2.61	*>0,68*	0.39	0.15	1.06	0.34	0.11	1.05
							ΣPBs	1.11	0.90	1.38	1.01	0.80	1.29
							>LOD-2,84	1.00			1.00		
							>2,84-4,62	1.24	0.46	3.31	1.09	0.38	3.15
							>4,62	1.39	0.53	3.68	0.84	0.28	2.50

** Adjusted for age (yr), body mass index (kg/m2), residence (rural/urban), parity (yes/no) and urinary creatinine (mg/dL). Bold values indicate statistically significant associations.*

Supplementary Table S4 displays associations observed between urinary concentrations of TAP or TBARS and endometriosis. A statistically significant inverse association was found between TAP (OR = 0.15, p-value = 0.048) and endometriosis risk in the adjusted model, although only the second *versus* first tercile of TAP concentrations was associated with a lower risk (OR = 0.26, p-value = 0.050). In contrast, there was a trend for an association between TBARS concentration and increased endometriosis risk, although it did not reach statistical significance (OR = 1.60, p-value = 0.070), whereas a significantly increased risk was found for women in the third *versus* first terciles of TBARS concentrations (OR = 3.80, p-value = 0.047).

Finally, analyses were conducted to assess the influence of TAP and TBARS concentrations on associations found between exposure to BPs or PBs and the risk of endometriosis (Table 5). TAP concentrations showed a non-significant trend towards a negative association with BP-1 [exp (β) 0.97, 95%CI (0.95–1.00); p-value 0.071] and

ΣBPs [exp (β) 0.97, 95% CI (0.95–1.00); p-value 0.071] (Supplementary Table S5). TBARS concentrations were positively associated with 4-OHBP [exp (β) 1.11, 95% CI (1.01–1.23); p-value 0.035] and EtP [exp (β) 1.05, 95%CI (0.99–1.12); p-value 0.079], although the latter did not reach statistical significance. Given the potential involvement of oxidative stress in the causal pathway between BPs/PBs and endometriosis, multivariate models on the relationship between BP or PB concentrations and endometriosis risk were also adjusted for TAP and TBARS to explore the mediating effect of the redox balance. When the p-value of TAP or TBARS was below 0.050, the p-values of the concentrations of BPs and PBs obtained in the model were compared with the p-values obtained in models not adjusted for TAP or TBARS concentrations, respectively, finding that neither adjustment changed the odds ratios obtained.

Table 5. Influence of TAP and TBARS in the associations between benzophenones and parabens and risk of endometriosis.

TAP influence

	BP-1					BP-3					4-OHBP					ΣBPs				
	aOR[3]	95% CI		p-value (BP-1)	p-value (TAP)	aOR[3]	95% CI		p-value (BP-3)	p-value (TAP)	aOR[3]	95% CI		p-value (4-OH-BP)	p-value (TAP)	aOR[3]	95% CI		p-value (ΣBPs)	p-value (TAP)
T1	1.00	-		-	**0.045**	1.00	-		-	0.101	1.00	-		-	**0.049**	1.00	-		-	0.053
T2	**7.47**	**1.57**	**35.63**	**0.012**		**4.70**	**1.19**	**18.49**	**0.027**		1.21	0.38	3.93	0.745		2.94	0.83	10.44	0.096	
T3	1.35	0.32	5.61	0.680		1.26	0.33	4.82	0.732		0.54	0.14	2.08	0.371		0.57	0.15	2.13	0.408	

	MeP					EtP					PrP					BuP				ΣPBs				
	aOR[3]	95% CI		p-value (MeP)	p-value (TAP)	aOR[3]	95% CI		p-value (EtP)	p-value (TAP)	aOR[3]	95% CI		p-value (PrP)	p-value (TAP)	aOR[3]	95% CI		p-value (BuP)	p-value (TAP)	aOR[3]	95% CI		p-value (ΣPBs)	p-value (TAP)
T1	1.00	-		-	**0.036**	1.00	-		-	0.071	1.00	-		-	**0.049**	1.00	-		-	0.057	1.00	-		-	0.053
T2	**6.98**	**1.84**	**26.51**	**0.004**		1.49	0.47	4.69	0.494		0.86	0.27	2.75	0.799		0.32	0.09	1.17	0.084		1.20	0.38	3.77	0.756	
T3	1.37	0.35	5.29	0.650		0.83	0.25	2.76	0.760		0.83	0.24	2.93	0.771		0.48	0.14	1.67	0.250		0.76	0.23	2.43	0.638	

TBARS influence

	BP-1					BP-3					4-OHBP					ΣBPs				
	aOR[3]	95% CI		p-value (BP-1)	p-value (TBARS)	aOR[3]	95% CI		p-value (BP-3)	p-value (TBARS)	aOR[3]	95% CI		p-value (4-OH-BP)	p-value (TBARS)	aOR[3]	95% CI		p-value (ΣBPs)	p-value (TBARS)
T1	1.00	-			0.979	1.00	-			0.753	1.00	-			0.753	1.00	-			0.700
T2	**4.95**	**1.40**	**17.51**	**0.013**		**4.88**	**1.47**	**16.19**	**0.010**		**4.88**	**1.47**	**16.19**	**0.010**		3.30	1.07	10.18	0.037	
T3	1.82	0.51	6.44	0.353		1.54	0.44	5.38	0.502		1.54	0.44	5.38	0.502		0.82	0.24	2.79	0.748	

	MeP					EtP					PrP					BuP				ΣPBs				
	aOR[3]	95% CI		p-value (MeP)	p-value (TBARS)	aOR[3]	95% CI		p-value (EtP)	p-value (TBARS)	aOR[3]	95% CI		p-value (PrP)	p-value (TBARS)	aOR[3]	95% CI		p-value (BuP)	p-value (TBARS)	aOR[3]	95% CI		p-value (ΣPBs)	p-value (TBARS)
T1	1.00	-			0.902	1.00	-			0.796	1.00	-			0.993	1.00	-			0.747	1.00	-			0.976
T2	**7.07**	**1.97**	**25.39**	**0.003**		1.59	0.51	4.94	0.419		0.97	0.32	2.89	0.950		0.43	0.13	1.36	0.150		1.22	0.41	3.60	0.723	
T3	1.99	0.54	1.99	0.303		1.12	0.37	3.42	0.843		1.00	0.32	3.05	0.994		0.35	0.11	1.15	0.084		0.92	0.30	2.83	0.891	

[3] *Adjusted for age (yr), body mass index (kg/m^2), residence (rural/urban), parity (yes/no) and urinary creatinine (mg/dL).*

4. DISCUSSION

To our best knowledge, this is the first study to address the possible association of exposure to PBs with the risk of endometriosis and one of the very first to explore the association of exposure to BPs. The use of different cosmetics and PCPs were associated with urinary concentrations of BPs and PBs, and positive associations were found between exposure to MeP, BP-1, BP-3, and ΣBPs and the risk of endometriosis in women of childbearing age, which was higher for women in the second tertiles of exposure. Oxidative stress did not play a significant role in the influence of these EDCs on endometriosis risk.

There is increasing epidemiological evidence of a relationship between exposure to EDCs and an increased risk of estrogen-dependent gynecological disorders, including: anovulation (Costa et al., 2014), lower antral follicle count (Souter et al., 2013; Ziv-Gal and Flaws, 2016), infertility (Ziv-Gal and Flaws, 2016), miscarriage (Lathi et al., 2014; Shen et al., 2015; Yuan et al., 2015), preterm birth (Peretz et al., 2014; Weinberger et al., 2014), polycystic ovarian syndrome (Palioura and Diamanti-Kandarakis, 2015; Rutkowska and Diamanti-Kandarakis, 2016; Wang et al., 2017), endometriosis (Kunisue et al., 2012), and fibroids (Pollack et al., 2015; Shen et al., 2013). Kunisue et al. (2012) evidenced a positive association between exposure to BP-3 and the risk of endometriosis, and an inverted U-shaped association was observed in the present study between tertiles of BP-1, BP-3 and ΣBPs and increased endometriosis risk, as commonly reported in endocrinology (Blumberg, 2007). The fact that that BP or PB exposure but not the self-reported frequency of use of certain cosmetics and PCPs was associated with endometriosis risk may reflect the contribution of other sources of exposure (e.g., diet or smoking habit) to the urinary burden of PBs and BPs, indicating an additive/synergistic effect of EDCs from multiple sources. In this regard, the participants' urinary concentrations of BPs and PBs were related to the frequency of use of certain products/treatments, including lipstick, face masks and creams, hair products (dyes, lacquers, creams, and foam) and pedicure. These results are in agreement with previous studies reporting the widespread presence of BPs and PBs in PCPs and cosmetics (Han et al., 2016; Janjua et al., 2008; Panico et al., 2019; Yazar et al., 2011). At least one PB was also found in 100% of feminine hygiene products, and both MeP and EtP were found in> 80% of them, mainly bactericidal wipes, creams, and solutions and deodorant sprays and powders (Gao and Kannan, 2020). Finally, other authors have

related urinary PB concentrations to the use of hair products, deodorants, and face and hand creams (Guo et al., 2014; Sakhi et al., 2018).

Our group recently reported the generalized presence of BPs and PBs in menstrual blood, a biological matrix in intimate contact with the endometrium (Iribarne-Durán et al., 2020). Our findings led us to suspect that these EDCs might alter the uterine microenvironment and trigger signaling pathways that could in turn favor the development of certain gynecological disorders, including endometriosis. There is a scarcity of studies addressing the effects of BP exposure in *in vivo/in vitro* models of endometriosis. However, in relation to other gynecological tissues, Santamaria et al. (2019) found that exposure to BP-1 and BP-3 perturbed early events in ovarian cells, affecting germ cell development and disrupting the expression of crucial genes related to follicular assembly. Likewise, Shin et al. (2016) described the BP-dependent induction of metastasis in an *in vivo* model of ovarian cancer. In regard to PBs, one group found that trace concentrations of these compounds were more frequently detected in the endometrial tissue of patients with endometrial carcinoma *versus* controls (Dogan et al., 2019). In the present study, MeP was the most frequently detected chemical and demonstrated, for the first time, a potential inverted U-shaped association with increased endometriosis risk.

Oxidative stress, is suspected to play a key role in the pathophysiology of endometriosis (Amreen et al., 2019; Gupta et al., 2006; Ito et al., 2017; Lambrinoudaki et al., 2009; Scutiero et al., 2017). The borderline positive association between TBARS concentrations and endometriosis risk found in the present study is in agreement with previous reports (Jackson et al., 2005; Nasiri et al., 2017; Singh et al., 2013). We detected an inverse relationship between TAP concentrations and the risk of endometriosis, in line with previously reported findings of lower TAP concentrations in the serum of women with endometriosis *versus* controls (Liu et al., 2013; Nasiri et al., 2017; Singh et al., 2013; Turgut et al., 2013; Turkyilmaz et al., 2016). These results are also supported by *in vitro* studies in which endometriotic cells displayed endogenous oxidative stress, with increased reactive oxygen species (ROS) production, altered ROS detoxification pathways, and a fall in catalase levels (Ngô et al., 2009). Emerging evidence suggests that BP and PB exposure may disrupt both the systemic and local redox balance; however, it has not been established whether this action is related to or independent of their endocrine-disrupting properties, and it has been suggested that

EDCs might induce oxidative stress, at least in part, *via* estrogen receptor-α signaling pathways (Cho et al., 2018). In this context, some authors have reported that urinary concentrations of BPs and PBs are positively associated with higher systemic lipid peroxidation levels (Kang et al., 2013; Watkins et al., 2015), and our group has described BP- and PB-driven tissue-specific disturbances in the local antioxidant system (Artacho-Cordón et al., 2019). Our group recently suggested that oxidative stress might mediate in the association between exposure to bisphenols and a higher risk of endometriosis (Peinado et al., 2020b); however, according to the present findings, it does not appear to mediate in the association of exposure to parabens or benzophenes with endometriosis risk. The potential influence of oxidative stress on the aggressiveness and progression of endometriosis warrants further investigation.

4.1. Limitations of the study

Study limitations include the relatively small sample size, reducing the statistical power and the possibility of exploring differences in risk among stages of endometriosis. Nevertheless, statistically significant associations could be identified, even after adjustment for multiple potential confounders. Another limitation is that only one urine sample was collected from each patient, preventing consideration of the variability in daily exposure to analytes with relatively short elimination half-lives. However, detected concentrations of BPs and PBs are comparable to those previously reported in similar female populations (Jimenez-Diaz et al., 2016a; Lee et al., 2019; Pollack et al., 2020; Song et al., 2020). A further study limitation is that the contribution of cosmetics and PCPs to the urinary burden of PBs and BPs was investigated but not the contribution of other lifestyle factors, such as the diet or smoking habits of participants. An additional limitation of our study was its utilization of biomarkers of systemic oxidative stress, and further research is needed to evaluate the potential mediating effect of *in situ* redox status on the association between BP and BP exposure and risk of endometriosis. Finally, although humans are simultaneously exposed to a very wide range of synthetic chemicals with endocrine-disrupting properties, only congeners from two families of EDCs were considered. Hence, there is a need to explore the effects on endometriosis risk of PBs and BPs in combination with other EDCs. Study strengths include the laparoscopic confirmation of the presence (cases) or absence (controls) of endometriosis, ensuring that the controls did not have lesions that were asymptomatic or

undetectable by magnetic resonance imaging. In addition, biological samples were gathered from cases and controls under the same conditions (i.e., fasting early morning samples before surgery) increasing the comparability between cases and controls in relation to previous habits. Finally, a major contribution of this study is the combined investigation of biomarkers of exposure, potential biomarkers of effect, and a health outcome, offering evidence of the potential influence of BPs and PBs on an adverse outcome pathway for endometriosis.

5. CONCLUSIONS

This study sheds some light on the potential risk of endometriosis posed by exposure to BP and PBs and on the contribution of certain cosmetics and PCPs to this exposure. Some BP and PB congeners were found to increase this risk in an oxidative stress-independent manner, and these novel findings warrant further investigation in the near future. Preventive measures against endometriosis are especially relevant, because it is difficult to establish a diagnosis and the treatment is largely symptomatic. These results suggest that restrictions in the use of cosmetics and PCPs that contain BPs or PBs might help to decrease exposure to these EDCs and thereby reduce the risk of endometriosis, among other diseases.

CREDIT AUTHOR STATEMENT

Francisco M. Peinado: Investigation, Formal analysis, Writing - original draft, Olga Ocón-Hernández: Conceptualization, Supervision, Funding acquisition, Writing-Review & Editing, Luz M. Iribarne-Dur´an: Investigation, Fernando Vela-Soria: Investigation, Alfredo Ubiña: Resources, Writing-Review & Editing, Carmen Padilla: Resources, Writing- Review & Editing, Jose C. Mora: Resources, Writing-Review & Editing, Jesús Cardona: Resources, Writing-Review & Editing, Josefa León: Investigation, Writing-Review & Editing, Mariana F. Fernández: Writing-Review & Editing, Nicolás Olea: Writing-Review & Editing, Francisco Artacho-Cordón: Conceptualization, Methodology, Supervision, Formal analysis, Funding acquisition, Writing-Review & Editing.

CONFLICT OF 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110342.

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ARTICLE 3

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Human exposure to bisphenols, parabens, and benzophenones, and its relationship with the inflammatory response: a systematic review

Francisco Manuel Peinado [1], Luz María Iribarne-Durán [1,*] and Francisco Artacho-Cordón [1,2,3]

[1] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), E-18012 Granada, Spain; franciscopeinado@correo.ugr.es (F.M.P.); fartacho@ugr.es (F.A.-C.)

[2] CIBER Epidemiology and Public Health (CIBERESP), E-28029 Madrid, Spain

[3] Radiology and Physical Medicine Department, University of Granada, E-18016 Granada, Spain

***** Correspondence: luzmaria.irdu@gmail.com

ABSTRACT

Bisphenols, parabens (PBs), and benzophenones (BPs) are widely used environmental chemicals that have been linked to several adverse health effects due to their endocrine disrupting properties. However, the cellular pathways through which these chemicals lead to adverse out-comes in humans are still unclear, suggesting some evidence that inflammation might play a key role. Thus, the aim of this study was to summarize the current evidence on the relationship between human exposure to these chemicals and levels of inflammatory biomarkers. A systematic review of peer-reviewed original research studies published up to February 2023 was conducted using the MEDLINE, Web of Science, and Scopus databases. A total of 20 articles met the inclu-sion/exclusion criteria. Most of the reviewed studies reported significant associations between any of the selected chemicals (mainly bisphenol A) and some pro-inflammatory biomarkers (including C-reactive protein and interleukin 6, among others). Taken together, this systematic review has identified consistent positive associations between human exposure to some chemicals and levels of pro-inflammatory biomarkers, with very few studies exploring the associations between PBs and/or BPs and inflammation. Therefore, a larger number of studies are required to get a better understanding on the mechanisms of action underlying bisphenols, PBs, and BPs and the critical role that inflammation could play.

Keywords: bisphenol, paraben, benzophenone, human, exposure, inflammation

1. INTRODUCTION

During the last decades, there is a growing public concern about the harmful effects that environmental phenols, including bisphenols, parabens (PBs) and benzophenones (BPs) could exert on human health (Diamanti-Kandarakis et al., 2009; Engdahl and Rüegg, 2020; Huo et al., 2016; Liu et al., 2019b; Sifakis et al., 2017; Wang et al., 2012).

Bisphenols are non-persistent phenolic compounds widely used in the synthesis of polycarbonate plastics and epoxy resins, and are frequently found in the linings of canned and packaged food containers, thermal receipts, and dental sealants (Andra et al., 2015; Rochester and Bolden, 2015). Bisphenol A (BPA) is the most studied congener and is one of the most produced chemicals in the world (Hu et al., 2019), reaching a global production volume of more than 5 million tons (Yamazaki et al., 2015), and with an annual growth rate that reached 4.6% between 2013 and 2019 (Song et al., 2019). Moreover, data from biomonitoring studies indicate that BPA exposure is ubiquitous and widespread in the population, with BPA concentrations found in 90.0% of the general population in industrialized countries (Becker et al., 2009; Casas et al., 2011). Due to the harmful effects inherent to exposure to BPA, some international government regulators have banned its use in baby bottles and cosmetics (Chen et al., 2016). As an alternative to BPA, bisphenol analogues structurally similar to BPA began to be produced, such as bisphenol S, bisphenol F, bisphenol AF, tetrabromobisphenol, bisphenol A-glycidyl methacrylate, bisphenol A diglycidyl ether and bisphenol F diglycidyl ether (Liu et al., 2021a; Thoene et al., 2020). However, previous evidence has suggested that these analogues may be even more harmful than the original BPA in some situations (Thoene et al., 2020). The family of PBs includes alkyl esters of p-hydroxybenzoic acid and is used in a wide range of cosmetics and personal care products (PCPs) as well as in food packaging due to their antimicrobial and preservative properties (Casas Ferreira et al., 2011; Fisher et al., 2017; Snodin, 2017; Zhao et al., 2021). The main congeners of PBs are methylparaben, ethylparaben, propylparaben, and butylparaben. BPs are aromatic ketones included in a wide variety of cosmetics, PCPs and textiles due to their properties as UV filters (Kim and Choi, 2014; Molins-Delgado et al., 2016), and include different congeners, such as benzophenone 1, benzophenone 2, benzophenone 3, 4-hydroxybenzophenone, benzophenone 6, and benzophenone 8. Therefore, derived from their uses, humans are widely exposed to these compounds through different pathways. While humans are mainly exposed to bisphenols through

the diet (Morgan and Clifton, 2021; Robles-Aguilera et al., 2021), PBs and BPs are suspected to reach body compartments primarily through dermal absorption or consumption of packaged foodstuff (Benech-Kieffer et al., 2000; Díaz-Cruz et al., 2012). Despite these compounds are rapidly metabolized and excreted by the body, the public concern regarding their potential health effects derives from daily pattern of this exposure.

Indeed, previous studies have suggested that daily exposure to different bisphenol congeners might be associated with risks of women miscarriage, endometriosis, polycystic ovary syndrome, thyroid disease, diabetes mellitus, obesity, cardiovascular disease and metabolic syndrome (Lathi et al., 2014; Li et al., 2019; Peinado et al., 2020b; Peretz et al., 2014; Shen et al., 2015; Wang et al., 2012). In addition, PBs and BPs could also origin adverse effects on human health, such as decreased body weight and height in children (Wu et al., 2019), decreased serum thyroid levels in humans (Aker et al., 2018), obesity (Kolatorova et al., 2018) and gynecological disorders (Peinado et al., 2021; Ziv-Gal and Flaws, 2016).

Nevertheless, despite the currently suspected harmful effects of these environmental phenols on human health, there are still several gaps of knowledge concerning their mechanisms of action. Currently, it is well-known that bisphenols, PBs and BPs have the ability to alter the homeostasis of the hormonal system due to their (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid actions (Boberg et al., 2010; Darbre and Harvey, 2008; Perez et al., 1998) and therefore are considered endocrine-disrupting chemicals (EDCs). In addition, it has been postulated that inflammation might act as an alternative or complementary mechanism of action to the endocrine disruption hypothesis, given that they could promote an inflammatory milieu through activation of ERα nuclear receptors (Khan and Ansar Ahmed, 2015; Thompson et al., 2015; Watkins et al., 2015). In this sense, previous evidence has reported the presence of estrogen-dependent nuclear receptors in promoter regions of genes related to the inflammatory response, such as ERα and ERβ (Andersson et al., 2011; Felty et al., 2010; Kassi and Moutsatsou, 2010; Khan and Ansar Ahmed, 2015), suggesting that the origin and development of an inflammatory response could be an indirect consequence of endocrine alterations promoted by these compounds with hormonal activity.

Inflammation is a regulatory mechanism for maintaining tissue homeostasis. It consists of a protective response of vascularized tissues to fight against a variety of challenges from the external environment, including those from infectious agents and tissue damage. It provides pathways for the rapid destruction of invading pathogens through the mobilization of immune cells across the vasculature and for the removal of damaged cells and tissues that may have been compromised in host defense (Dietert, 2012). A large number of biochemical reactions and mediators, such as cytokines, phagocytic leukocytes, antibodies, complement proteins and intracellular adhesion molecules, among others, are involved in this complex process. Like most immune responses, the inflammatory phenomenon is tightly regulated, and a proper and precise balance between proinflammatory and anti-inflammatory immune responses is required to effectively eliminate infectious pathogens while limiting immune damage in the host (Martinez-Espinosa et al., 2022). The regulation of inflammatory responses is complex, involves many different cell types (immune, epithelial, endothelial, and mesenchymal cells) (Dietert, 2012; Khan and Ansar Ahmed, 2015), and sometimes it may not be properly regulated. A misregulated inflammation could be originated when the response among innate immune cells is inappropriate for the type of defense needed against the invader, the response is misdirected based on the location of the strange agent, the response is overproduced, and/or the response is not beneficially resolved for the host (Dietert, 2012; Khan and Ansar Ahmed, 2015). Deviations from tightly regulated inflammation present a significant health risk because unresolved inflammation can compromise tissue function and increase the risk of several chronic cardiovascular diseases and metabolic disorders (Dietert, 2012).

In this sense, two previous systematic reviews have summarized the associations reported between exposure to different families of EDCs and inflammatory biomarkers (Liu et al., 2022; Peinado et al., 2020a). However, the majority of EDCs explored were persistent organic pollutants, such as organochlorine pesticides (OCPs), and polychlorinated biphenyls. Considering non-persistent EDCs, only phthalates and BPA were explored (Liu et al., 2022) and currently there are no previous systematic reviews exploring the associations between other bisphenol congeners, PBs, or BPs and biomarkers of inflammation.Therefore, given (i) the ubiquity of these families of environmental phenols, and (ii) their possible adverse effects on health, there is a growing interest in the elucidation of potential mechanisms of action of these

compounds. Thus, the aim of this study was to conduct a systematic review of published scientific evidence on associations between human exposure to bisphenols, PBs, and BPs and levels of inflammatory biomarkers.

2. MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement (Moher et al., 2009).

2.1. Data Sources and Search Strategy

The databases MEDLINE (through the PubMed search engine), Web of Science, and Scopus were used to search for published studies reporting associations between human exposure to bisphenols, PBs, and BPs and levels of inflammatory biomarkers. The last search was performed on 1 February 2023. The detailed search strategy is displayed in supplementary material (Supplementary Table S1).

Our objective was to answer the question: "Is there evidence on associations between human exposure to bisphenols, PBs and BPs, and levels of inflammatory biomarkers?" We developed a PECO statement (Participants, Exposure, Comparator, and Outcomes) (Morgan et al., 2018), which is used as an aid to developing an answerable question. Our PECO statement included the following:

Participants: Humans.

Exposure: Bisphenols [bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol A-glycidyl methacrylate (BisGMA), bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE)], PBs [methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP) and butylparaben (BuP)] and BPs (BP1-12).

Comparators: Not applicable.

Outcomes: Inflammatory biomarkers (cytokines, intracellular adhesion molecules, humoral mediators, C-reactive protein, inflammatory milieu, phagocytic leukocytes, antibodies, complement proteins, receptor activator of nuclear factor-kappa B, prostaglandin-endoperoxide synthases).

2.2. Study Selection and Data Extraction

Review inclusion criteria were: original scientific article; publication in English or Spanish; and the reporting of data on (i) the associations between human exposure to bisphenols, PBs, and BPs, and (ii) levels of inflammatory biomarkers. Exclusion criteria were: systematic and narrative reviews, case reports, conferences, meeting abstracts, and editorials; *in vitro* and *in vivo* studies.

Two researchers (LMID and FMP) independently conducted this systematic review. Firstly, the titles/abstracts of retrieved articles were screened, and duplicates and those not meeting the inclusion criteria were excluded. From the initially selected articles, the full text was reviewed and those that did not meet the inclusion criteria were then excluded. In case of discrepancy between reviewers, a third external reviewer (FAC) participated to make a decision about the inclusion or exclusion of the article at any step of the screening. The following data were collected from each article: (1) country; (2) type of study; (3) sample collection period of the exposure biomarker; (4) sample collection period of the inflammation biomarker; (5) sample size; (6) health condition; (7) gender; (8) age; (9) exposure (family and congeners) biomarkers; (10) inflammation biomarkers; (11) biological matrix; (12) chemical and biological quantification methodology; (13) extraction volume; (14) frequencies of detection (FD); (15) limit of detection of exposure biomarker; (16) units; (17) concentrations (arithmetic means, geometric means or percentile 50); (18) quality; (19) risk of bias; (20) statistical test; (21) magnitude of the reported associations, and (22) p-values of such associations. It is worth mentioning that in case of a variety of statistics reported to summarize EDC and/or inflammation biomarker concentrations, the median value was prioritized. Moreover, we have preserved units of measurements in tables, although we have appropriately unified them in order to make comparisons between studies.

2.3. Assessment of Reporting Quality and Risk of Bias

The reporting quality of the epidemiological studies was assessed using the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist (von Elm et al., 2008). This checklist consists of six blocks and a total of 23 items: 1) title and summary (2 items), 2) introduction (2 items), 3) method (9 items), 4) results (5 items), 5) discussion (4 items), and 6) other information (1 item). The

reporting quality of articles was categorized according to Alvarenga et al. (2021) as high (≥16 items checked), medium (15-8 items), or low (<8 items) (Table 1).

The risk of bias was estimated by using a modified version of Risk of Bias in Non-randomized Studies of Exposures (ROBINS-E) (Higgins et al., 2022). This tool comprises seven domains for the overall assessment of the risk of bias, including bias due to confounding; bias in selecting participants in the study; bias in exposure classification; bias due to departures from intended exposures; bias due to missing data; bias in outcome measurement; and bias in the selection of reported results. Each study was classified as "low", "some concerns", "high", or "very high" risk of bias after evaluating each domain.

The reporting quality and the risk of bias assessment were performed by two re-viewers (FMP and LMID). Any disagreement was resolved through a consensus discus-sion with the involvement of a third reviewer (FAC).

3. RESULTS

Figure 1 depicts the PRISMA flow chart of articles through the study. A total of 3508 articles (1524 scientific papers were identified from MEDLINE, 469 from WoS, and 1515 from the Scopus database) were identified after applying the search strategy, from which 1182 were excluded for being duplicates. Then, titles and abstracts of the remaining 2326 articles were reviewed, and 2304 were excluded because no bisphenols, PBs or BPs were measured, no inflammation parameters were quantified, no Spanish or English language was used or they were review articles, conference papers, notes, book chapters, or con-ference abstracts. After full-text review of the remaining 22 articles, another two were excluded because they did not measure bisphenols, PBs, BPs or inflammation parameters or they were no epidemiological studies. Finally, 20 articles were selected for this review.

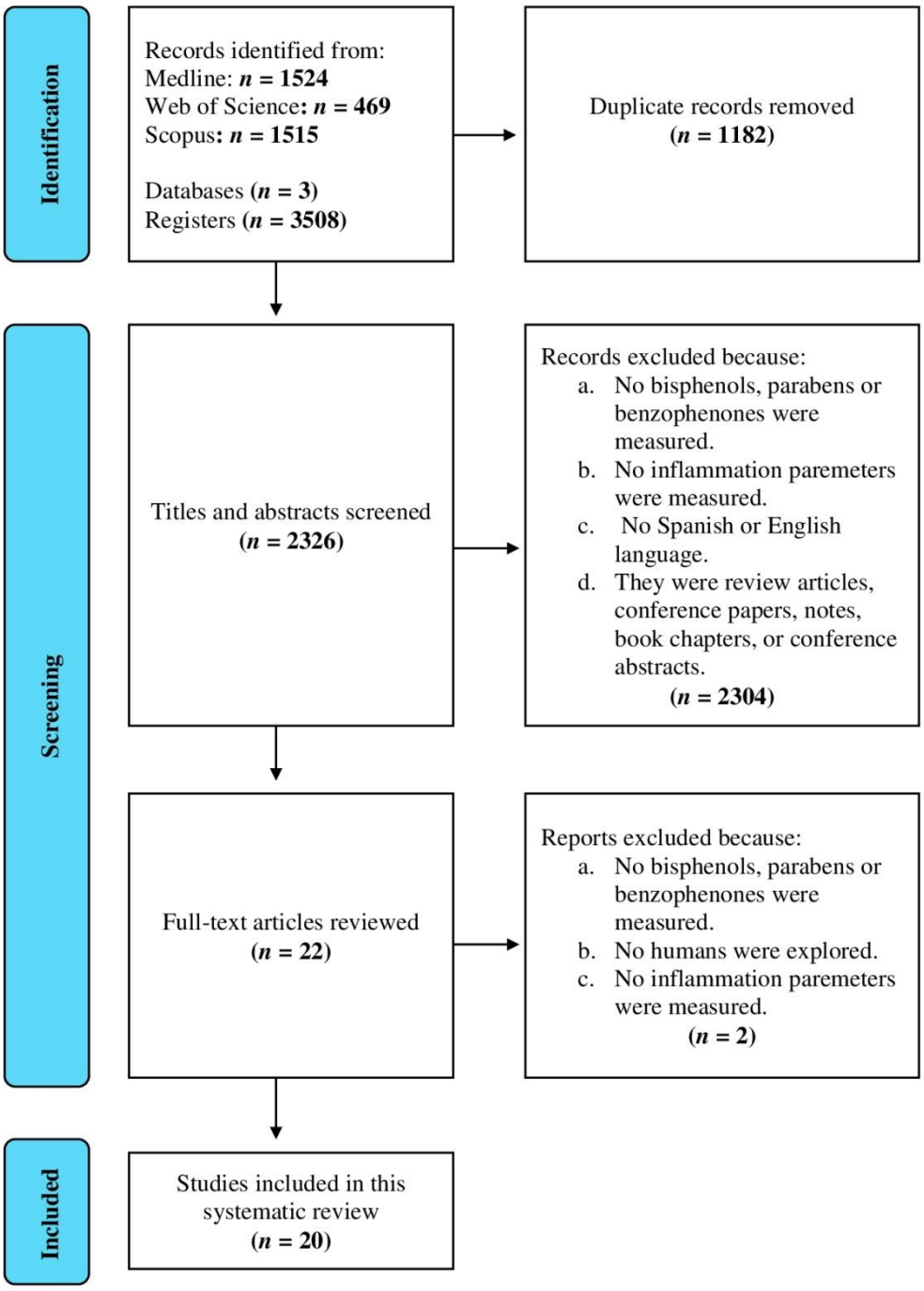

Figure 1. PRISMA flow chart for systematic review.

3.1. Characteristics of the studies

Table 1 exhibits the main characteristics of the 20 studies (Ashley-Martin et al., 2015; Aung et al., 2019; Choi et al., 2017; Ferguson et al., 2016; Haq et al., 2020; Huang et al., 2017; Jain et al., 2020; Kelley et al., 2019; Lang et al., 2008; Liang et al., 2020; Linares et al., 2021; Mohsen et al., 2018; Nalbantoğlu et al., 2021; Qu et al., 2023;

Savastano et al., 2015; Šimková et al., 2020; Song et al., 2017; Tsen et al., 2021; Watkins et al., 2015; Yang et al., 2009). All included studies were published in the last 15 years (2008-2023). Nine out of 20 studies (45.0%) had a cross-sectional design, while 6 (30.0%) and 4 (20.0%) were cohort and case-control studies. A final study had a prospective observational design. A total of 13 out of 20 studies (65.0%) reported the period of time when biological samples for exposure/outcome assessment were collected. Given that they reported similar periods of biological samples collection for exposure and outcome assessment, at least most of studies should be considered with a cross-sectional design (for the purpose of this systematic review) despite some of them declared a different study design. The sample size of the studies ranged from 39 to 1,455 participants, with a pooled sample size of 7,319 participants (10,339 samples). Studies were carried out in Asian (45.0%), American (30.0%) and European countries (20.0%). The reporting quality was classified as high in 15 studies (75.0%), while 5 studies (25.0%) had medium reporting quality (Jain et al., 2020; Linares et al., 2021; Šimková et al., 2020; Song et al., 2017; Tsen et al., 2021). The risk of bias was classified as very high in 2 studies (Choi et al., 2017; Šimková et al., 2020), high in 6 study (Ferguson et al., 2016; Jain et al., 2020; Liang et al., 2020; Linares et al., 2021; Savastano et al., 2015; Song et al., 2017), with some concerns in 3 studies (Ashley-Martin et al., 2015; Huang et al., 2017; Nalbantoğlu et al., 2021), and low in 9 studies (Aung et al., 2019; Haq et al., 2020; Jain et al., 2020; Kelley et al., 2019; Mohsen et al., 2018; Qu et al., 2023; Tsen et al., 2021; Watkins et al., 2015; Yang et al., 2009) (Figure 2). Taken together, the higher concerns were due to confounding, the measurement of the exposure and the missing data, as shown in Figure 3.

Characteristics of study participants in the included studies are depicted in Supplementary Table S2. Two studies (10.0%) (Mohsen et al., 2018; Nalbantoğlu et al., 2021) were focused on children (boys and girls) and 18 on adults [1 in men (5.0%), 8 in women (40%; 6 of them during pregnancy) and 9 (45.0%) included men and women]. The majority of studies included healthy population exclusively (n=13, 65.0%), 5 studies combined healthy and pathologic patients (people with diabetes, allergic rhinitis, rheumatoid arthritis and polycystic ovary syndrome), one study was focused on patients with Crohn's disease and other study on women with unexplained recurrent spontaneous abortion.

Table 1. General characteristics of the studies included in this systematic review.

Reference	Country	Study design	Period of sample collection		Sample size	Reporting quality*
			For exposure assessment	For outcome assessment		
Ashley-Martin et al., 2015	Canada	Cohort	2008-2011	2008-2011	1258	High
Aung et al., 2019	USA	Cohort	2006-2008	2006-2008	482 (1628 samples)	High
Choi et al., 2017	South Korea	Cohort	2013	2013	200	High
Ferguson et al., 2016	USA	Case-Control	2006-2008	2006-2008	482 (1695 samples)	High
Haq et al., 2020	Pakistan	Cross-sectional	N.R.	N.R.	400	High
Huang et al., 2017	Taiwan	Cohort	2014-2016	2014-2016	230	High
Jain et al., 2020	India	Cross-sectional	N.R.	N.R.	300	Medium
Kelley et al., 2019	USA	Cohort	2012-2015	2012-2015	56	High
Lang et al., 2008	USA	Cross-sectional	2003-2004	2003-2004	1455	High
Liang et al., 2020	China	Cross-sectional	2015-2016	2015-2016	111	High
Linares et al., 2021	Spain	Prospective observational	N.R.	N.R.	200	Medium
Mohsen et al., 2018	Egypt	Cross-sectional	N.R.	N.R.	167	High
Nalbantoğlu et al., 2021	Turkey	Case-Control	2018	2018	280	High
Qu et al., 2022	China	Case-Control	2018-2020	2018-2020	290	High
Savastano et al., 2015	Italy	Cross-sectional	N.R.	N.R.	76	High
Šimková et al., 2020	Czech Republic	Case-Control	N.R.	N.R.	39	Medium
Song et al., 2017	South Korea	Cross-sectional	2008-2012	2008-2012	612 (1141 samples)	Medium
Tsen et al., 2021	Taiwan	Cross-sectional	N.R.	N.R.	90	Medium
Watkins et al., 2015	Puerto Rico	Cohort	2010-2012	2010-2012	106 (238 samples)	High
Yang et al., 2009	Korea	Cross-sectional	2005	2005	485	High

*USA: United States of America; N.R.: Not reported. *STROBE checklist items <8: "low quality"; 14-8: "medium quality"; ≥15: "high quality".*

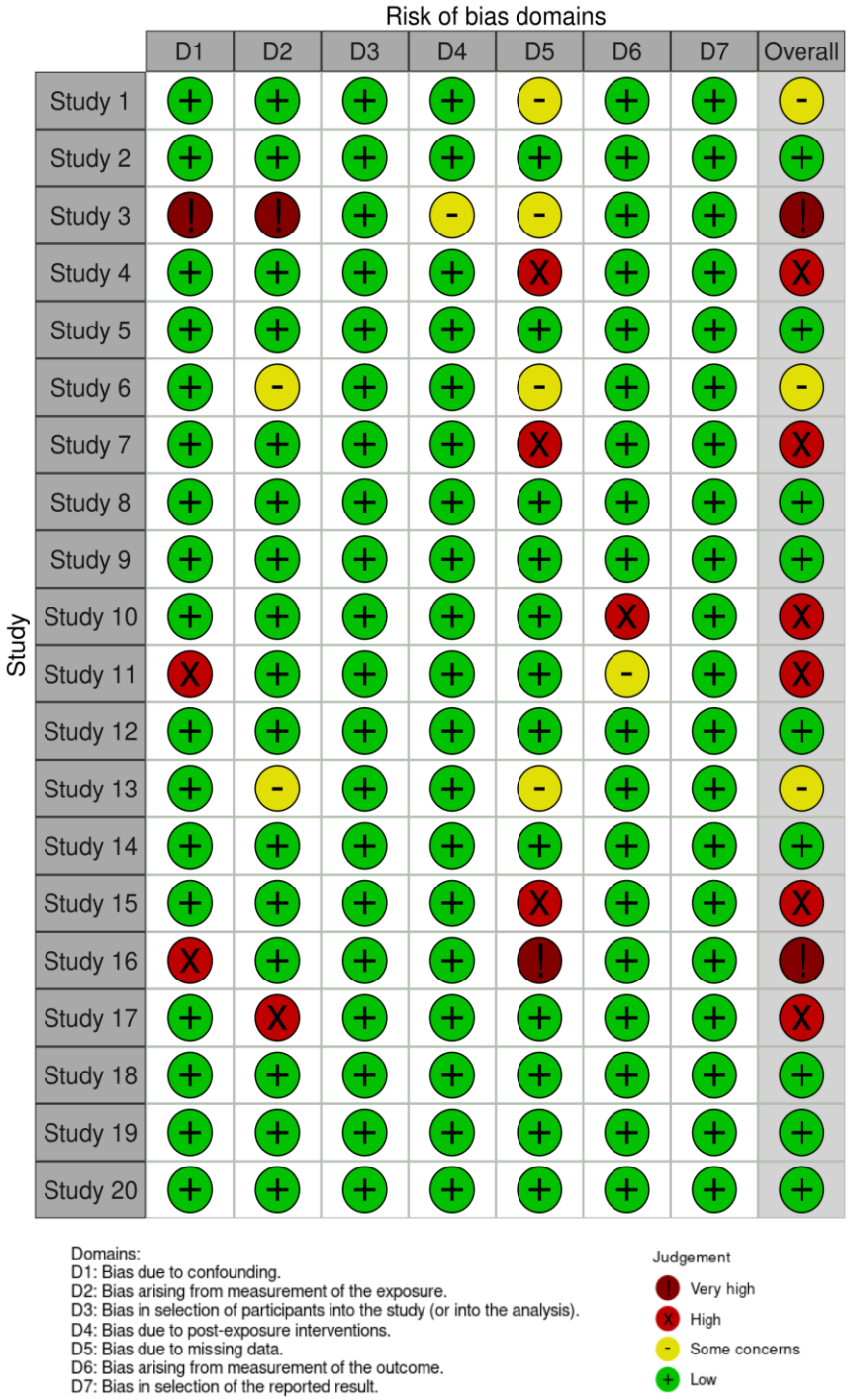

Figure 2. Assessment of the risk of bias of each study considering the ROBINS-E domains.

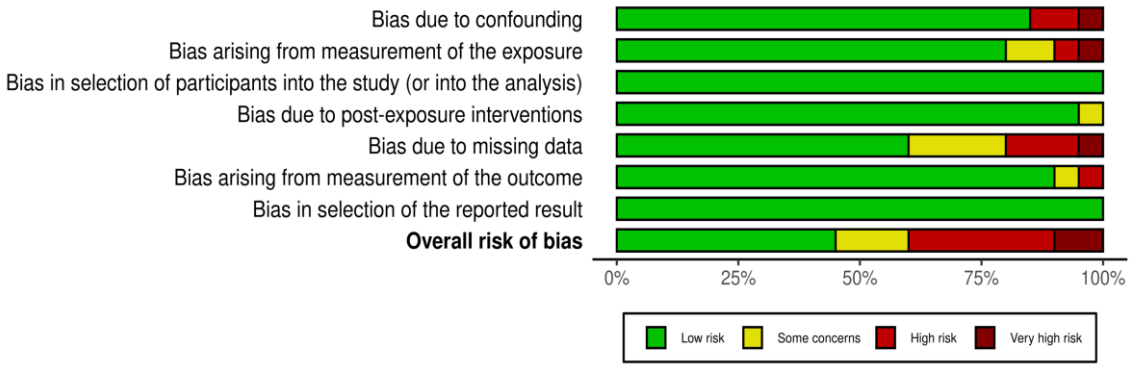

Figure 3. Assessment of the risk of bias of each ROBINS-E domain taking together all studies included in this review.

3.2. Exposure of Bisphenols, PBs, and BPs

Table 2 provides an overview of the main methodological characteristics related to the exposure assessment to bisphenols, PBs and BPs in the selected studies. Most of the studies used urine (13, 65.0%), while 7 studies (35.0%) assessed the exposure in serum/plasma/blood samples. Regarding the families of chemicals assessed, 14 out 20 studies (70.0%) were focused on the exposure to bisphenols (mainly BPA), 4 (20.0%) on the exposure of the three families (bisphenols, PBs and BPs) (Aung et al., 2019; Kelley et al., 2019; Linares et al., 2021; Watkins et al., 2015), one on bisphenols and PBs (Šimková et al., 2020) and another only assessed PB concentrations (Qu et al., 2023). The most frequently detected compounds were BPA (FD: 76.0-100%), MeP (FD: 97.0-100%), and BP-3 (FD: 99.7-100%). Concentrations of each studied chemical are also summarized in Table 2. Bisphenols, PBs, and BPs showed a median concentration ranging from <LOD-2.7 ng/mL, <LOD-186.0 ng/mL, and 34.5-42.6 ng/mL, respectively. BPA, MeP and BP-3 were the most detected congeners of each of the three EDCs families explored. Other characteristics of the exposure assessment are summarized in Supplementary Table S2, including details on quantification methodology, limits of detection and volume of sample used for the determination of the exposure in each study.

Table 2. Characteristics related to the exposure assessment to bisphenols, PBs and BPs in the selected studies.

Reference	EDC family	Compounds	Matrix	Frequency of detection (%)	Unit	Concentrations
Ashley-Martin et al., 2015	Bisphenols	BPA	Urine	86.6	µg/L	N.R.
Aung et al., 2019	Bisphenols	BPS	Urine	20.6	ng/mL	P50: 0.38
	PBs	MeP		99.9		P50: 186
		EtP		59.5		P50: 2.15
		PrP		99.0		P50: 45.60
		BuP		68.4		P50: 0.85
	BPs	BP-3		99.7		P50: 42.60
Choi et al., 2017	Bisphenols	BPA	Urine	N.R.	µg/L	N.R.
Ferguson et al., 2016	Bisphenols	BPA	Urine	83.4	ng/mL	GM: 1.32-1.38
Haq et al., 2020	Bisphenols	BPA	Urine	N.R.	ng/mL	Diabetic: 3.44 ± 1.82 * Healthy: 1.70 ± 0.43*
Huang et al., 2017	Bisphenols	BPA	Urine	82.2	ng/mL	P50: 1.77
Jain et al., 2020	Bisphenols	BPA	Serum	N.R.	N.R.	N.R.
Kelley et al., 2019	Bisphenols	BPA	Urine	N.R.	N.R.	N.R.
		BPS				
		BPF				
	PBs	MeP				
		EtP				
		PrP				
		BuP				
	BPs	BP-3				
Lang et al., 2008	Bisphenols	BPA	Urine	N.R.	ng/mL	Men weighted mean: 4.53 Women weighted mean: 4.66
Liang et al., 2020	Bisphenols	BPA	Urine	99.1	ng/mL	P50: 0.95
		BPS		41.4		P50: <LOD
Linares et al., 2021	Bisphenols	BPA	Serum	N.R.	µM	In remission: 5.57 ± 8.29 Active disease: 11.98 ± 20.25*
	PBs	MeP				In remission: 3.67 ± 5.72 Active disease: 3.26 ± 5.50*
		EtP				In remission: 0.90 ± 2.79 Active disease: 0.31 ± 0.55*
		PrP				In remission: 0.32 ± 0.82 Active disease: 0.15 ± 0.25*
		BuP				In remission: 0.07 ± 0.40 Active disease: 0.04 ± 0.13*
	BPs	BP-1				In remission: 0.10 ± 0.52 Active disease: 0.05 ± 0.14*
		BP-3				In remission: 0.21 ± 0.46 Active disease: 0.03 ± 0.09*
Mohsen et al., 2018	Bisphenols	BPA free	Urine	N.R.	ng/mL	P50 Boys: 0.20 P50 Girls: 0.21
		BPA conjugated				P50 Boys: 0.25 P50 Girls: 0.33
		BPA total				P50 Boys: 0.60 P50 Girls: 0.67
Nalbantoğlu et al., 2021	Bisphenols	BPA	Serum	N.R.	µg/L	Healthy: 445.38 ± 329.14 Allergic rhinitis: 2225.83 ± 1321.75*
Qu et al., 2022	PBs	MeP	Serum	Healthy: 97.0, Rheumatoid arthritis: 100.0	ng/mL	P50 Healthy: 2.60 P50 Rheumatoid arthritis: 4.70
		EtP		Healthy: 50.0, Rheumatoid arthritis: 63.0		P50 Healthy: 0.33 P50 Rheumatoid arthritis: 0.96
		PrP		Healthy: 53.0, Rheumatoid arthritis: 71.0		P50 Healthy: 0.49 P50 Rheumatoid arthritis: 0.74
		BuP		Healthy: 43.0, Rheumatoid arthritis: 55.0		P50 Healthy: <LOD P50 Rheumatoid arthritis: 0.98

Table 2 (continued).

Savastano et al., 2015	Bisphenols	BPA	Plasma	N.R.	ng/mL	1.04 ± 0.77*
Šimková et al., 2020	Bisphenols	BPA	Blood	Controls: 70.0 Normal weight Obesity PCOS:	nM/L	P50 Controls: 0.13 P50 Normal weight PCOS: 0.28 P50 Obesity PCOS: 0.13
		BPS		Controls: 25.0 Normal weigh Obesity PCOS: 40.0		P50 Controls: 0.00 P50 Normal weight PCOS: 0.00 P50 Obesity PCOS: 0.00
	PBs	BPF		N.R.		N.R.
		BPAF		N.R.		N.R.
		MeP		N.R.		N.R.
		EtP		N.R.		N.R.
		PrP		N.R.		N.R.
		BuP		N.R.		N.R.
		BzP		N.R.		N.R.
		Total PBs		Controls: 30.0 Normal weigh PCOS: 56.0 Obesity PCOS: 10.0		P50 Controls: 0.00 P50 Normal weight PCOS: 0.49 P50 Obesity PCOS: 0.00
Song et al., 2017	Bisphenols	BPA free BPA conjugated	Urine	N.R.	µg/L	N.R.
Tsen et al., 2021	Bisphenols	BPA	Plasma	100.0	ng/mL	4.50 ± 2.00*
Watkins et al., 2015	Bisphenols	BPA	Urine	98.7	ng/mL	P50: 2.67
	PBs	MeP		100.0		P50: 152.00
		PrP		100.0		P50: 45.40
		BuP		75.6		P50: 0.60
	BPs	BP-3		100.0		P50: 34.50
Yang et al., 2009	Bisphenols	BPA	Urine	76.0	µg/L	P50: 0.64

*EDC: endocrine-disrupting chemical; PBs: parabens; BPs: benzophenones; BPA: bisphenol A; BPS: bisphenol S; BPF: bisphenol F; BPAF: bisphenol AF; BzP: benzylparaben; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; BP-3: benzophenone 3; BP-1: benzophenone 1; PCOS: polycystic ovary syndrome; P50: percentile 50; GM: geometric mean; LOD: limit of detection; N.R.: Not reported. *Mean ± standard deviation.*

3.3. Inflammation Assessment

Table 3 provides an overview of the evaluation of the inflammation biomarkers assessed in the selected studies. All the studies quantified inflammation biomarkers in blood-related samples, half of them in the serum fraction of the blood (50.0%). Regarding the biomarkers assessed, C-reactive protein (CRP) was evaluated in 12 out 20 studies (60.0%); of these, half of them had CRP as the exclusive inflammation biomarker assessed. Interleukin 6 (IL-6) was the most common interleukin assessed (n = 11, 55.0%), followed by interleukin 10 (IL-10) (n = 6, 30.0%). Tumor necrosis factor-α (TNF-α) was quantified in nine studies (45.0%). The concentrations reported in each study are also summarized in Table 3. Briefly, CRP levels showed a mean concentration ranging from 2.6–678.0 ng/mL, and IL-6, IL-10, and TNF-α levels showed a median concentration range of <0.1–770.0 ng/mL, <0.1–0.2 ng/mL, and <0.1–1900.0 ng/mL, respectively. Other characteristics of outcome assessment are depicted in

Supplementary Table S2, including the quantification methodology used and the frequency of detection of each biomarker.

Table 3. Characteristics related to the outcome assessment in the selected studies.

Reference	Inflammation parameter	Matrix	Unit	Concentrations
Ashley-Martin et al., 2015	IL-33	Cord blood	pg/mL	GM: 0.90 GM: 0.90
Aung et al., 2019	CRP	Plasma	µg/mL	P50: 5.26
	IL-10		pg/mL	P50: 13.20
	IL-6			P50: 1.33
	TNF-α			P50: 2.99
	IL-1β			P50: 0.26
Choi et al., 2017	CRP	Serum	mg/L	0.63-4.57
Ferguson et al., 2016	CRP IL-1β IL-6 IL-10 TNF-α	Plasma	N.R.	N.R.
Haq et al., 2020	CRP	Blood	ng/mL	Diabetic BPA detected: Mean:10.63 Diabetic BPA non detected: Mean: 7.50 Non- diabetic BPA detected: Mean: 5.29 Non-diabetic BPA non detected: Mean: 2.63
	IL-6		pg/mL	Diabetic BPA detected: Mean: 14.87 Diabetic BPA non detected: Mean: 10.49 Non- diabetic BPA detected: Mean: 4.62 Non-diabetic BPA non detected:Mean: 2.75
Huang et al., 2017	CRP	Plasma and cord serum	µg/mL	P50 Plasma: 2.60 P50 Cord serum: -
	IL-6		pg/mL	P50 Plasma: 6.26 P50 Cord serum: 3.70
	TNF-α		pg/mL	P50 Plasma: 3.65 P50 Cord serum: 5.47
Jain et al., 2020	TNF-α	Serum	pg/mL	Diabetic population: 87.88 ± 26.77* Control: 82.12 ± 27.45*
	IL-6			Diabetic population: 103.89 ± 16.83* Control: 101.76 ± 13.37*
	IL-1α			Diabetic population: 62.42 ± 10.53* Control: 60.15 ± 7.73*
Kelley et al., 2019	GM-CSF IFN-γ MCP-1 MCP-3 MIP-1α MIP-1β TNFα VEGF IL-1β IL-6 IL-8 IL-17A	Blood and cord blood	pg/mL	N.R.
Lang et al., 2008	CRP	Serum	N.R.	N.R.

Table 3 (continued).

Liang et al., 2020	IL-1β	Serum	ng/mL	P50: 0.08
	IL-2			P50: <LOD
	IL-4			P50: <LOD
	IL-6		µg/mL	P50: 0.70
	IL-8			P50: 0.06
	IL-10			P50: 0.17
	IL-12p70		ng/mL	P50: 0.01
	IL-13			P50: 0.24
	TNF-α			P50: 1.82
	TGF-β		µg/mL	P50: 17.13
	IFN-γ		ng/mL	P50: 5.54
Linares et al., 2021	IL-12	Serum	µg/mL	In remission: 38.60 ± 17.20, Active disease: 42.50 ± 16.90*
	IFN-γ			In remission: 21.10 ± 10.90, Active disease: 26.13 ± 11.50*
	IL-6			In remission: 28.90 ± 16.30, Active disease: 27.70 ± 13.50*
	IL-23			In remission: 12.60 ± 10.40, Active disease: 16.50 ± 8.90*
	IL-17A			In remission: 26.6 ± 11.60, Active disease: 32.0 ± 16.60*
Mohsen et al., 2018	CRP	Serum	ng/mL	Boys: 5.17 ± 7.01 Girls: 4.13 ± 5.75*
Nalbantoğlu et al., 2021	IL-4	Serum	µg/mL	Healthy: 14.28 ± 10.17 Allergic rhinitis: 32.03 ± 26.45*
	IL-13			Healthy: 9.09 ± 5.13 Allergic rhinitis: 9.27 ± 5.44*
	IFN-γ			Healthy: 5.12 ± 3.79 Allergic rhinitis: 5.79 ± 4.13*
Qu et al., 2022	CRP	Serum	mg/L	P25-P75 Controls: 1.60-2.40 P25-P75 Cases: 4.30-55.30
Savastano et al., 2015	MCP1	Plasma	µg/mL	27.40 ± 23.50*
	IL-6			P50: 0.77
	TNF-α			P50: 1.90
Šimková et al., 2020	FGF basic	Plasma	pg/mL	N.R.
	Eotaxin			N.R.
	GM-CSF			N.R.
	IFN-γ			P50 Controls: 19.90 P50 Normal weigh PCOS: 13.40 P50 Obesity PCOS: 32.80
	IL-1β			N.R.
	IL-1ra			N.R.
	IL-2			P50 Controls: 18.00 P50 Normal weigh PCOS: 12.50 P50 Obesity PCOS: 22.20
	IL-4			N.R.
	IL-5			N.R.
	IL-6			P50 Controls: 23.10 P50 Normal weigh PCOS: 56.70 P50 Obesity PCOS: 82.10
	IL-7			N.R.
	IL-8			N.R.
	IL-9			N.R.
	IL-10			N.R.
	IL-12 (p70)			N.R.

Table 3 (continued).

Šimková et al., 2020	IL-13	Plasma	pg/mL	P50 Controls: 7.38 P50 Normal weigh PCOS: 5.82 P50 Obesity PCOS: 8.85
	IL-15			N.R.
	IL-17A			N.R.
	IP-10			N.R.
	MCP-1			N.R.
	MIP-1α			N.R.
	MIP-1β			N.R.
	PDGF-BB			P50 Controls: 216.00 P50 Normal weigh PCOS: 328.00 P50 Obesity PCOS: 291.00
	RANTES			N.R.
	TNF-α			N.R.
	VEGF			P50 Controls: 459.00 P50 Normal weigh PCOS: 1028.00 P50 Obesity PCOS: 1120.00
Song et al., 2017	CRP IL-10 ALT AST γ-GTP	Blood and serum	N.R.	N.R.
Tsen et al., 2021	CRP	Plasma	ng/mL	678.00 ± 918.10*
Watkins et al., 2015	CRP IL-1β IL-6 IL-10 TNF-α	Serum	N.R.	N.R.
Yang et al., 2009	CRP	Serum	mL/dL	Men: 0.08 ± 2.45 Premenopausal women: 0.06 ± 3.63 Postmenopausal women: 0.08 ± 3.00

*IL: interleukin; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; GM-CSF: granulocyte macrophage colony-stimulating factor; IFN-γ: interferon-γ; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor-β; FGF: fibroblast growth factor; PDGF-BB: platelet-derived growth factor-BB; RANTES: regulated upon activation, normal T-cell expressed, and secreted; ALT: alanine aminotransferase, AST: aspartate aminotransferase; γ-GTP: γ-glutamyl transferase; GM: geometric mean; BPA: bisphenol A; LOD: limit of detection; P50: percentile 50; P25: percentile 25; P75: percentile 75; PCOS: polycystic ovary syndrome; N.R.: Not reported. *Mean ± standard deviation.*

3.4. Association between Exposure to Bisphenols, PBs and BPs, and Inflammation Biomarkers

As shown in Table 4, positive associations were identified between exposure to all bisphenols, PB and BP congeners, and levels of some inflammatory biomarkers. The great majority of the studies assessing BPA (12 out 18 studies, 66.7%) reported BPA-related increased levels of some proinflammatory cytokines, including CRP, MCP-1, CRP, IFN-γ, IL-23, IL-17A, IL-6, TNF-α, ALT, AST, and γ -GTP. Regarding PBs, only half of the studies assessing the influence of PBs on inflammation biomarkers (3 out 6 studies) reported significant associations with any inflammation biomarker. However, despite elevated levels of CRP and IL-6 were found to be related to MeP and increased CRP levels were related to PrP and BuP, Aung et al. (2019) and Watkins et al. (2015)

also found inverse association between EtP exposure and IL-1β levels and between BuP exposure and CRP levels, respectively. Finally, 2 out 4 studies addressing exposure to BPs (Aung et al., 2019; Watkins et al., 2015) identified significant inverse association with TNF-α and CRP, respectively (Table 4).

Table 4. Association between concentrations of bisphenols, parabens, and benzophenones and levels of inflammation biomarkers.

Reference	Exposure biomarker-inflammation biomarker	Statistical test	Magnitude of the association	p-value
Ashley-Martin et al., 2015	BPA-IL-33	Bayesian hierarchical logistic regression models [OR (95% CI)]	1.00 (0.70-1.30)	0.050
Aung et al., 2019	MeP-CRP	Percent change (95% CI)	5.56 (−1.49, 13.1)	0.130
	EtP-CRP		3.36 (−4.31, 11.6)	0.400
	PrP-CRP		6.40 (−0.25, 13.5)	0.060
	BuP-CRP		7.17 (−2.22, 17.5)	0.140
	BP-3-CRP		0.79 (−6.44, 8.59)	0.840
	MeP-IL-1β		−0.15 (−6.37, 6.48)	0.960
	EtP-IL-1β		−7.70 (−14.1, −0.86)	0.030
	PrP-IL-1β		−2.36 (−8.01, 3.63)	0.430
	BuP-IL-1β		−6.28 (−13.9, 2.04)	0.130
	BP-3-IL-1β		1.05 (−5.83, 8.43)	0.770
	MeP-IL-6		6.69 (0.02, 13.8)	0.049
	EtP-IL-6		−4.20 (−10.9, 2.95)	0.240
	PrP-IL-6		2.94 (−3.05, 9.30)	0.340
	BuP-IL-6		−3.59 (−11.5, 5.03)	0.400
	BP-3-IL-6		−1.60 (−8.32, 5.61)	0.650
	MeP-IL-10		0.34 (−4.38, 5.29)	0.890
	EtP-IL-10		−3.33 (−8.37, 2.00)	0.220
	PrP-IL-10		−1.53 (−5.82, 2.97)	0.500
	BuP-IL-10		0.80 (−5.42, 7.44)	0.800
	BP-3-IL-10		−0.34 (−5.47, 5.07)	0.900
	MeP-TNF-α		1.42 (−1.85, 4.80)	0.400
	EtP-TNF-α		−3.14 (−6.61, 0.46)	0.090
	PrP-TNF-α		−0.05 (−3.05, 3.03)	0.970
	BuP-TNF-α		−0.42 (−4.66, 4.00)	0.850
	BP-3-TNF-α		−3.69 (−7.09, −0.17)	0.040
Choi et al., 2017	BPA-CRP	Multiple logistic regression [OR (95% CI)]	2.85 (1.16-6.97)	0.022
Ferguson et al., 2016	BPA-CRP	Percent change (95% CI)	-1.64 (-8.63-5.88)	0.660
	BPA-IL-1β		3.36 (-3.41-10.60)	0.340
	BPA-IL-6		8.95 (1.81-16.60)	0.010
	BPA-IL-10		3.05 (-1.98-8.35)	0.240
	BPA -TNF-α		0.30 (-3.18-3.91)	0.860
Haq et al., 2020	BPA Detected-CRP	Two-tailed Student's t test (mean ± SEM).	Diabetes: 10.63±0.66	<0.05
	BPA Non-detected-CRP		Diabetic: 7.50±1.51	
	BPA Detected-CRP		Non-diabetic: 5.29±0.59	
	BPA Non-detected-CRP		Non-diabetic: 2.63±0.34	
	BPA Detected-IL-6		Diabetes: 14.84±0.63	<0.001
	BPA Non-detected-IL-6		Diabetic: 10.49±0.76	
	BPA Detected-IL-6		Non-diabetic: 4.62±0.37	
	BPA Non-detected-IL-6		Non-diabetic: 2.75±0.21	
Huang et al., 2017	BPA-CRP (plasma)	Multivariate linear regression [β (SE)]	-0.06 (0.10)	0.570
	BPA-CRP (cord serum)		N.R.	N.R.
	BPA-Il-6 (plasma)		-0.82 (0.98)	0.400
	BPA-Il-6 (cord serum)		-0.74 (2.30)	0.750
	BPA-TNF-α (plasma)		-0.16 (0.32)	0.620
	BPA-TNF-α (cord serum)		-0.14 (0.26)	0.590

Table 4 (continued).

Jain et al., 2020	BPA-TNF-α (control population)	Spearman correlation (Sρ)	-0.07	0.940
	BPA-TNF-α (diabetes population)		-0.05	0.560
	BPA-IL-6 (control population)		-0.11	0.180
	BPA-IL-6 (diabetes population)		-0.04	0.660
	BPA-IL-1α (control population)		-0.05	0.510
	BPA-IL-1α (diabetes population)		0.04	0.660
Kelley et al., 2019	BuP-IL-6	Linear regression. Effect size (standard deviation)	-0.32 (0.11)	0.097
	BPA-MCP-1		0.82 (0.21)	0.019
	BPA, BPS, BPF, MeP, EtP, PrP, BuP, BP-3 - GM-CSF, IFN-γ, MCP-1, MCP-3, MIP-1α, MIP-1β, TNFα, VEGF, IL-1β, IL-6, IL-		No significant correlations	N.R.
Lang et al., 2008	BPA-CRP	Multivariate linear regression [β (95% CI)]	0.09 (0.02 to 0.15)	0.020
Liang et al., 2020	BPA-IL-1β	Multivariate linear regression [β (95% CI)]	0.31 (-0.48-1.10)	0.439
	BPA-IL-2		N.R.	N.R.
	BPA-IL-4		N.R.	N.R.
	BPA-IL-6		0.15 (-0.14-0.44)	0.314
	BPA-IL-8		0.06 (-0.31-0.46)	0.776
	BPA-IL-10		0.03 (-0.18-0.23)	0.801
	BPA-IL-12p70		-0.09 (-0.40-0.22)	0.573
	BPA-IL-13		0.26 (-0.17-0.69)	0.225
	BPA-TNF-α		0.00 (-0.16-0.16)	0.996
	BPA-TGF-β		-0.00 (-0.07-0.07)	0.981
	BPA-IFN-γ		0.18 (0.00-0.36)	0.045
	BPS-IL-1β		0.17 (-0.27-0.61)	0.433
	BPS-IL-2		N.R.	N.R.
	BPS-IL-4		N.R.	N.R.
	BPS-IL-6		0.03 (-0.13-0.19)	0.724
	BPS-IL-8		0.05 (-0.17-0.27)	0.644
	BPS -IL-10		0.06 (-0.06-0.17)	0.328
	BPS-IL-12p70		0.08 (-0.09-0.25)	0.340
	BPS-IL-13		0.07 (-0.17-0.31)	0.572
	BPS-TNF-α		-0.00 (-0.09-0.09)	0.984
	BPS-TGF-β		0.01 (-0.03-0.05)	0.658
	BPS-IFN-γ		-0.01 (-0.11-0.09)	0.890
Linares et al., 2021	BPA IL-23	Multivariate linear regression [β (95%	1.69 (1.60-1.77)	0.001
	BPA IL-17A		1.15 (1.00-1.29)	0.001
	MeP, EtP, PrP, BuP, BP-1, BP-3 - IL-12, IFN-γ, IL-6, IL-23, IL-17A	N.R.	N.R.	N.R.
Mohsen et al., 2018	BPA-CRP	Spearman correlation coefficients (Sρ)	N.R.	N.R.
Nalbantoğlu et al., 2021	BPA-IL-4	Multivariate linear regression [β (95% CI)]	0.31 (3.47-7.40)	0.000
	BPA-IL-13		N.R.	N.R.
	BPA-IFN-γ		N.R.	N.R.
Qu et al., 2022	MeP-CRP	Multivariate linear regression [β (95% CI)]	0.15 (0.04-0.28)	<0.05
	EtP-CRP		0.23 (-0.11-0.56)	>0.05
	PrP-CRP		0.20 (0.10-0.32)	<0.05
	BuP-CRP		0.27 (-0.10-0.80)	>0.05

Table 4 (continued).

Savastano et al., 2015	BPA-MCP-1 BPA-IL-6 BPA-TNF-α	Multivariate linear regression (β)	N.R. 0.24 N.R.	N.R. 0.037 N.R.
Šimková et al., 2020	BPA, BPS, BPF, BPAF, Mep, EtP, PrP, BuP, BzP, total PBs - FGF basic, eotaxin, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF	Spearman correlation coefficients (Sρ)	No significant correlations	N.R.
Song et al., 2017	BPA-CRP	Linear mixed effect model and a generalized additive mixed models (GAMM)	Positive non-linear association	0.081
	BPA-IL-10		Negative non-linear association	0.083
	BPA-ALT		Positive non-linear association	0.001
	BPA-AST		Positive non-linear association	0.056
	BPA-γ-GTP		Positive non-linear association	0.018
Tsen et al., 2021	BPA-CRP	Multiple logistic regression [OR	1.82 (0.58-5.36)	0.283
Watkins et al., 2015	BPA-CRP	Percent change (95% CI)	5.10 (-7.47-19.40)	0.440
	BPA-IL-1β		4.65 (-7.91-18.90)	0.480
	BPA-IL-6		12.50 (-2.50-29.70)	0.110
	BPA-IL-10		-1.20 (-13.40-12.70)	0.850
	BPA-TNF-α		4.85 (-1.70-11.80)	0.150
	MeP-CRP		-6.75 (-19.00-7.38)	0.330
	MeP-IL-1β		-3.63 (-17.10-12.10)	0.630
	MeP-IL-6		4.90 (-11.20-23.90)	0.570
	MeP-IL-10		6.66 (-8.62-24.50)	0.410
	MeP-TNF-α		2.00 (-6.18-10.90)	0.640
	PrP-CRP		-13.60 (-25.80-0.50)	0.060
	PrP-IL-1β		-1.76 (-16.50-15.60)	0.830
	PrP-IL-6		3.70 (-13.40-24.20)	0.690
	PrP-IL-10		-0.09 (-15.40-18.00)	0.990
	PrP-TNF-α		-0.83 (-9.29-8.42)	0.850
	BuP-CRP		-17.50 (-30.30-(-2.27)	0.030
	BuP-IL-1β		10.50 (-8.11-32.80)	0.290
	BuP-IL-6		15.80 (-5.47-41.70)	0.150
	BuP-IL-10		5.28 (-12.90-27.20)	0.590
	BuP-TNF-α		5.69 (-4.67-17.20)	0.290
	BP-3-CRP		-16.30 (-27.50-(-3.42)	0.020
	BP-3-IL-1β		-0.75 (-15.10-16.10)	0.920
	BP-3-IL-6		-4.81 (-19.90-13.10)	0.570
	BP-3-IL-10		-3.88 (-18.10-12.80)	0.620
	BP-3-TNF-α		-2.13 (-10.30-6.77)	0.620

Table 4 (continued).

Yang et al., 2009	BPA-CRP	Multivariate linear regression (β)	Men: -0.02	0.418
	BPA-CRP		Premenopausal women: 0.09	0.268
	BPA-CRP		Postmenopausal women: 0.11	0.029

BPA: bisphenol A; BPS: bisphenol S; BPF: bisphenol F; BPAF: bisphenol AF; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; BzP: benzylparaben; PBs: parabens; BP-1: benzophenone-1; BP-3: benzophenone 3; EDC: endocrine-disrupting chemical; IL: interleukin; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; EDC: endocrine-disrupting chemical; TGF-β: transforming growth factor-β; INF-γ: interferon-γ; MCP: monocyte chemoattractant protein; FGF: fibroblast growth factor; GM-CSF: granulocyte macrophage colony-stimulating factor; IP-10: interferon-γ-inducible protein 10; MIP: macrophage inflammatory protein; PDGF-BB: platelet-derived growth factor-BB; RANTES: regulated upon activation, normal T-cell expressed and secreted; VEGF: vascular endothelial growth factor; ALT: alanine aminotransferase, AST: aspartate aminotransferase; γ-GTP: γ-glutamyl transferase; OR: odds ratio; CI: confidence interval; SEM: standard error of mean, SE: standard error; Sρ: Spearman's correlation coefficient; N.R.: Not reported.

4. DISCUSSION

To date, this is the first systematic review gathering epidemiological studies exploring associations between exposure to bisphenols, PBs and BPs, and levels of inflammatory biomarkers. Most of the 20 included studies focused on the associations between BPA and inflammation, while the relationship between PBs/BPs and inflammation was only addressed in few studies (n=6 and n=4, respectively). Moreover, although most studies were focused on well-known inflammatory biomarkers such as CRP, IL-6, IL-10, IL-1α or IL-1β, more than 30 biomarkers of inflammations have been addressed (CRP, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 IL-10, IL-12, IL-13, IL-15, IL-17a, IL-23, IL-33, TNF-α, TGF-β, IFN-γ, GM-CSF, MCP-1, MCP-3, MIP1-a, MIP1-b, VEGF, FGF-basic, eotaxin, PDGF-BB, RANTES, ALT, AST and γ-GTP). More than a half of the studies included in this review (n=13, 65.0%) reported significant associations between any of the target EDCs included in this review and different inflammation parameters (Ashley-Martin et al., 2015; Aung et al., 2019; Choi et al., 2017; Ferguson et al., 2016; Kelley et al., 2019; Liang et al., 2020; Linares et al., 2021; Nalbantoğlu et al., 2021; Qu et al., 2023; Savastano et al., 2015; Song et al., 2017; Watkins et al., 2015; Yang et al., 2009). However, one-quarter of selected studies were classified as medium reporting quality, and a certain degree of risk of bias was observed in more than half of the selected studies.

Briefly, BPA exposure was positively associated with a variety of pro-inflammatory biomarkers [CRP (Choi et al., 2017; Haq et al., 2020; Lang et al., 2008; Yang et al., 2009), IL-6 (Ferguson et al., 2016; Haq et al., 2020; Savastano et al., 2015), IL-4

(Nalbantoğlu et al., 2021), IL-17A (Linares et al., 2021), IL-23 (Linares et al., 2021), IL-33 (Ashley-Martin et al., 2015), MCP-1 (Kelley et al., 2019), ALT (Song et al., 2017) and γ-GTP (Song et al., 2017)]. Moreover, both MeP and PrP were associated with higher CPR levels (Qu et al., 2023), while MeP concentrations were also related to increased serum levels of IL-6 (Aung et al., 2019). These results are in line with previous *in vitro* and *in vivo* studies reporting consistent positive associations between inflammation and exposure to bisphenols (Loffredo et al., 2020; Lu et al., 2019; Priego et al., 2021; Wang et al., 2021) and parabens (Inderbinen et al., 2022). Thus, the epidemiological findings summarized in this review, together with the *in vitro* and *in vivo* evidence, strongly support our hypothesis on the relationship between exposure to bisphenols, PBs and BPs, and the development of an inflammatory response. Moreover, the underlying mechanisms explaining this association between exposure to these chemicals and inflammation might be related to the xenoestrogenic activity exhibited by bisphenols, PBs and BPs. In this regard, it has been reported that low estrogenic activity promotes the production of type I interferon and pro-inflammatory cytokines (Kovats, 2015). Since these chemicals has nearly 1000-fold weaker affinity for estrogen receptors (ERs) than estradiol, they can bind with ERs more actively when estrogen levels are low, which would in turn trigger physiological responses associated with inflammation (Yang et al., 2009).

However, few inverse associations were also observed in previous studies. For instance, Watkins et al. (2015) found that exposure to BuP and BP-3 was related to lower CRP in pregnant women. Similarly, despite Aung et al. (2019) reported some positive associations between exposure and pro-inflammatory biomarkers, they also observed that EtP exposure during pregnancy was related to lower IL-1β levels and BP-3 to reduced TNF-α production. Nevertheless, sensitivity analyses of interaction terms between individual exposure analytes and study visits indicated that the association between EtP and IL-1β differed across study visits, becoming positive by visit 4 (33-38 gestational weeks). Moreover, a previous *in vivo* study revealed that inhibition of edema, an anti-inflammatory effect, was associated with topical BP-3 application (Couteau et al., 2012).

To date, there is currently growing concern about the effects that human exposure to bisphenols, PBs and BPs may have on health, and the adverse effects on human health of these chemicals are suggested to be related to their disruption of the endocrine system

due to xenoestrogenic, xenoandrogenic and xeno-thyroid activities (Boberg et al., 2010; Darbre and Harvey, 2008; Molina-Molina et al., 2013; Perez et al., 1998). However, the exact mechanisms of action are not fully elucidated, with some evidence suggesting that these chemicals could exert adverse effect for human health though the perturbation of the oxidative microenvironment via ER-dependent pathways (Artacho-Cordón et al., 2019; Watkins et al., 2015). It is suspected that exposure to bisphenols, PBs and BPs could have an immunotoxic effect, producing alterations in the immune system and deregulating inflammatory pathways through interactions with immune cells and peripheral tissues (Kiyama and Wada-Kiyama, 2015; Kovats, 2012; Rogers et al., 2013). In this sense, a deregulation of the inflammation pathway is becoming increasingly important as there is a growing number of evidence reporting a relationship between disturbances in the inflammatory milieu and a multitude of allergic, autoimmune and reproductive diseases, as well as obesity, metabolic syndrome and cancer, (Dietert, 2011; Dietert, 2012). In fact, some of the studies of this review (n=6, 20%) included in their study population participants with different diseases, such as diabetes (Haq et al., 2020; Jain et al., 2020), Crohn's disease (Linares et al., 2021), allergic rhinitis (Nalbantoğlu et al., 2021), rheumatoid arthritis (Qu et al., 2023) and polycystic ovary syndrome (PCOS) (Šimková et al., 2020). Haq et al. (2020) and Jain et al. (2020) reported higher urinary BPA levels in diabetic participants compared with non-diabetics, and urinary BPA levels were correlated with elevated levels of CRP (Haq et al., 2020), TNF-α, IL-6 and IL-1α (Jain et al., 2020). Linares et al. (2021) reported higher BPA levels in participants with active Crohn's disease compared to participants with this disease in remission, along with positive correlations between BPA concentrations and IL-23 and IL-17a levels. Nalbantoğlu et al. (2021) also evidenced an association between BPA concentrations and allergic rhinitis in children, with increased levels of both BPA and IL-4 in more severe stages of the disease. Qu et al. (2023) reported significant associations among MeP and PrP exposure, increased CRP levels, and risk of rheumatoid arthritis, and Šimková et al. (2020) showed higher levels of BPA and IL-6, VEGF, and PDGFBB in PCOS women compared to controls. Taken together, these results suggest that a deregulated inflammatory response could be the nexus between the association between bisphenols, PBs and BPs and the development and/or progression of diseases related to an altered immune system.

It is also important to highlight that humans are exposed to several toxicants and complex mixtures of EDCs and that their effects are difficult to predict given the possible synergistic, additive or antagonistic actions between chemical residues (Carpenter et al., 2002), suspecting that they may be acting through immunological mechanisms (Dietert, 2011). Therefore, studies exploring associations between complex mixtures of bisphenols, PBs and BPs with inflammatory biomarkers levels acquire high importance. In this review, only one study (Kelley et al., 2019) explored the combined effect of exposure to multiple chemicals. However, the inclusion of different families of chemicals than those considered for this systematic review (heavy metals, phthalates and other environmental phenols) hampered the elucidation of the specific contribution of bisphenols, PBs and BPs to the inflammatory response (data not shown). Therefore, future studies are required to address this combined effect on the inflammatory response. In addition, the great majority of studies (16, 80.0%) considered spot urine/blood samples for exposure assessment. Given that bisphenols, PBs and BPs have a very short urinary elimination half-life in human body (Boberg et al., 2010; Yuan et al., 2015), and thus spot samples may not be representative of the overall exposure of an individual, future studies considering pooled samples for exposure assessment (i.e. collecting 24-h urine/blood sample or repeated measurements) would show a more realistic scenario in relation of human exposure. In fact, this approach might yield stronger and more consistent associations between exposure and inflammatory biomarkers.

Moreover, it is plausible a differential interference of exposure to these chemicals on inflammatory milieu according to specific characteristics of study participants. For instance, inflammatory disruption might be more pronounced in vulnerable populations. Previous evidences suggest that EDCs could have a greater effect on health when human exposure occurs during critical periods of individual development, such as pregnancy, lactation, childhood or puberty (Dietert et al., 2000; Holsapple et al., 2004). In this sense, less than half of the studies included in this review (n=8, 40.0%) considered these critical periods, of which six explored associations between exposure and inflammation in pregnant women (Ashley-Martin et al., 2015; Aung et al., 2019; Ferguson et al., 2016; Huang et al., 2017; Kelley et al., 2019; Watkins et al., 2015) and two in children (Mohsen et al., 2018; Nalbantoğlu et al., 2021). The results of these studies showed discrepant associations between exposure and inflammation, requiring a

larger number of studies to be able to establish reliable conclusions between exposure and inflammation considering critical windows of vulnerability. Furthermore, previous evidence suggested the existence of gender differences related to inflammatory diseases (Shakil et al., 2022). For this reason, the inclusion of a study population gathering both genders becomes more important. In this review, almost half of the studies (n=9, 45.0%) did not study gender differences (Ashley-Martin et al., 2015; Aung et al., 2019; Ferguson et al., 2016; Huang et al., 2017; Kelley et al., 2019; Liang et al., 2020; Savastano et al., 2015; Šimková et al., 2020; Watkins et al., 2015), requiring further research in this regard.

Thus, the variability on the characteristics of study participants prevented the identification of subgroups of people with a higher risk for dysregulation of the inflammatory response with the exposure to bisphenols, PBs and BPs. Additionally, the great methodological heterogeneity in terms of assessed inflammatory biomarkers and statistical analyses hampered the performance of a meta-analysis. In addition, taken together, the great heterogeneity in terms of study population (i.e. healthy adult subjects, healthy boys and girls, infant allergic rhinitis patients, arthritis rheumatoid patients, women diagnosed with PCOS, and diabetic and Crohn's patients…), the applied methodological for both EDC (i.e. gas chromatography-mass spectrometry (GC-MS/MS), liquid chromatography-mass spectrometry (LC-MS/MS), isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), enzyme-linked immunosorbent assay (ELISA)…) and inflammation assessment (i.e. ELISA, immunoturbidimetry, high performance liquid chromatography (HPLC)…), and the use of different biological matrices for exposure assessment (urine and serum) could explain, at least in part, the wide ranges reported in EDC and inflammatory biomarker levels shown in the different studies included in this review. Finally, concerns related to reporting quality and risk of bias were identified in one-quarter and two-quarters of included studies, respectively. Thus, well-conducted studies are needed in the close future in order to get a more realistic overview on the contribution of these families of chemicals, to which humans are daily exposed, on the dysregulation of the inflammatory response.

Considering the limitations of this systematic review, the selection of the studies was based on the implementation of the search strategy in only three public databases. However, these databases are considered among the most relevant in the field of human

health, and only a small number of specific publications that are only available in other databases could have been lost. On the other hand, only epidemiological studies were included in this review. This could limit the number of quantified inflammation biomarkers that could be included in other types of studies (*in vivo* and *in vitro*). Finally, although only three families of environmental phenols with endocrine-disrupting properties were selected for investigation, these include the phenolic EDCs in widest daily use, and a systematic review has already been carried out on the influence of organochlorine pesticides and PCBs on inflammation biomarkers (Peinado et al., 2020a).

In conclusion, this systematic review summarizes the current evidence on the association between human exposure to bisphenols, PBs and BPs, and alterations in the inflammatory milieu. Despite some concerns related to reporting quality and risk of bias, selected studies showed consistent positive associations between human exposure to BPA and levels of some pro-inflammatory biomarkers, while very few studies explored associations between PBs and/or BPs and inflammation. Therefore, well-conducted studies in general but also vulnerable populations assessing exposure to both individual and mixtures of EDCs are required in the close future to clarify whether inflammation could act as a nexus between exposure to these EDCs and human health.

Supplementary Materials: The supporting information can be downloaded at: www.mdpi.com/xxx/s1.

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ARTICLE 4

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Expression profiles of genes related to development and progression of endometriosis and their association with paraben and benzophenone exposure

Peinado FM[1*], Olivas-Martínez A[1,2], Lendínez I[3], Iribarne-Durán LM[1], León J[1,4,5], Fernández MF[1,6,7], Sotelo R[8], Vela-Soria F[1], Olea N[1,6,7,9], Freire C[1,6], Ocón-Hernández O[1,8], Artacho-Cordón F[1,6,7*].

[1]Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), E-18012 Granada, Spain

[2] University of Granada, Centre for Biomedical Research, E-18016, Granada, Spain; Department of Radiology and Physical Medicine, School of Medicine, University of Granada, E-18071, Granada, Spain

[3] General surgery, San Cecilio University Hospital, Granada, E-18016, Spain

[4]Digestive Medicine Unit, 'San Cecilio' University Hospital, E-18012 Granada, Spain

[5]CIBER Hepatic and Digestive Diseases (CIBEREHD), E-28029 Madrid, Spain

[6]CIBER Epidemiology and Public Health (CIBERESP), E-28029 Madrid, Spain

[7]Radiology and Physical Medicine Department, University of Granada, E-18016 Granada, Spain

[8]Gynaecology and Obstetrics Unit, 'San Cecilio' University Hospital. Granada, E-18016, Spain

[9]Nuclear Medicine Unit, 'San Cecilio' University Hospital, E-18016 Granada, Spain

[*]Corresponding author:

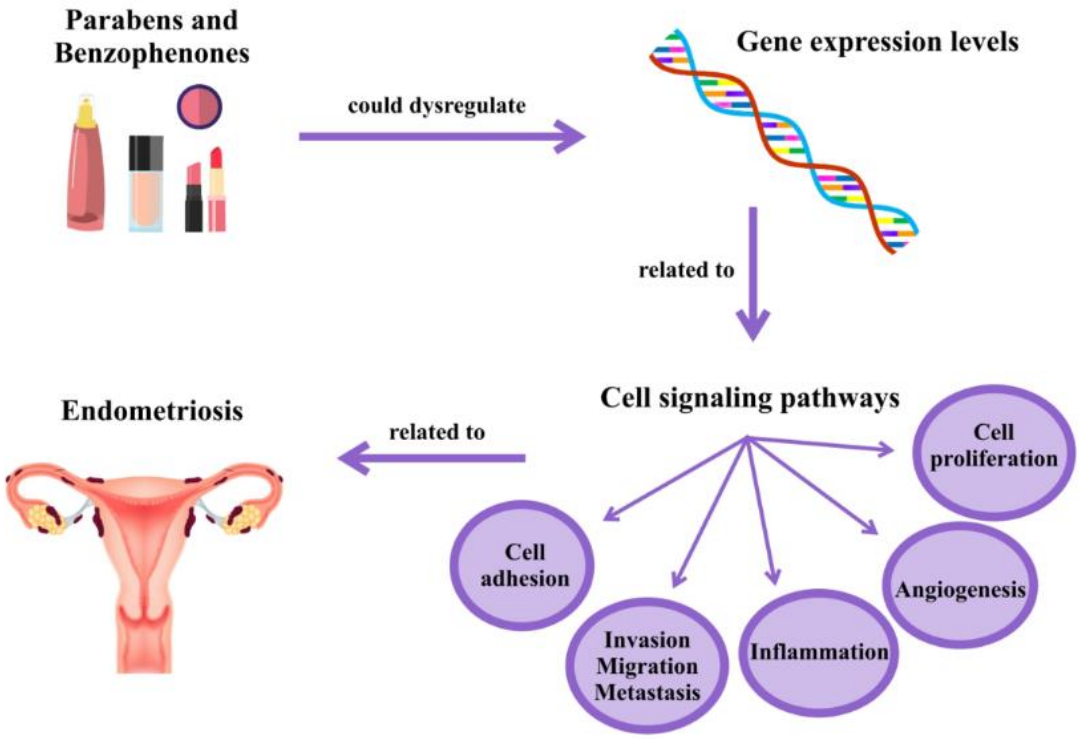
Francisco Manuel Peinado. Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), E-18012 Granada, Spain. Phone: +34 958 249983; E-mail: franciscopeinado@correo.ugr.es; ORCID (0000-0001-6517-1941).

Francisco Artacho-Cordón. University of Granada, Department of Radiology and Physical Medicine. 18071 Granada, Spain. Phone: +34 958 249983; E-mail: fartacho@ugr.es; ORCID (0000-0003-3850-6173).

HIGHLIGHTS

- Endometriotic tissue gene expression profiles and urinary PB/BP levels were studied
- PB/BP exposure was related to overexpression of adhesion- and invasion-related genes
- Increased expression of pro-inflammatory genes was related to PB/BP exposure
- PB exposure was positively related to pro-angiogenic gene expression
- Upregulation of cell proliferation-related genes was related to PB/BP exposure

GRAPHICAL ABSTRACT



ABSTRACT

Aim: To describe the endometriotic tissue expression profile of a panel of 23 genes related to crucial cell signaling pathways for the development and progression of endometriosis (cell adhesion, invasion/migration, inflammation, angiogenesis, and cell proliferation/hormone stimulation) and explore its relationship with exposure of patients to parabens (PBs) and benzophenones (BPs).

Methods: This cross-sectional study included a subsample of 33 women with endometriosis from the EndEA study, measuring their urinary concentrations of methyl-, ethyl-, propyl-, butyl-paraben, benzophenone-1, benzophenone-3, and 4-hydroxybenzophenone and their endometriotic tissue expressions of 23 genes. Spearman's correlations test and linear and logistic regression analyses were performed to evaluate the association of gene expressions with exposure to PBs and BPs.

Results: Expression of 52.2% of studied genes was observed in >75% of endometriotic tissue samples and expression of 17.4% (n=4) of them in 50-75%. Exposure to certain PB and BP congeners was positively associated with the expression of key genes for the development and proliferation of endometriosis, including integrin β2, matrix metalloproteinase 1, interleukin 6 signal transducer, soluble vascular endothelial growth factor receptor-1, and estrogen receptor-α.

Conclusions: Genes related to the development and progression of endometriosis were expressed in most endometriotic tissue samples studied, suggesting that exposure of women to PBs and BPs may be associated with the altered expression profile of genes related to cellular pathways involvement in the development of endometriosis. Given the novelty of these findings, further studies are warranted to confirm the role of exposure to these compounds in the pathophysiology of endometriosis.

Keywords: paraben, benzophenone, endometriosis, gene expression profile, inflammation

1. INTRODUCTION

Endometriosis is a gynecological disease characterized by the presence of endometrial-like tissue outside the uterine cavity, primarily in the abdominopelvic cavity (peritoneum, ovaries, or rectovaginal septum) (Giudice, 2010; Vercellini et al., 2014). This functionally active ectopic tissue is sensitive to hormonal stimulation and can originate cyclic bleeding, promoting the appearance of local inflammatory reactions and triggering pain (dysmenorrhea, dyspareunia, or chronic pelvic pain), gastrointestinal disorders, or infertility, among others (Eskenazi and Warner, 1997; Giudice and Kao, 2004).

The pathogenesis and pathophysiology of endometriosis have yet to be fully elucidated, although various theories have been proposed. Increasing evidence has been published over recent years on the implication of endocrine disrupting chemicals (EDCs) (Signorile et al., 2022) in an increased risk of endometriosis, which has been associated with human exposure to several families of EDCs (Kunisue et al., 2012; Peinado et al., 2020b; Peinado et al., 2021), including parabens (PBs) and benzophenones (BPs) (Peinado et al., 2021). PBs, which are widely used as preservatives in personal care products (PCPs), pharmaceuticals, and food (Błędzka et al., 2014), include methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butyl-paraben (BuP) congeners. BPs, which frequently serve as UV-filters in PCPs (Kim and Choi, 2014), include benzophenone-1 (BP-1), benzophenone-3 (BP-3), and 4-hydroxybenzophenone (4-OHBP) congeners.

In vitro and *in vivo* studies have demonstrated the estrogen-like effects of PBs and BPs, supporting the hypothesis that they promote endometriosis, an estrogen-dependent disease (Charles and Darbre, 2013; Molina-Molina et al., 2008). EDC exposure has also been associated with inflammation and oxidative stress (Thompson et al., 2015; Watkins et al., 2015), although its role in the pathophysiology remains unknown. Seli et al. (2003) describes five key steps in the development and progression of endometriotic lesions: cell adhesion to the peritoneum; invasiveness into the mesothelium; recruitment of inflammatory cells; angiogenesis in endometriotic tissue; and cell proliferation. Changes in mediators of the corresponding cell signaling pathways have been observed in studies of endometriosis. Thus, in comparison to normal endometrial tissue, ectopic tissue has evidenced upregulation of: integrins and claudins, key members of tight junctions (Horné et al., 2019; Li et al., 2022); metalloproteases (*MMPs*), involved in

extracellular matrix cleavage and therefore invasiveness (Gottschalk et al., 2000; Matsuzaki et al., 2010); proinflammatory interleukins (Kato et al., 2019; Mu et al., 2018); certain angiogenesis-related genes (Cho et al., 2012; Machado et al., 2010); and genes related to cell proliferation (Bulun et al., 2010; Szaflik et al., 2020). To our best knowledge, no study has been published on the ways in which exposure to EDCs-might affect these cell signaling pathways. Following the observation by our group that exposure to PB and BP is associated with endometriosis risk (Peinado et al., 2021), the present study was designed to determine the expression profile of a panel of 23 genes related to five key cell signaling pathways for endometriosis development (cell adhesion; invasion, migration and metastasis; inflammation; angiogenesis; and cell proliferation and hormone stimulation) in women with endometriosis and to explore its relationship with their exposure to PBs and BPs.

2. MATERIAL AND METHODS

2.1. Study population and sample collection

This cross-sectional study, which forms part of the hospital-based case-control EndEA study, included 33 women with endometriosis undergoing surgery at the Gynecology and Obstetrics Units of San Cecilio and Virgen de las Nieves University Hospitals (Granada, Southern Spain) from January 2018 through July 2019 (Peinado et al., 2020b; Peinado et al., 2021). All cases were diagnosed with endometriosis by laparotomy or laparoscopic surgery and histological confirmation. Inclusion criteria were premenopausal status, age between 20 and 54 years, receipt of abdominal surgery, and body mass index (BMI) below 35 kg/m^2. Exclusion criteria were history of cancer (except non-melanoma skin cancer), pregnancy at study enrolment, and inability to read and sign the informed consent document. Women were staged according to the Revised American Fertility Society classification (Canis et al., 1997). Written informed consent was provided by all participants, and the study was approved by the Research Ethics Committee of Granada.

Endometriotic tissue samples were gathered during surgery and kept in QIAazol reagent (Qiagen, Hilden, Germany) to ensure RNA stability, and first-morning spot urine samples were collected in fasting conditions (fasting time >8 h) on the same day as the surgery and were kept in PB- and BP-free glass tubes. All samples were immediately stored at -80 ºC at the Biobank of the Public Andalusian Health Care System until their

analysis. The BMI of participants was calculated, and data were gathered on sociodemographic, lifestyle, and clinical variables from epidemiological and clinical questionnaires completed by participants and on surgical variables from questionnaires completed by surgeons (Table 1).

2.2. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Endometriotic tissue samples were maintained in QIAzol reagent (Qiagen, Hilden, Germany) during the homogenization process to ensure RNA stability. Total RNA was extracted from 30 mg of sample with the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Final RNA concentration and quality (260/280 ratio) were determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1000 ng) was transcribed into cDNA with the iScriptAdvanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions.

Real-time PCR was carried out with a CFX96 Real-time PCR detection system (Bio-Rad Laboratories, Hercules, California, USA) using SsoAdvanced SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, California, USA). The manufacturer's protocol was followed to measure the expression of 23 genes involved in the five cell signaling pathways: cell adhesion [integrin beta-2 (*ITGB2*) and claudin 7 (*CLDN7*)], migration/invasion [matrix metallopeptidase 1 (*MMP1*), matrix metallopeptidase 7 (*MMP7*), fucosyltransferase 8 (*FUT8*), ribonucleotide reductase M2 (*RRM2*), midkine (*MDK*), ras homolog gene, family, member B (*RHOB*), and sprout homolog 2 (*SPRY2*)], inflammation [interleukin 1 receptor, type I (*IL1R1*), interleukin 1 receptor, type II (*IL1R2*), interleukin 6 cytokine family signal transducer (*IL6ST*), nuclear receptor subfamily 3 group C member 1 (*NR3C1*), and tumor necrosis factor receptor superfamily member 1B (*TNFRSF1B*)], angiogenesis [angiogenin (*ANG*), angiopoietin 1 (*ANGPT1*), soluble vascular endothelial growth factor receptor-1 (*sVEGFR-1*), and vascular endothelial growth factor A (*VEGFA*)], and cell proliferation/hormone stimulation [cytochrome P450 family 19 subfamily A member 1 (*CYP19A1*), dual specificity phosphatase 6 (*DUSP6*), estrogen receptor alpha (*ERα*), progesterone receptor (*PGR*), and steroidogenic acute regulatory protein (*STAR*)]. Primers used for these studies were purchased from Bio-Rad Laboratories (Hercules, California, USA), and their sequences are detailed in Supplementary Table S1. The criteria for the

selection of these genes was based on previous evidence relating exposure to EDCs with the gene expression of these genes, their participation in the different cell signaling pathways and their relationship with endometriosis (Chung et al., 2002; Roy et al., 2015).

All values were normalized using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) RNA expression levels. Gene expression was analyzed by Bio-Rad CFX 96 Manager software to determine the cycle of quantification (Ct) and was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.3. Chemical analysis

Dispersive liquid-liquid microextraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) were performed to determine urinary concentrations of MeP, EtP, PrP, BuP, BP-1, BP-3, and 4-OHBP as previously described (Vela-Soria et al., 2014).

After thawing urine samples at room temperature and centrifuging at 2600xg for 10 min, 1.0 mL was taken for analysis. The total amount of these benzophenones and parabens (free and conjugated) was obtained by enzymatically treating samples with an enzyme solution of β-glucuronidase/sulfatase, pre-prepared by dissolving 10 mg of β-glucuronidase/sulfatase ($3 \cdot 10^6$ U g solid^{-1}) in 1.5 mL of 1M ammonium acetate/acetic acid buffer solution (pH 5.0). Enzymatically treated samples were incubated at 37 ºC for 24 h. Next, 20 µL of standard replacement solution (5 mg / L of EP–$^{13}C_6$, 2 mg/L of BPA-D_{16}, and 2 mg/L of BP-d_{10}) were added, and samples were diluted with 10 mL of 10% aqueous NaCl solution (pH 2.0, adjusted with 0.5 M HCl). Samples were then mixed with a solution of 1 mL of acetone (dispersing solvent) and 0.5 mL of trichloromethane (extraction solvent), shaken manually for 30 sec, and centrifuged at 4000xg for 10 min. The organic phase was then collected with care from the bottom of the glass tube using a 1 mL pipette and was placed in 2 mL glass vials. All of the extracted fluid was evaporated under a nitrogen stream, and the residue was dissolved with 100 µL of an acetonitrile/water mixture (0.1% ammonia, 70:30 [v/v]) and vortexed for 30 sec. The extract was then ready for analysis by UHPLC-MS/MS. The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a

signal-to-noise ratio ≥3. LODs were 0.05 ng/mL for BP-1, 0.06 ng/mL for BP-3 and 4-OHBP, and 0.10 ng/mL for MeP, EtP, PrP, and BuP.

2.4. Statistical analysis

In a descriptive analysis, arithmetic means and standard deviations were calculated for continuous variables and relative frequencies for categorical variables. Urinary concentrations of PBs, BPs, and gene expression levels were summarized as arithmetic means with standard deviations and as 25, 50, and 75 percentiles. The Shapiro-Wilk test was applied to check the normality of variable distributions, and data found to be non-normally distributed underwent logarithmic transformation.

Spearman's rank correlation coefficient was used to evaluate monotonic correlations between urinary concentrations of PBs/BPs and the expression of genes with detection/expression frequencies above 75%. Simple linear regression models were also developed to evaluate the relationship between PB/BP exposure and gene expression levels, expressing the results as β with 95% confidence intervals. In parallel, simple linear regression models were constructed after patient stratification (i) into lower or higher PB and BP exposure, i.e., below or above the median concentration of each congener; and (ii) into low, moderate, and high exposure, based on tertiles of PB and BP concentrations. Genes expressed in 25-75% of samples were considered as dichotomous variables (detected/not detected), and simple logistic regression models were used to evaluate their association with PBs and BPs, while no analyses were performed on genes expressed in <25% of samples.

Statistical analysis was performed with SPSS Statistics 23.0 (IBM, Armonk, NY) analysis, and the level of significance was $p<0.05$ in all tests; however, associations with p-values between 0.05 and 0.10 are cautiously considered, given the limited sample size.

3. RESULTS

3.1 Characteristics of the study population and urinary PB and BP concentrations

Sociodemographic and reproductive characteristics of all participants are summarized in Table 1. Out of the 33 women in the study, an adequate sample for exposure and gene expression measurements was obtained from 22, whose characteristics are reported in Supplementary Table S2. Mean age of study participants was 38.0±7.3 years, 60.6%

(n=20) were in a normal weight range [BMI <25kg/m^2], 57.6% (n=19) lived in a rural area, 63.6% (n=21) did not have a university degree, 72.7% (n=24) were employed outside the home, 45.5% (n=15) were nulliparous, 63.6% (n=21) had moderate/severe menstrual bleeding, and 75.8% (n=25) had a diagnosis of ovarian/peritoneal endometriosis, with 63.6% (n=21) being in stage I/II (Table 1).

Table 1. Characteristics of study population (n=33).

	n		%
Sociodemographic characteristics			
Age *(years)**	38.0	±	7.3
Weight *(kg)**	67.6	±	14.7
Height *(m)**	1.6	±	0.1
Body mass index *(kg/m^2)**	25.3	±	5.3
Normal weight (BMI < 25)	20		60.6
Overweight (BMI 25-30)	6		18.2
Obese (BMI > 30)	7		21.2
Residence			
Rural	19		57.6
Urban /sub-urban	14		42.4
Parity			
Nulliparous	15		45.5
Primiparous/Multiparous	18		54.5
Educational level			
Less than university degree	21		63.6
University degree	12		36.4
Working outside home			
Yes	24		72.7
No	9		27.3
Current smoker			
Yes	10		30.3
No	23		69.7
Urinary creatinine *(ng/mL)**	152.3	±	68.3
Reproductive characteristics			
Intensity of menstrual bleeding			
Mild	12		36.4
Moderate/severe	21		63.6
Endometrioma location			
Deep infiltrating endometriosis	8		24.2
Ovarian/peritoneal endometriosis	25		75.8
Endometriosis stage			
I/II	21		63.6
III/IV	12		36.4

**Mean ± standard deviation.*

Our group previously reported the urinary PB and BP concentrations obtained for participants in the EndEA study (Peinado et al., 2021). All compounds were detected in all samples from our subsample of 22 women (Supplementary Table S3). The PB and BP congeners with highest concentrations were MeP and BP-3, respectively.

3.2 Gene expression levels and associations with PB and BP concentrations

Gene expression levels in the endometriotic tissues are exhibited in Table 2, showing that 12 genes (52.2%) were expressed in >75% of samples and 4 (17.4%) in 50-75%. Both genes related to cell adhesion were expressed in all samples, whereas only four of the seven invasion, migration, and metastasis-related genes (*MMP1*, *RRM2*, *RHOB* and *SPRY2*) and three of the five inflammation-related genes (*IL1RL1*, *IL6ST* and *NR3C1*) were expressed in more than half of samples. Finally, all angiogenesis-related genes and three of the genes related to cell proliferation and hormonal stimulation (*DUSP6*, *ERα* and *STAR*) were expressed in more than half of samples. The sole difference in expression between endometriosis stages was observed for two genes related to the invasion, migration, and metastasis pathway (*FUT8* and *SPRY2* genes), which were both overexpressed in patients with stages III/IV (data not shown). In addition, a close-to-significant overexpression of *DUSP6* (p-value=0.092) was observed in patients with stages III/IV (data not shown). Supplementary Table 4 summarizes the gene expression profile of the subset of women with exposure information.

Spearman correlation coefficients between PBs/BPs and gene expression levels are displayed in Supplementary Table 5. Associations between PB/BP exposure and gene expression levels are reported in Supplementary Table S6 and Tables 3-6. The concentration of at least one PB congener was associated with the expression of gene(s) involved in each of the five pathways under study, while BPs were associated with genes related to invasion, migration/metastasis, inflammation, cell proliferation, and hormonal stimulation cell signaling pathways but not the adhesion pathway.

Table 2. Gene expression levels in endometriotic tissue (n=33).

Cell pathway	Gene	n	%	Mean	St. Dev.	Percentiles		
						25	50	75
Cell adhesion	ITGB2	33	100	4.89E+11	1.18E+13	8.67E+10	1.74E+11	4.65E+11
	CLDN7	33	100	1.49E+12	2.56E+13	5.24E+11	8.96E+11	1.52E+12
Invasion, migration and metastasis	MMP1	21	64	6.77E+11	3.40E+13	*n.e.*	1.70E+09	1.94E+10
	MMP7	4	12	1.31E+12	7.18E+13	*n.e.*	*n.e.*	*n.e.*
	FUT8	11	33	1.18E+09	2.82E+10	*n.e.*	*n.e.*	4.75E+08
	RRM2	33	100	1.37E+12	4.55E+13	2.51E+11	4.48E+11	7.79E+11
	MDK	14	43	1.61E+10	5.29E+11	*n.e.*	*n.e.*	1.38E+09
	RHOB	33	100	7.32E+12	2.61E+14	5.12E+11	1.06E+12	2.95E+12
	SPRY2	21	64	8.14E+10	1.39E+12	*n.e.*	4.21E+10	1.01E+11
Inflammation	IL1R2	4	12	7.06E+07	2.16E+09	*n.e.*	*n.e.*	*n.e.*
	IL1RL1	27	82	1.10E+11	3.81E+12	1.36E+09	9.56E+09	3.19E+10
	IL6ST	33	100	1.13E+13	5.06E+14	5.10E+11	1.12E+12	2.41E+12
	NR3C1	30	91	6.78E+11	3.21E+13	6.23E+09	2.98E+10	1.21E+11
	TNFRSF1B	11	33	1.91E+09	4.92E+10	*n.e.*	*n.e.*	1.03E+09
Angiogenesis	ANG	33	100	2.91E+11	8.33E+12	5.28E+10	9.42E+10	2.13E+11
	ANGPT1	28	85	4.82E+10	1.17E+12	1.15E+09	1.32E+10	4.12E+10
	sVEGFR-1	23	70	2.07E+11	1.15E+13	*n.e.*	2.42E+09	7.00E+09
	VEGFA	23	70	4.37E+11	5.15E+12	*n.e.*	3.02E+11	6.65E+11
Cell proliferation and hormonal stimulation	CYP19A1	5	15	1.67E+12	9.28E+13	*n.e.*	*n.e.*	*n.e.*
	DUSP6	31	94	3.84E+11	5.12E+12	1.99E+09	1.95E+11	5.44E+11
	ERα	33	100	2.99E+12	8.51E+13	7.01E+11	1.28E+12	2.12E+12
	PGR	2	6.1	1.94E+09	1.08E+11	*n.e.*	*n.e.*	*n.e.*
	STAR	28	85	1.49E+12	4.95E+13	1.37E+09	1.56E+11	4.34E+11

St. Dev.: standard deviation; ITGB2: integrin beta-2; CLDN7: claudin 7; MMP1: matrix metallopeptidase 1; MMP7: matrix metallopeptidase 7; FUT8: fucosyltransferase 8; RRM2: ribonucleotide reductase M2; MDK: midkine; RHOB: ras homolog gene family, member B; SPRY2: sprout homolog 2; IL1RL1: interleukin 1 receptor, type I; IL1R2: interleukin 1 receptor, type II; IL6ST: interleukin 6 cytokine family signal transducer; NR3C1: nuclear receptor subfamily 3 group C member 1; TNFRSF1B: tumor necrosis factor receptor superfamily member 1B; ANG: angiogenin; ANGPT1: angiopoietin 1; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; VEGFA: vascular endothelial growth factor A; CYP19A1: cytochrome P450 family 19 subfamily A member 1; DUSP6: dual specificity phosphatase 6; ERα: estrogen receptor 1; PGR: progesterone receptor; STAR: steroidogenic acute regulatory protein.; n.e.: not expressed.

3.2.1 Cell adhesion

Urinary concentrations of MeP and ∑PBs showed significant positive correlations with ectopic tissue *ITGB2* and *CLDN7* gene expression (Supplementary Table S6). When concentrations were considered as dichotomous variables (higher *vs.* lower), expression

of *ITGB2* was related to higher concentrations of MeP, BuP and ∑PBs, and its positive association with EtP was close to statistical significance (p-value=0.091). A similar relationship was observed between ectopic overexpression of *CLDN7* and higher concentrations of MeP, BuP, and ∑PBs. Furthermore, *CLDN7* gene expression was greater for the third exposure tertile of both ∑PBs and BuP, almost reaching statistical significance in the case of BuP (p-value=0.066) (data not shown). No significant associations were found between BP exposure and *ITGB2* or *CLDN7* gene expression levels.

3.2.2 Invasion, migration, and metastasis

Urinary MeP and ∑PBs concentrations were associated with the overexpression of *MMP1* and *RHOB* genes, and EtP and BuP concentrations were also related to the overexpression of *RHOB* gene (Table 3). Similar associations were observed when exposure was dichotomized, with increased risk of detectable *MMP1* and *RHOB* expression levels in patients with higher exposure to MeP and ∑PBs and of detectable *RHOB* expression in patients with higher exposure to EtP and BuP. In addition, *RHOB* gene expression levels gradually increased when BuP was categorized into tertiles (data not shown). A similar trend was observed for tertiles of ∑PBs and *MMP1*. Moreover, detectable expression of *FUT8* was observed in the women with higher exposure to PrP, while the higher expression levels of RRM2 in women with a higher concentration of MeP was close to statistically significant (p-value=0.088). By contrast, MeP and ∑PBs concentrations were inversely associated with the detectable expression of *SPRY2*. A positive association was found between concentrations of ∑BPs and detectable expression of *MMP1*. *MMP7* expression was detected in <25% of the study population; therefore, no linear or logistic regression was performed.

Table 3. Associations between urinary concentrations of PBs and BPs and expression levels of genes involved in invasion, migration, and metastasis.

	MMP1				FUT8				RRM2			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**	**β**	**95% CI**		**p-value**
Parabens												
MeP	***2.96***	***1.14***	***7.72***	***0.026***	0.90	0.55	1.46	0.668	0.10	-0.094	0.303	0.283
<39.26 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>39.26 ng/mL	***12.00***	***1.12***	***128.84***	***0.040***	0.66	0.11	4.00	0.648	0.63	-0.10	1.36	0.088
EtP	1.27	0.83	1.95	0.277	1.17	0.77	1.76	0.466	0.09	-0.72	0.26	0.252
<4.51 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>34.51 ng/mL	3.75	0.54	26.05	0.181	3.75	0.54	26.05	0.181	0.41	-0.36	1.18	0.280
PrP	1.63	0.93	2.84	0.086	1.26	0.81	1.96	0.306	0.05	-0.14	0.23	0.609
<2.54 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>2.54 ng/mL	1.52	0.25	9.30	0.648	***12.00***	***1.12***	***128.84***	***0.040***	0.18	-0.61	0.96	0.647
BuP	1.46	0.73	2.19	0.285	0.67	0.33	1.35	0.262	0.03	-0.23	0.29	0.827
<0.14 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>0.14 ng/mL	5.00	0.70	35.50	0.108	1.17	0.19	7.12	0.867	0.71	-0.34	1.75	0.174
ΣPBs	***2.68***	***0.09***	***6.59***	***0.031***	0.93	0.56	1.52	0.760	0.10	-0.11	0.32	0.317
<53.31 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>53.31 ng/mL	***12.00***	***1.12***	***128.84***	***0.040***	0.66	0.11	4.00	0.648	0.33	-0.45	1.10	0.387
Benzophenones												
BP-1	0.59	0.22	1.55	0.281	1.41	0.56	3.54	0.467	0.077	-0.302	0.455	0.677
<1.42 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>1.42 ng/mL	0.66	0.11	4.00	0.648	1.52	0.25	9.30	0.648	0.44	-0.32	1.21	0.239
BP-3	1.23	0.63	2.39	0.542	1.06	0.54	2.09	0.872	-0.13	-0.42	0.15	0.343
<2.53 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>2.53 ng/mL	1.52	0.25	9.30	0.648	3.75	0.54	26.045	0.181	0.40	-0.67	1.48	0.443
4-OHBP	1.86	0.55	6.34	0.322	1.476	0.475	4.583	0.501	-0.13	-0.604	0.352	0.587
<0.73 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>0.73 ng/mL	1.52	0.25	0.30	0.648	1.52	0.25	9.30	0.648	-0.44	-1.20	0.32	0.243
ΣBPs	***1.22***	***0.02***	***0.88***	***0.036***	1.29	0.47	3.58	0.620	-0.07	-0.50	0.35	0.725
<7.43 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>7.43 ng/mL	0.66	0.11	4.00	0.648	1.52	0.25	9.30	0.648	0.55	-0.51	1.61	0.289

	MDK				RHOB				SPRY2			
	OR	**95% CI**		**p-value**	**β**	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Parabens												
MeP	0.77	0.47	1.27	0.309	***0.27***	***0.11***	***0.43***	***0.002***	***0.48***	***0.24***	***0.97***	***0.040***
<39.26 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>39.26 ng/mL	0.45	0.08	2.67	0.379	***1.02***	***0.08***	***1.96***	***0.035***	0.31	0.05	1.85	0.200
EtP	0.88	0.60	1.31	0.529	***0.18***	***0.03***	***0.32***	***0.022***	0.89	0.60	1.30	0.535
<4.51 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>34.51 ng/mL	1.00	0.18	5.68	1.000	0.30	-0.76	1.35	0.565	1.46	0.26	8.05	0.665
PrP	0.94	0.62	1.42	0.772	0.11	-0.07	0.28	0.206	0.79	0.52	1.21	0.274
<2.54 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>2.54 ng/mL	1.00	0.18	5.68	1.000	-0.50	-1.84	0.83	0.441	0.69	0.12	3.78	0.665
BuP	0.72	0.38	1.36	0.308	***0.40***	***0.11***	***0.69***	***0.010***	0.63	0.34	1.16	0.137
<0.14 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>0.14 ng/mL	0.75	0.13	4.29	0.746	***1.68***	***0.56***	***2.79***	***0.005***	0.43	0.07	2.50	0.346
ΣPBs	0.76	0.45	1.27	0.293	***0.28***	***0.12***	***0.45***	***0.002***	***0.51***	***0.26***	***0.98***	***0.044***
<53.31 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>53.31 ng/mL	0.45	0.08	2.67	0.379	***1.11***	***0.19***	***2.03***	***0.021***	0.31	0.05	1.85	0.200

Table 3 (continued).

Benzophenones												
BP-1	0.71	0.30	1.70	0.438	-0.02	-0.42	0.38	0.923	1.03	0.45	2.35	0.954
<1.42 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>1.42 ng/mL	0.45	0.08	2.67	0.379	0.30	-1.06	1.65	0.653	0.69	0.12	3.78	0.665
BP-3	0.83	0.43	1.58	0.567	0.03	-0.36	0.42	0.865	0.83	0.42	1.61	0.572
<2.53 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>2.53 ng/mL	2.22	0.38	13.18	0.379	0.85	-0.45	2.15	0.187	1.46	0.26	8.05	0.665
4-OHBP	0.44	0.13	1.53	0.197	0.234	-0.267	0.735	0.340	0.70	0.24	2.05	0.510
<0.73 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>0.73 ng/mL	1.00	0.18	5.68	1.000	0.04	-1.32	1.40	0.947	0.69	0.12	3.78	0.665
ΣBPs	0.82	0.31	2.15	0.689	-0.01	-0.44	0.42	0.957	0.98	0.38	2.51	0.967
<7.43 ng/mL	1.00	-	-		0.00	-	-		1.00	-	-	
>7.43 ng/mL	0.45	0.08	2.67	0.379	0.85	-0.34	2.04	0.151	0.69	0.12	3.78	0.665

OR: odds ratio; CI: confidence interval; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones; MMP1: matrix metallopeptidase 1; FUT8: fucosyltransferase 8; RRM2: ribonucleotide reductase M2; MDK: midkine; RHOB: ras homolog gene family, member B; SPRY2: sprout homolog 2.

3.2.3 Inflammation

MeP, BuP and ∑PBs concentrations were significantly correlated with *NR3C1* and *IL6ST* expression levels, and higher EtP concentrations were also correlated with *IL6ST* expression levels (Table 4). Women with higher concentrations of BuP also showed increased *IL1RL1* expression, although statistical significance was not reached (p-value=0.055). Moreover, the expression of *IL6ST* and *NR3C1* gradually increased with higher tertiles of BuP concentrations (data not shown). *TNFRSF1B* overexpression was associated with urinary concentrations of BP3 and ΣBPs, and a similar association was observed between *TNFRSF1B* and concentrations of BP3 and ΣBPs when the exposure was dichotomized (p-value=0.080). *IL1RL1* overexpression was also observed in the participants with higher 4-OHBP concentrations. *IL1R2* expression was detected in <25% of the study population; therefore, no linear or logistic regression was performed.

Table 4. Associations between urinary concentrations of PBs and BPs and expression levels of genes involved in inflammation.

	IL1RL1				IL6ST				NR3C1				TNFRSF1B			
	β	95% CI		p-value	β	95% CI		p-value	β	95% CI		p-value	OR	95% CI		p-value
Parabens																
MeP	-0.21	-0.77	0.35	0.440	***0.35***	***0.12***	***0.58***	***0.005***	**0.57**	**0.131**	**1.02**	**0.014**	0.81	0.47	1.37	0.426
<39.26 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>39.26 ng/mL	2.00	-2.32	6.31	0.345	***1.26***	***0.38***	***2.13***	***0.007***	1.09	-1.05	3.22	0.300	0.39	0.06	2.77	0.346
EtP	-0.12	-0.58	0.33	0.575	***0.23***	***0.07***	***0.40***	***0.008***	0.30	-0.07	0.67	0.107	0.99	0.65	1.49	0.950
<4.51 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>34.51 ng/mL	-1.32	-3.31	0.67	0.179	0.25	-0.80	1.31	0.620	-0.42	-2.61	1.77	0.692	1.00	0.15	6.53	1.000
PrP	-0.20	-0.69	0.29	0.391	0.05	-0.19	0.29	0.662	0.21	-0.21	0.63	0.305	1.02	0.66	1.59	0.929
<2.54 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>2.54 ng/mL	-1.31	-5.68	3.06	0.538	-0.42	-1.86	1.02	0.549	-0.69	-2.86	1.48	0.513	2.57	0.36	18.33	0.346
BuP	0.43	-0.20	1.06	0.165	***0.47***	***0.20***	***0.73***	***0.002***	***0.74***	***0.27***	***1.21***	***0.004***	0.42	0.147	1.18	0.101
<0.14 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>0.14 ng/mL	3.91	-0.08	7.91	0.055	***1.71***	***0.49***	***2.94***	***0.008***	***2.61***	***0.78***	***4.44***	***0.007***	0.30	0.04	2.17	0.232
ΣPBs	-0.25	-0.83	0.33	0.379	***0.36***	***0.13***	***0.60***	***0.004***	***0.60***	***0.14***	***1.05***	***0.013***	0.78	0.45	1.36	0.379
<53.31 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>53.31 ng/mL	2.29	-1.99	6.57	0.278	***1.27***	***0.40***	***2.14***	***0.006***	1.26	-0.85	3.37	0.225	0.39	0.06	2.77	0.346
Benzophenones																
BP-1	-0.13	-1.19	0.93	0.797	-0.03	-0.50	0.44	0.886	-0.33	-1.262	0.594	0.459	0.58	0.21	1.62	0.298
<1.42 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>1.42 ng/mL	-1.39	-4.97	2.19	0.425	0.32	-1.13	1.77	0.649	-1.18	-4.74	2.39	0.499	2.57	0.36	18.33	0.346
BP-3	-0.17	-1.10	0.77	0.714	-0.03	-0.42	0.36	0.862	0.31	-0.48	1.09	0.428	***10.09***	***1.34***	***76.29***	***0.025***
<2.53 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>2.53 ng/mL	2.31	-2.45	7.06	0.324	0.60	-0.83	2.03	0.389	2.15	-1.32	5.62	0.211	***11.00***	***1.05***	***120.40***	***0.049***
4-OHBP	2.23	-0.57	5.03	0.112	-0.04	-0.688	0.606	0.895	0.31	-0.779	1.404	0.555	1.58	0.48	5.17	0.452
<0.73 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>0.73 ng/mL	***4.71***	***0.36***	***9.06***	***0.035***	0.17	-1.29	1.62	0.810	1.87	-1.63	5.38	0.278	1.00	0.15	6.53	1.000
ΣBPs	-0.15	-1.46	1.16	0.812	-0.13	-0.64	0.38	0.593	-0.35	-1.57	0.87	0.558	***14.15***	***1.27***	***157.38***	***0.031***
<7.43 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>7.43 ng/mL	1.87	-1.67	5.42	0.281	0.40	-1.04	1.84	0.569	0.54	-1.65	2.71	0.614	8.33	0.78	89.47	0.080

OR: odds ratio; CI: confidence interval; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: butylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones; IL1RL1: interleukin 1 receptor, type I; IL6ST: interleukin 6 cytokine family signal transducer; NR3C1: nuclear receptor subfamily 3 group C member 1; TNFRSF1B: tumor necrosis factor receptor superfamily member 1B.

3.2.4 Angiogenesis

Urinary concentrations of MeP and ∑PBs were significantly correlated with *ANGPT1* exposure, both as a continuous and dichotomous variable (Table 5). MeP and ∑PBs were close to significantly associated with *ANG* expression levels (p-values=0.062 and 0.063, respectively). PrP concentrations were related to the overexpression of *sVEGFR-1*, and BuP showed a similar association when considered as a dichotomous variable. Finally, reduced *VEGFA* expression was observed in the women with higher EtP concentration, although statistical significance was not reached (p-value=0.080). No association was observed between concentrations of BP congeners and the expression of angiogenesis-related genes, except for lower *VEGFA* expression in the women with higher concentrations of BP-1 and BP-3, although these associations did not reach statistical significance (p-values=0.080 in both cases).

3.2.5 Cell proliferation and hormonal stimulation

DUSP6 gene expression was inversely associated with concentrations of MeP and ΣPBs. *ERα* expression was significantly and positively related to MeP and ΣPBs concentrations, both as a continuous and dichotomous variable, and was also positively and close-to-significantly associated with EtP and BuP (p-values=0.074 and 0.076, respectively). Lower *STAR* gene expression was associated with higher MeP concentrations, although statistical significance was not reached when considered as a continuous variable (p-value=0.086), while *STAR* overexpression was associated with higher concentrations of BP-1 and 4-OHBP (Table 6). Expression of *CYP19A1* and *PGR* was detected in <25% of the study population; therefore, no linear or logistic regression was performed.

Table 5. Associations between urinary concentrations of PBs and BPs and expression levels of genes involved in angiogenesis.

	ANG				ANGPT1				sVEFGR-1				VEGFA			
	β	95% CI		p-value	β	95% CI		p-value	β	95% CI		p-value	OR	95% CI		p-value
Parabens																
MeP	0.22	-0.01	0.46	0.062	***0.49***	***0.18***	***0.81***	***0.004***	1.53	0.87	2.67	0.139	0.88	0.53	1.44	0.602
<39.26 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>39.26 ng/mL	***0.99***	***0.14***	***1.84***	***0.025***	***1.62***	***0.27***	***2.97***	***0.021***	2.22	0.38	13.18	0.379	1.00	0.15	6.53	1.000
EtP	0.15	-0.05	0.35	0.141	0.22	-0.10	0.55	0.160	1.14	0.77	1.69	0.508	0.82	0.53	1.27	0.369
<4.51 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>34.51 ng/mL	0.35	-0.85	1.55	0.549	0.15	-1.43	1.73	0.842	2.22	0.38	13.18	0.379	0.12	0.01	1.29	0.080
PrP	0.14	-0.08	0.35	0.201	0.26	-0.09	0.60	0.132	***1.86***	***1.01***	***3.40***	***0.045***	0.85	0.54	1.33	0.465
<2.54 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>2.54 ng/mL	0.21	-1.00	1.41	0.722	0.38	-1.19	1.95	0.620	5.40	0.78	37.51	0.088	0.39	0.06	2.77	0.346
BuP	0.24	-0.02	0.51	0.071	-0.25	-1.81	1.30	0.736	1.147	0.64	2.073	0.650	0.95	0.514	1.748	0.865
<0.14 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>0.14 ng/mL	0.94	-0.20	2.07	0.100	-2.04	-6.69	2.61	0.371	***7.50***	***1.04***	***54.12***	***0.046***	0.50	0.07	3.55	0.488
ΣPBs	0.23	-0.01	0.48	0.063	***0.51***	***0.19***	***0.84***	***0.004***	1.54	0.87	2.74	0.140	0.89	0.53	1.49	0.664
<53.31 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>53.31 ng/mL	0.58	-0.60	1.76	0.320	***1.78***	***0.49***	***3.07***	***0.010***	2.22	0.38	13.18	0.379	1.00	0.15	6.53	1.000
Benzophenones																
BP-1	0.05	-0.42	0.52	0.837	-0.55	-1.23	0.13	0.103	1.08	0.46	2.52	0.865	0.67	0.25	1.78	0.419
<1.42 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>1.42 ng/mL	0.68	0.48	1.84	0.240	0.48	-4.25	5.20	0.836	2.22	0.38	13.18	0.379	0.12	0.01	1.29	0.080
BP-3	0.03	-0.32	0.39	0.843	0.05	-0.60	0.70	0.879	0.76	0.37	1.56	0.453	0.55	0.22	1.41	0.215
<2.53 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>2.53 ng/mL	0.79	-0.36	1.94	0.169	0.53	-1.03	2.09	0.482	2.22	0.38	13.18	0.379	0.12	0.01	1.29	0.080
4-OHBP	-0.25	-0.83	0.34	0.388	-0.148	-1.18	0.89	0.767	1.47	0.48	4.55	0.501	1.16	0.35	3.77	0.810
<0.73 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>0.73 ng/mL	-0.16	-1.37	1.04	0.779	-0.03	-1.62	1.55	0.964	2.22	0.38	13.18	0.379	2.57	0.36	18.33	0.346
ΣBPs	0.03	-0.49	0.56	0.894	-0.43	-1.30	0.45	0.316	0.80	0.30	2.13	0.654	0.50	0.15	1.63	0.250
<7.43 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		1.00	-	-	
>7.43 ng/mL	0.47	-0.72	1.66	0.421	0.83	-2.55	4.20	0.613	1.00	0.18	5.68	1.000	0.39	0.06	2.77	0.346

OR: odds ratio; CI: confidence interval; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones; ANG: angiogenin; ANGPT1: angiopoietin 1; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; VEGFA: vascular endothelial growth factor A.

Table 6. Associations between low and high urinary concentrations of PBs and BPs and expression levels of genes involved in cell proliferation and hormonal stimulation.

	DUSP6				ERα				STAR			
	β	95% CI		p-value	β	95% CI		p-value	β	95% CI		p-value
Parabens												
MeP	***-0.90***	***-1.49***	***-0.30***	***0.005***	***0.18***	***0.04***	***0.31***	***0.015***	-1.35	-2.908	0.211	0.086
<39.26 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>39.26 ng/mL	-3.13	-6.99	0.73	0.106	***0.78***	***0.30***	***1.26***	***0.003***	***-5.64***	***-11.08***	***-0.20***	***0.043***
EtP	0.01	-0.63	0.65	0.968	0.10	-0.11	0.21	0.074	-0.51	-1.84	0.83	0.437
<4.51 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>34.51 ng/mL	-1.74	-6.52	3.03	0.450	0.29	-0.31	0.87	0.325	0.02	-6.02	6.06	0.994
PrP	0.16	-0.56	0.89	0.640	0.06	-0.08	0.19	0.406	-0.07	-1.31	1.16	0.902
<2.54 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>2.54 ng/mL	-1.38	-6.18	3.42	0.560	0.05	-0.56	0.65	0.871	0.38	-5.66	6.42	0.898
BuP	-0.49	-1.65	0.68	0.391	0.16	-0.02	0.35	0.076	0.57	-1.25	2.39	0.520
<0.14 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>0.14 ng/mL	-3.01	-7.67	1.64	0.192	***0.70***	***0.19***	***1.20***	***0.009***	1.50	-4.53	7.52	0.610
ΣPBs	***-0.83***	***-1.46***	***-0.19***	***0.014***	***0.19***	***0.05***	***0.33***	***0.011***	-1.28	-2.82	0.27	0.100
<53.31 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>53.31 ng/mL	1.37	-8.24	0.81	0.102	***0.39***	***0.21***	***1.21***	***0.008***	-3.47	-9.29	2.35	0.228
Benzophenones												
BP-1	0.97	-0.28	2.21	0.122	-0.09	-0.35	0.17	0.463	***2.94***	***0.38***	***5.50***	***0.027***
<1.42 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>1.42 ng/mL	1.37	-3.43	6.18	0.558	0.39	-0.50	1.29	0.372	4.20	-1.51	9.92	0.140
BP-3	0.17	-0.85	1.18	0.737	-0.12	-0.33	0.10	0.262	0.41	-1.68	2.51	0.683
<2.53 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>2.53 ng/mL	-0.05	-4.90	4.79	0.982	-0.01	-0.92	0.91	0.988	2.45	-3.49	8.38	0.400
4-OHBP	-0.30	-2.00	1.40	0.713	0.017	-0.351	0.386	0.923	2.53	-0.923	5.983	0.141
<0.73 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>0.73 ng/mL	-0.47	-5.31	4.37	0.841	-0.01	-0.62	0.60	0.971	***5.94***	***0.94***	***10.93***	***0.022***
ΣBPs	0.85	-0.60	2.29	0.234	-0.17	-0.49	0.15	0.273	2.10	-0.84	5.03	0.151
<7.43 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-	
>7.43 ng/mL	-0.58	-5.41	4.26	0.807	0.09	-0.82	1.01	0.832	4.61	-0.70	9.92	0.085

CI: confidence interval; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones; DUSP6: dual specificity phosphatase 6; ERα: estrogen receptor 1; STAR: steroidogenic acute regulatory protein.

4. DISCUSSION

To our best knowledge, this is the first study to describe the expression profile of key genes involved in the main cell signaling pathways related to endometriosis in human endometriotic tissue and its potential association with exposure to EDCs. Most of the studied genes were expressed in the majority of samples, and exposure to certain PB and BP congeners was associated with the overexpression of genes involved in cell adhesion; invasion, migration and metastasis; inflammation; angiogenesis; and cell proliferation and hormonal stimulation, critical signaling pathways for the onset and progression of this disease.

Adhesion of endometrial cells to the peritoneal surface is considered a primary hallmark of endometriosis in its initial stages (Jensen and Coddington, 2010). Exposure to various PBs was associated with adhesion-related *ITGB2* and *CLDN7* genes in the present study, in line with recent *in vitro* findings of enhanced adhesive capacity in human umbilical vein endothelial cells after exposure to different EDCs (Kenda et al., 2022). Upregulation of *CLDN7* has also been reported in ovarian cancer cell lines (Dahiya et al., 2011), although the evidence is contradictory, given that downregulation of *CLDN7* has been observed in human endometriotic lesions (Gaetje et al., 2008) and endometrial cancer, promoting proliferation and metastasis (Li et al., 2013).

Adhesion of endometrial cells to the peritoneal surface is followed by a process of invasion, migration, and metastasis that involves the overexpression of metalloproteinases (Krikun, 2012). *MMP1* was expressed in most of the present endometriotic samples, in agreement with previous reports of *MMP* overexpression in women with endometriosis (Pino et al., 2009; Sillem et al., 2001) and more severe stages of this disease (Gilabert-Estellés et al., 2007; Matsuzaki et al., 2010). An increase in *MMP* activation has been detected in ovarian cancer, enhancing cell invasiveness (Dahiya et al., 2011). In the present investigation, *MMP1* overexpression was associated with exposure to MeP, ∑PBs, and ΣBPs, which may therefore favor this endometriosis-related cell signaling pathway. *In vitro* studies have linked PB exposure to increased *MMP* expression and activity in dermal fibroblasts (Majewska et al., 2017) and breast cancer cells (Khanna et al., 2014). Exposure to other EDCs also increased *MMP* expression in ovarian cancer cell lines (Ptak et al., 2014). *RHOB*, *FUT8*, and *RRM2* genes may also participate in invasion, migration, and metastasis. The expression of

RHOB has not been described in endometriosis, but upregulation of this gene has been reported in human breast tumors (Fritz et al., 2002). The present study describes for the first time the presence of *RHOB* in endometriotic tissue and its positive association with exposure to most PB congeners studied. This suggests that exposure to these EDCs may contribute to the invasiveness of endometriotic tissue in a similar manner to the observed effects of EDCs on metastasis in breast cancer. *FUT8* has not previously been studied in endometriosis tissue samples but has been implicated in the epithelial-mesenchymal transition and metastasis (Wang et al., 2006; Zhao et al., 2006). PrP concentrations were positively associated with *FUT8* expression, suggesting that exposure to PrP may increase endometriotic tissue *FUT8* and promote metastasis and progression to more advanced stages of disease. It is also suspected that *RRM2* upregulation may promote cell invasion and metastasis in cancer and ovarian endometriosis (Liu et al., 2007; Yang et al., 2021). Its expression was increased in the present women with higher MeP exposure, which may therefore play a potential role in boosting invasiveness. Finally, although the importance of *SPRY2* in endometriosis is unknown, it plays a crucial role in the regulation of cancer cell invasion (Mei et al., 2013; Wang et al., 2015). According to the present findings, MeP and ΣPBs might enhance *SPRY2* expression and thereby favor invasiveness and metastasis in women with endometriosis.

Another hallmark of endometriosis is a complex proinflammatory microenvironment, which favors cellular adhesion and invasion processes and the vascularization and proliferation of endometriotic lesions (García-Gómez et al., 2019). Upregulation of *IL1RL1*, *IL6ST*, *TNFRSF1B,* and *NR3C1* has been described in women with endometriosis (Monsivais et al., 2012; Sherwin et al., 2008). In line with the proposal that human exposure to PBs/BPs may be related to inflammation (Linares et al., 2021; Watkins et al., 2015), the present results suggest that the upregulation of *IL1RL1*, *IL6ST*, *TNFRSF1B* and *NR3C1* genes associated with exposure to various congeners of PBs and BPs could contribute to the generation of an inflammatory environment in endometriotic tissue.

Angiogenesis plays an important role in the pathogenesis and pathophysiology of endometriosis, and various genes are involved in this cell signaling pathway (Taylor et al., 2002). Increased *ANG*, *VEGFA,* and *sVEGFR-1* expression was previously observed in the peritoneal fluid of women with endometriosis (Cho et al., 2012; McLaren et al.,

1996). In the present study, urinary concentrations of various PB congeners were positively associated with *ANG*, *ANGPT1*, and *sVEGFR-1* expression. In contrast, exposure to BP-3 was previously found to be negatively associated with *VEGFA* levels (Prashanth et al., 2014). The present findings support the hypothesis that exposure to these EDCs could favor the development of pro-angiogenic properties by endometriotic lesions, as suggested by the observation of increased microvessel density after exposure to bisphenol A in a model of neuroblastoma (Zhu et al., 2009).

Estradiol (E_2) promotes the persistence, multiplication, and progression of endometriotic lesions, acting as a growth factor for this tissue (Attar et al., 2009), and endometriosis is closely associated with steroid metabolism and associated pathways (Giudice and Kao, 2004; Greene et al., 2016). E_2 production requires the action of *STAR*, which facilitates the entry of cytosolic cholesterol into the mitochondrion, and *CYP19A1*, which is responsible for aromatization of androgens into estrogens (Tang et al., 2019; Zeitoun and Bulun, 1999). In the present study, *STAR* gene was expressed in most of the ectopic tissue samples, and exposure to BP-1 and 4-OHBP was associated with STAR overexpression, indicating that exposure to these EDCs might be related to increased E_2 production in endometriotic tissue. These results support the previous suggestion of a potential role for EDCs in the upregulation of *CYP19A1* (Williams and Darbre, 2019). In contrast, a reduction in *STAR* expression was observed in the women with higher concentrations of MeP. Endometriotic lesions are very sensitive not only to the production of E_2 but also to the presence of circulating estrogens. In this regard, E_2 membrane receptors (*ERα* and *ERβ*) have been widely reported in endometriotic tissue (Todorow et al., 2017), with some researchers describing *ERβ* as the predominant receptor on the cell surface (Attar and Bulun, 2006). Hence, although *ERβ* gene expression was not evaluated, *ERα* gene expression in endometriotic tissue was found to be positively related to exposure to most PB congeners examined, indicating that this exposure might contribute to the sensitivity of this tissue to circulating E_2. The influence of BPs or PBs on ERα expression has not previously been studied, but the present results are in line with *in vivo* findings of *ERα* overexpression after exposure to BPA (Cao et al., 2013).

DUSP6 gene encodes a protein that counteracts cellular proliferation and is dysregulated in various diseases, including cancer. The number of cells expressing *DUSP6* was reported to be lower in ectopic *versus* eutopic endometrium (Yu et al.,

2021), suggesting this gene might also play a crucial role alongside E_2 in enhancing cell proliferation in endometriotic tissue. In the present investigation, MeP and ΣPBs were associated with a lower expression of this gene in endometriotic tissue, suggesting that these EDCs might contribute to the downregulation of *DUSP6* in this tissue.

Study limitations include the small sample size, reducing the statistical power, although it is highly challenging to gather endometriotic tissue samples for investigation. Furthermore, the utilization of spot urine samples prevented consideration of the variability in daily exposure to analytes with a relatively short elimination half-life. Nevertheless, samples were all first-morning urine samples taken during hospitalization before endometriosis surgery. In addition, only two families of EDCs were measured, with no evaluation of the combined effect of PBs and BPs alongside other EDCs. Finally, the ability to include covariates in regression analyses was limited by the small study population. Study strengths include the measurement for the first time in endometriotic tissue of numerous genes involved in cellular pathways related to the pathophysiology of endometriosis. Importantly, many cases revealed consistent associations between gene expression and exposure to certain PB/BP congeners. The combined investigation of biomarkers of exposure and potential biomarkers of effect yielded evidence of different pathways for adverse outcomes in endometriosis.

5. CONCLUSIONS

Genes related to the development and progression of endometriosis were expressed in most of this series of endometriotic tissue samples. Results obtained indicate that the exposure of women to PBs and BPs may be associated with the expression of genes involved in cell signaling pathways that play key roles in the development of endometriosis, including cell adhesion; invasion, migration, and metastasis; inflammation; angiogenesis; and cell proliferation and hormonal stimulation. Given the novelty of these results, further studies with larger sample sizes are warranted to confirm the impact of human exposure to EDCs on the pathophysiology of endometriosis.

AUTHOR CONTRIBUTIONS

Francisco M. Peinado: Investigation, Formal analysis, Writing - original draft, Alicia Olivas-Martínez: Investigation, Formal analysis, Inmaculada Lendínez: Resources,

Writing-Review & Editing, Luz M. Iribarne-Durán: Investigation, Josefa León: Investigation, Writing-Review & Editing, Mariana F. Fernández: Writing-Review & Editing, Rafael Sotelo: Resources, Writing-Review & Editing, Fernando Vela-Soria: Investigation, Nicolás Olea: Writing-Review & Editing, Carmen Freire: Investigation, Writing-Review & Editing, Olga Ocón-Hernández: Conceptualization, Supervision, Funding acquisition, Writing-Review & Editing, Francisco Artacho-Cordón: Conceptualization, Methodology, Supervision, Formal analysis, Funding acquisition, Writing-Review & Editing.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

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ARTICLE 5

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Cell cycle, apoptosis, cell differentiation, and lipid metabolism gene expression in endometriotic tissue and exposure to parabens and benzophenones

Peinado FM[1,2*], Olivas-Martínez A[1,2], Iribarne-Durán LM[1], Ubiña A[3], León J[1,4,5], Vela-Soria F[1], Fernández-Parra J[6], Fernández MF[1,7,8], Olea N[1,7,8,9], Freire C[1,7], Ocón-Hernández O[1,10], Artacho-Cordón F[1,7,8*] .

[1]Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), E-18012 Granada, Spain

[2]University of Granada, Centre for Biomedical Research, E-18016, Granada, Spain; Department of Radiology and Physical Medicine, School of Medicine, University of Granada, E-18071, Granada, Spain

[3]General surgery, San Cecilio University Hospital, Granada, E-18016, Spain

[4]Digestive Medicine Unit, 'San Cecilio' University Hospital, E-18012 Granada, Spain

[5]CIBER Hepatic and Digestive Diseases (CIBEREHD), E-28029 Madrid, Spain

[6]Gynaecology and Obstetrics Unit, 'Virgen de las Nieves' University Hospital, E-18014 Granada, Spain.

[7]CIBER Epidemiology and Public Health (CIBERESP), E-28029 Madrid, Spain

[8]Radiology and Physical Medicine Department, University of Granada, E-18016 Granada, Spain

[9]Nuclear Medicine Unit, 'San Cecilio' University Hospital, E-18016 Granada, Spain

[10]Gynaecology and Obstetrics Unit, 'San Cecilio' University Hospital. Granada, E-18016, Spain

***Corresponding author:**

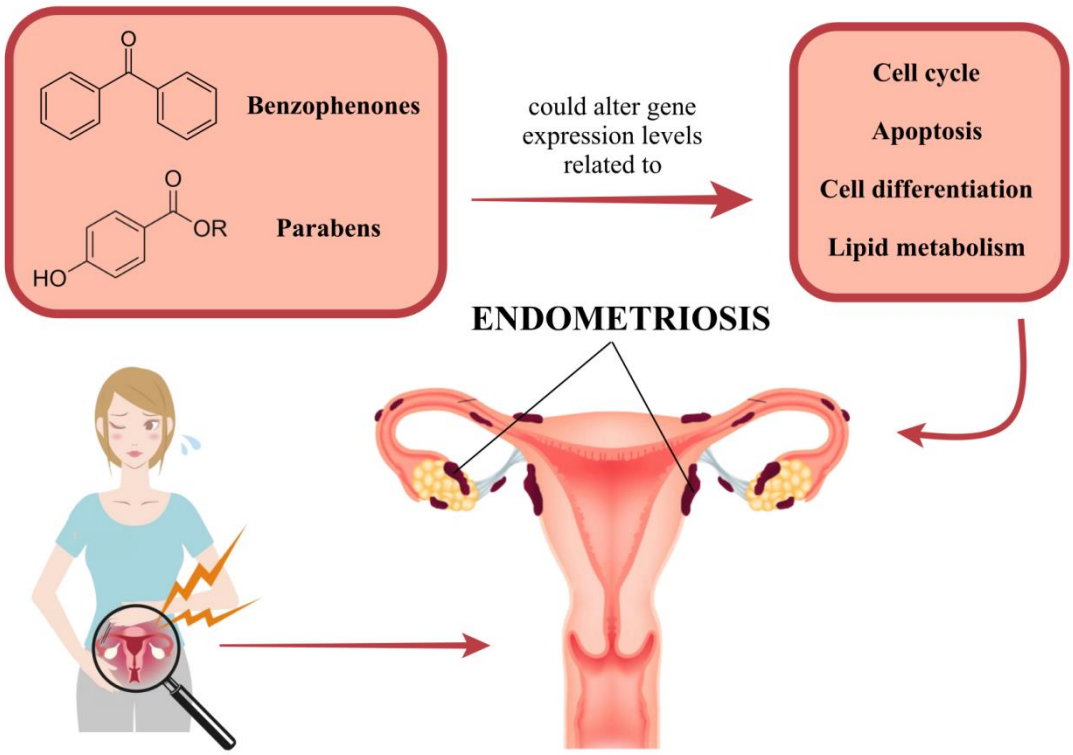
Francisco Manuel Peinado. Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), E-18012 Granada, Spain. Phone: +34 690992259; E-mail: franciscopeinado@correo.ugr.es; ORCID (0000-0001-6517-1941).

Francisco Artacho-Cordón. University of Granada, Department of Radiology and Physical Medicine. 18071 Granada, Spain. Phone: +34 958 249983; E-mail: fartacho@ugr.es; ORCID (0000-0003-3850-6173).

HIGHLIGHTS

- Endometriotic tissue gene expression profiles and urinary PB/BP levels were studied
- PB exposure was related to overexpression of cell cycle-related genes
- Increased expression of cell differentiation genes was related to PB exposure
- Upregulation of lipid metabolism-related genes was related to PB/BP exposure

GRAPHICAL ABSTRACT



ABSTRACT

Aim: To describe the expression profile in endometriotic tissue of genes involved in four signaling pathways related to the development and progression of endometriosis (cell cycle, apoptosis, cell differentiation and lipid metabolism) and to explore its relationship with the women exposure to chemicals with hormonal activity released from cosmetics and personal care products (PCPs).

Methods: This cross-sectional study, encompassed within the EndEA study, comprised a subsample of 33 women with endometriosis. Expression levels of 13 genes (*BMI1*, *CCNB1*, *CDK1*, *BAX*, *BCL2L1*, *FOXO3*, *SPP1*, *HOXA10*, *PDGFRA*, *SOX2*, *APOE*, *PLCG1* and *PLCG2*) in endometriotic tissue and urinary concentrations of 4 paraben (PB) and 3 benzophenone (BP) congeners were quantified. Bivariate linear and logistic regression analyses were performed to explore the associations between exposure and gene expression levels.

Results: A total of 8 out 13 genes (61.5%) were expressed in more than 75% of the samples. Exposure to congeners of PBs and/or BPs was associated with the overexpression of *CDK1* gene (whose protein drives cells through G2 phase and mitosis), *HOXA10* and *PDGFRA* genes (whose proteins favor pluripotent cell differentiation to endometrial cells), and *APOE* (whose protein regulates the transport and metabolism of cholesterol, triglycerides and phospholipids in multiple tissues) and *PLCG2* genes (whose protein creates 1D-myo-inositol 1,4,5-trisphosphate and diacylglycerol, two important second messengers).

Conclusions: Our findings suggest that women exposure to cosmetic and PCP-released chemicals might be associated with the promotion of cell cycle and cell differentiation as well as with lipid metabolism disruption in endometriotic tissue, three crucial signaling pathways in the development and progression of endometriosis. However, further studies should be accomplished to confirm these preliminary data.

Keywords: parabens, benzophenones, endometriosis, cell cycle, cell differentiation, lipid metabolism

1. INTRODUCTION

Endometriosis is a common gynecological disease that affects 10-15% of women of childbearing age (Zondervan et al., 2020). It is characterized by the presence of endometrial-like tissue outside the uterine cavity, commonly in the abdomino-pelvic cavity such as the pelvic peritoneum and the ovaries (Bulun, 2009; Giudice, 2010; Vercellini et al., 2014). Although the symptomatic burden varies among endometriosis patients, most of them often experience dysmenorrhea, dyspareunia, chronic pelvic pain, menstrual irregularities and infertility, leading to a significant decrease in their quality of life (Bulun, 2009).

To date, various theories, including retrograde menstruation, coelomic metaplasia, embryonic cell rest, induction, and lymphatic and vascular dissemination, have been proposed to explain the origin of this disease (Nezhat et al., 2008). However, its etiology and pathophysiology have not been fully elucidated. It has been reported aberrations in the regulation of the cell cycle and cell differentiation, such as *CDK1* and *HOXA10* genes that could be involved in the proliferation and malignant transformation, two processes linked to endometriosis (Charrasse et al., 2000; Ito et al., 2005; Tang et al., 2009; Taylor et al., 1998; Taylor et al., 1997; Van Langendonckt et al., 2010). Moreover, a reduced apoptotic index with overexpression of anti-apoptotic and downregulation of pro-apoptotic genes has been shown in endometriotic lesions (Dmowski et al., 2001; Gebel et al., 1998; Harada et al., 2004; Meresman et al., 2000; Nasu et al., 2011). In addition, an alteration of the lipid metabolism signaling pathway, which is closely linked to the inflammatory process, has been also reported in women with endometriosis (Liu et al., 2021b; Lo Vasco et al., 2012).

Currently, it is suspected that the interaction of genetic and environmental factors could be crucial in the onset and development of the disease since there is a growing number of women with this estrogen-dependent disease. For that reason, women exposure to chemicals with hormonal activity (so called endocrine-disrupting chemicals, EDCs) has been postulated as potential risk factors for endometriosis (Smarr et al., 2016). In fact, we have previously showed that the magnitude of women exposure to various congeners of parabens (PBs) and benzophenones (BPs) was related to a higher consumption of cosmetics and personal care products (PCPs) and that they could be associated with an increased risk of endometriosis (Peinado et al., 2021). The family of

PBs, which includes methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butyl-paraben (BuP) congeners, are alkyl esters of p-hydroxybenzoic acid commonly used in a wide range of PCPs, pharmaceuticals, food, and beverages for their antimicrobial and preservative properties (Błędzka et al., 2014; Darbre and Harvey, 2008; Iribarne-Durán et al., 2020; Moos et al., 2015). The congeners of the BPs, which includes BP-1, BP-3 and 4-OHBP congeners, are frequently added as synthetic UV filters and used in a wide range of cosmetics and PCPs, food packaging materials, and textiles (Molins-Delgado et al., 2016). The main routes of human exposure to PBs and BPs are dermal absorption of PCPs and consumption of pharmaceuticals and foods (Benech-Kieffer et al., 2000; Díaz-Cruz et al., 2012; Janjua et al., 2008). Both of them are considered EDCs because of their (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid actions (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Molina-Molina et al., 2008). However, the mechanisms of action of these EDCs in the pathophysiology of endometriosis are still unknown.

Therefore, the aim of this study was to describe the expression levels in endometriotic tissue of 13 genes involved in four signaling pathways related with the development and progression of endometriosis (cell cycle, apoptosis, cell differentiation and lipid metabolism), and to explore their potential associations with women exposure to PBs and BPs. Despite the variety of chemicals released from cosmetics and PCPs (including bisphenols, phthalates, camphenes, dimeticones and oxycinnamates, among others), the selection of these two families of chemicals was based on (i) the large evidence on the relationship between cosmetic and PCP consumption - PB/BP exposure - health adverse effects in women, (ii) the previous evidence supporting a relationship between PB/BP exposure and endometriosis risk (Kunisue et al., 2012; Peinado et al., 2021) and (iii) the lack of knowledge on the adverse outcome pathways between PB/BP exposure and endometriosis.

2. MATERIAL AND METHODS

2.1. Study population and sample collection

This study is enclosed in a wider research project (EndEA study, **End**ometriosis y **E**xposición **A**mbiental) focused on the elucidation of the contribution of EDCs to endometriosis and their potential mechanisms of action, which has been described elsewhere (Peinado et al., 2020b; Peinado et al., 2021). This cross-sectional study was conducted in a subsample of 33 women with confirmed endometriosis by laparotomy or laparoscopic surgery and further histological confirmation, recruited between January 2018 and July 2019 in two public hospitals ('San Cecilio' and 'Virgen de las Nieves') in Granada, Southern Spain. Inclusion criteria were: premenopausal woman aged between 20 and 54 years, receipt of abdominal surgery, and body mass index (BMI) below 35 kg/m^2. Exclusion criteria were: history of cancer (except non-melanoma skin cancer), pregnancy at study enrolment, and inability to read and sign the informed consent document. Cases were categorized in stages I/II and III/IV according to Revised American Fertility Society's classification (Canis et al., 1997). Informed consent was obtained from each participant, and the study was approved by the Research Ethics Committee of Granada.

Before surgery, all participants were clinically examined and anthropometric characteristics were collected, recording their height and weight for calculation of their body mass index (BMI). In addition, epidemiological and clinical questionnaires were completed by the women to collect sociodemographic, lifestyle and clinical information, and surgical questionnaires were completed by the surgeons, gathering data on: age (years), residence (rural or urban/sub-urban), educational level (university degree or less), working outside the home (yes or no), current smoker (yes or no), parity (nulliparous or primiparous/multiparous), average intensity of menstrual bleeding (spotting/light or moderate/heavy), and endometrioma location (deep infiltrating endometriosis or ovarian/peritoneal endometriosis). First morning urine samples were collected the same day immediately before surgery and divided into 1 mL aliquots. Endometriotic tissue samples (3 g) were collected intraoperatively by the surgeon, avoiding any potential risk to the patient's health. Both urine and endometriotic tissue samples were placed in PB- and BP-free glass tubes, anonymously coded, and immediately stored at -80 ºC at the Biobank of the Public Andalusian Healthcare

System until further laboratory analyses. Banking of biological samples and completion of questionnaires followed the standardized procedures of the EHPect project (http://endometriosisfoundation.org/ephect/).

2.2. Chemical extraction and analysis

A total of four PB [methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butyl-paraben (BuP)] and 3 BP congeners [benzophenone-1 (BP-1), benzophenone-3 (BP-3), and 4-hydroxybenzophenone (4-OHBP)] were analyzed in urine samples. Chemical extraction of PBs and BPs were carried out through dispersive liquid-liquid microextraction (DLLME), while chemical quantification was performed with a ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS), as previously described (Vela-Soria et al., 2014).

Briefly, samples were centrifuged (2600 x g for 10 min) and 1.0 mL sample was used. Then, samples were enzymatically treated with β-glucuronidase/sulfatase, previously prepared by dissolving 10 mg of β-glucuronidase/sulfatase ($3 \cdot 10^6$ U g solid^{-1}) in 1.5 mL of 1M ammonium acetate/acetic acid buffer solution (pH 5.0), in order to determine the total amount (free and conjugated) of the aforementioned chemicals. After incubation (at 37 ºC for 24 h), 20 µL of labeled standards solution (5 mg·L^{-1} of EtP–$^{13}C_6$, 2 mg·L^{-1} of BPA-D_{16}, and 2 mg·L^{-1} of BP-D_{10}) were added and the samples were diluted with 10 mL of 10% aqueous NaCl solution (pH 2.0, adjusted with 0.5 M HCl). Then a mixture of 1 mL of acetone (dispersing solvent) and 0.5 mL of trichloromethane (extraction solvent) was added, and samples were shaken manually for 30 seconds and centrifuged at 4000 x g for 10 minutes. Next, the organic phase was carefully collected from the bottom of the glass tube using a 1 mL pipette and placed in 2 mL glass vials. The extract was evaporated under a nitrogen stream, and the residue was dissolved with 100 µL of an acetonitrile/water mixture and vortexed for 30 seconds. The extract was then ready for analysis by UHPLC-MS/MS. The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a signal-to-noise ratio ≥3. The LODs obtained were 0.05 ng/mL for BP-1, 0.06 ng/mL for BP-3 and 4-OHBP, and 0.10 ng/mL for MeP, EtP, PrP, and BuP.

2.3. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from 30 mg of endometriotic tissue samples was extracted using QIAzol reagent (Qiagen, Germany) and RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA concentration was determined by spectrophotometer using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA) and the A260/A280 ratio was comprised between 1.8 and 2.2 for each sample. Reverse transcription was performed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions. For qRT-PCR, cDNA samples, as well as SsoAdvanced Universal SYBR Green Supermix and specific primers (Supplementary Table S1) purchased from Bio-Rad (Bio-Rad Laboratories, Hercules, California, USA) were used. Gene expression levels were detected using a CFX96 RealTime PCR detection system (Bio-Rad Laboratories, Hercules, California, USA), and they were standardized to the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the the $2^{-\Delta\Delta Ct}$ method.

Differential expression levels of 13 genes from 4 different cell signaling pathways was explored: cell cycle [BMI1 Proto-Oncogene, Polycomb Ring Finger (*BMI1*), Cyclin B1 (*CCNB1*) and Cyclin Dependent Kinase 1 (*CDK1*)], apoptotsis [BCL2-associated X Protein (*BAX*), BCL2 Like 1 (*BCL2L1*), Forkhead Box P3 (*FOXO3*) and Secreted Phosphoprotein 1 (*SPP1*)], cell differentiation [Homeobox A10 (*HOXA10*), Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*) and SRY-Box Transcription Factor 2 (*SOX2*)], and lipid metabolism [Apolipoprotein E (*APOE*), Phospholipase C Gamma 1 (*PLCG1*) and Phospholipase C Gamma 2 (*PLCG2*)].

2.4. Statistical analysis

Categorical variables were expressed as percentages, while urinary concentrations of individual BP and PB congeners, ΣPBs and ΣBPs, and gene expression levels were expressed as means±standard deviation and percentiles (25, 50 and 75). Given their non-normal distribution, both exposure and gene expression variables were log-transformed.

Correlations between PB and BP concentrations and gene expression levels were calculated by Spearman's rank correlation coefficient. In addition, bivariate linear regression models were used to explore associations between gene expression levels and

PB/BP exposure concentrations. Finally, alternative models were created after exposure categorization into high and low exposure (based on median value of each PB/BP congener), expressing the results as β with 95% confidence intervals. Those genes expressed in 25-75% of the samples were considered as dichotomous variables (detected/not detected), and associations with PBs and BPs were evaluated using bivariate logistic regression models. Associations with PB/BPs were not explored for those genes expressed in <25% of the samples. Moreover, the potential confounding effect of sociodemographic and reproductive characteristics was explored by introducing individual variables in the regression models.

All analyses were performed using SPSS Statistics 23.0 (IBM, Chicago, IL). Associations with p-values <0.050 were considered significant, although given the limited sample size, associations yielding p-values between 0.050 and 0.100 were also cautiously discussed. The *post-hoc* analysis to estimate the power (1-β) of the statistical analysis was conducted using G*Power 3.1.9.7 statistical software (Düsseldorf University, Düsseldorf, Germany). Considering the Spearman correlation analyses, the power of the associations found ranged from 65% to 98% (ρ coefficients ranging from 0.48 to 0.65; n=22).

3. RESULTS

3.1 Characteristics of the study population and PB and BP concentrations

Sociodemographic and reproductive characteristics of the study population are shown in Table 1. Considering the 33 cases included in this study, the mean (±standard deviation) age was 38.0 (±7.3) years old, and the majority had a normal weight (body mass index <25 Kg/m^2; n=20, 60.6%). A total of 19 women (57.6%) lived in rural areas, 24 (72.7%) hold a job outside home and 12 (36.4%) had a university degree. A total of 21 women (63.6%) reported a moderate/heavy menstrual bleeding intensity, 15 (45.5%) were nulliparous, 25 (75.8%) had ovarian/peritoneal endometriosis and 21 (63.6%) were diagnosed with stage I/II endometriosis.

Table 1. Characteristics of study population (n=33).

	n	%		n	%
Sociodemographic characteristics					
Age *(years)**	38.0 ± 7.3		**Educational level**		
Weight *(kg)**	67.6 ± 14.7		*Less than university degree*	21	63.6
Height *(m)**	1.6 ± 0.1		*University degree*	12	36.4
Body mass index *(kg/m^2)**	25.3 ± 5.3		**Working outside home**		
Normal weight (BMI < 25)	20	60.6	*Yes*	24	72.7
Overweight/Obese (BMI > 25)	13	39.4	*No*	9	27.3
Residence			**Current smoker**		
Rural	19	57.6	*Yes*	10	30.3
Urban /sub-urban	14	42.4	*No*	23	69.7
			Urinary creatinine *(ng/mL)**	152.3 ± 68.3	
Reproductive characteristics					
Parity			**Endometrioma location**		
Nulliparous	15	45.5	*Deep infiltrating endometriosis*	8	24.2
Primiparous/Multiparous	18	54.5	*Ovarian/peritoneal endometriosis*	25	75.8
Intensity of menstrual bleeding			**Endometriosis stage**		
Mild	12	36.4	*I/II*	21	63.6
Moderate/severe	21	63.6	*III/IV*	12	36.4

**Mean ± standard deviation.*

From those 33 women that comprises the entire study population, adequate urine sample volume for exposure assessment was only available from 22 cases, and therefore, associations between exposure and gene expression were accomplished in this subsample. No statistically significant differences in sociodemographic and reproductive characteristics were found between this subset of the population included in the analyzes (n=22) and the subset of the population not included in the analyses (n=11), except for the higher percentage of participants who work outside the home in the latter (Supplementary Table S2). Urinary concentrations of PBs and BPs in endometriosis patients participating in the EndEA study were previously reported (Peinado et al., 2021). Regarding our subsample (n=22), detectable concentrations of the four PB and the three BP congeners were found in all the analyzed samples, with MeP and BP-3 as the PB and BP congeners found in highest concentrations, respectively (Supplementary Table S3).

3.2 Gene expression levels and associations with PB and BP concentrations

Arithmetic means, standard deviations and percentiles of gene expression levels in endometriotic tissue from the total population are shown in Table 2. A total of 7 genes (53.8%) were expressed in more than 75% of the samples, while 3 (23.1%) were expressed in 50-75% of samples. In particular, 2 of 5 genes related to cell cycle (*BMI* and *CDK1*), 3 of 4 genes related to apoptosis (*BAX*, *BCL2L1* and *FOXO3*), 2 out 3

genes related to cell differentiation (*HOXA10* and *PDGFRA*) and 2 of 3 genes related to lipid metabolism (*APOE* and *PLCG2*) were expressed in >50% of the samples. Gene expression levels in the subsample of patients with both measurements (exposure and gene expression) (n=22) are summarized in Supplementary Table S4.

Table 2. Gene expression levels in endometriotic tissue.

Cell pathway	Gene	Total cases (n=33)						
		Expression frequency (n,%)		Mean	St. Dev	Percentiles		
						25	50	75
Cell cycle	**BMI1**	20	60.6	8.61E+07	1.14E+08	*n.e.*	7.03E+07	1.22E+08
	CCNB1	9	27.3	1.16E+05	3.02E+05	*n.e.*	*n.e.*	9.44E+04
	CDK1	30	90.9	1.66E+08	8.67E+08	7.06E+05	1.97E+06	6.98E+06
Apoptosis	**BAX**	26	78.8	2.66E+07	2.81E+07	4.78E+05	2.09E+07	4.12E+07
	BCL2L1	29	87.9	2.42E+08	7.47E+08	4.71E+07	1.03E+08	1.89E+08
	BAX/BCL2L1	26	78.8	1.13E+12	6.42E+12	1.38E-02	2.59E-01	3.59E-01
	FOXO3	32	97.0	1.54E+08	4.70E+08	2.44E+07	6.12E+07	1.09E+08
	SPP1	10	30.3	9.65E+06	4.44E+07	*n.e.*	*n.e.*	2.69E+04
Cell diferentiation	**HOXA10**	33	100.0	1.49E+08	4.31E+08	2.94E+07	5.67E+07	1.14E+08
	PDGFRA	32	97.0	7.85E+08	3.77E+09	1.13E+07	3.15E+07	1.07E+08
	SOX2	16	48.5	3.23E+06	5.26E+06	*n.e.*	4.52E+05	5.08E+06
Lipid metabolism	**APOE**	19	57.6	5.09E+07	1.86E+08	*n.e.*	1.59E+05	1.29E+06
	PLCG1	1	3.0	3.43E+03	1.91E+04	*n.e.*	*n.e.*	*n.e.*
	PLCG2	25	75.8	1.21E+06	2.44E+06	7.73E+03	1.52E+05	1.49E+06

St. Dev.: standard deviation; BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2; ApoE: Apolipoprotein E; PLCG1: Phospholipase C Gamma 1; PLCG2: Phospholipase C Gamma 2; n.e.: not expressed.

Results from the linear (or logistic) regression analyses carried out to assess the associations between urinary concentrations of PBs and BPs and gene expression levels in endometriotic tissue samples are shown in Tables 3-6. Moreover, results from Spearman correlations analyses are shown in Supplementary Table S5.

Urinary concentrations of MeP, BuP and ΣPBs were associated with increased *CDK1* expression levels (Table 3). These associations were also corroborated after exposure dichotomization, with higher expression levels of *CDK1* in those women with higher exposure to these PB congeners. A close-to-significant positive association was also observed between 4-OHBP and *BMI1* when exposure was dichotomized (p-value = 0.088). No significant associations were observed between PB exposure and *CCNB1* gene expression levels. Similarly, no associations were found between exposure to BPs and selected cell cycle-related genes.

Table 3. Association betwen PB and BP concentrations and expression of genes involved in cell cycle.

	BMI1				CCNB1				CDK1			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**	**β**	**95% CI**		**p-value**
Parabens												
MeP	0.73	0.44	1.21	0.223	0.71	0.39	1.30	0.711	***0.63***	***0.26***	***1.00***	***0.002***
<39.26 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>39.26 ng/mL	0.45	0.08	2.67	0.379	0.18	0.02	0.92	0.154	***1.99***	***0.42***	***3.56***	***0.016***
EtP	0.94	9.64	1.32	0.747	0.83	0.53	1.31	0.415	0.23	-0.54	1.00	0.535
<4.51 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>34.51 ng/mL	0.45	0.08	2.67	0.379	1.69	0.22	12.81	0.613	1.17	-0.59	2.92	0.179
PrP	1.01	0.67	1.52	0.974	1.21	0.75	1.95	0.438	0.27	-0.18	0.62	0.263
<2.54 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>2.54 ng/mL	2.22	0.38	13.18	0.379	5.71	0.52	62.66	0.154	0.08	-1.57	1.72	0.925
BuP	1.27	0.69	2.36	0.441	0.52	0.20	1.367	0.183	***0.72***	***0.23***	***1.21***	***0.007***
<0.14 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>0.14 ng/mL	1.33	0.23	7.63	0.746	0.47	0.06	3.57	0.463	***2.31***	***0.85***	***3.77***	***0.004***
ΣPBs	0.74	0.45	1.23	0.250	0.70	0.37	1.32	0.267	***0.67***	***0.30***	***1.05***	***0.001***
<53.31 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>53.31 ng/mL	0.45	0.08	2.67	0.379	0.18	0.02	1.92	0.154	***2.05***	***0.50***	***3.61***	***0.012***
Benzophenones												
BP-1	0.66	0.27	1.64	0.369	1.41	0.51	3.96	0.510	-0.25	-1.12	0.62	0.551
<1.42 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>1.42 ng/mL	0.45	0.08	2.67	0.379	1.69	0.22	12.81	0.613	-0.02	-1.83	1.88	0.980
BP-3	0.76	0.37	1.56	0.453	0.91	0.44	1.88	0.792	0.17	-0.54	0.87	0.627
<2.53 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>2.53 ng/mL	2.22	0.38	13.18	0.379	1.69	0.22	12.81	0.613	0.86	-0.95	2.67	0.330
4-OHBP	1.72	0.54	5.50	0.362	2.05	0.56	7.53	0.278	0.26	-0.85	1.38	0.626
<0.73 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>0.73 ng/mL	5.40	0.78	37.51	0.088	5.71	0.52	62.66	0.154	-0.03	-1.88	1.82	0.740
ΣBPs	0.67	0.25	1.84	0.441	1.16	0.38	3.54	0.796	-0.08	-1.13	0.97	0.877
<7.43 ng/mL	1.00	-	-		1.00	-	-		0.00	-	-	
>7.43 ng/mL	1.00	0.18	5.68	1.000	0.59	0.08	4.50	0.613	-0.39	-2.24	1.46	0.661

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1.

Regarding apoptosis-related genes, no association was observed between any PB/BP congener and *BAX*, *BCL2L1*, *BAX*/*BCL2L1* ratio nor *FOXO3* when exposure was considered as a continuous variable (Table 4). However, we observed a close-to-statistical association between increased expression levels of *BCL2L1* and higher concentrations of BuP (p-value = 0.095). In relation to *FOXO3*, decreased expression levels were observed in those with higher EtP concentrations, although the association was close to the statistical significance (p-value = 0.097). Moreover, Spearman correlation analyses also revealed a close-to-significance inverse correlation between *FOXO3* expression levels and exposure to both EtP and PrP (p-value = 0.074 and 0.096, respectively) (Supplementary Table S5).

Table 4. Association betwen PB and BP concentrations and expression of genes involved in apoptosis.

	BAX				BCL2L1				BAX/BCL2				FOXO3			
	β	95% CI		p-value	β	95% CI		p-value	β	95% CI		p-value	β	95% CI		p-value
Parabens																
MeP	0.01	-0.21	0.24	0.898	-0.04	-1.07	0.99	0.938	-0.43	-2.45	1.59	0.660	-0.11	-0.40	0.17	0.424
<39.26 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>39.26 ng/mL	0.14	-0.75	1.04	0.739	-1.17	-5.08	2.74	0.539	-3.25	-11.28	4.77	0.403	-0.06	-1.51	1.40	0.938
EtP	0.03	-0.18	0.25	0.752	-0.27	-1.11	0.57	0.505	0.21	-1.75	2.17	0.822	-0.15	-0.42	0.12	0.264
<4.51 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>34.51 ng/mL	0.26	-0.63	1.15	0.543	-1.93	-5.78	1.91	0.305	5.17	-2.57	12.91	0.176	-1.12	-2.47	0.22	0.097
PrP	0.00	-0.21	0.21	0.997	-0.02	-0.97	0.93	0.967	-0.91	-2.74	0.93	0.309	-0.22	-0.50	0.06	0.122
<2.54 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>2.54 ng/mL	-0.18	-1.06	0.69	0.661	1.29	-2.62	5.19	0.499	-2.58	-10.52	5.36	0.501	-0.92	-2.30	0.46	0.179
BuP	0.08	-0.19	0.35	0.541	0.89	-0.34	2.12	0.147	-1.20	-3.61	1.20	0.304	-0.50	-0.57	0.46	0.835
<0.14 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>0.14 ng/mL	0.52	-0.31	1.35	0.200	3.11	-0.59	6.81	0.095	-4.12	-11.82	3.58	0.273	0.21	-1.23	1.66	0.760
ΣPBs	0.03	-0.21	0.27	0.803	-0.07	-1.14	1.00	0.898	-0.48	-2.64	1.67	0.642	-0.14	-0.49	0.21	0.418
<53.31 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>53.31 ng/mL	0.01	-0.89	0.90	0.990	-1.28	-5.19	2.62	0.500	-3.26	-11.28	4.77	0.403	-0.74	-2.15	0.66	0.281
Benzophenones																
BP-1	0.15	-0.24	0.54	0.436	0.33	-1.57	2.24	0.717	1.16	-2.45	4.78	0.505	0.15	-0.54	0.85	0.652
<1.42 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>1.42 ng/mL	2.78	-0.98	6.55	0.137	1.81	-2.04	5.67	0.337	3.32	-4.70	11.34	0.393	0.95	-0.43	2.33	0.166
BP-3	-0.11	-0.46	0.24	0.505	-0.10	-1.64	1.44	0.893	2.37	-0.61	5.35	0.111	-0.13	-0.57	0.31	0.530
<2.53 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>2.53 ng/mL	-0.23	-1.10	0.64	0.585	1.56	-2.32	5.44	0.410	2.98	-4.92	10.88	0.436	0.61	-0.81	2.03	0.383
4-OHBP	0.38	-0.16	0.92	0.153	0.70	-1.7365	3.145	0.553	-2.51	-7.59	2.58	0.312	-0.06	-0.69	0.57	0.833
<0.73 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>0.73 ng/mL	0.49	-0.35	1.33	0.236	2.22	-1.59	6.03	0.237	-4.18	-11.93	3.57	0.269	0.12	-1.33	1.57	0.863
ΣBPs	0.00	-0.51	0.50	0.992	0.33	-1.95	2.61	0.766	3.38	-0.86	7.62	0.111	0.09	-0.69	0.87	0.815
<7.43 ng/mL	0.00	-	-		0.00	-	-		0.00	-	-		0.00	-	-	
>7.43 ng/mL	0.46	-0.40	1.33	0.272	1.69	-2.18	5.56	0.372	3.22	-4.81	11.26	0.407	0.60	-0.82	2.02	0.386

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1: FOXO3: Forkhead Box P3: SPP1: Secreted Phosphoprotein 1.

HOXA10 expression levels were increased in those women with higher exposure to MeP and ΣPBs, the latter association being close to the statistical significance (p-value = 0.068) (Table 5). Similarly, *PDGFRA* expression levels were increased in those women with higher concentrations of BuP, while the positive association found with the concentrations of MeP and ΣPBs did not reach the statistical significance (p-values = 0.058 and 0.052, respectively). Finally, no association was observed between PB/BP exposure and *SOX2* expression levels.

Table 5. Association betwen PB and BP concentrations and expression of genes involved in cell differentiation.

	HOXA10				PDGFRA				SOX2			
	β	95% CI		p-value	β	95% CI		p-value	OR	95% CI		p-value
Parabens												
MeP	0.13	-0.05	0.30	0.145	0.21	-0.09	0.50	0.159	0.75	0.36	1.57	0.446
<39.26 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>39.26 ng/mL	***0.79***	***0.20***	***1.38***	***0.012***	1.04	-0.04	2.11	0.058	0.43	0.06	2.97	0.391
EtP	0.03	-0.12	0.17	0.720	0.11	-0.14	0.35	0.376	1.02	0.58	1.81	0.936
<4.51 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>34.51 ng/mL	-0.05	-0.75	0.66	0.893	-0.10	-1.30	1.09	0.858	0.22	0.03	1.71	0.148
PrP	-0.02	-0.19	0.14	0.785	0.13	-0.12	0.36	0.287	1.23	0.72	2.13	0.450
<2.54 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>2.54 ng/mL	-0.37	-1.06	0.31	0.268	-0.04	-1.23	1.16	0.948	4.50	0.59	34.61	0.148
BuP	0.08	-0.15	0.31	0.459	***0.51***	***0.12***	***0.91***	***0.014***	0.55	0.25	1.17	0.118
<0.14 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>0.14 ng/mL	0.47	-0.20	1.14	0.160	1.02	-0.31	2.35	0.123	0.67	0.10	4.35	0.672
ΣPBs	0.13	-0.06	0.31	0.163	0.22	-0.08	0.52	0.142	0.76	0.35	1.62	0.471
<53.31 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>53.31 ng/mL	0.60	-0.05	1.24	0.068	1.06	-0.01	2.13	0.052	0.43	0.06	2.97	0.391
Benzophenones												
BP-1	-0.28	-0.73	0.18	0.217	-0.56	-1.42	0.29	0.185	2.02	0.74	5.48	0.168
<1.42 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>1.42 ng/mL	-0.45	-1.41	0.50	0.330	-0.78	-2.57	1.02	0.378	4.50	0.59	34.61	0.148
BP-3	-0.13	-0.39	0.14	0.332	-0.09	-0.60	0.43	0.733	0.89	0.47	1.70	0.725
<2.53 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>2.53 ng/mL	-0.39	-1.08	0.29	0.241	0.06	-1.36	1.47	0.936	1.50	0.23	9.80	0.672
4-OHBP	0.03	-0.40	0.46	0.900	-0.27	-1.13	0.58	0.509	1.30	0.39	4.38	0.667
<0.73 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>0.73 ng/mL	0.00	-0.70	0.71	0.997	-0.39	-1.80	1.01	0.564	1.02	0.16	6.42	0.958
ΣBPs	-0.53	-1.24	0.17	0.126	-0.45	-1.04	0.14	0.129	1.42	0.53	3.80	0.479
<7.43 ng/mL	0.00	-	-		0.00	-	-		1.00	-	-	
>7.43 ng/mL	-0.27	-1.52	0.98	0.658	-0.63	-1.78	0.52	0.265	2.50	0.37	16.89	0.347

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2.

A close-to-significant association was found between *APOE* overexpression and 4-OHBP concentrations (p-value = 0.061) (Table 6). A similar but significant association was observed when 4-OHBP exposure was considered as a dichotomous variable. Finally, overexpression of *PLCG2* was found to be associated with PrP concentrations,

which was also was corroborated in Spearman correlation analyses (Supplementary Table S5). Moreover, overexpression of *PLCG2* was observed in those women with higher BP-3 concentrations.

No confounding effect was observed for sociodemographic and reproductive characteristics in associations found, except for educational level and working outside home (Supplementary Table S6).

Table 6. Association betwen PB and BP concentrations and expression of genes involved in lipid metabolism.

	APOE				PLCG2			
	OR	**95% CI**		**p-value**	**β**	**95% CI**		**p-value**
Parabens								
MeP	0.90	0.57	1.42	0.652	0.02	-0.62	0.66	0.942
<39.26 ng/mL	1.00	-	-		0.00	-	-	
>39.26 ng/mL	0.31	0.05	1.85	0.200	0.72	-1.33	2.78	0.462
EtP	1.01	0.69	1.47	0.967	-0.05	0.54	0.44	0.826
<4.51 ng/mL	1.00	-	-		0.00	-	-	
>34.51 ng/mL	1.46	0.26	8.05	0.665	-0.50	-2.57	1.58	0.617
PrP	1.09	0.73	1.63	0.682	***0.50***	***0.02***	***0.99***	***0.043***
<2.54 ng/mL	1.00	-	-		0.00	-	-	
>2.54 ng/mL	3.20	0.54	18.98	0.200	1.73	-0.12	3.58	0.064
BuP	0.79	0.45	1.392	0.412	0.59	-0.28	1.47	0.167
<0.14 ng/mL	1.00	-	-		0.00	-	-	
>0.14 ng/mL	0.93	0.17	5.15	0.937	1.07	-0.94	3.07	0.272
ΣPBs	0.89	0.55	0.42	0.612	0.05	-0.60	0.70	0.872
<53.31 ng/mL	1.00	-	-		0.00	-	-	
>53.31 ng/mL	0.69	0.12	3.78	0.665	1.29	-0.69	3.27	0.185
Benzophenones								
BP-1	1.63	0.67	3.97	0.283	0.20	-0.77	1.17	0.667
<1.42 ng/mL	1.00	-	-		0.00	-	-	
>1.42 ng/mL	1.46	0.26	8.05	0.665	0.08	-2.03	2.19	0.935
BP-3	1.05	0.56	1.97	0.887	0.20	-0.47	0.87	0.538
<2.53 ng/mL	1.00	-	-		0.00	-	-	
>2.53 ng/mL	3.20	0.54	18.98	0.200	***1.93***	***0.14***	***3.73***	***0.037***
4-OHBP	4.13	0.94	18.21	0.061	0.28	-0.87	1.43	0.613
<0.73 ng/mL	1.00	-	-		0.00	-	-	
>0.73 ng/mL	***7.88***	***1.11***	***56.12***	***0.039***	0.15	-1.95	2.24	0.884
ΣBPs	1.55	0.59	4.11	0.378	0.31	-0.71	1.32	0.530
<7.43 ng/mL	1.00	-	-		0.00	-	-	
>7.43 ng/mL	3.20	0.54	28.98	0.200	0.68	-1.37	2.74	0.487

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; APOE: Apolipoprotein E; PLCG2: Phospholipase C Gamma 2.

4. DISCUSSION

To our best knowledge, this is the first study to explore the potential association between exposure to EDCs released from cosmetics and PCPs, and disruption in the expression profiles of genes involved in cell cycle, apoptosis, cell differentiation, and lipid metabolism in the ectopic tissue from women with endometriosis. Most of the genes studied were expressed in the majority of the samples, and exposure to certain PB and BP congeners were related to the overexpression of genes involved in cell cycle promotion, cell differentiation and lipid metabolism disruption.

Cell cycle has been identified as a crucial cell signaling pathway altered in women with endometriosis (Sahraei et al., 2022) with overexpression of cell cycle promoting genes in ectopic tissue (Tang et al., 2009). Recent studies have also related exposure to PBs/BPs with deregulation of a variety of cell cycle genes in *in vivo* models of gynecological disorders such as ovarian cancer (Park et al., 2013) and altered steroidogenesis and antral follicle growth (Gal et al., 2019). In line with these previous studies, we have observed that *CDK1* gene overexpression was associated with exposure to PBs. Lee et al. (2014) also reported a positive association between exposure to bisphenol A (BPA), another estrogenic EDC, and *CDK1* expression levels in breast cancer cell lines. *CDK1* is a crucial gene for cell cycle regulation, orchestrating the G2 to M transition (Jin et al., 1998) and has been reported to be involved in the development of endometriosis (Chen et al., 2021; Tang et al., 2009). Moreover, we have also detected a close-to-significant overexpression of *BMI1* in those women with higher exposure to 4-OHBP. *BMI1* is another key gene involved in cell cycle regulation, which is negatively regulated by p16 and p19 tumor suppressor proteins (Bracken et al., 2007; Bruggeman et al., 2005; Jacobs et al., 1999; Molofsky et al., 2005). Although the role of *BMI1* in the pathophysiology of endometriosis is still unknown, increased *BMI1* levels have been detected in endometriotic tissues (Forte et al., 2009; Ponandai-Srinivasan et al., 2020). In line with these previous findings, our results suggest that exposure to 4-OHBP might contribute to the upregulation of *BMI1* gene, deregulating the cell cycle and, thus, favoring the proliferation of endometriosis. A recent study also described the disruptive potential of BPA in *BMI1* gene regulation (Li et al., 2021).

Cell differentiation is another important process in the endometriosis pathophysiology, with some theories suggesting that the origin of this disease might be the result of the

differentiation of various cell types into endometrial-like cells (Klemmt and Starzinski-Powitz, 2018). Several genes could be involved in this process, including the *HOXA10* gene. *HOXA10* gene has been related to the differentiation of embryonic tissues to endometrial tissue and it was shown to be abnormally expressed in different types of endometriotic lesions (Browne and Taylor, 2006; Van Langendonckt et al., 2010; Zanatta et al., 2015). The results of our study showed increased expression in endometriotic tissues from those women with higher concentrations of MeP. *PDGFRA* gene, that encodes a protein that participates in the activation of a series of proteins involved in a variety of signaling pathways, such as multipotent stem cell differentiation as well as cell growth, proliferation and survival (Bartoletti et al., 2020), is also overexpressed in ectopic tissues in comparison with eutopic endometrium (Matsuzaki et al., 2006). Our findings showed a positive association between exposure to BuP and *PDGFRA* and close-to-statistical associations with exposure to MeP and ΣPBs. Thus, taken together, these findings might support the hypothesis of a potential role of exposure to cosmetic- and PCP-released EDCs favouring differentiation of multiponent stem cells into endometriotic cells.

Alterations in lipid metabolism have also been related to the onset and progression of endometriosis (Lu et al., 2023), identifying the deregulation of several lipids such as phosphatidylcholines, sphingomyelins, phosphatidylethanolamines, and triglycerides (Adamyan et al., 2018; Dutta et al., 2016; Ortiz et al., 2021). In fact, some of these deregulated lipids have proposed as potential biomarkers for early diagnosis of endometriosis (Li et al., 2018). In this sense, ApoE protein, that plays an important role in lipid metabolism (Chen et al., 2005) have been found to be highly expressed in the follicular fluid of patients with endometriosis in comparison with controls without endometriosis (Liu et al., 2021b). The results of our study showed increased odds of *APOE* gene expression in endometriotic tissue in women with higher concentrations of 4-OHBP. Similarly, we have also observed that women exposure to PrP and BP-3 was associated with increased expression levels of the *PLCG2* gene, which encodes a crucial enzyme in the phosphoinositide signal transduction system. This is in agreement with a previous studies that demonstrated that *PLCG2* was upregulated in endometrial cancer cell lines in response to exposure other EDCs used in cosmetics such as phthalates (Song and Cho, 2014). Therefore, our findings suggest that exposure to different

congers of PBs and BPs might deregulate lipid metabolism in endometriotic lesions (Lu et al., 2023).

Apoptosis, i.e. the efficient elimination of cells from tissue without eliciting an inflammatory response (Kerr et al., 1972), is another physiological process that has been found to be deregulated in endometriotic tissues (Béliard et al., 2004). Hence, various genes involved in apoptosis have been shown to be deregulated in endometriotic tissues, with a decreased expression of the pro-apoptotic genes *BAX* (Meresman et al., 2000), and an overexpression of the anti-apoptotic gene *BCL2L1* (Meresman et al., 2000). Despite a variety of apoptosis-related genes have been explored in this study, only few close-to-significant associations was detected between exposure to BuP and overexpression of *BCL2L1* anti-apoptotic gene and lower expression of the pro-apoptotic *FOXO3* gene suggesting that apoptosis might not be crucially deregulated by women exposure to these chemicals.

The findings of this study should be taken with caution because it has some limitations: firstly, the sample size was relatively small that could decrease the statistical power of explored associations. However, it is worth to mention that it is highly challenging to gather endometriotic tissue samples for investigation. Secondly, the fact that undergoing elective surgery for endometriosis was considered one of the inclusion criteria might have introduced a selection bias. Therefore, despite our study population included 63.6% of women with stage I/II endometriosis and 37.4% with stage III/IV endometriosis, the extrapolation of these study findings should be made with caution. Moreover, the assessment of exposure through a spot urine specimen prevented consideration of the variability in daily exposure to analytes with a relatively short elimination half-life. Nevertheless, samples were all first-morning urine samples, evidenced to be representative of the exposure (Deng et al., 2023), taken during hospitalization before endometriosis surgery. In addition, only two families of EDCs were measured, with no evaluation of the combined effect of PBs and BPs alongside other EDCs. This is of relevance, given the increasing interest in the mixed impact of multiple EDC exposures on human health. Regarding outcome assessment, the random bias that might be attributable to the selected genes was reduced, at least in part, using a wide panel of genes from four different cell signaling pathways. Finally, the epidemiological findings of this study were not corroborated through *in vivo* studies. This study have some strengths that should be also considered such as the measurement

of numerous genes involved in cellular pathways related to the pathophysiology of endometriosis in endometriotic tissue. Moreover, this study has identified plausible and consistent associations between gene expression and exposure to certain PB/BP congeners. The combined investigation of biomarkers of exposure and potential biomarkers of effect yielded evidence of different pathways for adverse outcomes in endometriosis.

Taken together, our findings suggest that women exposure to cosmetic- and PCP-released EDCs (including PBs and BPs) might be associated with altered gene expression profiles related to the cell cycle, cell differentiation and lipid metabolism in endometriotic tissues. Given the novelty of these results, further studies with larger sample sizes and *in vivo* corroboration of findings are warranted to shed some light on the impact of human exposure to EDCs on the pathophysiology of endometriosis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Francisco M. Peinado: Investigation, Formal analysis, Writing - original draft, Alicia Olivas-Martínez: Investigation, Formal analysis, Luz M. Iribarne-Durán: Investigation,

Alfredo Ubiña: Resources, Writing-Review & Editing, Josefa León: Investigation, Writing-Review & Editing, Fernando Vela-Soria: Investigation, Jorge Fernández-Parra: Resources, Writing-Review & Editing, Mariana F. Fernández: Writing-Review & Editing, Nicolás Olea: Writing-Review & Editing, Carmen Freire: Investigation, Writing-Review & Editing, Olga Ocon-Hernández: Conceptualization, Supervision, Funding acquisition, Writing-Review & Editing, Francisco Artacho-Cordón: Conceptualization, Methodology, Supervision, Formal analysis, Funding acquisition, Writing-Review & Editing.

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Chemical analysis

Liquid and semisolid samples

Detectable concentrations of MeP, EtP and PrP were only found in one cream sample, with a maximum concentration of 61.13 ng/g. On the other hand, detectable concentrations of BP-3 were found in four of the received cosmetic products, albeit at low concentrations (0.53-0.70 ng/g). The rest of the PB and BP congeners were not detected (Table 4).

Solid samples

No detectable concentrations of PBs and/or BPs were found in any of the plastic containers tested, regardless of the methodological condition used (Table 4).

Table 4. PB and BP concentrations of Inves Biofarm samples (ng/g).

Type of sample	Sample ID	Paraben congeners				Benzophenone congeners				
		MeP	EtP	PrP	BuP	BP-1	BP-3	4-OHBP	BP-6	BP-8
Liquid samples	L2639	ND	ND	ND	ND	ND	ND	ND	ND	ND
Semisolid samples	F182	ND	**3.48**	**3.04**	ND	ND	**0.70**	ND	ND	ND
	F128	ND	ND	ND	ND	ND	**0.59**	ND	ND	ND
	F192	**61.13**	ND	ND	ND	ND	**0.53**	ND	ND	ND
	F86	ND	ND	ND	ND	ND	ND	ND	ND	ND
	F89	ND	ND	ND	ND	ND	**0.53**	ND	ND	ND
	F53	ND	ND	ND	ND	ND	ND	ND	ND	ND
Solid samples	B30 (Condition A)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B30 (Condition B)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B30 (Condition C)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B30 (Condition D)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B50 (Condition A)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B50 (Condition B)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B50 (Condition C)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B50 (Condition D)	ND	ND	ND	ND	ND	ND	ND	ND	ND

MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP-6: benzophenone-6 and BP-8: benzophenone-8; ND: not detected.

Biological analysis

Liquid and semisolid samples

Some of the cosmetic products showed both estrogenic and anti-androgenic activities, showing maximum values of 527.10 E_2Eq/g and 3.87 ProcEq/g, respectively. The liquid sample did not show any type of hormonal activity (Table 5).

Solid samples

The plastic containers showed both estrogenic and antiandrogenic activity only when the chemical extraction methodology had been performed with chloroform and acetonitrile (conditions A and B) (Table 5).

Table 5. Estrogenic (E-Screen) and anti-androgenic (PALM) activities of Inves Biofarm samples.

Type of sample	Sample ID	E-Screen bioassay	PALM luciferase assay
		E_2Eq/g (pM)	ProcEq/g (mM)
Liquid samples	L2639	ND	ND
Semisolid samples	F53	527.10	2.96
	F81	ND	ND
	F89	317.02	2.28
	F128	317.85	3.42
	F182	503.81	3.87
	F192	ND	ND
Solid samples	B30 (Condition A)	459.88	1.52
	B30 (Condition B)	345.52	1.39
	B30 (Condition C)	ND	ND
	B30 (Condition D)	ND	ND
	B50 (Condition A)	562.64	1.89
	B50 (Condition B)	183.26	2.48
	B50 (Condition C)	ND	ND
	B50 (Condition D)	ND	ND

E_2Eq: estradiol equivalents; ProcEq: procymidone equivalents; ND: not detected.

6. GENERAL DISCUSSION

This PhD thesis has evaluated from a holistic point of view the possible effect of exposure to EDCs through cosmetics and PCPs on the risk of endometriosis, investigating some of the underlying mechanisms involved in both the genesis and development of this disease. The results obtained suggest that: (1) concentrations of hormone-active chemicals, namely PBs and BPs, present in menstrual blood, a matrix in intimate contact with the endometrium, could promote the generation of an appropriate microenvironment for the onset and development of endometriosis; (2) the frequency of use of cosmetics and PCPs is a strong predictor of exposure to specific PB and BP congeners; (3) urinary concentrations of PBs and BPs are associated with an increased risk of endometriosis in women of childbearing age, in an oxidative stress-independent manner; (4) exposure of women to PBs and BPs is associated with an altered expression profile of genes related to cell signaling pathways involvement in the development of endometriosis.

Although several theories have been postulated to explain the origin of endometriosis, the theory of retrograde menstruation is the most widely accepted (Peinado et al., 2021; Sampson, 1927a). However, given that this is a common physiological process that appears in 90% of women of menstrual age, it has been suggested that the existence of a permissive uterine microenvironment would be necessary for the disease to originate. The results obtained in this PhD thesis showed for the first time the presence of PBs and BPs in menstrual blood and, therefore, in gynecological tissues in intimate contact with the endometrium. Moreover, these concentrations were not correlated (or were weakly correlated) with serum concentrations in peripheral blood, which were higher, suggesting that menstrual blood could provide additional information to that obtained in matrices widely used to measure exposure, such as urine or serum [**article 1**, (Iribarne-Durán et al., 2020)]. Therefore, menstrual blood could be a useful matrix for exploring the possible effects of EDCs on gynecological disorders, including endometriosis. Few studies have previously explored differences in the composition of menstrual blood and peripheral blood (van der Molen et al., 2014; Yang et al., 2012), finding differences between both matrices and showing lower levels of PBs and BPs in menstrual blood, in agreement with our results.

Although there is a growing number of studies exploring associations between EDC exposure and the risk of estrogen-dependent gynecological disorders, such as anovulation, miscarriage, preterm birth, polycystic ovarian syndrome, fybroids, and

infertility among others (Costa et al., 2014; Lathi et al., 2014; Palioura and Diamanti-Kandarakis, 2015; Peretz et al., 2014; Pollack et al., 2015; Shen et al., 2013; Ziv-Gal and Flaws, 2016), studies on the possible effects that EDC exposure could have on endometriosis are still scarce. To date, only two studies have explored the relationship between BP exposure and endometriosis risk (Kunisue et al., 2012; Lee and Eata, 2022). Kunisue et al. (2012) suggested that BP-3 exposure may be associated with an increased risk of endometriosis, with a 65% increase in women with the highest BP-3 concentrations compared to those women with lower concentrations. Lee and Eata (2022), however, found no significant results after exploring this association. These contradictory results suggested us to evaluate the possible associations between urinary concentrations of several PBs and BPs and the risk of endometriosis, thus being the first study to explore the exposure to PBs and their association with this disease. The results obtained in this thesis corroborated those reported by Kunisue et al. (2012), which suggested that higher urinary concentrations of MeP, BP-1, BP-3 and ΣBPs could increase the risk of endometriosis in women of childbearing age [**article 2**, (Peinado et al., 2021)].

The mechanisms of action through which EDCs may exert their effect are still unknown. Previous evidence has suggested that they alter the homeostasis of the endocrine system by interfering with a variety of hormone-signaling pathways (La Merrill et al., 2020), exerting, for example, (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid activities (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Molina-Molina et al., 2008). In recent decades, complementary mechanisms of action have been suggested, such as inflammation or oxidative stress, which could play an important role (Artacho-Cordón et al., 2019; Mustafa et al., 2015; Thompson et al., 2015; Watkins et al., 2015). It is also suspected that EDC exposure could trigger cell signaling pathways that could promote the development of certain gynecological disorders, including endometriosis. Therefore, after exploring the possible associations between exposure to PBs and BPs and the risk of endometriosis, the next objective of this PhD thesis was to explore the different mechanisms of action through which exposure to these families of EDCs could induce the development of endometriosis. First of all, oxidative stress was evaluated, investigating the role of oxidative biomarkers in the endometriosis risk. Our results showed a borderline positive association between TBARS concentrations and the

disease, and an inverse relationship between TAP concentrations and the risk of disease [**article 2**, (Peinado et al., 2021)]. These results were in line with previous evidence, reporting positive associations between TBARS and endometriosis (Jackson et al., 2005; Nasiri et al., 2017; Singh et al., 2013), and of lower serum TAP concentrations in women with endometriosis *versus* controls (Liu et al., 2013; Nasiri et al., 2017; Singh et al., 2013; Turgut et al., 2013; Turkyilmaz et al., 2016). Oxidative stress is suspected to play a key role in the pathophysiology of endometriosis (Amreen et al., 2019; Gupta et al., 2006; Ito et al., 2017; Lambrinoudaki et al., 2009; Scutiero et al., 2017), and emerging evidence indicates that BP and PB exposure could alter systemic and local redox balance. It has also been suggested that EDCs might induce oxidative stress, at least in part, through estrogen receptor-α signaling pathways (Cho et al., 2018). In this regard, our group has also proposed that oxidative stress could mediate the association between exposure to bisphenols exposure and increased risk of endometriosis (Peinado et al., 2020b), although it does not appear to mediate the association of exposure to PBs or BPs with risk of endometriosis. However, the possible mediating effect of oxidative stress on the aggressiveness and progression of endometriosis warrants further investigation.

Although the pathophysiology of endometriosis is still unknown, it has been also suggested that, together with a depressed immune system, different molecular processes must occur for the generation of a uterine microenvironment that allow the proliferation of endometriotic lesions. In this regard, Seli et al. (2003) described five key steps in the development and progression of endometriotic lesions, including (1) cell adhesion, (2) invasion, migration and metastasis, (3) inflammation, (4) angiogenesis and (5) cell proliferation. Alterations in other molecular processes could also favor disease development, such as cell cycle, cell differentiation, apoptosis and lipid metabolism (Liu et al., 2021b; Lo Vasco et al., 2012; Nasu et al., 2011; Sahraei et al., 2022; Van Langendonckt et al., 2010).

This PhD thesis has described, for the first time, the expression profile of several key genes involved in some of the main cell signaling pathways related to endometriosis in human endometriotic tissue, as well as their potential association with EDC exposure. Thus, the association between urinary PB and BP concentrations and the expression profiles of 33 different genes related to the the cell signaling pathways mentioned above and involved in the pathophysiology of endometriosis were evaluated [**article 4**

(submitted to Ecotoxicology and Environmental Safety) **& article 5** (Peinado et al., 2023b)]. The results showed that different PB congeners are positively associated with the expression levels of genes involved in cell adhesion processes, such as, for example, with *ITGB2* and *CLDN7* levels. Other studies have shown, however, opposite studies, reporting down-regulated *CLDN7* levels in human endometriotic lesions (Gaetje et al., 2008), and upregulated *CLDN7* levels, but in ovarian cancer cell lines (Dahiya et al., 2011). When we considered invasion, migration and metastasis processes, an overexpression of *MMP1* was positively associated with exposure to MeP, ∑PBs, and ΣBPs [**article 4**, (submitted to Ecotoxicology and Environmental Safety)]. These results are supported by *in vitro* studies, which reported positive associations between exposure to PBs and increased levels of *MMPs* in dermal fibroblasts (Majewska et al., 2017), and in breast cancer cells (Khanna et al., 2014). Other genes related to this molecular process, such as *RHOB*, *FUT8*, *RRM2* and *SPRY2*, were also associated with exposure to certain PB congeners. However, these genes have not been previously studied in endometriosis and future studies are needed to elucidate their role in the development of the disease.

Inflammation plays a central and fundamental role in the pathophysiology of endometriosis, and both cell adhesion and invasion processes are promoted by a complex inflammatory microenvironment, which is also important for the development of angiogenesis and proliferation of endometriotic lesions. For that reason, in this PhD thesis a systematic review was carried out, summarizing the current evidence on the relationship between human exposure to non-persistent EDCs, including PBs and BPs, and inflammatory biomarkers [**article 3**, (Peinado et al., 2023a)]. Although inflammation has been postulated as a possible mechanism of action of EDCs, and plays a fundamental role in the development of numerous diseases (Mustafa et al., 2015), only a very few studies have explored the associations between PBs and/or BPs and inflammation (n=6 and n=4, respectively). MeP and PrP were associated with higher CRP levels (Huang et al., 2017), while MeP concentrations were related to increased serum levels of IL-6 (Tsen et al., 2021). These results were supported by an *in vitro* study, reporting consistent positive associations between inflammation and exposure to PBs (Inderbinen et al., 2022). The mechanisms of action that could explain the associations between environmental pollutant exposure and inflammation may be related to the xenoestrogenic activity exhibited by some families of EDCs. Previous

evidence has shown that low endogenous estrogenic activity may promote the production of proinflammatory cytokines (Kovats, 2015). Because these chemicals have lower affinity for ERs than estradiol (almost 1000-fold), they may bind to ERs more actively when estrogen levels are low, which in turn would trigger physiological responses associated with inflammation (Ashley-Martin et al., 2015). Nevertheless, inverse associations between exposure to some EDCs, such as EtP and BP-3, and inflammation have also been reported (Tsen et al., 2021). Given the limitations of the included studies, well-conducted studies in the general population, but also in vulnerable groups, that futher assess exposure to both individual EDCs and mixtures of EDCs are required in the near future to clarify whether inflammation could act as a nexus between exposure to these EDCs and human health.

In this PhD thesis, the associations between PB and BP exposure and the expression levels of inflammation-related genes were explored. In this regard, an upregulation of the *IL1RL1*, *IL6ST*, *TNFRSF1B* and *NR3C1* genes was found to be associated with PB and BP exposure. Furthermore, in line with previous studies reporting increased *ANG*, *VEGFA*, and *sVEGFR-1* expression in the peritoneal fluid of women with endometriosis (Cho et al., 2012), the results of this PhD thesis showed that urinary concentrations of various PB congeners were positively associated with *ANG*, *ANGPT1*, and *sVEGFR-1* expression levels, suggesting that exposure may promote the angiogenesis process. For the proliferation of endometriotic lesions to occur, a specific hormonal microenvironment, closely related to steroid metabolism and associated pathways, is necessary (Giudice and Kao, 2004; Greene et al., 2016). Positive associations were reported between BP-1 and 4-OHBP concentrations and *STAR* gene levels, indicating that exposure to these families of EDCs could be related to increased E_2 production in endometriotic tissue. Our results indicate that this exposure contributes to the sensitivity of this tissue to circulation E_2 [**articles 4**, (submitted to Ecotoxicology and Environmental Safety)].

Exposure to PBs and BPs has also been associated with overexpression of cell cycle-related genes, such as *CDK1* gene, whose protein drives cell through G2 phase and mitosis. Genes related to cell differentiation, such as *HOXA10* and *PDGFRA*; and to lipid metabolism, such as *APOE* and *PLCG2*, are also positively associated with EDC exposure [**article 5**, (Peinado et al., 2023b)]. Our results follow the same trend of previous *in vivo* studies, which have linked PB/BP exposure with alterations of genes

involved in the cell cycle (Gal et al., 2019; Park et al., 2013). Some theories on the etiology of endometriosis suggest differentiation of different cell types to endometrial-like cells (Klemmt and Starzinski-Powitz, 2018). In this regard, previous studies have shown that the *HOXA10* gene is involved in the differentiation of embryonic tissues to endometrial tissue and aberrant expressions of this gene have been observed in endometriotic tissue (Browne and Taylor, 2006; Van Langendonckt et al., 2010; Zanatta et al., 2015). *PDGFRA* has also been shown to be overexpressed in ectopic tissue compared to eutopic endometrium (Matsuzaki et al., 2006). Therefore, in summary, the results of this PhD thesis, together with the results of previous studies supporting them, suggest a potential role of exposure to PBs and BPs in the differentiation of multipotent stem cells into endometriotic cells. Finally, alterations in lipid metabolism have been linked to the onset and progression of endometriosis (Lu et al., 2023). Our results showed a positive association between 4-OHBP exposure and *APOE* gene levels. Previous studies have shown increased APOE protein expression in the follicular fluid of patients with endometriosis compared to controls. A positive association has also been observed between PrP and BP-3 exposure and increased *PLCG2* gene expression levels, suggesting that exposure to these families of EDCs may deregulate lipid metabolism in endometriotic lesions (Lu et al., 2023).

Although previous evidence suggests that decreased apoptosis may be associated with endometriosis, showing decreased expression of pro-apoptotic genes, such as *BAX* (Meresman et al., 2000) and increased expression of anti-apoptotic genes, such as *BCL2L1* (Meresman et al., 2000), the results shown in this PhD thesis showed no significant associations between exposure to PBs and BPs and levels of apoptosis-related genes.

Finally, taken together, the results shown in this PhD thesis suggest that human exposure to EDCs present in cosmetics and PCPs (including PBs and BPs) may be associated with altered gene expression profiles involving major cell signaling pathways related to endometriosis.

In recent decades, due to the modernization of society and changes in lifestyle, the frequency of use of cosmetics and PCPs has increased considerably. Given the wide variety of products and usage patterns, different formulations and individual susceptibility (Darbre and Harvey, 2008), it is difficult to establish the potential effect

of the use of cosmetics and PCPs on human health. Currently, it is suspected that the daily use of these products could be related to exposure to EDCs and thus to increased risk of various hormonal diseases, such endometriosis (Buck Louis et al., 2011; Cohn et al., 2015; Pastor-Barriuso et al., 2016; Reaves et al., 2015). Chemical analyses of these products have revealed a large number of EDCs present in cosmetics and PCPs, among which the PBs and BPs families are commonly found (Gao and Kannan, 2020; Panico et al., 2019), and it is suggested that these hormone-active chemicals would be responsible for causing negative health effects as a consequence of the use to these products. The results of this PhD thesis have shown positive associations between the use of cosmetics and PCPs and the concentrations of some congeners of PBs and BPs in menstrual blood, findings supported by previous studies, which showed associations between the frequency of PCPs use and higher urinary concentrations of MeP and BPs (Berger et al., 2019; Ko et al., 2016). In addition, the frequency of use of certain products/treatments, including lipstick, face masks and creams, hair products (dyes, lacquers, creams, and foam) and pedicure was positively associated with urinary concentrations of PBs and BPs. These results follow the same line of previous studies, evidencing the presence of PBs and BPs in cosmetics and PCPs (Gao and Kannan, 2020; Han et al., 2016; Panico et al., 2019). It would therefore be of utmost interest to establish preventive measures to reduce exposure to EDCs in cosmetics and PCPs as much as possible in order to reduce the risk of numerous hormone-dependent diseases, including endometriosis.

The Spanish Royal Decree (85/2018) for the regulation of the production of cosmetic products, in line with the European Cosmetic Regulation (July 11, 2013), tightened the quality control requirements for the production and marketing of cosmetics. Therefore, quality control of the products marketed by companies acquires great importance, avoiding the use of EDCs in their manufacture, which would favor the decrease of human exposure to these compounds. An important part of this PhD thesis has been carried out in collaboration with the private company Inves Biofarm, dedicated to the development of cosmetic and therapeutic products. The aim of the collaboration was to determine the presence of EDCs and to evaluate the hormonal activity (estrogenic and antiandrogenic) of different cosmetic products, active ingredients, and packaging used by the company. The results of the chemical analyses performed showed that no plastic packaging or active ingredient had detectable concentrations of PBs and BPs. Detectable concentrations of MeP, EtP and PrP were only found in one cosmetic

product supplied, and BP-3 was detected in four others. Regarding biological analyses, the plastic containers showed estrogenic and anti-androgenic activity when the sample extraction methodology included acetonitrile and dichloromethane. Under less aggressive conditions, no hormonal activity was detected. The active ingredient also showed no hormonal activity, but more than half of the cosmetic samples showed estrogenic and antiandrogenic activity. The hormonal activity in cosmetics and packaging with undetected concentrations of PBs and BPs could be due to the existence of other families of EDCs that could be part of the composition of these compounds, such as bisphenols, pftalates, oxycinnamates, camphenes and dimethicones, among others. The collaboration with the company continues with the aim of developing and commercializing EDC-free cosmetic products, essential to reduce the exposure of the population to these compounds and thus prevent the risk of certain diseases.

This PhD thesis has several limitations that should be taken into account when interpreting the results obtained. First, the sample size was limited, reducing the statistical power of the studies. The difficulty in collecting menstrual blood samples from the general population and the decreasing number of patients with endometriosis undergoing surgery made both case selection and collection of endometriotic tissue samples difficult, limiting the possibility of achieving a larger sample size. The collection of endometriotic tissue samples is highly challenging and, although surgery is considered the “gold standard” for a reliable diagnosis, in recent years fewer women with endometriosis are undergoing surgery and current medicine tends to use more conservative and less invasive treatments. Furthermore, to avoid possible selection bias, only women with a surgical diagnosis and subsequent histological confirmation were selected as potential study participants. This confers additional value to our work, although the sample size is limited. Nevertheless, statistically significant associations could be identified, even after adjustment for possible confounders. Another limitation is the collection of only one urine sample from each participant, which precludes analysis of variability in daily exposure to the selected pollutants with relatively short elimination half-lives. Nevertheless, all samples were collected early in the morning during hospitalization prior to endometriosis surgery, and concentrations of both PBs and BPs were comparable with those previously reported in similar female population (Pollack et al., 2020). The use of systemic oxidative stress biomarkers, such as TBARS and TAP, could constitute an additional limitation. In this regard, future studies are

needed to evaluate the potential mediating effect of *in situ* redox status on the association between PB and BP exposure and risk of endometriosis. Finally, only two families of EDCs present in cosmetics and PCPs were explored, and therefore future research is needed to explore the effects on endometriosis risk of other families of EDCs in combination with PBs and BPs.

Despite the previously mentioned limitations, this PhD thesis is the first study to determine the presence of PBs and BPs in menstrual blood, address the possible association of PBs exposure with endometriosis risk and one of the first to explore the association of BPs exposure. Moreover, given that the evidence on gene expression profile in endometriotic tissues is very limited, and to date no study has correlated the expression profile with chemical exposure, this PhD thesis acquires special relevance, constituting a first approach on this issue, shedding some light on the impact of human exposure to EDCs on the pathophysiology of endometriosis. The combined investigation of biomarkers of exposure and potential biomarkers of effect yielded evidence of different pathways for adverse outcomes in endometriosis.

Therefore, given the novel and interesting results reported, this PhD thesis may constitute the starting point for future studies with larger sample sizes exploring these possible associations and increasing knowledge of endometriosis, a disease that considerably decreases the quality of life in an increasing number of women worldwide.

7. CONCLUSIONS

Conclusion 1

Concentrations of PBs and BPs, two families of EDCs commonly present in cosmetics and PCPs, were detected in menstrual blood samples. Therefore, menstrual blood could be provided as a potential matrix for characterizing exposure to PBs and BPs. These families of hormone-mimicking chemicals could disrupt physiology of estrogen-sensitive tissues, such as the endometrium, promoting the generation of a uterine microenvironment that could favor the origin and development of different gynecological diseases, including endometriosis. Menstrual blood concentrations of the selected pollutants did not correlate with those in peripheral blood from the same women.

Conclusion 2

In the population-based case-control study conducted in Granada (Spain), the frequency of use of certain cosmetics and PCPs was found to be a strong predictor of exposure to different PB and BP congeners. After adjustment for potential confounders, the risk of endometriosis was increased in women in the second tertile of exposure to MeP, BP-3 and ΣBPs compared to those in the first tercile of exposure. A close-to-significant relationship was also observed between biomarkers of lipid peroxidation and increased endometriosis risk, as well as an inverse association with total antioxidant power concentrations. Oxidative stress did not modify the observed associations between paraben/benzophenone exposure and endometriosis risk. Given the modest sample size analyzed in this study and the novelty of these findings, future studies are warranted in order to corroborate previous results.

Conclusion 3

The results of this doctoral thesis support the multifactorial nature of endometriosis, in which genetic, immunological, hormonal and environmental factors interact. Genes related to the development and progression of endometriosis, were expressed in most of the endometriotic tissue samples studied. Exposure of women to EDCs present in cosmetics and PCPs (including PBs and BPs) might be associated with altered gene expression profiles in endometriotic tissues related to key cellular signaling pathways for the development of endometriosis, such as cell adhesion; invasion, migration and metastasis; inflammation; angiogenesis; proliferation and hormonal stimulation; cell

cycle; cell differentiation; and lipid metabolism. In contrast, no significant associations were found between PB and BP exposure and apoptosis. Given the novelty of these findings, further studies with larger sample sizes are warranted to shed some light on the impact of human exposure to PBs and BPs on the pathophysiology of endometriosis.

Conclusion 4

Given the strict quality controls carried out by Inves Biofarm to ensure the quality of its products, no plastic packaging or active ingredient tested from this company showed detectable concentrations of PBs and BPs, and only a few cosmetic product samples showed detectable concentrations of MeP, EtP, PrP and/or BP-3. However, after applying a chemical extraction methodology with chloroform and acetonitrile, the plastic containers showed both estrogenic and antiandrogenic activity, and some cosmetic products also showed both types of hormonal activity. This hormonal activity could be a consequence of the presence of other families of EDCs in the chemical composition of these products. Further research and exploration of the chemical composition of cosmetics and their potential content of EDCs is therefore required to ensure that the products marketed are free of these hormonally active compounds and do not pose a health risk to consumers.

Conclusion 5

The results collected in this PhD thesis support the need to establish preventive measures against endometriosis, a disease whose incidence is progressively increasing, whose diagnosis, mainly surgical, is difficult to establish, and whose treatment is mostly symptomatic. Our results suggest that restrictions on the use of cosmetics and PCPs containing PBs or BPs, and the use of environmental friendly cosmetics could help to reduce human exposure to these EDCs and, therefore, reduce the risk of onset and development of endometriosis.

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9. ANNEXES

ANNEX I. SUPPLEMENTARY MATERIAL. ARTICLE 1

Supplementary Table 1. Bivariate linear associations of sociodemographic, reproductive, dietary, and personal care product data with menstrual blood levels of parabens and benzophenones.

		MeP					PrP					BP3				
		β	exp(β)	95% CI		p-value	β	exp(β)	95% CI		p-value	β	exp(β)	95% CI		p-value
Sociodemographic and reproductive characteristics	**Age** *(years)*	-0.001	0.999	0.953	1.048	0.982	0.008	1.008	0.959	1.060	0.742	0.003	1.003	0.970	1.038	0.843
	BMI *(kg/m^2)*	-0.022	0.978	0.911	1.051	0.540	-0.003	0.997	0.927	1.072	0.932	-0.025	0.975	0.927	1.026	0.324
	Residence *(urban)*	0.076	1.079	0.626	1.862	0.780	-0.400	0.670	0.379	1.185	0.165	0.019	1.020	0.677	1.535	0.924
	Time living in residence *(years)*	0.001	1.001	0.980	1.022	0.928	-0.011	0.989	0.968	1.011	0.326	0.010	1.010	0.995	1.025	0.174
	Greenhouse proximity = *Yes*	0.351	1.421	0.563	3.588	0.451	-0.474	0.622	0.237	1.636	0.330	0.168	1.183	0.609	2.295	0.614
	Agriculture proximity = *Yes*	0.332	1.394	0.793	2.451	0.244	0.045	1.046	0.575	1.903	0.880	-0.025	0.976	0.649	1.467	0.904
	Industry proximity = *Yes*	0.308	1.361	0.773	2.398	0.280	-0.286	0.752	0.415	1.362	0.340	-0.123	0.884	0.588	1.328	0.547
	Currently working = *Yes*	-0.364	0.695	0.360	1.339	0.271	***-0.595***	***0.551***	***0.280***	***1.084***	***0.083***	0.059	1.061	0.661	1.704	0.802
	Currently studing = *Yes*	-0.124	0.883	0.447	1.744	0.716	***0.749***	***2.115***	***1.067***	***4.190***	***0.032***	-0.122	0.885	0.545	1.438	0.616
	Currently working and/or studying = *Yes*	-0.272	0.762	0.361	1.609	0.469	0.572	1.772	0.819	3.834	0.143	-0.226	0.798	0.468	1.360	0.400
	Type of work = *Manual worker*	-0.052	0.949	0.518	1.740	0.864	0.506	1.658	0.892	3.082	0.108	-0.155	0.856	0.557	1.317	0.474
	Educational level = *University*	-0.269	0.764	0.425	1.376	0.364	-0.185	0.831	0.448	1.542	0.551	-0.010	0.990	0.648	1.511	0.961
	Age at first menstruation = *After 12 years old*	-0.161	0.851	0.481	1.507	0.575	***-0.501***	***0.606***	***0.338***	***1.087***	***0.091***	0.228	1.257	0.839	1.882	0.262
	Parity =*Yes*	0.208	1.232	0.696	2.180	0.468	0.257	1.293	0.712	2.348	0.392	-0.031	0.969	0.643	1.459	0.878
	Time since last pregnancy *(months)*	-0.006	0.994	0.976	1.013	0.547	-0.008	0.992	0.974	1.012	0.434	0.001	1.001	0.988	1.014	0.886
	Cumulative breastfeeding time *(months)*	0.002	1.002	0.990	1.013	0.795	0.001	1.001	0.989	1.013	0.897	-0.002	0.998	0.990	1.007	0.668
	Perceived weight loss = *Yes*	-0.057	0.945	0.519	1.719	0.850	***-0.599***	***0.549***	***0.300***	***1.007***	***0.053***	-0.265	0.767	0.503	1.169	0.213
	Dental filling = *Yes*	-0.113	0.893	0.352	2.265	0.808	0.387	1.473	0.559	3.883	0.427	-0.049	0.952	0.490	1.851	0.883
	Current smoker = *Yes*	***-0.856***	***0.425***	***0.248***	***0.729***	***0.002***	-0.254	0.776	0.421	1.429	0.409	-0.162	0.850	0.561	1.290	0.439
Dietary habits	**Frequency of fish consumption** *(At least 2times/week)*	***0.509***	***1.664***	***0.945***	***2.931***	***0.077***	-0.397	0.672	0.369	1.226	0.191	0.170	1.185	0.611	2.299	0.609
	Frequency of dairy products consumption *(everyday)*	0.450	1.568	0.887	2.772	0.119	-0.063	0.939	0.516	1.710	0.835	0.221	1.247	0.832	1.868	0.278
	Frequency of cheese consumption *(More than 2 times/week)*	-0.072	0.931	0.523	1.655	0.803	-0.079	0.924	0.433	1.973	0.836	0.098	1.103	0.658	1.848	0.706
	Frequency of cold meat consumption *(At least 1 time/week)*	0.280	1.324	0.627	2.795	0.455	-0.473	0.623	0.287	1.354	0.227	***0.443***	***1.558***	***0.923***	***2.627***	***0.095***
	Frequency of meat consumption *(At least 2 times/week)*	0.527	1.694	0.853	3.364	0.130	-0.164	0.849	0.464	1.554	0.589	***0.588***	***1.800***	***1.229***	***2.636***	***0.003***
	Frequency of fat consumption *(More than 3 times/week)*	0.256	1.291	0.593	2.810	0.512	***-0.761***	***0.467***	***0.209***	***1.043***	***0.063***	***0.430***	***1.537***	***1.027***	***2.300***	***0.037***
	Frequency of pulse consumption *(At least 2 times/week)*	-0.021	0.980	0.552	1.739	0.943	-0.395	0.674	0.373	1.217	0.186	-0.091	0.913	0.607	1.374	0.658
	Frequency of fresh vegetable consumption *(More than 2 times/week)*	0.161	1.174	0.646	2.134	0.592	-0.076	0.927	0.495	1.735	0.809	-0.133	0.876	0.572	1.341	0.535
	Frequency of cooked vegetable consumption *(More than 2 times/week)*	0.046	1.047	0.539	2.033	0.890	-0.141	0.868	0.434	1.739	0.685	-0.063	0.939	0.585	1.507	0.789
	Frequency of fruit consumption *(More than 2 times/week)*	0.601	1.824	0.876	3.800	0.106	0.167	1.182	0.648	2.156	0.581	-0.038	0.963	0.638	1.452	0.854
	Frequency of egg consumption *(More than 2 times/week)*	0.170	1.185	0.611	2.299	0.609	-0.360	0.698	0.373	1.307	0.255	***0.390***	***1.477***	***0.971***	***2.247***	***0.068***
	Frequency of pasta consumption *(At least 1 time/week)*	0.262	1.300	0.632	2.672	0.469	-0.294	0.745	0.351	1.584	0.438	0.164	1.179	0.704	1.973	0.525
	Frequency of bread consumption *(everyday)*	0.146	1.157	0.620	2.159	0.641	0.161	1.174	0.623	2.212	0.613	0.142	1.153	0.749	1.774	0.512
	Consumption of canned food = *Yes*	0.123	1.131	0.630	2.033	0.675	-0.385	0.681	0.371	1.248	0.209	-0.178	0.837	0.552	1.270	0.396
	Frequency of cans *(times/week)*	0.142	1.153	0.954	1.392	0.138	-0.117	0.890	0.730	1.084	0.241	***-0.110***	***0.896***	***0.786***	***1.020***	***0.096***
	Consumption of organic food = *Yes*	-0.493	0.611	0.335	1.114	0.106	-0.199	0.820	0.432	1.557	0.537	-0.258	0.773	0.501	1.191	0.238
	Proportion of organic food *(%)*	-0.835	0.434	0.147	1.282	0.128	-0.016	0.984	0.309	3.135	0.978	-0.540	0.582	0.268	1.266	0.169
PCP habits	**Use of hair dye** = *Yes*	-0.006	0.994	0.546	1.809	0.983	0.156	1.168	0.625	2.184	0.620	0.259	1.295	0.850	1.974	0.224
	Frequency of hair dye *(times/year)*	0.005	1.005	0.925	1.092	0.907	-0.023	0.978	0.897	1.066	0.601	***0.055***	***1.057***	***0.998***	***1.119***	***0.060***
	Weekly use of dermal oil and cream = *Yes*	0.023	1.023	0.467	2.240	0.954	0.147	1.158	0.510	2.629	0.721	***0.493***	***1.637***	***0.951***	***2.818***	***0.075***
	Weekly use of cosmetics = *Yes*	***0.511***	***1.666***	***0.946***	***2.935***	***0.076***	-0.335	0.715	0.391	1.308	0.271	***0.384***	***1.468***	***0.621***	***3.473***	***0.375***

Supplementary Table 2. Bivariate logistic associations of sociodemographic, reproductive, dietary, and personal care product data with menstrual blood levels of parabens and benzophenones.

		EtP				BuP				BP-1				BP-6				4-OHBP			
		OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value
Sociodemographic and reproductive characteristics	**Age** *(years)*	***0.92***	***0.83***	***1.02***	***0.098***	0.95	0.86	1.04	0.254	0.93	0.85	1.02	0.121	0.98	0.90	1.07	0.673	0.95	0.87	1.04	0.243
	BMI *(kg/m^2)*	0.41	0.11	1.50	0.177	0.45	0.12	1.65	0.228	1.75	0.48	6.34	0.394	1.83	0.48	6.96	0.378	2.71	0.73	9.99	0.135
	Residence *(urban)*	1.00	0.34	2.92	0.999	1.55	0.54	4.46	0.421	***0.35***	***0.11***	***1.05***	***0.061***	1.00	0.35	2.86	0.997	1.16	0.40	3.36	0.786
	Time living in residence *(years)*	0.98	0.95	1.02	0.449	1.04	0.99	1.08	0.097	0.98	0.94	1.02	0.353	0.98	0.95	1.02	0.382	1.00	0.96	1.04	0.881
	Greenhouse proximity = *Yes*	3.79	0.41	34.83	0.239	2.86	0.48	17.06	0.249	1.40	0.23	8.36	0.712	0.56	0.09	3.35	0.527	3.79	0.41	34.83	0.239
	Agriculture proximity = *Yes*	0.51	0.17	1.49	0.217	2.19	0.75	6.37	0.149	0.45	0.15	1.33	0.148	0.60	0.21	1.73	0.347	0.61	0.21	1.76	0.359
	Industry proximity = *Yes*	1.03	0.36	2.98	0.955	0.59	0.20	1.70	0.326	0.72	0.25	2.09	0.551	1.62	0.57	4.62	0.371	1.75	0.60	5.13	0.307
	Currently working = *Yes*	3.19	0.78	13.06	0.107	0.72	0.21	2.42	0.595	1.69	0.50	5.70	0.399	1.72	0.49	5.97	0.395	1.15	0.34	3.89	0.826
	Currently studing = *Yes*	0.90	0.25	3.21	0.874	1.13	0.33	3.91	0.850	0.73	0.21	2.56	0.628	1.54	0.44	5.32	0.499	0.49	0.14	1.71	0.264
	Currently working and/or studying = *Yes*	0.98	0.24	3.96	0.980	0.83	0.21	3.31	0.787	1.73	0.40	7.52	0.466	1.24	0.32	4.86	0.759	1.02	0.25	4.10	0.980
	Type of work = *Manual worker*	***0.27***	***0.07***	***0.95***	***0.042***	1.70	0.56	5.17	0.347	0.65	0.21	1.98	0.446	0.58	0.19	1.80	0.349	1.25	0.40	3.87	0.703
	Educational level = *University*	***3.09***	***0.85***	***11.21***	***0.087***	1.10	0.31	3.83	0.887	1.94	0.51	7.33	0.327	0.99	0.28	3.47	0.991	1.76	0.47	6.65	0.404
	Age at first menstruation = *After 12 years old*	***6.60***	***1.96***	***22.25***	***0.002***	0.40	0.14	1.17	0.094	1.03	0.36	2.98	0.955	1.09	0.38	3.11	0.866	***4.78***	***1.52***	***14.98***	***0.007***
	Parity =*Yes*	0.47	0.16	1.41	0.180	2.12	0.73	6.16	0.167	0.86	0.30	2.49	0.783	0.78	0.27	2.23	0.646	0.86	0.30	2.49	0.783
	Time since last pregnancy *(months)*	***0.96***	***0.93***	***1.00***	***0.036***	1.02	0.99	1.05	0.273	0.98	0.95	1.02	0.286	0.99	0.96	1.03	0.637	0.99	0.96	1.03	0.614
	Cumulative breasfeeding time *(months)*	1.01	0.99	1.04	0.298	1.00	0.97	1.02	0.713	1.00	0.98	1.03	0.768	0.99	0.97	1.01	0.406	1.01	0.99	1.03	0.439
	Perceived weight loss = *Yes*	0.50	0.16	1.60	0.245	1.07	0.36	3.21	0.899	0.39	0.13	1.20	0.101	***0.25***	***0.08***	***0.85***	***0.026***	1.02	0.34	3.10	0.968
	Dental filling = *Yes*	0.65	0.12	3.52	0.613	0.76	0.14	4.13	0.749	1.55	0.28	8.45	0.613	1.78	0.30	10.59	0.527	1.55	0.28	8.45	0.613
	Current smoker = *Yes*	1.92	0.65	5.69	0.242	2.03	0.69	6.00	0.200	1.31	0.44	3.93	0.627	1.33	0.46	3.89	0.598	1.31	0.44	3.93	0.627
Dietary habits	**Frequency of fish consumption** *(At least 2times/week)*	1.68	0.57	4.94	0.346	0.97	0.34	2.83	0.962	0.60	0.20	1.75	0.346	0.47	0.16	1.41	0.180	0.60	0.20	1.75	0.346
	Frequency of dairy products consumption *(everyday)*	0.68	0.23	2.02	0.482	1.31	0.45	3.80	0.620	0.44	0.15	1.30	0.138	1.56	0.54	4.52	0.415	1.09	0.37	3.21	0.877
	Frequency of cheese consumption *(More than 2 times/week)*	0.76	0.26	2.22	0.620	0.74	0.26	2.13	0.578	0.97	0.34	2.83	0.962	1.12	0.39	3.21	0.829	1.31	0.45	3.80	0.620
	Frequency of cold meat consumption *(At least 1 time/week)*	0.62	0.16	2.45	0.496	1.21	0.30	4.86	0.787	***0.13***	***0.01***	***1.08***	***0.059***	0.81	0.21	3.17	0.759	1.61	0.41	6.35	0.496
	Frequency of meat consumption *(At least 2 times/week)*	0.39	0.11	1.45	0.160	1.75	0.46	6.65	0.411	0.42	0.10	1.75	0.231	0.52	0.14	1.90	0.324	0.68	0.18	2.61	0.578
	Frequency of fat consumption *(More than 3 times/week)*	1.29	0.31	5.44	0.730	3.22	0.72	14.52	0.128	0.50	0.12	2.10	0.341	0.57	0.13	2.55	0.460	0.85	0.20	3.57	0.822
	Frequency of pulse consumption *(At least 2 times/week)*	1.56	0.54	4.52	0.415	2.12	0.73	6.16	0.167	2.11	0.71	6.28	0.180	0.44	0.15	1.28	0.130	0.86	0.30	2.49	0.783
	Frequency of fresh vegetable consumption *(More than 2 times/week)*	0.39	0.13	1.20	0.101	1.76	0.57	5.41	0.324	0.50	0.16	1.60	0.245	1.04	0.35	3.10	0.945	1.34	0.45	4.05	0.599
	Frequency of cooked vegetable consumption *(More than 2 times/week)*	1.98	0.54	7.31	0.306	1.57	0.45	5.45	0.481	0.77	0.22	2.69	0.684	0.54	0.16	1.83	0.322	1.69	0.50	5.70	0.399
	Frequency of fruit consumption *(More than 2 times/week)*	0.62	0.16	2.45	0.496	1.21	0.30	4.86	0.787	0.98	0.24	3.96	0.980	2.24	0.51	9.71	0.283	0.98	0.24	3.96	0.980
	Frequency of egg consumption *(More than 2 times/week)*	0.51	0.14	1.87	0.306	0.95	0.28	3.20	0.931	1.30	0.37	4.52	0.684	1.26	0.38	4.23	0.705	0.87	0.26	2.96	0.826
	Frequency of pasta consumption *(At least 1 time/week)*	0.77	0.20	2.91	0.701	0.59	0.16	2.20	0.429	0.49	0.11	2.08	0.331	1.01	0.27	3.78	0.991	1.30	0.34	4.88	0.701
	Frequency of bread consumption *(everyday)*	0.48	0.15	1.52	0.210	2.40	0.71	8.08	0.157	***0.05***	***0.01***	***0.42***	***0.006***	0.92	0.29	2.87	0.886	0.51	0.15	1.72	0.276
	Consumption of canned food = *Yes*	0.96	0.33	2.85	0.946	2.27	0.74	6.92	0.150	1.04	0.35	3.08	0.946	1.01	0.35	2.95	0.985	1.41	0.48	4.17	0.534
	Frequency of cans *(times/week)*	1.08	0.75	1.56	0.681	0.96	0.67	1.35	0.799	1.05	0.73	1.49	0.805	0.72	0.47	1.10	0.126	0.73	0.46	1.16	0.184
	Consumption of organic food = *Yes*	2.23	0.67	7.46	0.194	2.74	0.82	9.15	0.102	0.91	0.29	2.87	0.879	***0.28***	***0.09***	***0.91***	***0.034***	***0.30***	***0.08***	***1.08***	***0.065***
	Proportion of organic food *(%)*	0.31	0.04	2.44	0.266	0.37	0.05	2.84	0.339	0.28	0.03	2.39	0.243	3.61	0.44	29.54	0.231	2.32	0.30	17.96	0.421
PCP habits	**Use of hair dye** = *Yes*	0.98	0.32	2.97	0.968	0.41	0.13	1.29	0.126	1.02	0.34	3.10	0.968	0.70	0.23	2.12	0.532	1.41	0.46	4.36	0.546
	Frequency of hair dye *(times/year)*	1.02	0.88	1.19	0.779	1.15	0.96	1.38	0.137	0.98	0.84	1.14	0.809	0.95	0.82	1.11	0.527	0.89	0.74	1.07	0.209
	Weekly use of dermal oil and cream = *Yes*	0.82	0.19	3.44	0.785	0.97	0.23	4.08	0.969	2.08	0.49	8.78	0.317	***8.70***	***1.01***	***75.00***	***0.049***	2.08	0.49	8.78	0.317
	Weekly use of cosmetics = *Yes*	1.09	0.37	3.21	0.877	***0.11***	***0.03***	***0.37***	***<0.001***	1.68	0.57	4.94	0.346	1.16	0.40	3.35	0.790	2.28	0.77	6.78	0.138

ANNEX II. SUPPLEMENTARY MATERIAL. ARTICLE 2

Supplementary Table S1. Occupational activity of the study population.

	Total (n=99)		Cases (n=25)		Controls (n=74)	
	n	**%**	**n**	**%**	**n**	**%**
Occupational activity						
Health	6	6.1	1	4.0	5	6.8
Office work	17	17.2	6	24	11	14.9
Education	12	12.1	4	16	8	10.8
Commerce	11	11.1	2	8	9	12.2
Hostelry	4	4.0	0	0.0	4	5.4
Student	7	7.1	0	0.0	7	9.5
Beauty salon	4	4.0	1	4.0	3	4.1
Unemployed	31	31.3	10	40	21	28.4
Not specified	7	7.1	1	4.0	6	8.1

Supplementary Table S2. Frequency of use of cosmetics and personal care products and endometriosis risk (n=99).

	n	**%**	**aOR**	**95% CI**	
Cosmetics and personal care products					
Use of lipstick = *More than 1 time/day*[a]	13	13.1	0.78	0.18	3.36
Use of makeup = *Weekly*[b]	59	59.6	1.35	0.47	3.87
Use of eye pencil = *Everyday*[c]	46	46.5	1.12	0.40	3.13
Use of eyeshadow = *Everyday*[c]	24	24.2	0.53	0.15	1.87
Use of nail polish = *Everyday*[c]	20	10.1	0.80	0.27	2.41
Use of mask = Weekly[b]	28	28.3	0.42	0.10	1.70
Use of face cream = *Everyday*[c]	64	64.6	1.25	0.41	3.81
Use of hair lacquer, cream or foam [d]					
Less than everyday	20	20.2	1.95	0.56	6.81
Everyday	17	17.2	0.49	0.11	2.25
Use of body lotion = *Everyday*[c]	43	43.4	1.24	0.44	3.46
Use of handcream = *More than 1 time/day*[b]	27	27.3	0.95	0.29	3.08

Use of facial tonic = *Weekly*[b]	37	37.4	1.04	0.34	3.23
Use of facial milk = *Every day*[c]	13	13.1	0.25	0.03	2.23
Use of shampoo = *At least 3 times/week*[e]	56	56.6	1.18	0.40	3.51
Use of gel = *Every day*[c]	70	70.7	1.40	0.44	4.48
Use of deodorant = *Every day*[c]	82	82.8	1.34	0.30	5.89
Use of conditioner = *At least 3 times/week*[e]	47	47.5	0.68	0.24	1.89
Use of cologne, perfume = *Everyday*[c]	77	77.8	1.28	0.34	4.85
Use of toothpaste = *More than 1 time/day*[b]	63	63.6	1.40	0.45	4.38
Use of mouthwash = *More than 1 time/day*[b]	21	21.2	0.45	0.12	1.75
Use of sunscreen = *Yes*[d]	46	46.5	1.05	0.37	3.02
Activities related to cosmetic and personal care products					
Hair dye[d]					
Less than 1 time/month	32	32.3	1.00	0.30	3.33
Monthly	29	29.3	0.34	0.07	1.69
Acrylic nails = *Monthly*[f]	13	13.1	1.97	0.48	8.08
Pedicure [f]					
Monthly	16	16.2	0.25	0.05	1.41
More than 1 time/month	10	10.1	1.35	0.17	10.52
Facial treatment = *Monthly*[f]	13	13.1	1.69	0.34	8.41
Manicure = *Monthly*[f]	30	30.3	0.47	0.13	1.73
Massage = *Monthly*[f]	23	23.2	0.85	0.23	3.17

[a]Reference category= ≤ 1time/day; Rarely/never; [b]Reference category= < Weekly; [c]Reference category= <Every day; [d]Reference category= No ; [e]Reference category= < 3 times/week; [f]Reference category= <Monthly.

Supplementary Table S3. Urinary levels of parabens, benzophenones, and oxidative stress biomarkers according to rASRM stage.

		Stages I/II (n=23)					Stages III/IV (n=12)					p-value
		Mean	St. Dev	Percentiles			Mean	St. Dev	Percentiles			
				25	50	75			25	50	75	
BPs	**BP-1**	2.54	2.39	0.94	1.45	3.47	4.96	7.83	0.80	1.76	6.87	0.677
	BP-3	13.73	39.78	1.72	3.83	7.61	5.32	8.44	0.58	2.26	5.61	0.348
	4-OHBP	0.88	0.78	0.36	0.66	1.05	1.20	1.36	0.26	0.66	1.27	0.835
	ΣBPs	17.15	41.16	3.84	7.66	10.34	11.48	15.77	1.90	7.62	11.97	0.794
PBs	**MeP**	174.88	222.98	11.93	51.61	393.89	280.16	839.87	7.01	31.27	77.32	0.348
	EtP	38.68	93.33	1.13	2.41	7.79	28.47	43.91	0.45	7.66	37.30	0.555
	PrP	18.26	36.01	0.15	1.32	17.78	6.85	11.66	0.48	2.69	7.68	0.664
	BuP	1.40	4.01	0.05	0.16	0.91	0.68	1.91	0.05	0.08	0.16	0.324
	ΣPBs	233.22	312.11	17.20	73.55	477.50	316.16	866.04	9.54	54.36	159.05	0.404
Ox. stress	**TAP**	4.07	2.82	1.80	4.18	7.02	4.91	4.22	1.42	4.26	6.78	0.957
	TBARS	1.68	0.45	1.38	1.70	2.08	1.92	0.32	1.83	2.00	2.10	0.211

St. Dev: standard deviation; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; TAP: total antioxidant power; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxideoxiguanosine.

Supplementary Table S4. Relationship between urinary TAP, TBARS and endometriosis. Logistic regression analyses (n=124).

	OR	95% CI		aOR[3]	95% CI	
TAP	0.55	0.12	2.45	**0.15**	**0.02**	**0.98**
<1,75	1.00			1.00		
1,75-2,06	0.57	0.20	1.63	**0.26**	**0.07**	**1.00**
>2,06	1.00	0.38	2.66	0.61	0.19	1.96
TBARS	1.40	0.92	2.12	1.60	1.00	2.80
<1,5	1.00			1.00		
1,5-4,23	1.51	0.54	4.26	2.10	0.60	7.70
>4,23	3.54	0.94	6.85	**3.80**	**1.00**	**13.90**

[3]Adjusted for age (yr), body mass index (kg/m2), residence (rural/urban), parity (yes/no) and urinary creatinine (mg/dL); TAP: total antioxidant power; TBARS: thiobarbituric acid reactive substances; OR: odd ratio; CI: confidence interval; Bold values indicate statistically significant associations.

Supplementary Table S5. Relationship between urinary levels of benzophenones and parabens and oxidative stress biomarkers. Linear regression (n=124).

	TAP					TBARS				
	β	**exp(β)**	**95% CI**		**p-value**	**β**	**exp(β)**	**95% CI**		**p-value**
BP-1	-0.03	0.97	0.95	1.00	0.071	-0.01	0.99	0.92	1.05	0.646
BP-3	-0.02	0.98	0.96	1.01	0.286	0.02	1.02	0.95	1.09	0.630
4-OHBP	-0.01	0.99	0.95	1.03	0.630	**0.11**	**1.11**	**1.01**	**1.23**	**0.035**
ΣBPs	-0.03	0.97	0.95	1.00	0.071	0.02	1.02	0.94	1.09	0.686
MeP	0.00	1.00	0.97	1.02	0.738	0.03	1.03	0.97	1.09	0.354
EtP	-0.01	0.99	0.97	1.01	0.420	0.05	1.05	0.99	1.12	0.079
PrP	-0.01	0.99	0.96	1.02	0.403	0.00	1.00	0.94	1.06	0.919
BuP	0.01	1.01	0.98	1.04	0.536	0.05	1.05	0.98	1.13	0.176
ΣPBs	0.00	1.00	0.97	1.02	0.782	0.05	1.05	0.98	1.12	0.150

Adjusted for age (yr), body mass index (kg/m2), residence (rural/urban), parity (yes/no) and urinary creatinine (mg/dL). TAP: total antioxidant power; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxideoxiguanosine; CI: confidence interval.

ANNEX III. SUPPLEMENTARY MATERIAL. ARTICLE 3

Supplementary Table S1. Search strategies for Pubmed, Scopus and Web of Science.

Database	**Pubmed (Medline)**
Date	**01/02/2023**
Strategy	**#1 AND #2**
#1	("bisphenols" [Title/Abstract] OR "bisphenol A" [Title/Abstract] OR "bisphenol S" [Title/Abstract] OR "bisphenol F" [Title/Abstract] OR "bisphenol A-glycidyl methacrylate" [Mesh] OR "bisphenol A-glycidyl methacrylate" [Title/Abstract] OR "bisphenol A diglycidyl ether" [Title/Abstract] OR "bisphenol F diglycidyl ether" [Title/Abstract] OR "parabens" [Mesh] OR "parabens" [Title/Abstract] OR "methylparaben" [Title/Abstract] OR "ethylparaben" [Title/Abstract] OR "prophylparaben" [Title/Abstract] OR "buthylparaben" [Title/Abstract] OR "benzophenones" [Mesh] OR "benzophenones" [Title/Abstract] OR "benzophenone 1" [Title/Abstract] OR "2,4-dihydroxy-benzophenone" [Title/Abstract] OR "benzophenone 2" [Title/Abstract] OR "2,2',4,4'-tetrahydroxybenzophenone" [Title/Abstract] OR "benzophenone 3" [Title/Abstract] OR "oxybenzone" [Title/Abstract] OR "benzophenone 4" [Title/Abstract] OR "sulisobenzone" [Title/Abstract] OR "4-hydroxybenzophenone" [Title/Abstract] OR "benzophenone 5" [Title/Abstract] OR "benzophenone 6" [Title/Abstract] OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" [Title/Abstract] OR "benzophenone 7" [Title/Abstract] OR "5-chloro-2-hydroxybenzophenone" [Title/Abstract] OR "benzophenone 8" [Title/Abstract] OR "dioxybenzone" [Title/Abstract] OR "benzophenone 9" [Title/Abstract] OR "benzophenone 10" [Title/Abstract] OR "benzophenone 12" [Title/Abstract] OR "octabenzone" [Title/Abstract])
#2	("Inflammation Mediators" [Mesh] OR “inflammation mediators” [Title/Abstract] OR "Inflammation" [Mesh] OR "Inflammation" [Title/Abstract] OR “cytokines” [Mesh] OR “cytokines” [Title/Abstract] OR “intracellular adhesion molecules” [Title/Abstract] OR “humoral mediators” [Title/Abstract] OR “c reactive protein” [Title/Abstract] OR “inflammatory milieu” [Title/Abstract] OR “phagocytic leukocytes” [Title/Abstract] OR “antibodies” [Title/Abstract] OR “complement proteins” [Title/Abstract] OR "receptor Activator of Nuclear Factor-kappa B" [Mesh] OR "receptor Activator of Nuclear Factor-kappa B" [Title/Abstract] OR “prostaglandin-Endoperoxide Synthases” [Mesh] OR “prostaglandin-Endoperoxide Synthases” [Title/Abstract])
Database	**Scopus**
Date	**01/02/2023**
Strategy	**#1 AND #2**
#1	TITLE-ABS-KEY ("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#2	TITLE-ABS-KEY ("Inflammation Mediators" OR "Inflammation" OR "cytokines" OR "intracellular adhesion molecules" OR "humoral mediators" OR "c reactive protein" OR "inflammatory milieu" OR "phagocytic leukocytes" OR "antibodies" OR "complement proteins" OR "receptor Activator of Nuclear Factor-kappa B" OR "prostaglandin-Endoperoxide Synthases")

Database	Web of Science
Date	01/02/2023
Strategy	(#1 OR #2) AND (#3 OR #4)
#1	TI=("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#2	AB=("bisphenols" OR "bisphenol A" OR "bisphenol S" OR "bisphenol F" OR "bisphenol A-glycidyl methacrylate" OR "bisphenol A diglycidyl ether" OR "bisphenol F diglycidyl ether" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "benzophenone 2" OR "2,2',4,4'-tetrahydroxybenzophenone" OR "benzophenone 3" OR "oxybenzone" OR "benzophenone 4" OR "sulisobenzone" OR "4-hydroxybenzophenone" OR "benzophenone 5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "benzophenone 8" OR "dioxybenzone" OR "benzophenone 9" OR "benzophenone 10" OR "benzophenone 12" OR "octabenzone" OR "parabens" OR "methylparaben" OR "ethylparaben" OR "prophylparaben" OR "buthylparaben")
#3	TI=("Inflammation Mediators" OR "Inflammation" OR “cytokines” OR “intracellular adhesion molecules” OR “humoral mediators” OR “c reactive protein” OR “inflammatory milieu” OR “ phagocytic leukocytes” OR “antibodies” OR “complement proteins” OR "receptor Activator of Nuclear Factor-kappa B" OR “ prostaglandin-Endoperoxide Synthases”)
#4	AB=("Inflammation Mediators" OR "Inflammation" OR “cytokines” OR “intracellular adhesion molecules” OR “humoral mediators” OR “c reactive protein” OR “inflammatory milieu” OR “ phagocytic leukocytes” OR “antibodies” OR “complement proteins” OR "receptor Activator of Nuclear Factor-kappa B" OR “ prostaglandin-Endoperoxide Synthases”)

Supplementary Table S2. Additional information collected from the included studies in this systematic review.

Reference	Population characteristics			Characteristics of exposure assessment			Characteristics of outcome assessment	
	Health condition	Gender	Age	Quantification methodology	Volume	LOD	Quantification methodology	FD (%)
Ashley-Martin et al., 2015	Healthy	Pregnant women	>30 (most of them)	GC-MS-MS	N.R.	0.20	ELISA	N.R.
Aung et al., 2019	Healthy	Pregnant women	>30 (most of them)	ID-LC-MS/MS	N.R.	N.R.	ELISA and Milliplex MAP High Sensitivity Human Cytokine Magnetic Bead Panel	78.0-99.9
Choi et al., 2017	Healthy	Men and women	30-64	LC-MS/MS	N.R.	N.R.	TIA	N.R.
Ferguson et al., 2016	Healthy	Pregnant women	N.R.	ID-LC-MS/MS	N.R.	N.R.	Enzyme-linked immunosorbent assay and sensitivity Human Cytokine Magnetic Bead Panel	N.R.
Haq et al., 2020	Healthy/diabetes	Men and women	43.86 ± 13.61*	ELISA	N.R.	N.R.	ELISA	N.R.
Huang et al., 2017	Healthy	Pregnant women	18-45	UPLC	N.R.	0.16	ELISA and SYNCHRON Systems reagent with the highly sensitive near infrared particle immunoassay rate methodology	0.0-100.0
Jain et al., 2020	Healthy/diabetes	Men and women	44.12 ± 8.5*	N.R.	N.R.	N.R.	ELISA	N.R.
Kelley et al., 2019	Healthy	Pregant women	18-42	LC-MS/MS	N.R.	N.R.	N.R.	N.R.
Lang et al., 2008	Healthy	Men and women	18-74	HPLC	N.R.	N.R.	Latexenhanced nephelometry	N.R.
Liang et al., 2020	URSA	Women	20-40	UPLC-MS/MS	N.R.	0.01-0.10	Multiplex assay U-PLEX proinflammatory panel 1 kit and single-plex assay V-PLEX assay.	9.0-100.0
Linares et al., 2021	Crohn´s disease	Men and women	40	UPLC-MS/MS	N.R.	N.R.	N.R.	N.R.
Mohsen et al., 2018	Healthy	Boys and girls	6-16	LC-MS/MS	500 µL	N.R.	ELISA	N.R.
Nalbantoğlu et al., 2021	Healthy/allergic rhinitis	Boys and girls	Healthy children: 8.32 ± 1.29* Allergic rhinitis children: 8.78 ± 1.51*	ELISA	10 mL	N.R.	ELISA	N.R.
Qu et al., 2022	Healthy/rheumatoid arthritis	Men and women	55	UPLC-MS/MS	500 µL	0.05-0.12	Immunoturbidimetry	N.R.
Savastano et al., 2015	Healthy	Men	53.5 ± 5.7*	ELISA	500 µL	N.R.	Human BIO-PLEX Suspension Array Multi-panel System	N.R.
Šimková et al., 2020	Healthy/PCOS	Women	Healthy women : 29.9 PCOS women: 28.9-29.5	LC-MS/MS	500 µL	N.R.	Multiples immunoanalytic xMAP technology - kit #M500KCAF0Y Bio-Plex Pro™ Human Cytokine 27-plex	N.R.
Song et al., 2017	Healthy	Men and women	≥60	HPLC	N.R.	0.01	High sensitive latex turbidimetric immunoassay, ELISA and autobiochemical analyser	N.R.
Tsen et al., 2021	Healthy	Men and women	20-45	LC-MS/MS	1 mL	0.10	Quantikine® human immunoassay kits	N.R.
Watkins et al., 2015	Healthy	Pregnant women	18-40	ID-LC-MS/MS	N.R.	0.20-0.40	ELISA and Milliplex MAP High Sensitivity Human Cytokine Magnetic Bead Panel	64.0-100.0
Yang et al., 2009	Healthy	Men and women	45.9-57.0	LC-MS/MS	500 µL	0.06	High sensitive assay	100.0

*URSA: unexplained recurrent spontaneous abortion; PCOS: Polycystic Ovary Syndrome; GC-MS-MS: gas chromatography-mass spectrometry; ID-LC-MS/MS: isotope dilution-liquid chromatography-tandem mass spectrometry; LC-MS/MS: liquid chromatography-mass spectrometry; UPLC: acquity ultraperformance liquid chromatography; UPLC-MS/MS: ultra-performance liquid chromatography-tandem mass spectrometry; ELISA: enzyme-linked immunosorbent assay; HPLC: high performance liquid chromatography; TIA: turbido-immunometric assay; LOD: limit of detection; FD: frequency of detection; N.R.: Not reported. *Mean ± standard deviation.*

ANNEX IV. SUPPLEMENTARY MATERIAL. ARTICLE 4

Supplementary Table S1. The primers used in this study.

Cell pathway	Gene	Assay ID
Cell adhesion	ITGB2	qHsaCED0056513
	CLDN7	qHsaCED0047491
Invasion, migration, and metastasis	MMP1	qHsaCED0048106
	MMP7	qHsaCED0044775
	FUT8	qHsaCID0008117
	RRM2	qHsaCED0002264
	MDK	qHsaCED0043317
	RHOB	qHsaCED0045167
	SPRY2	qHsaCED0034752
Inflammation	IL1R1	qHsaCID0010015
	IL1R2	qHsaCID0036435
	IL6ST	qHsaCID0007540
	NR3C1	qHsaCED0043506
	TNFRSF1B	qHsaCED0056907
Angiogenesis	ANG1	qHsaCED0037602
	ANGPT1	qHsaCED0045626
	sVEGFR-1	qHsaCED0042939
	VEGFA	qHsaCED0043454
Cell proliferation and hormonal stimulation	CYP19A1	qHsaCID0007396
	DUSP6	qHsaCED0036582
	ERα	qHsaCED0033920
	PGR	qHsaCID0036346
	STAR	qHsaCED0045371
	GAPDH*	qHsaCED0038674

** Housekeeping gene. ITGB2: integrin beta-2; CLDN7: claudin 7; MMP1: matrix metallopeptidase 1; MMP7: matrix metallopeptidase 7; FUT8: fucosyltransferase 8; RRM2: ribonucleotide reductase M2; MDK: midkine; RHOB: ras homolog gene family, member B; SPRY2: sprout homolog 2; IL1RL1: interleukin 1 receptor, type I; IL1R2: interleukin 1 receptor, type II; IL6ST: interleukin 6 cytokine family signal transducer; NR3C1: nuclear receptor subfamily 3 group C member 1; TNFRSF1B: tumor necrosis factor receptor superfamily member 1B; ANG: angiogenin; ANGPT1: angiopoietin 1; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; VEGFA: vascular endothelial growth factor A; CYP19A1: cytochrome P450 family 19 subfamily A member 1; DUSP6: dual specificity phosphatase 6; ERα: estrogen receptor 1; PGR: progesterone receptor; STAR: steroidogenic acute regulatory protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase*

Supplementary Table S2. Characteristics of study population (n=22).

	n	%		n	%
Sociodemographic characteristics					
Age *(years)**	37.0 ± 7.5		**Educational level**		
Weight *(kg)**	67.0 ± 15.1		*Less than university degree*	15	68.2
Height *(m)**	1.6 ± 0.1		*University degree*	7	31.8
Body mass index *(kg/m^2)**	25.3 ± 5.2		**Working outside home**		
Normal weight (BMI < 25)	12	54.5	*Yes*	13	59.1
Overweight (BMI 25-30)	5	22.7	*No*	9	40.9
Obese (BMI > 30)	5	22.7	**Current smoker**		
Residence			*Yes*	6	27.3
Rural	12	54.5	*No*	16	72.7
Urban /sub-urban	10	45.5	**Urinary creatinine** *(ng/mL)**	152.3 ± 68.3	
Reproductive characteristics					
Intensity of menstrual bleeding	9	40.9	**Endometrioma location**		
Mild	13	59.1	*Deep infiltrating endometrios*	7	31.8
Moderate/severe			*Ovarian/peritoneal endometr*	15	68.2
Parity			**Endometriosis stage**		
Nulliparous	9	40.9	*I/II*	13	59.1
Primiparous/Multiparous	13	59.1	*III/IV*	9	40.9

**Mean ± standard deviation.*

Supplementary Table S3. Urinary concentrations of parabens and benzophenones (ng/mL) (n=22).

	FD	Mean	St. Dev.	Percentiles		
				25	50	75
MeP	22 (100.0%)	236.56	631.95	11.45	45.44	90.13
EtP	22 (100.0%)	44.20	95.28	1.61	4.66	23.11
PrP	22 (100.0%)	16.43	35.16	0.48	2.69	10.38
BuP	22 (100.0%)	0.81	1.63	0.05	0.15	0.61
ΣPBs	22 (100.0%)	298.00	681.23	15.64	60.81	158.71
BP1	22 (100.0%)	2.59	2.55	0.89	1.44	3.61
BP3	22 (100.0%)	5.21	6.45	1.70	2.74	5.76
4-OHBP	22 (100.0%)	1.10	1.06	0.44	0.74	1.29
ΣBPs	22 (100.0%)	8.90	7.84	3.36	7.50	11.26

FD: frequency of detection; St. Dev.: standard deviation; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones.

Supplementary Table S4. Gene expression levels (n=22).

Cell pathway	Gene	n	%	Mean	St. Dev	Percentiles		
						25	50	75
Cell adhesion	ITGB2	22	100.0	6.09E+07	1.43E+08	8.68E+06	1.96E+07	4.61E+07
	CLDN7	22	100.0	1.76E+08	3.07E+08	5.25E+07	1.04E+08	1.64E+08
Invasion, migration and metastasis	MMP1	15	68.2	9.59E+07	4.16E+08	*n.e.*	2.00E+05	2.85E+06
	MMP7	3	13.6	1.83E+08	8.52E+08	*n.e.*	*n.e.*	*n.e.*
	FUT8	7	31.8	6.71E+04	1.76E+05	*n.e.*	*n.e.*	3.38E+04
	RRM2	22	100.0	1.80E+08	5.55E+08	2.54E+07	4.88E+07	1.08E+08
	MDK	8	36.4	8.56E+05	3.84E+06	*n.e.*	*n.e.*	8.89E+04
	RHOB	22	100.0	1.00E+09	3.18E+09	5.97E+07	1.06E+08	3.06E+08
	SPRY2	13	59.1	6.13E+06	1.10E+07	*n.e.*	5.27E+05	9.47E+06
Inflammation	IL1R2	3	13.6	5.15E+03	1.35E+04	*n.e.*	*n.e.*	*n.e.*
	IL1RL1	19	86.4	1.52E+07	4.64E+07	1.42E+05	9.76E+05	2.95E+06
	IL6ST	22	100.0	1.60E+09	6.19E+09	5.25E+07	1.18E+08	2.75E+08
	NR3C1	21	95.5	9.53E+07	3.93E+08	7.79E+05	2.36E+06	1.53E+07
	TNFRSF1B	6	27.3	9.81E+04	2.97E+05	*n.e.*	*n.e.*	4.06E+04
Angiogenesis	ANG	22	100.0	3.52E+07	1.01E+08	5.08E+06	1.06E+07	2.14E+07
	ANGPT1	19	86.4	4.69E+06	1.28E+07	1.97E+05	1.18E+06	2.82E+06
	sVEGFR-1	14	63.6	3.02E+07	1.41E+08	*n.e.*	2.24E+05	3.28E+05
	VEGFA	16	72.7	4.67E+07	5.67E+07	*n.e.*	3.84E+07	6.68E+07
Cell proliferation and hormonal stimulation	CYP19A1	4	18.2	2.35E+08	1.10E+09	*n.e.*	*n.e.*	*n.e.*
	DUSP6	20	90.9	3.47E+07	5.01E+07	1.67E+05	4.70E+06	7.03E+07
	ERα	22	100	3.75E+08	1.03E+09	9.33E+07	1.32E+08	2.20E+08
	PGR	22	100.0	2.73E+05	1.28E+06	*n.e.*	*n.e.*	*n.e.*
	STAR	18	81.8	2.04E+07	2.66E+07	3.02E+04	1.04E+07	3.73E+07

St. Dev.: standard deviation; ITGB2: integrin beta-2; CLDN7: claudin 7; MMP1: matrix metallopeptidase 1; MMP7: matrix metallopeptidase 7; FUT8: fucosyltransferase 8; RRM2: ribonucleotide reductase M2; MDK: midkine; RHOB: ras homolog gene, family, member B; SPRY2: sprout homolog 2; IL1RL1: interleukin 1 receptor, type I; IL1R2: interleukin 1 receptor, type II; IL6ST: interleukin 6 cytokine family signal transducer; NR3C1: nuclear receptor subfamily 3 group C member 1; TNFRSF1B: tumor necrosis factor receptor superfamily member 1B; ANG: angiogenin; ANGPT1: angiopoietin 1; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; VEGFA: vascular endothelial growth factor A; CYP19A1: cytochrome P450 family 19 subfamily A member 1; DUSP6: dual specificity phosphatase 6; ERα: estrogen receptor 1; PGR: progesterone receptor; STAR: steroidogenic acute regulatory protein; n.e.: not expressed.

Supplementary Table S5. Spearman correlation test betwen gene expression and PB and BP concentrations.

		MeP	EtP	PrP	BuP	Sum PBs	BP1	BP3	4-OHBP	Sum BPs
Cell adhesion	**ITGB2**	0.50*	0.47*	0.05	0.28	0.52*	0.05	0.01	-0.17	0.02
	CLDN7	0.55*	0.16	0.02	0.59*	0.56*	-0.25	-0.29	-0.15	-0.35
Invasion, migration and metastasis	**RRM2**	0.16	0.21	0.13	0.23	0.19	0.18	-0.02	-0.15	0.02
	RHOB	0.55*	0.43·	0.19	0.67*	0.56*	-0.07	-0.02	-0.03	-0.10
Inflammation	**IL1RL1**	0.01	-0.13	0.02	0.32	-0.05	-0.15	0.09	0.52*	-0.05
	IL6ST	0.44*	0.25	0.21	0.67*	0.44*	-0.13	-0.14	0.08	-0.24
	NR3C1	0.50*	0.32	0.29	0.65*	0.48*	-0.19	0.02	0.23	-0.19
Angiogenesis	**ANG**	0.46*	0.24	0.21	0.53*	0.28	0.04	0.07	-0.24	-0.02
	ANGPT1	0.52*	0.15	0.19	0.57*	0.48*	-0.16	-0.02	0.01	-0.16
Cell proliferation and hormonal stimulation	**CYP19A1**	-0.02	0.11	0.26	-0.02	-0.02	0.23	0.21	0.03	0.22
	DUSP6	-0.37·	-0.06	-0.09	-0.33	-0.35	0.21	0.12	-0.15	0.24
	ERα	0.41·	0.26	0.13	0.47*	0.56*	0.05	-0.15	-0.06	-0.12
	STAR	-0.21	-0.16	0.09	0.06	-0.15	0.24	0.18	0.45*	0.26

** p-value < 0.05; ·p-value < 0.10; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; ITGB2: integrin beta-2; CLDN7: claudin 7; RRM2: ribonucleotide reductase M2; RHOB: ras homolog gene, family, member B; IL1RL1: interleukin 1 receptor, type I; IL6ST: interleukin 6 cytokine family signal transducer; NR3C1: nuclear receptor subfamily 3 group C member 1; ANG: angiogenin; ANGPT1: angiopoietin 1; CYP19A1: cytochrome P450 family 19 subfamily A member 1; DUSP6: dual specificity phosphatase 6; ERα: estrogen receptor 1; STAR: steroidogenic acute regulatory protein.*

Supplementary Table S6. Associations between urinary concentrations of PBs and BPs and expression levels of genes involved in cell adhesion.

	ITGB2				CLDN7			
	β	**95% CI**		**p-value**	**β**	**95% CI**		**p-value**
Parabens								
MeP	0.30	0.11	0.49	0.004	0.17	0.02	0.32	0.026
<39.26 ng/mL	0.00	-	-		0.00	-	-	
>39.26 ng/mL	***1.13***	***0.36***	***1.90***	***0.006***	***0.85***	***0.35***	***1.35***	***0.002***
EtP	0.23	0.05	0.40	0.013	0.08	-0.06	0.21	0.260
<4.51 ng/mL	0.00	-	-		0.00	-	-	
>4.51 ng/mL	0.74	-0.13	1.61	0.091	-0.11	-0.93	0.71	0.780
PrP	0.04	-0.18	0.25	0.723	0.01	-0.14	0.16	0.869
<2.54 ng/mL	0.00	-	-		0.00	-	-	
>2.54 ng/mL	-0.01	-0.95	0.93	0.986	-0.22	-0.86	0.42	0.478
BuP	0.17	-0.19	0.53	0.343	0.15	-0.05	0.35	0.135
<0.14 ng/mL	0.00	-	-		0.00	-	-	
>0.14 ng/mL	***1.07***	***0.05***	***2.09***	***0.040***	***0.91***	***0.21***	***1.62***	***0.014***
ΣPBs	0.29	0.08	0.51	0.011	0.18	0.02	0.33	0.029
<53.31 ng/mL	0.00	-	-		0.00	-	-	
>53.31 ng/mL	***0.85***	***0.01***	***1.70***	***0.049***	***0.67***	***0.11***	***1.23***	***0.022***
Benzophenones								
BP-1	0.10	-0.45	0.66	0.697	-0.24	-0.53	0.05	0.101
<1.42 ng/mL	0.00	-	-		0.00	-	-	
>1.42 ng/mL	0.70	-0.39	1.78	0.848	0.06	-0.76	0.88	0.882
BP-3	0.04	-0.38	0.46	0.848	-0.16	-0.38	0.06	0.150
<2.53 ng/mL	0.00	-	-		0.00	-	-	
>2.53 ng/mL	0.48	-0.63	1.59	0.380	-0.10	-0.91	0.72	0.810
4-OHBP	0.06	-0.65	0.76	0.870	-0.133	-0.52	0.254	0.482
<0.73 ng/mL	0.00	-	-		0.00	-	-	
>0.73 ng/mL	-0.17	-1.30	0.96	0.754	0.12	-0.70	0.94	0.768
ΣBPs	0.07	-0.55	0.70	0.809	-0.28	-0.61	0.04	0.079
<7.43 ng/mL	0.00	-	-		0.00	-	-	
>7.43 ng/mL	0.63	-0.47	1.72	0.245	-0.16	-0.97	0.66	0.696

CI: confidence interval; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; ΣPB: sum of parabens; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; ΣBP: sum of benzophenones; ITGB2: integrin beta-2; CLDN7: claudin 7.

ANNEX V. SUPPLEMENTARY MATERIAL. ARTICLE 5

Supplementary Table S1. Information of the primers used in this study.

Cell pathway	Gene	Assay ID
Cell cycle	**BMI1**	qHsaCED0002041
	CCNB1	qHsaCED0044529
	CDK1	qHsaCID0036777
Apoptosis	**BAX**	qHsaCED0037943
	BCL2L1	qHsaCED0036793
	FOXO3	qHsaCID0023235
	SPP1	qHsaCID0012060
Cell diferentiation	**HOXA10**	qHsaCED0007868
	PDGFRA	qHsaCID0007202
	SOX2	qHsaCED0036871
Lipid metabolism	**APOE**	qHsaCED0044297
	PLCG1	qHsaCID0010865
	PLCG2	qHsaCID0016190
Housekeeping gene	**GAPDH**	qHsaCED0038674

BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2; APOE: Apolipoprotein E; PLCG1: Phospholipase C Gamma 1 and PLCG2: Phospholipase C Gamma 2; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

Supplementary Table S2. Comparison of the characteristics of the population between the subset of women included (n=22) and not in the final analyses (n=11).

	Women included (n=22)		Women not included (n=11)		
	n	**%**	**n**	**%**	**p-value**
Sociodemographic characteristics					
Age *(years)**	37.0 ± 7.5		40.0 ± 6.8		0.366
Weight *(kg)**	67.0 ± 15.1		68.7 ± 14.7		0.458
Height *(m)**	1.6 ± 0.1		1.6 ± 0.1		0.129
Body mass index *(kg/m^2)**	25.3 ± 5.2		25.4 ± 5.7		0.826
Normal weight (BMI < 25)	12	54.5	8	72.7	0.456
Overweight/Obese (BMI > 25)	10	45.5	3	27.3	
Residence					0.719
Rural	12	54.5	7	63.6	
Urban /sub-urban	10	45.5	4	36.4	
Parity					0.488
Nulliparous	9	40.9	6	54.5	
Primiparous/Multiparous	13	59.1	5	45.5	
Educational level					0.471
Less than university degree	15	68.2	6	54.5	
University degree	7	31.8	5	45.5	
Working outside home					**0.015**
Yes	13	59.1	11	100.0	
No	9	40.9	0	0	
Current smoker					0.696
Yes	6	27.3	4	36.4	
No	16	72.7	7	63.6	
Reproductive characteristics					
Intensity of menstrual bleeding					0.703
Spotting/light	9	40.9	3	27.3	
Moderate/heavy	13	59.1	8	72.7	
Endometrioma location					0.218
Deep infiltrating endometriosis	7	31.8	1	9.1	
Ovarian/peritoneal endometriosis	15	68.2	10	90.9	
Endometriosis stage					0.703
I/II	13	59.1	8	72.7	
III/IV	9	40.9	3	27.3	

**Mean ± standard deviation. Bold: p-value<0.05.*

Supplementary Table S3. Urinary concentrations of parabens and benzophenones (ng/mL).

EDC	Mean	St. Dev	Percentiles		
			25	50	75
MeP	236.56	631.95	11.45	45.44	90.13
EtP	44.20	95.28	1.61	4.66	23.11
PrP	16.43	35.16	0.48	2.69	10.38
BuP	0.81	1.63	0.05	0.15	0.61
ΣPBs	298.00	681.23	15.64	60.81	158.71
BP1	2.59	2.55	0.89	1.44	3.61
BP3	5.21	6.45	1.70	2.74	5.76
4-OHBP	1.10	1.06	0.44	0.74	1.29
ΣBPs	8.90	7.84	3.36	7.50	11.26

St. Dev: standard deviation; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone.

Supplementary Table S4. Gene expression levels (n=22).

Cell pathway	Gene	n	%	Mean	St. Dev	Percentiles		
						25	50	75
Cell cycle	**BMI1**	14	63.6	6.91E+07	7.56E+07	0.00E+00	5.53E+07	1.27E+08
	CCNB1	5	22.7	3.75E+04	8.23E+04	0.00E+00	0.00E+00	2.36E+04
	CDK1	21	95.5	2.40E+08	1.06E+09	6.04E+05	1.51E+06	6.43E+06
Apoptosis	**BAX**	18	81.8	2.68E+07	2.51E+07	5.69E+06	2.13E+07	4.63E+07
	BCL2L1	20	90.9	3.09E+08	9.12E+08	5.04E+07	1.06E+08	1.91E+08
	BAX/BCL2L1	18	81.8	1.68E+12	7.86E+12	2.95E-02	2.63E-01	3.18E-01
	FOXO3	21	95.5	1.92E+08	5.75E+08	1.62E+07	6.29E+07	1.42E+08
	SPP1	5	22.7	1.57E+06	6.74E+06	0.00E+00	0.00E+00	4.03E+03
Cell diferentiation	**HOXA10**	22	100.0	1.86E+08	5.26E+08	3.04E+07	5.79E+07	1.32E+08
	PDGFRA	22	100.0	1.12E+09	4.61E+09	1.20E+07	3.05E+07	1.12E+08
	SOX2	10	45.5	3.21E+06	5.78E+06	0.00E+00	0.00E+00	3.33E+06
Lipid metabolism	**APOE**	13	59.1	3.04E+07	1.27E+08	0.00E+00	1.80E+05	1.22E+06
	PLCG1	1	4.5	4.83E+03	2.26E+04	0.00E+00	0.00E+00	0.00E+00
	PLCG2	17	77.3	8.44E+05	2.23E+06	1.55E+09	6.02E+04	7.52E+05

BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2; APOE: Apolipoprotein E; PLCG1: Phospholipase C Gamma 1 and PLCG2: Phospholipase C Gamma 2.

Supplementary Table S5. Spearman correlation test betwen gene expression and PB and BP concentrations.

		MeP	EtP	PrP	BuP	ΣPBs	BP1	BP3	4-OHBP	ΣBPs
Cell cycle	**CDK1**	**0.48***	0.38	0.40	**0.65***	**0.52***	-0.09	-0.05	0.27	-0.13
Apoptosis	**BAX**	-0.05	0.02	-0.05	0.19	-0.05	0.31	-0.15	0.25	0.10
	BCL2L1	0.35	0.26	0.05	0.16	0.37	-0.09	-0.35	-0.07	-0.29
	BAX/BCL2L1	-0.16	0.18	0.34	-0,17	-0.16	0.44	0.31	-0.10	0.08
	FOXO3	-0.32	-0.39	-0.36	-0.06	-0.34	0.04	-0.02	-0.15	0.07
Cell diferentiation	**HOXA10**	0.25	-0.01	0.01	0.33	0.23	-0.15	-0.30	0.11	-0.35
	PDGFRA	0.40	0.24	0.27	**0.48***	0.41	-0.37	-0.16	-0.03	-0.39
Lipid metabolism	**PLCG2**	0.12	-0.08	**0.53***	0.25	0.10	0.06	0.27	0.04	0.11

** p-value < 0,05; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; CDK1: Cyclin Dependent Kinase 1; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha and PLCG2: Phospholipase C Gamma 2.*

Supplementary Table S6. Associations between PB and BP concentrations and gene expression levels.

Adjusted for educational level

	BMI									BCL2L1							
	OR	95% CI		p-value	OR[1]	95% CI		p-value		β	95% CI		p-value	β[1]	95% CI		p-value
4-OHBP	1.72	0.54	5.50	0.362	1.27	0.33	4.96	0.731	**BuP**	0.89	-0.34	2.12	0.147	0.09	-0.18	0.37	0.463
<0.73 ng/mL	1.00	-	-		1.00	-	-		*<0.14 ng/mL*	0.00	-	-		0.00	-	-	
>0.73 ng/mL	5.40	0.78	37.51	0.088	3.17	0.31	32.59	0.332	*>0.14 ng/mL*	3.11	-0.59	6.81	0.095	0.55	-0.27	1.37	0.165
	CDK1									**APOE**							
	β	95% CI		p-value	β[1]	95% CI		p-value		OR	95% CI		p-value	OR[1]	95% CI		p-value
ΣPBs	***0.67***	***0.30***	***1.05***	***0.001***	***0.65***	***0.03***	***1.27***	***0.042***	**4-OHBP**	4.13	0.94	18.21	0.061	2.15	0.48	9.68	0.320
<53.31 ng/mL	0.00	-	-		0.00	-	-		*<0.73 ng/mL*	1.00	-	-		1.00	-	-	
>53.31 ng/mL	***2.05***	***0.50***	***3.61***	***0.012***	1.11	-0.79	5.20	0.133	*>0.73 ng/mL*	***7.88***	***1.11***	***56.12***	***0.039***	5.77	0.52	64.10	0.153
	PDGFRA									**PLCG2**							
	β	95% CI		p-value	β[1]	95% CI		p-value		β	95% CI		p-value	β[1]	95% CI		p-value
MeP	0.21	-0.09	0.50	0.159	0.06	-0.32	0.45	0.732	**PrP**	***0.50***	***0.02***	***0.99***	***0.043***	0.42	-0.42	1.27	0.276
<39.26 ng/mL	0.00	-	-		0.00	-	-		*<2.54 ng/mL*	0.00	-	-		0.00	-	-	
>39.26 ng/mL	1.04	-0.04	2.11	0.058	0.31	-1.21	0.83	0.663	*>2.54 ng/mL*	1.73	-0.12	3.58	0.064	1.39	-2.00	4.78	0.366
ΣPBs	0.22	-0.08	0.52	0.142	0.09	-0.31	0.49	0.633	**BP-3**	0.20	-0.47	0.87	0.538	0.20	-1.13	1.53	0.727
<53.31 ng/mL	0.00	-	-		0.00	-	-		*<2.53 ng/mL*	0.00	-	-		0.00	-	-	
>53.31 ng/mL	1.06	-0.01	2.13	0.052	0.74	-0.99	2.47	0.365	*>2.53 ng/mL*	***1.93***	***0.14***	***3.73***	***0.037***	1.68	-1.60	4.96	0.266

Adjusted for working outside home

	PDGFRA									PLCG2							
	β	95% CI		p-value	β[2]	95% CI		p-value		β	95% CI		p-value	β[2]	95% CI		p-value
MeP	0.21	-0.09	0.50	0.159	0.22	-0.19	0.64	0.257	**PrP**	***0.50***	***0.02***	***0.99***	***0.043***	0.81	-0.543	2.17	0.193
<39.26 ng/mL	0.00	-	-		0.00	-	-		*<2.54 ng/mL*	0.00	-	-		0.00	-	-	
>39.26 ng/mL	1.04	-0.04	2.11	0.058	0.76	-0.96	2.47	0.348	*>2.54 ng/mL*	1.73	-0.12	3.58	0.064	1.55	-2.455	5.56	0.380
	BCL2L1								**BP-3**	0.20	-0.47	0.87	0.538	0.20	-1.24	2.646	0.741
	β	95% CI		p-value	β[2]	95% CI		p-value	*<2.53 ng/mL*	0.00	-	-		0.00	-	-	
BuP	0.89	-0.34	2.12	0.147	0.06	-0.218	0.345	0.626	*>2.53 ng/mL*	***1.93***	***0.14***	***3.73***	***0.037***	2.39	-1.185	5.96	0.153
<0.14 ng/mL	0.00	-	-		0.00	-	-										
>0.14 ng/mL	3.11	-0.59	6.81	0.095	0.55	-0.36	1.45	0.209									

[1]Adjusted for the covariate "educational level". [2]Adjusted for the covariate "working outside home"; OR: odds ratio; CI: confidence interval; 4-OHBP: 4-hydroxibenzophenone; PB: paraben; BuP: buthylparaben; MeP: methylparaben; PrP: propylparaben; BP-3: benzophenone-3; BMI: BMI1 Proto-Oncogene; CDK1: Cyclin Dependent Kinase 1; BCL2L1: BCL2 Like 1; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; APOE: Apolipoprotein E and PLCG2: Phospholipase C Gamma 2. Bold and italics: p-value<0.05.