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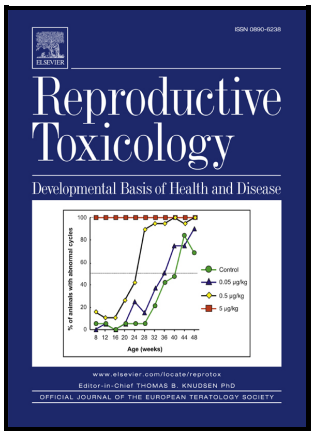

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The fate of bisphenol A, bisphenol S, and their respective glucuronide metabolites in ovarian cells

Authors:

Elodie Person[1], Sandrine Bruel[1], Trishabelle I Manzano[2], Emilien L Jamin[1,3], Daniel Zalko[1], Catherine MH Combelles[2*]

Author Affiliations:

[1] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, 31027, Toulouse, France.
[2] Biology Department, Middlebury College, Middlebury, VT 05753, USA.
[3] MetaboHUB-Metatoul, National Infrastructure of Metabolomics and Fluxomics, Metatoul-AXIOM, Toulouse, 31077, France.

*****Corresponding author: ccombell@middlebury.edu

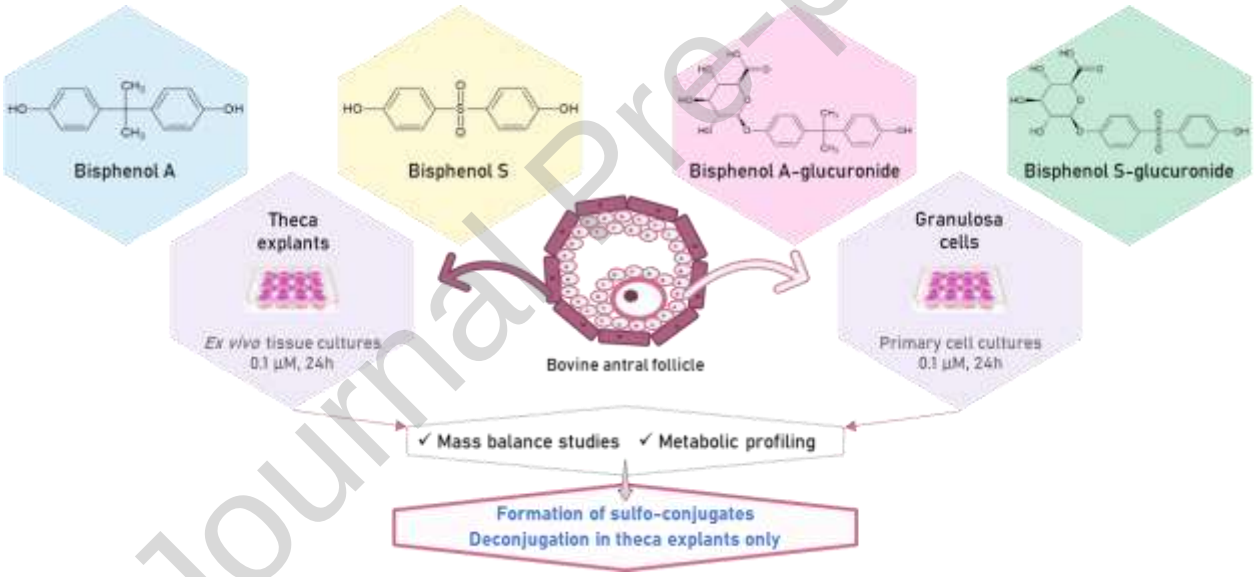
Abstract

Ovarian cells are critical for reproduction and steroidogenesis, which are functions that can be impacted by exposure to xenobiotics. As in other extra-hepatic tissues, biotransformation events may occur at the ovarian level. Such metabolic events deserve interest, notably as they may modulate the overall exposure and toxicity of xenobiotics. In this study, the comparative metabolic fate of two bisphenols was investigated in ovarian cells. Bisphenol A (BPA), a model endocrine disruptor, and its major substitute bisphenol S (BPS) were selected. Bovine granulosa cells (primary cultures) and theca explants (*ex vivo* tissue) were exposed for 24 hr to tritium-labeled BPA, BPS and their respective glucuronides (*i.e.* their major circulating forms), at concentrations consistent with low-dose exposure scenarios. Mass balance studies were performed, followed by radio-HPLC profiling. The capability of both cell compartments to biotransform BPA and BPS into their respective sulfo-conjugates was demonstrated, with sulfation being the predominant metabolic route. In theca, there was a significantly higher persistence of BPA (compared to BPS) residues over 24 hr. Moreover, only theca explants were able to deconjugate inactive BPA-glucuronide and BPS-glucuronide back into their biologically

active aglycone forms. Deconjugation rates were demonstrated to be higher for BPS-G than for BPA-G. These findings raise concerns about the *in situ* direct release of bisphenols at the level of the ovary and demonstrate the relevance of exploring the biotransformation of bisphenols and their circulating metabolites in different ovarian cells with specific metabolic capabilities. This work also provides essential knowledge for the improved risk assessment of bisphenols.

Keywords: Bisphenol A; Bisphenol S; Endocrine disruptors; Biotransformation; Ovarian follicle; Theca; Granulosa cells

Graphical abstract



1. Introduction

Bisphenol A (BPA) is a common plasticizer and one of the largest volume chemicals produced worldwide. It is used to manufacture polymers (epoxy resins and polycarbonates), and free BPA (unpolymerized) is also used in many countries as a color developer in thermal papers [1,2]. BPA has been detected in sediments, sewage, waters, indoor dust, and a variety of food samples [3,4]. Such pervasiveness helps explain that BPA has been detected in 74-99 % of urine samples of adults in the U.S. [5]. With growing concerns and regulations for BPA, substitutes

such as bisphenol S (BPS) are now on the rise [5]. BPS has similar applications as BPA, including its increasing use in thermal papers [1,2,6]. Its presence was demonstrated in 81 % of urinary samples in the U.S. and Asian countries [7]. Most bisphenols are suspected or proven endocrine disrupting chemicals (EDCs). That is, exogenous chemicals (xenobiotics) able to interfere with the body's hormonal system through various means, whether it be by mimicking hormone action, inhibiting signals, or hijacking hormone regulation [8,9]. Specifically, bisphenols such as BPA and BPS can act as xeno-estrogens that affect estrogens action, and they have known health effects, including compromised female reproductive function and fertility [10,11]. Together, impairments in sex steroids, oocyte production, and conception rates point to the need to focus more studies on the impact of bisphenols on the ovary.

Available data on the ability of BPA and its major substitutes to reach the ovaries are scarce, although of high interest. Mass balance studies carried out in laboratory animals after exposure to low doses of radio-labeled bisphenols have demonstrated their wide distribution in the body, including in the reproductive tract. Specifically, administered bisphenols were found to reach the ovaries of mice (BPA, [12]), rats (BPS, [13]), and even rhesus monkeys (BPA, [14]). The high residual levels of BPA in the ovaries of rhesus monkeys (particularly when compared to levels in rodents) point to the ovary as a main target tissue of BPA in primates. In biomonitoring studies, the presence of BPA has also been reported in human follicular fluid (the fluid that bathes cells of the follicle) at average concentrations around 1.5-4.7 ng/mL for BPA [15–17], and 1.9 ng/mL for BPS [18]. Given the known exposure of the ovary to bisphenols, an impact of these xeno-estrogens on essential functions must be considered; these include the production of steroids as well as follicle and oocyte development.

To support fertile reproductive cycles, complex relationships between all parts of the ovarian follicle are required [19], and the granulosa and theca cells of the antral follicle play major roles. Granulosa cells not only provide nutrients and local signals for oocyte development, but also convert the androgens (produced by the theca cells themselves) into estrogens [20]. In the follicle, the biosynthesis and metabolism of estrogens, androgens, and progestins depend on the extensive interplay of steroidogenic enzymes. Steroidogenic enzymes include cytochrome P450 enzymes (CYP) and hydroxysteroid dehydrogenases (HSD) that are expressed in a cell-specific manner in granulosa and theca cells and that catalyze each of the steps in the steroid conversion pathway of the follicle [21]. The proper functions of granulosa and theca cells can however be threatened by EDCs, including bisphenols. A large body of evidence has now shown the many negative impacts of BPA on oocyte and follicle development; steroidogenesis is also affected with for example changes in the expression and activity of steroidogenic enzymes in the follicle [11,22]. Findings to date also indicate that BPS negatively affects ovarian functions, including abnormalities in oocyte development [23–25], inhibition of proper follicle formation [26,27], and disruption of steroidogenesis [18]. In addition, evidence is mounting for the negative impacts of not just BPS but also other bisphenols (*e.g.* bisphenol F) on reproductive function, albeit with some differences in the type and extent of effects [28]. Together, there is thus an unequivocal need to study not only the effects of BPA but also that of its major substitutes on the ovary.

Central to assessing the toxicity of compounds is the need to understand their fate. As for many other xenobiotics, very little is known about the biotransformation of bisphenols at the level of target organs. This is certainly the case for the ovary. Current knowledge about the conjugation pathways of BPA *in vivo*, including in primates [14,29–31], and rodents [12], clearly

demonstrate that conjugated metabolites (mainly via glucuronidation or sulfation, with BPA glucuronidation predominating in primates) are by far the major forms under which this substance circulates in blood prior to its distribution in tissues. Such conversion into more hydrophilic metabolites facilitates their elimination. Following the early study by Matthews et al. (2001) examining the activity of BPA glucuronide [32], conjugated forms of BPA or BPS were generally assumed to lack biological (specifically nuclear estrogen receptor) activity [33]. However, later work suggested that some bisphenols (and potentially their metabolites) could exert part of their effects through the activation of membrane estrogen receptors [34]. Regardless, conjugated metabolites of BPA or BPS may be converted back to the parent, free, and biologically active forms, at the level of tissues or in the fetal sphere, as demonstrated by Corbel et al. in sheep [35]. There is thus a critical need to consider both conjugation and deconjugation activities, along with how the balance, regulation, and location of these activities may influence the actual threats posed by bisphenols. Although conjugation routes (phase II metabolism) undoubtedly predominate in the metabolism of BPA and are perceived as a detoxification process, other metabolites may be formed *in vivo*, as shown with hydroxylated intermediates of BPA in rodents [12]. BPA cytotoxicity may result from metabolites and intermediates produced by phase I oxidative metabolism (usually involving CYP450) [36,37]. In fact, for endogenous estrogens, cellular impairments have already been demonstrated to be caused by metabolites that originate from oxidative metabolism [38–40]. The possible formation of different metabolites of bisphenols must thus be investigated in reproductive organs, including the ovary.

While the liver plays a prominent role in the biotransformation of most xenobiotics, including bisphenols, extrahepatic organs can also express functional xenobiotic metabolizing

enzymes (XME), albeit in more limited variety and capacity. For example, the lung and brain can locally modify drugs or xenobiotics by glucuronidation or sulfation [41–43]. The kidney and testis are other organs that can express glucuronidation activities [44,45], including towards environmental or pharmaceutical estrogens such as BPA or diethylstilbestrol [46]. For some xenobiotics, the expression of XME has been reported in the ovary [47,48]; however, their conjugation activities have not yet been directly investigated. For endogenous compounds (such as steroids), enzymes catalyzing the glucuronidation (UDP-glucuronosyltransferases: UGTs) or sulfate conjugation (sulfotransferases: SULTs), as well as enzymes capable of hydrolyzing such conjugates have been shown to be functional in the ovary [44,45,49,50]. Since these metabolizing enzymes can accept both endogenous and exogenous substances as substrates, it can be hypothesized that bisphenols (or their conjugates) may undergo biotransformation in the ovary. In support of that possibility, there exists to date a single pilot study reporting on the conjugation of BPA and the deconjugation of BPA-glucuronide in explants of whole ovaries from fetal and adult sheep [35].

In this context, the present study addresses critical gaps that aim to better understand the possible role of the ovary in the biotransformation of bisphenols. Specifically, we focused our attention on two specific key structures: the granulosa and theca compartments of antral follicles. We selected for our study BPA (as a model EDC) and also explored the fate of its major substitute BPS. For this, we took advantage of the possibilities offered by [^{3}H] radioactive labeling. Bovine granulosa (cultured primary cells) or theca explants (*ex vivo* explants) were exposed to radiolabeled BPA, BPS, and their respective glucuronides (0.1 µM) for 24 hours. These studies were followed by full mass balance studies that enabled the determination of the location of residues and the assessment of their extractability. Metabolic profiling using high

performance liquid chromatography (radio-HPLC) was performed to monitor parent compounds and major metabolites of BPA and BPS. Our experimental design thus aimed for a direct evaluation of the biotransformation (with a focus on the conjugation/deconjugation balance) of a class of compound (bisphenols) for which metabolism has never been examined in specific cell compartments of the ovary.

2. Materials and methods

2.1. Chemicals, reagents, and media

Acetic acid, androstenedione (A4), dimethyl sulfoxide (DMSO), penicillin, streptomycin, amphotericin B, insulin, transferrin, sodium selenite, bisphenol A (reference 239658, ≥ 99 %) and bisphenol S (4,4′-Sulfonyldiphenol, reference 103039, 98 %) were purchased from Sigma-Aldrich Merck (Saint Quentin Fallavier, France). Dulbecco's Modified Eagle's Medium/Ham's F-12 50/50 mix media (DF12) with L-Glutamine and without phenol red was obtained from Corning (Corning, NY, USA). HEPES, Ca^{2+} and Mg^{2+}-free Hank's balanced salt solution (HBSS), TrypLE express enzyme, and HPLC grade acetonitrile were purchased from Thermo Fisher Scientific (Illkirch, France), fetal bovine serum (FBS) from Hyclone (Logan, Utah, USA), Ca^{2+} and Mg^{2+}-free Dulbecco's phosphate-buffered saline (DPBS) from PAN-Biotech (Aidenbach, Germany), ammonium acetate from VWR (Fontenay-sous-Bois, France), Flo-Scint™ II, Ultima Gold™ liquid scintillation cocktails and Soluene® 350 from PerkinElmer (Courtabœuf, France). Ultrapure water, produced using a Milli-Q system (Millipore, Saint-Quentin-en-Yvelines, France), was used for the preparation of HPLC mobile phases.

[^{3}H]-bisphenol A ([^{3}H]-BPA; specific activity: 92.5 GBq/mmol) and [^{3}H]-bisphenol S ([^{3}H]-BPS; specific activity: 62.9 GBq/mmol) were purchased from Moravek (Brea, CA, USA). They were further purified by radio-HPLC to reach a radiopurity of 99.4 % ([^{3}H]-BPA) and

100.0 % ([^{3}H]-BPS), respectively. The biochemical synthesis of [^{3}H]-BPA-glucuronide ([^{3}H]-BPA-G; specific activity: 22 GBq/mmol) and of [^{3}H]-BPA-sulfate ([^{3}H]-BPA-S; specific activity: 28.3 GBq/mmol) were carried out from [^{3}H]-BPA, as described previously [35]. Similar biochemical synthesis methods were used to synthesize and purify [^{3}H]-BPS-glucuronide ([^{3}H]-BPS-G; specific activity: 37.9 GBq/mmol) and [^{3}H]-BPS-sulfate ([^{3}H]-BPS-S; specific activity: 50 GBq/mmol). Briefly, *in vitro* incubations were carried out using guinea pig liver microsomal and cytosolic fractions; subsequent purification steps allowed the synthesis of 100 % pure radiolabeled metabolites with their structures confirmed by mass spectrometry.

2.2. Confirmation of BPS-G and BPS-S standards

In vitro incubations with non-labeled BPS followed by high resolution mass spectrometry (HRMS) analyses were performed to produce then confirm the structures of BPS-S and BPS-G. These two conjugated metabolites produced by biochemical means were characterized using an LTQ-Orbitrap-XL mass spectrometer (Thermo Scientific, Les Ulis, France) working with an electrospray ionization source. The following negative ionization parameters were applied during infusion at 5 µL/min of metabolites collected after liquid chromatography and prepared in methanol/water 1:1 (v/v): source voltage 3 kV, sheath gas (N_2) flow rate 10 *a.u.*, capillary temperature 300 °C, tube lens voltage -90 V. Mass spectra were acquired at a resolution of 30000, after an external calibration according to supplier's protocol. BPS-G was detected at *m/z* 425.0539 ([M-H]$^-$ 2 ppm) and displayed the neutral loss of the glucuronide moiety at 249.0225 and the corresponding dipole ion at *m/z* 175.0246 after collision induced fragmentations achieved at a normalized collision energy (NCE) of 12 %. The MS^3 spectrum obtained at NCE=25 % of the *m/z* 249 ion displayed the fragmentation pattern of the BPS standard with

daughter ions detected at *m/z* 185, 184, 156 and 108. BPS-S was detected at *m/z* 328.9795 ($[M-H]^-$ 0.05 ppm). The loss of sulfate was observed at *m/z* 249.0223 on its MS^2 spectrum achieved at NCE=12 %, and the MS^3 spectrum of *m/z* 249 displayed *m/z* 185, 184, 156 and 108 fragment ions at NCE=25 %.

2.3. Collection of ovaries

Ovaries from 38 non-pregnant cows (2-9 years old) were obtained from a local abattoir: 18 cows were used for isolating granulosa cells, and 20 were used for the isolation of theca explants. Only cows without ovarian cysts and with evidence of estrous cycling were included. This assessment was based on follicle characteristics and the presence of an active corpus luteum [51]. Each pair of ovaries (on a per animal basis) was kept in DPBS on ice for about 2 (and a maximum of 3) hours prior to cell/explant isolation.

2.4. Theca collection and preparation

Theca sheets were isolated from the largest antral follicle (13-20 mm; 15.7 ± 2.1, mean ± SD) on each ovarian pair using a validated methodology that allows the clean isolation of the entire theca interna layer from a single follicle [52,53]. Follicles were dissected clean of surrounding stroma prior to opening with a 4-way incision pattern. Granulosa cells and oocytes were removed by gentle scraping of the inner wall of the follicle with an inoculation loop and by flushing with DPBS supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 µg/mL amphotericin B (DPBS-S). Theca sheets (free of any other ovarian parts) were gently peeled using fine forceps. After separation, the intact theca sheets were rinsed in DPBS-S and dried of excess solution onto a sterile gauze. Theca sheets were weighed and only sheets of

at least 80 mg mass were included in the study. Theca sheets were then cut into theca pieces or explants which mass ranges between 18 and 34 mg (24.3 ± 4.7 mg, mean ± SD). Aiming for theca explants between 20 to 30 mg in mass each, we intentionally performed single cuts with no follow-up resizing. This approach allowed minimal tissue handling and helped maximize tissue viability and integrity. As a result, exact masses varied slightly between individual explants; however, there were no statistical differences between any of the treatment groups for which formation rates were compared: namely, between BPA (26.0 ± 5.8 mg) and BPS (20.8 ± 2.5 mg) and between BPA-G (21.8 ± 3.5 mg) and BPS-G (26.7 ± 3.1 mg). To minimize the potential impact of cell dissociation or any other processing steps, the cut theca explants were cultured intact *ex vivo*. Instead of considering the relative contribution of individual components of the theca layer, this culture system was specifically chosen to allow the assessment of metabolic capacity of the theca, that is as an intact unit with all its parts (including thecal cells, immune cells, vascular tissue, and extracellular matrix). Each theca explant was moved into DPBS-S in individual wells of 12-well plates (sterile polystyrene plates, TPP, Trasadingen, Switzerland). Such *ex vivo* system maintains viability and function of the theca explant, as previously shown from steroidogenic outputs and responses to gonadotropins throughout a 3-day culture period [54,55].

2.5. Granulosa cell collection and preparation

Using a 23-gauge needle, granulosa cells were aspirated from antral follicles (3 to 5 mm in diameter) at the surface of each ovary. Follicular aspirates were gathered into warm DF12 supplemented with 5 mM HEPES, 10 % (v/v) FBS and 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 µg/mL amphotericin B (DF12+S). After all visible 3-5 mm antral follicles

were aspirated from the surface of the ovary, the ovary was bisected lengthwise to access and aspirate additional 3-5 mm follicles. All the granulosa cell aspirates from an animal were pooled and centrifuged for 10 minutes at 82 x *g* and each cell pellet was resuspended with DF12+S medium for culture in a T-75 flask. Cells were cultured to confluency for 4 days (with one media change after 3 days). Cells were then detached by exposure to HBSS and then TrypLE enzymes, followed by neutralization with DF12-S and centrifugation for 10 minutes at 82 x *g*. Each cell pellet was resuspended with 1 mL DF12 supplemented with 5 % charcoal-stripped FBS, 10 ng/mL insulin, 5 µg/mL transferrin, 5 ng/mL sodium selenite, 5 mM HEPES, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 µg/mL amphotericin B, and 100 nM androstenedione (DF12+CS). Using Trypan blue and an automated cell counter (Countess II, ThermoFisher Scientific), the number of viable cells was counted. 400,000 viable cells were seeded per well in 12-well plates (sterile polystyrene plates, TPP, Trasadingen, Switzerland) and cultured in DF12+CS for 24 hours prior to exposure to radiolabeled compounds. Isolated, processed, and cultured under these conditions (as well as under the serum-free conditions used for biotransformation assays), granulosa cells were previously shown to express *STAR*, *FSHR*, and *CYP19A1*, produce estradiol, and adequately respond to FSH and IGF-1 [56], together confirming the identity of the cells as granulosa.

2.6. [^{3}H]-BPA and [^{3}H]-BPS biotransformation assays

Biotransformation assays were carried out on 7 and 8 granulosa cell biological replicates (each obtained from different cows) for [^{3}H]-BPA and [^{3}H]-BPS, respectively, and on 9 theca biological replicates (also from different cows) for [^{3}H]-BPA as well as for [^{3}H]-BPS. All concentrations were set at 0.1 µM (BPA: 22.8 ng/mL and BPS: 25.0 ng/mL), a concentration

consistent with previously detected levels in human follicular fluid [15–18]. [^{3}H]-BPA (3.33 kBq per incubation), fortified with unlabeled BPA to reach the required concentration, and [^{3}H]-BPS (6.29 kBq per incubation) were prepared in DMSO. After a 24-hr initial culture period (granulosa cells) or immediately after the addition of theca explants into wells, incubations with the radio-labeled compounds were started. For both primary culture models, incubations were performed in 12-well plates, in a final volume of 1 mL DF12 in the absence of serum and with supplementation with 10 ng/mL insulin, 5 µg/mL transferrin, 5 ng/mL sodium selenite, 5 mM HEPES, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 µg/mL amphotericin B. For granulosa cell cultures, 100 nM androstenedione was also added. Each well contained 0.5 % DMSO. Granulosa cells and theca explants were cultured for 24 hours at 37 °C, 5 % CO_2 and 95 % relative humidity. To detect potential degradation under the culture conditions, "no-cell" controls (consisting of wells with no cultured cells/explants) were also performed for each cellular system and each compound.

2.7. [^{3}H]-BPA-glucuronide and [^{3}H]-BPS-glucuronide deconjugation assays

Deconjugation assays were carried out on 9 and 7 granulosa cell biological replicates (each obtained from different cows) for [^{3}H]-BPA-G and [^{3}H]-BPS-G, respectively, and on 10 and 11 theca biological replicates (also from different cows) for [^{3}H]-BPA-G and [^{3}H]-BPS-G respectively. All concentrations were again set at 0.1 µM (BPA-G: 40.4 ng/mL and BPS-G: 42.6 ng/mL). [^{3}H]-BPA-G (2.2 kBq per incubation) and [^{3}H]-BPS-G (3.79 kBq per incubation) were prepared in DMSO. Incubations were performed in the same conditions as described above for biotransformation assays. All wells contained 0.5 % DMSO. "No-cell" controls were

performed for each cellular system to assess the potential degradation and/or spontaneous deconjugation of [^{3}H]-BPA-G or [^{3}H]-BPS-G.

2.8. Mass balance studies

Radioactivity mass balance studies were carried out for each cellular system (theca, granulosa cells). Theca culture media were individually collected in glass vials, with 10 µL sampled for radioactivity measurement and the rest immediately stored at -20 °C for later radio-HPLC analyses. Wells were then washed with 1 mL cold (4 °C) DPBS, and theca explants were individually transferred into glass vials. After addition of 1 mL of water-acetonitrile mix 50:50 (v/v), vials were vortexed for 5 min, followed by 10 µL collection for radioactivity measurement. The radioactivity remaining in each explant after extraction was quantified following theca explant solubilization in 500 µL of Soluene® 350 for 2 hours at 50 °C.

Granulosa cell culture media were individually collected in glass vials, with 10 µL sampled for radioactivity measurement before storage at -20 °C and pending radio-HPLC analyses. Each cell monolayer was washed with 1 mL cold (4 °C) DPBS, followed by the addition of 500 µL water-acetonitrile mix 50:50 (v/v). A cell scraper was used to recover the cells from the wells, and cell suspensions were individually transferred into glass vials. This step was repeated once, with the cells collected in a final volume of 1 mL. 10 µL were sampled for radioactivity determination. All radioactivity measurements were carried out using a Tri-Carb 2910TR (PerkinElmer) liquid scintillation analyzer, using Ultima Gold™ as the scintillation cocktail. Sample quenching was compensated by the use of quench curves and external standardization.

2.9. Radio-HPLC metabolic profiling

Culture media were individually analysed by reversed-phase high-performance liquid chromatography coupled with radio-detection (R-HPLC) for profiling and quantification. Analyses were performed on an Ultimate-3000 system (Thermo Fisher Scientific) coupled with a flow scintillation analyzer Flo-One Radiomatic™ 610TR (PerkinElmer) equipped with a 500-µL detection cell. Flo-Scint™ II was used as scintillation cocktail, at a flow rate of 2 mL/min. The HPLC system consisted of a Zorbax SB-C18 column (250 x 4.6 mm, 5 µm, Agilent, Santa Clara, CA, USA) coupled to a C18 guard precolumn (EC 4/2 Universal RP, Macherey-Nagel, Dueren, Germany). Mobile phases were A: ammonium acetate buffer (20 mM, adjusted to pH 3.5 with acetic acid) and acetonitrile 95:5 (v/v*)* and B: ammonium acetate buffer (20 mM, adjusted to pH 3.5 with acetic acid) and acetonitrile 10:90 (v/v*)*. Flow rate was 1 mL/min and the injection volume was 500 µL.

For the metabolic fate of [^{3}H]-BPA and [^{3}H]-BPA-G, the column was maintained at 25 °C, and the gradient was as follows: 0-4 min 100 % A; 4-6 min from 100 % A to A:B 85:15 (v/v); 6-16 min A:B 85:15; 16-18 min from 85:15 to 75:25; 18-28 min A:B 75:25; 28-30 min from 75:25 to 70:30; 30-37 min A:B 70:30; 37-39 min from 70:30 to 30:70; 39-50 min A:B 30:70; 50-52 min from 30:70 to 100 % B; 52-62 min 100 % B. System returned to its initial condition at 64 min and was held for another 6 min. Each culture medium (250 µL, corresponding to *ca.* 500 Bq) was mixed with mobile phase A prior to HPLC injection.

For the metabolic fate of [^{3}H]-BPS and [^{3}H]-BPS-G, the column was maintained at 30 °C and the gradient was as follows: 0-4 min 100 % A; 4-6 min from 100 % A to A:B 90:10 (v/v); 6-16 min A:B 90:10; 16-18 min from 90:10 to 80:20; 18-28 min A:B 80:20; 28-30 min from 80:20 to 70:30; 30-40 min A:B 70:30; 40-42 min from 70:30 to 30:70; 42-50 min A:B 30:70; 50-

51 min from 30:70 to 100 % B; 51-57 min 100 % B. System returned to its initial condition at 58 min and was held for another 7 min. Each culture medium (150 µL, corresponding to *ca.* 500 Bq) was mixed with mobile phase A prior to HPLC injection.

Radio-HPLC profiles were processed with the A500 software (PerkinElmer) using an efficiency correction (35 % for [^{3}H]) and a background suppression of 20 cpm (counts per minute). Compounds were quantified by integrating the area under the peaks monitored by radioactivity detection. Values are expressed as mean ± SD.

2.10. Statistical analysis

Statistical analyses were performed using the GraphPad Prism software V9. One sample t-test (radioactivity recovery, with theoretical value 100), one-way (deconjugation rates) and two-way (radioactivity distribution) analysis of variance (ANOVA) were performed, followed by Tukey's *post hoc* test. Differences were considered as significant when p-value was < 0.05 (*: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001, ****: p-value < 0.0001).

3. Results

Radio-labeled BPA, BPS, as well as their corresponding conjugated metabolites (BPA-G and BPS-G), were individually cultured with either theca explants or granulosa cells at a concentration of 0.1 µM. 24 hr mass balance studies were carried out, and all culture media were individually analysed by radio-HPLC.

3.1. Recovery and distribution of radioactivity

Radioactivity recovery (Fig. 1 and **Error! Reference source not found.**) ranged between 90 % and 110 % whatever the cellular system or molecule assayed. It significantly differed from

100 % only for theca explant cultures carried out with [^{3}H]-BPA (108.8 ± 5.0 %, p-value = 0.0008), [^{3}H]-BPS (92.1 ± 2.6 %, p-value < 0.0001), and [^{3}H]-BPA-G (91.4 ± 4.9 %, p-value = 0.0004).

For all theca explants assays (Fig. 1A), the largest part of the radioactivity was recovered in culture media after 24 hr. However, at that time, theca explants were also found to contain a substantial part of the radioactivity, unambiguously demonstrating the internalization of assay molecules. The total amount of radioactivity present in explants was 11.0 ± 4.0 %, 7.0 ± 2.7 % and 4.5 ± 1.5 %, for cultures carried out with BPS, BPA-G or BPS-G, respectively, and most of this radioactivity was found to be extractable following explant extraction with water-acetonitrile mix 50:50 (v/v) (Fig. 1A and **Error! Reference source not found.**). In cultures carried out with BPA, the proportion of radioactivity contained in the theca explants themselves was much higher (42.5 ± 5.9 %), and a consistent part of it (6.6 ± 1.5 %) was determined not to be extractable in our conditions, *i.e.* it remained in the tissue after extraction and was quantified following theca explant solubilization (Fig. 1 and **Error! Reference source not found.**). Statistical comparisons between BPA and BPS were all found to be highly significant. Both the extractable and the non-extractable fractions of the radioactivity remaining in theca explants by the end of the study were higher for BPA than for BPS (p < 0.0001, two-way ANOVA). In cultures carried out with their respective glucuronic acid conjugates (BPA-G or BPS-G, Fig. 1A), minor yet significant differences in the distribution of radioactivity were also observed, with a slightly lower proportion of the radioactivity recovered in culture media for BPA-G, compared to BPS-G (p = 0.013). Conversely, a higher proportion of non-extractable residues was measured in cultures carried out with BPA-G, compared to BPS-G (p = 0.021); this was as observed for the incubations carried out with the parent compounds.

In assays carried out with granulosa cells (Fig. 1B), most of the radioactivity was found in culture media at 24 hr, again with higher values recorded for BPS than for BPA ($p < 0.001$). The proportion of radioactivity present inside granulosa cells also appeared to be higher for BPA than for BPS. However, due to marked inter-individual variability, this difference was not found to be statistically significant (Fig. 1B; **Error! Reference source not found.**).

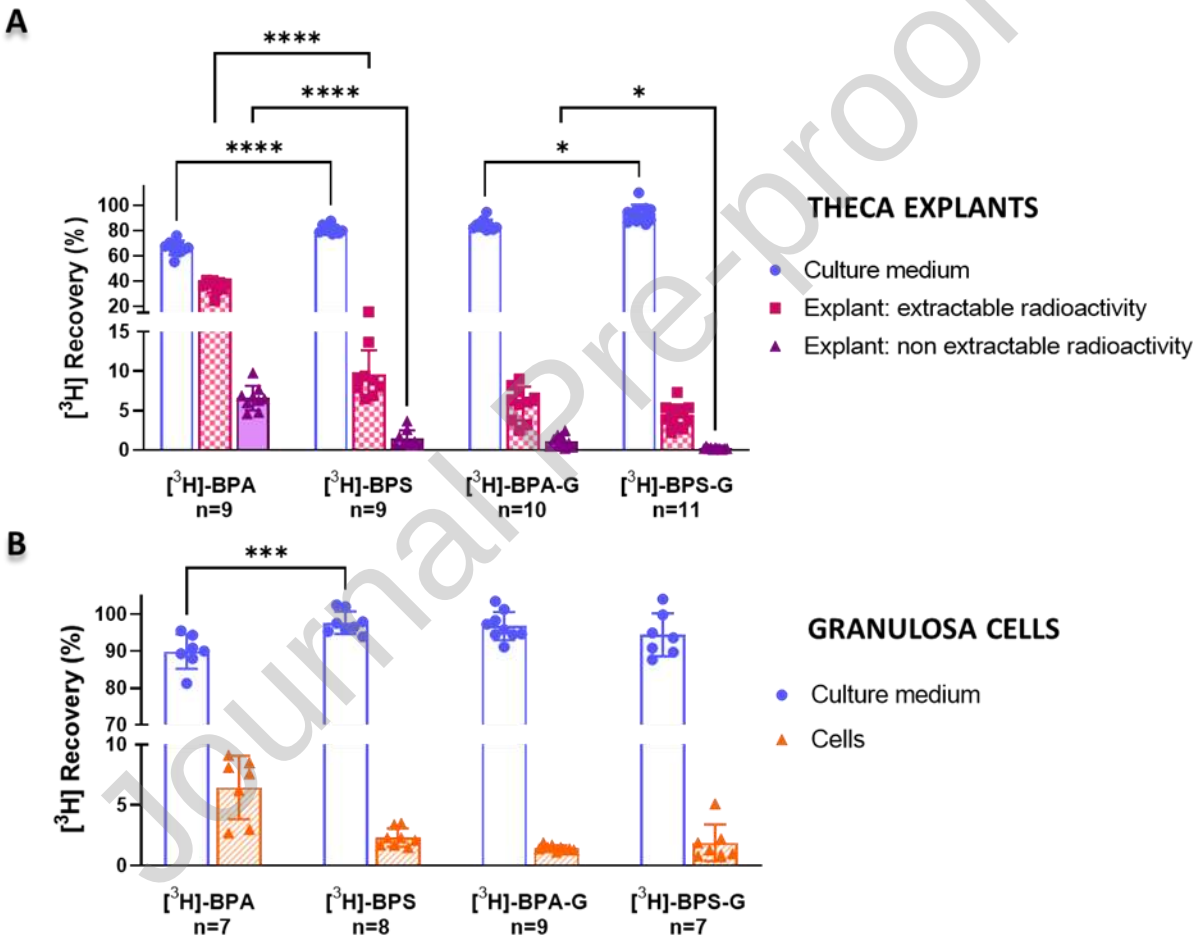


Fig. 1. Radioactivity mass balance of [^{3}H]-BPA (n=9 and n=7), [^{3}H]-BPS (n=9 and n=8), [^{3}H]-BPA-G (n=10 and n=9) and [^{3}H]-BPS-G (n=11 and n=7) cultured for 24 hr with (A) theca explants or (B) granulosa cells at 0.1 µM. [^{3}H] Recovery results are expressed in percentage ± SD, 2-way ANOVA analysis and Tukey's *post hoc* test, *: p-value < 0.05, ***: p-value < 0.001, ****: p-value < 0.0001.

3.2. Biotransformation of BPA and BPS

The retention times (Rt) of BPA and BPS, using the specific HPLC conditions developed for each of these bisphenols, were 42.9 min and 29.4 min respectively. In all "no-cell" control culture media samples, parent compounds accounted for more than 98 % of the radioactivity put in incubations, demonstrating that no significant degradation of the assay molecules occurred over 24 hr. Furthermore, none of the very minor impurities detected eluted at a Rt similar to one of the metabolites of these bisphenols.

Radio-HPLC analyses (Fig. 2; Table 1) demonstrated that both theca explants and granulosa cells were able to biotransform BPA, as well as BPS, into their corresponding sulfated conjugates, namely BPA-S (Rt: 34.9 min) and BPS-S (Rt: 23.3 min). These conjugates were found to coelute with their respective pure radio-labeled standards produced by biochemical synthesis. In addition, in 6 out of 9 cows, low amounts of a second [^{3}H]-BPA metabolite, eluted at a Rt of 22.4 min, were also detected in the theca explant culture media (Fig. 2A; Table 1). This metabolite (1.3 ± 1.5 pmol/24 hr, **Error! Reference source not found.**) was not BPA-G (Rt: 24.5 min), but HRMS investigations did not allow its identification.

Theca explants were found to convert 2.9 ± 1.8 % of the BPA put in culture into BPA-S. In cultures carried out with BPS, 2.8 ± 1.4 % of BPS-S were detected (Fig. 2 A-B; Table 1). Notable inter-individual variability was observed. Radio-HPLC results, combined with mass balance data, allowed to calculate that 2.0 ± 1.2 pmol/24 hr of BPA-S were formed by theca explants and detected in culture media (range: 0.2-3.4 pmol/24 hr). The rate of formation of BPS-S was identical: 2.2 ± 1.1 pmol/24 hr (range: 0.7-4.2 pmol/24 hr, **Error! Reference source not found.**). Granulosa cells also converted BPA into BPA-S (1.3 ± 0.9 %) and BPS into BPS-S (1.3 ± 0.6 %) (Fig. 2D; Table 1), with the formation of 1.1 ± 0.9 pmol/24 hr BPA-S (range: 0-

2.6 pmol/24 hr) and 1.3 ± 0.5 pmol/24 hr BPS-S (range: 0.5-2.1 pmol/24 hr) detected in culture media (**Error! Reference source not found.**).

Table 1. Radioactive peaks quantification and retention times for 24 hr cultures carried out with 0.1 µM [^{3}H]-BPA, 0.1 µM [^{3}H]-BPS, 0.1 µM [^{3}H]-BPA-G, or 0.1 µM [^{3}H]-BPS-G for theca explants or granulosa cells. Results are expressed in percentage of total radioactivity in the HPLC profile ± SD, ND: Not Detected, major peak in bold.

Radio-HPLC peaks (%)	[^{3}H]-BPA				[^{3}H]-BPS			[^{3}H]-BPA-G			[^{3}H]-BPS-G		
	Unidentified 22.4 min	BPA-S 34.9 min	**BPA 43.0 min**	Others	BPS-S 23.3 min	**BPS 29.4 min**	Others	**BPA-G 24.5 min**	BPA 42.9 min	Others	**BPS-G 13.9 min**	BPS 29.1 min	Others
Theca explants	1.9 ± 2.0	2.9 ± 1.8	**92.1 ± 5.4**	3.1 ± 3.0	2.8 ± 1.4	**97.2 ± 1.4**	ND	**95.9 ± 1.7**	4.0 ± 1.7	0.09 ± 0.3	**85.8 ± 5.2**	14.0 ± 5.3	0.2 ± 0.3
Granulosa cells	ND	1.3 ± 0.9	**97.1 ± 0.7**	1.6 ± 0.6	1.3 ± 0.6	**98.7 ± 0.6**	ND	**99.5 ± 0.8**	ND	0.5 ± 0.8	**100.0**	ND	ND

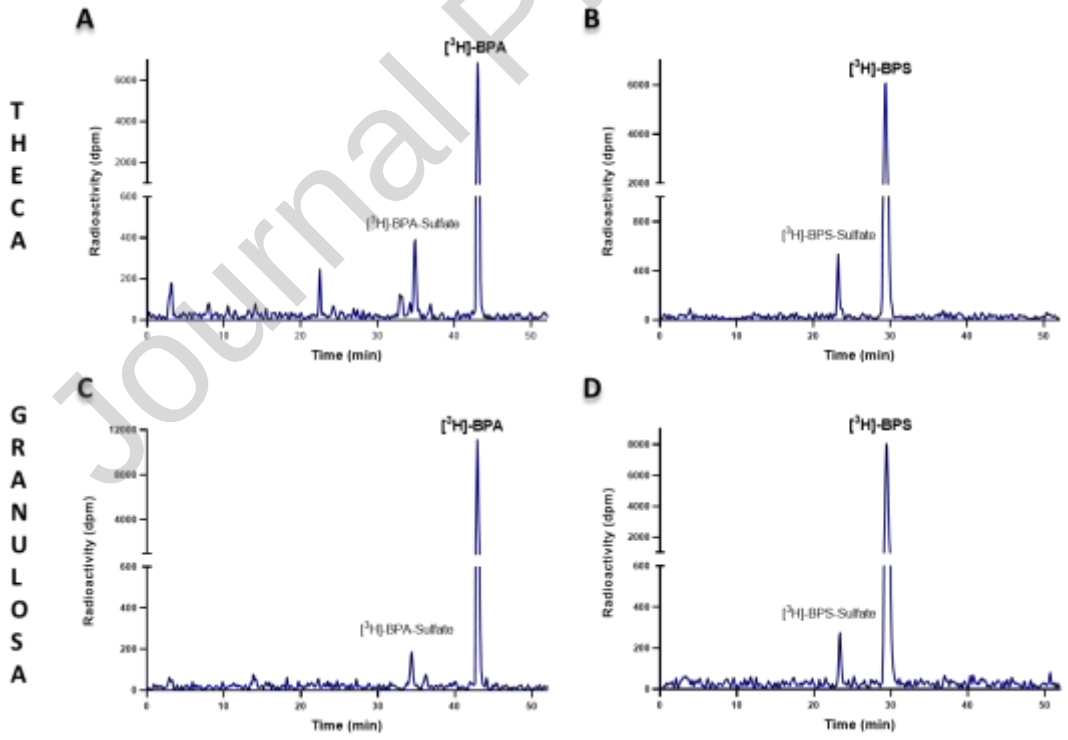


Fig. 2. Typical radio-HPLC metabolic profiles following culture of theca explants (A, B) or granulosa cells (C, D) for 24 hr with 0.1 µM [^{3}H]-BPA (A, C) or 0.1 µM [^{3}H]-BPS (B, D).

3.3. Deconjugation of BPA-G and BPS-G

Using the HPLC systems developed for BPA and BPS studies, the respective Rt of BPA-G and BPS-G were 24.5 min and 13.9 min. Again, in all "no-cell" control culture media samples, the recovery of assayed compounds was higher than 98 %, with no parent compound detected; over 24 hr, there was thus no degradation into the unconjugated forms.

Theca explants were able to deconjugate BPA-G and BPS-G into BPA and BPS, respectively (Fig. 3 A-B). Significant differences were found between cultures carried out with BPA-G (4.0 ± 1.7 % converted to BPA; *i.e.* 3.4 ± 1.5 pmol/24 hr) or with BPS-G (14.0 ± 5.3 % converted to BPS; *i.e.* 12.9 ± 4.7 pmol/24 hr) ($p = 0.0004$ on formation rates). In contrast, assays conducted with granulosa cells demonstrated that these cells were not able to achieve any detectable deconjugation of either glucuronide (Fig. 3 C-D).

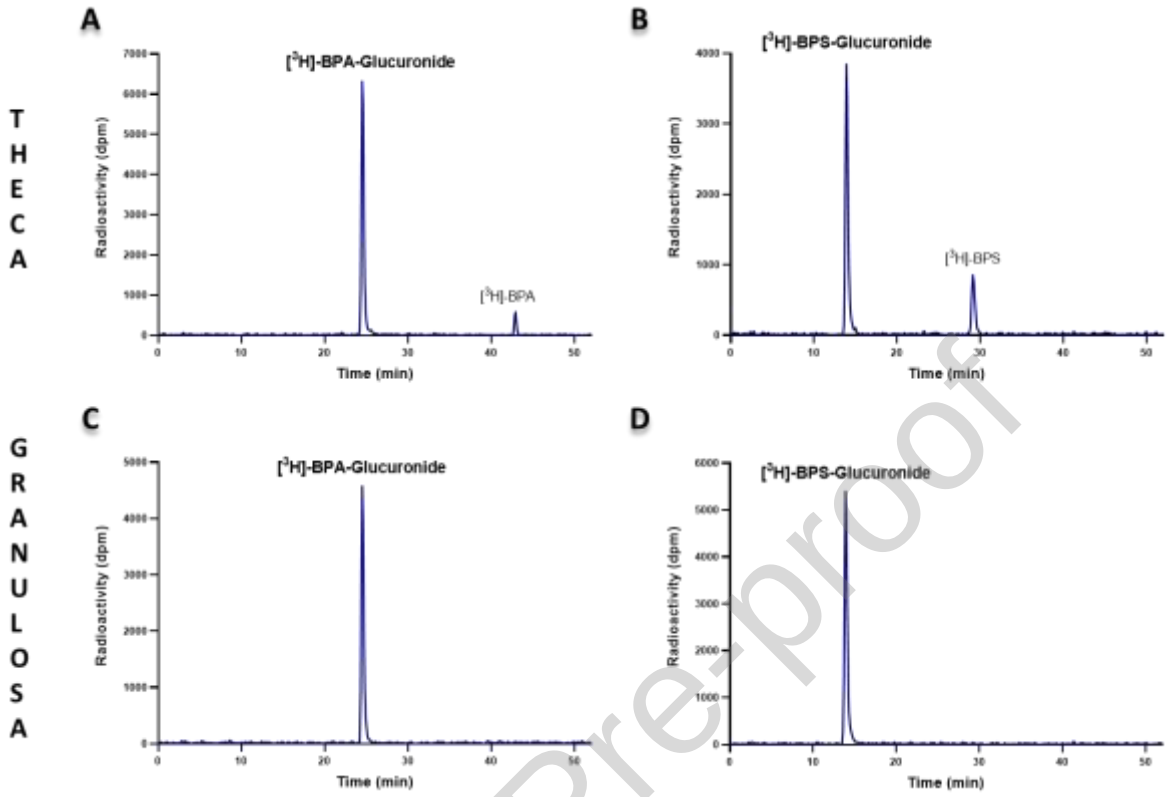


Fig. 3. Typical radio-HPLC metabolic profiles following culture of theca explants (A, B) or granulosa cells (C, D) for 24 hr with 0.1 µM [^{3}H]-BPA-G (A, C) or 0.1 µM [^{3}H]-BPS-G (B, D).

4. Discussion

This study is the first to investigate and compare the capacity of the granulosa and theca compartments of the ovarian follicle to metabolize BPA and BPS. Our findings demonstrate that these ovarian cells can metabolize BPA and BPS. In contrast, only theca explants were found to be able to deconjugate the glucuronides of these bisphenols. Of note, key differences were not only evidenced between cell compartments, but also according to the substance under study.

4.1. Distribution of radioactivity

The comprehensive analyses of all sample fractions showed an increased retention of BPA (compared to BPS) in the cellular compartments of both granulosa ($p = 0.0803$, not significant) and theca explants ($p < 0.0001$). This finding suggests that depending on their chemical structure, there exist differences in the entry of bisphenols (and/or metabolites) in follicular cells, and/or in the capacity of these cells to excrete these substances. Whatever the mechanism involved, this may result in an extended period of time during which these substances can impact cells. Mechanistically, the selective uptake of a bisphenol may result from its sulfation, followed by an inward transport that is desulfation-dependent. Such pathway was demonstrated at least for BPA in breast cancer cells [57], and it involves the same type of membrane uptake carriers used for the transport of sulfated steroids into cells of the testis [58,59]. Given the differences reported here, mechanisms and factors (both intra- and extra-cellular) that may influence the distribution or partitioning of each bisphenol should thus be examined within the multiple compartments and barriers of the ovarian follicle. Such knowledge would help inform efforts into physiologically based toxicokinetic (PBTK) modeling.

4.2. Conjugation activities

Our results indicate that within the ovary, sulfation is expected to be the predominant metabolic pathway for bisphenols. Indeed, only sulfated metabolites (with no detectable traces of glucuronides) were observed in granulosa and theca explants, both for BPA and BPS. These findings are in accordance with a pilot study demonstrating a conversion of BPA to BPA-S in sheep fetal and adult whole ovaries *in vitro* [35], with our current study now extending to BPS and to the examination of different cell compartments of the developing bovine follicle. While in

most species both the sulfation and glucuronidation of bisphenols co-exist, glucuronides are usually the major circulating forms in primates *in vivo* [12,14,30,31]. However, bisphenol sulfation/glucuronidation ratios may also vary according to the target tissue [60], a situation that is often overlooked, as is the case in the ovary.

The extrahepatic metabolism of bisphenols reported herein for the ovary isn't surprising since specific conjugation pathways (notably sulfation) exist to control steroid production and availability [50,59,61,62]. Interestingly, Prévost and Bélanger [63] found that estrogen glucuronidation is a minor pathway in granulosa cells, and given that estrone sulfate is a major precursor of estradiol, sulfation may be the prevalent pathway in the ovary. Possibly, the sulfated bisphenol metabolites found in our study result from sulfotransferases known to be involved in steroid metabolism in the ovary. Our findings on the sulfation of BPA and BPS highlight the need to look for BPA-S and BPS-S within the ovary *in vivo*, as well as the need to consider their biological effects on follicle and oocyte development.

It is generally assumed that BPA conjugated metabolites (including sulfates) lack estrogenic activity [33,64], although this is still questionable given that no exhaustive study of the activity of bisphenol conjugates has been carried out yet at the level of membrane estrogen receptors. Still, given the sulfation pathways highlighted for BPA and BPS, our findings suggest the ability of follicle cells to provide some degree of protection. Indeed, by conjugating bisphenols, follicle cells may help neutralize the potential impact of these EDCs on the oocyte. In future studies on the metabolic fate of EDCs, there would thus be a need to consider all parts of the follicle. As expected, the capacity of theca explants and granulosa cells to conjugate BPA and BPS into their corresponding sulfates was quantitatively limited. In theory, these low conversion rates rule out the possibility that BPA or BPS may be significantly deactivated

through conjugation at the level of the ovary. However, given the known impact of bisphenols on oocyte and follicle development and the occurrence of low-dose responses for xeno-estrogens [11,23,25,65,66], even low rates of biotransformation may matter. Further, sulfated bisphenols may act as a reservoir of "inactive" bisphenols. By returning the compound to an active form, desulfation activities may increase the hazards associated with bisphenols. With steroid sulfatases (STS) that catalyze the hydrolysis of steroid sulfates known to be active in the ovarian follicle [67,68], it cannot be ruled out that these same enzymes would also accept bisphenols as substrates.

A central new finding of our study is the lack of BPA or BPS glucuronidation by granulosa and theca explants. This is spite of the known expression of UDP-glucuronosyltransferases (UGTs) in the ovary, including in granulosa and theca cells [49,69], and the physiological role that UGTs play in the conjugation of endogenous steroids. In addition, UGTs are involved in the metabolism of xenobiotics; for instance, rat follicle cells rely on glucuronidation as a major conjugation pathway for 1-naphthol [70]. Our findings suggest that, at least in cows, no UGT conjugating activity can be detected for BPA or BPS, in either ovarian granulosa or theca explants; this is in accordance with the previous failure to detect any BPA glucuronidation by whole sheep ovaries *ex vivo* [35]. Follow up studies should thus consider whether ovarian UGTs can conjugate bisphenols, with particular attention as to whether differences in substrate specificity may exist between endogenous steroids and such xenobiotics.

It is also possible that in the ovary, the metabolism of bisphenols mirrors the dynamic and developmentally regulated situation of the placenta-fetal unit, with initially an immature glucuronidation system and the early activity of sulfotransferases [35]. Our work didn't examine different stages during follicle development; indeed, granulosa cells and theca explants each

originated from only a specific developmental window. But with the differential cell-type specific expression of UGTs in early *versus* late stages in monkey follicles [71], potential changes in xenobiotic conjugating activities may need consideration during follicle development. Interestingly, BPS-G has previously been detected in human follicular fluid [18]. However, to date, no study like ours has been carried out in primates, and it is possible that in Amar et al.'s study [18], the BPS-G detected may be from non-ovarian sources rather than produced by the follicle itself.

4.3. Other metabolites

Our work highlights the predominance of ovarian sulfation for BPA and BPS, as well as the need to better understand the ins and outs of conjugation and deconjugation processes at the level of the ovary. However, it does not rule out the possible formation of other metabolites. Both for BPA [12,36] and BPS [37], prior studies have demonstrated the occurrence of metabolites, the formation of which relies on phase I oxidative metabolism (*e.g*. hydroxylated metabolites). Such pathways, usually initiated by CYP450-mediated oxidation, may also imply the formation of reactive intermediates, at least in the case of BPA [36,72]. Oxidative pathways leading to the formation of reactive species, have previously been demonstrated for natural estrogens such as estradiol [40], but also for bisphenols able to undergo *ipso*-substitution following first-step oxidation. For chemical reasons, solely bisphenols containing a central asymmetric carbon (*i.e.* BPA but not BPS) can undergo this specific mechanism [72]. Human and rodent ovaries have previously been shown to possess functional phase I activities [73–76], and these activities provide support for the possible occurrence of BPA oxidative metabolism in ovarian cells. In theca explant assays carried out with [^{3}H]-BPA, several minor metabolites were

detected. This included a metabolite eluted at 22.4 min, which structure could not be confirmed by HRMS due to its low formation rate, but for which we were able to rule out correspondence with BPA glucuronide. This metabolite may well result from oxidative metabolism. In parallel, we demonstrated that theca explants contained a significantly higher proportion of non-extractable radioactivity after incubations with BPA compared to with BPS (see Section 4.1). The formation of reactive species via oxidative routes could lead to minor BPA metabolites that are prone to bind cellular macromolecules, in turn resulting in an increase in non-extractable radioactivity. Since BPS cannot undergo *ipso*-substitution, no comparable oxidative pathways are expected. Such expectation is consistent with the lack of observation of metabolites other than BPS-S in our study, and the lower proportion of non-extractable radioactivity recorded in comparison to BPA assays in theca explants.

4.4. Deconjugation activities

Beside the demonstration of conjugation activities in theca explants and granulosa cells, another major and novel finding of our study is that theca explants can deconjugate the glucuronidated forms of BPA and BPS. This may rely on β-glucuronidase, that are enzymes known to be widely distributed in the body (including in the ovary) and can deconjugate both endogenous compounds or xenobiotic glucuronides [77,78]. One single study previously reported the occurrence of BPA deconjugation in ovaries, in fetal as well as adult sheep [35], but it did not include a consideration of cell-type specific activities. Our study now provides invaluable additional information. It extends these findings to BPS and reveals that theca - but not granulosa - can deconjugate bisphenols' glucuronides (their major circulating form) back into the active parent molecules. Our findings may reflect the fact that the granulosa and theca

represent distinct compartments of the follicle that are characterized by specialized steroid biosynthetic and metabolic activities [20,21]. Further, the two cell compartments also occupy different positions in relation to chemical insults that are coming from the circulating blood. Notably, the outer theca layer anatomically stands amidst blood vessels, while the granulosa cells are separated from the theca cells by a basement membrane and reside within the confines of the avascular follicle. Since deconjugation of BPA-G and BPS-G is now demonstrated in the outer layers of the follicle (*i.e.* in the theca), future studies may consider the activity of its individual components (*e.g.* theca cells, immune cells, vascular tissue, extracellular matrix) as well as the exact localization of bisphenols along with transfer mechanisms across the follicle barriers (acellular and cellular).

Strikingly, we showed that the extent of deconjugation by the theca was much higher for BPS-G than for BPA-G at 24 hr. This finding reveals the need to assess the metabolism of individual bisphenols. The difference in deglucuronidation rates may be due to a higher affinity of theca β-glucuronidase for BPS-G than for BPA-G, and it also correlates with the increased proportion of non-extractable radioactivity in BPA-G theca explants. Due to the known effects that BPA and BPS have on reproduction and ovarian function, any local bisphenol deconjugation (*i.e.* reactivation) at the level of the ovary raises concerns. Indeed, it is well established that for such EDCs, even low doses can disrupt normal follicle and oocyte function; in fact, low doses may even be more detrimental than higher doses [11,23,65,79].

4.5. Significance of the study model

This study pays attention to distinct compartments of the follicle. Further, our findings rely on the use of intact cellular systems rather than subcellular fractions. To minimize deviations

from the *in vivo* situation, primary cultures (for granulosa cells) and an *ex vivo* tissue model (for the theca) were used, along with previously established and defined culture conditions known to model physiological conditions. Also innovative was the use of the bovine model, which offers unique advantages. Granulosa cells can be obtained in sufficient quantity within a single animal for direct exposures *in vitro*, and an entire theca layer can be isolated as an intact sheet from an individual follicle for testing *ex vivo*. Given the prior attention of toxicokinetic studies to rodent and non-human primate models and yet reported species differences in the metabolism of xenobiotics [80–82], our focus on a ruminant species is novel and relevant. Further, the use of the bovine as a model species is of high value since it is a relevant mammalian system for both agricultural and human health concerns, including reproductive impairments. Bovine and human species also share key characteristics in ovarian physiology and follicle and oocyte development [83].

5. Conclusions

This is the first study reporting detailed data on the metabolic fate of key bisphenols in the ovary, and specifically considering two essential cell layers of the follicle: the granulosa and the theca. Radio-HPLC analyses revealed that both granulosa and theca explants can metabolize BPA and BPS into their respective sulfo-conjugates (BPA-S, BPS-S). In addition, theca explants were shown to be able to deconjugate both BPA-G and BPS-G back into their respective aglycones BPA and BPS. Interestingly, over 24 hr, the deconjugation rate of BPS was higher than that of BPA. Since these glucuronides, unlike their respective parent compounds, lack estrogenic activity (at least via nuclear estrogen receptors), these deconjugation pathways should be considered as a reactivation of both EDCs *in situ* in the ovary. This result may be crucial

given the unique location and functions of the theca layer. In contrast, a lack of BPA-G and BPS-G deconjugating activity was observed in granulosa cells. A notable difference between the two bisphenols was also observed in theca explants, with a significant higher cellular retention reported in BPA compared to BPS experiments. This may be related to the formation of a metabolite (possibly originating from oxidative pathways), for BPA only and just in theca explants. These results on the different capacities of the theca and the granulosa to serve as extrahepatic routes of bisphenol metabolism reveal the need to pay particular attention to both cell compartments in future studies on the fate and effects of EDCs in the ovary. These novel findings provide essential knowledge for risk assessment purposes.

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Reference list

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

[2] Y. Yang, Y. Yang, J. Zhang, B. Shao, J. Yin, Assessment of bisphenol A alternatives in paper products from the Chinese market and their dermal exposure in the general population, Environ. Pollut. 244 (2019) 238–246. https://doi.org/10.1016/j.envpol.2018.10.049.

[3] C.J. Catenza, A. Farooq, N.S. Shubear, K.K. Donkor, A targeted review on fate, occurrence, risk and health implications of bisphenol analogues, Chemosphere. 268 (2021). https://doi.org/10.1016/j.chemosphere.2020.129273.

[4] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.L. Feng, Y. Wu, M. Widelka, Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity - A Review, Environ. Sci. Technol. 50 (2016) 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

[5] X. Ye, L.Y. Wong, J. Kramer, X. Zhou, T. Jia, A.M. Calafat, Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000-2014, Environ. Sci. Technol. 49 (2015) 11834–11839. https://doi.org/10.1021/acs.est.5b02135.

[6] M.K. Björnsdotter, J. de Boer, A. Ballesteros-Gómez, Bisphenol A and replacements in thermal paper: A review, Chemosphere. 182 (2017) 691–706. https://doi.org/10.1016/j.chemosphere.2017.05.070.

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

[8] A.C. Gore, V.A. Chappell, S.E. Fenton, J.A. Flaws, A. Nadal, G.S. Prins, J. Toppari, R.T. Zoeller, EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals, Endocr. Rev. 36 (2015) 1–150. https://doi.org/10.1210/er.2015-1010.

[9] M.A. La Merrill, L.N. Vandenberg, M.T. Smith, W. Goodson, P. Browne, H.B. Patisaul, K.Z. Guyton, A. Kortenkamp, V.J. Cogliano, T.J. Woodruff, L. Rieswijk, H. Sone, K.S. Korach, A.C. Gore, L. Zeise, R.T. Zoeller, Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification, Nat. Rev. Endocrinol. 16 (2020) 45–57. https://doi.org/10.1038/s41574-019-0273-8.

[10] S. Sifakis, V.P. Androutsopoulos, A.M. Tsatsakis, D.A. Spandidos, Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems, Environ. Toxicol. Pharmacol. 51 (2017) 56–70. https://doi.org/10.1016/j.etap.2017.02.024.

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

[12] D. Zalko, A.M. Soto, L. Dolo, C. Dorio, E. Rathahao, L. Debrauwer, R. Faure, J.P. Cravedi, Biotransformations of bisphenol A in a mammalian model: Answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice, Environ. Health Perspect. 111 (2003) 309–319. https://doi.org/10.1289/ehp.5603.

[13] S. Waidyanatha, S.R. Black, R.W. Snyder, Y.L. Yueh, V. Sutherland, P.R. Patel, S.L. Watson, T.R. Fennell, Disposition and metabolism of the bisphenol analogue, bisphenol S, in Harlan Sprague Dawley rats and B6C3F1/N mice and in vitro in hepatocytes from rats,

mice, and humans, Toxicol. Appl. Pharmacol. 351 (2018) 32–45. https://doi.org/10.1016/j.taap.2018.05.008.

[14] C.A. VandeVoort, R.R. Gerona, F.S. Vom Saal, A.F. Tarantal, P.A. Hunt, A. Hillenweck, D. Zalko, Maternal and fetal pharmacokinetics of oral radiolabeled and authentic bisphenol a in the rhesus monkey, PLoS One. 11 (2016). https://doi.org/10.1371/journal.pone.0165410.

[15] Y. Ikezuki, O. Tsutsumi, Y. Takai, Y. Kamei, Y. Taketani, Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure, Hum. Reprod. 17 (2002) 2839–2841. https://doi.org/10.1093/humrep/17.11.2839.

[16] D. Paoli, F. Pallotti, A. Pia Dima, E. Albani, C. Alviggi, F. Causio, C. Conca Dioguardi, A. Conforti, R. Ciriminna, G. Fabozzi, G. Giuffrida, R. Gualtier, M. Giulia Minasi, S. Ochetti, V. Pisaturo, C. Racca, L. Rienzi, E. Sarcina, C. Scarica, G. Tomasi, C. Verlengia, R. Villeggia, F. Zullo, A. Lenzi, F. Botrè, L. De Santis, Phthalates and bisphenol a: Presence in blood serum and follicular fluid of italianwomen undergoing assisted reproduction techniques, Toxics. 8 (2020) 1–15. https://doi.org/10.3390/toxics8040091.

[17] S. Aftabsavad, Z. Noormohammadi, A. Moini, M. Karimipoor, Effect of bisphenol A on alterations of ICAM-1 and HLA-G genes expression and DNA methylation profiles in cumulus cells of infertile women with poor response to ovarian stimulation, Sci. Rep. 11 (2021). https://doi.org/10.1038/s41598-021-87175-1.

[18] S. Amar, A. Binet, O. Téteau, A. Desmarchais, P. Papillier, M.Z. Lacroix, V. Maillard, F. Guérif, S. Elis, Bisphenol S impaired human granulosa cell steroidogenesis in vitro, Int. J. Mol. Sci. 21 (2020). https://doi.org/10.3390/ijms21051821.

[19] M.L. Hennet, C.M.H. Combelles, The antral follicle: A microenvironment for oocyte differentiation, Int. J. Dev. Biol. 56 (2012) 819–831. https://doi.org/10.1387/ijdb.120133cc.

[20] J.M. Young, A.S. McNeilly, Theca: The forgotten cell of the ovarian follicle, Reproduction. 140 (2010) 489–504. https://doi.org/10.1530/REP-10-0094.

[21] R.J. Rodgers, Steroidogenic cytochrome p450 enzymes and ovarian steroidogenesis, Reprod. Fertil. Dev. 2 (1990) 153–163. https://doi.org/10.1071/RD9900153.

[22] A.M. Neff, J.A. Flaws, The effects of plasticizers on the ovary, Curr. Opin. Endocr. Metab. Res. 18 (2021) 35–47. https://doi.org/10.1016/j.coemr.2021.01.004.

[23] K.A. Campen, K.M. Kucharczyk, B. Bogin, J.M. Ehrlich, C.M.H. Combelles, Spindle abnormalities and chromosome misalignment in bovine oocytes after exposure to low doses of bisphenol A or bisphenol S, Hum. Reprod. 33 (2018) 895–904. https://doi.org/10.1093/humrep/dey050.

[24] T. Žalmanová, K. Hošková, J. Nevoral, K. Adámková, T. Kott, M. Šulc, Z. Kotíková, Š. Prokešová, F. Jílek, M. Králíčková, J. Petr, Bisphenol S negatively affects the meotic maturation of pig oocytes, Sci. Rep. 7 (2017). https://doi.org/10.1038/s41598-017-00570-5.

[25] Š. Prokešová, K. Ghaibour, F. Liška, P. Klein, T. Fenclová, M. Štiavnická, P. Hošek, T.

Žalmanová, K. Hošková, H. Řimnáčová, J. Petr, M. Králíčková, J. Nevoral, Acute low-dose bisphenol S exposure affects mouse oocyte quality, Reprod. Toxicol. 93 (2020) 19–27. https://doi.org/10.1016/j.reprotox.2019.12.005.

[26] M.Y. Zhang, Y. Tian, Z.H. Yan, W.D. Li, C.J. Zang, L. Li, X.F. Sun, W. Shen, S.F. Cheng, Maternal Bisphenol S exposure affects the reproductive capacity of F1 and F2 offspring in mice, Environ. Pollut. 267 (2020). https://doi.org/10.1016/j.envpol.2020.115382.

[27] W.X. Liu, F. Donatella, S.J. Tan, W. Ge, J.J. Wang, X.F. Sun, S.F. Cheng, W. Shen, Detrimental effect of Bisphenol S in mouse germ cell cyst breakdown and primordial follicle assembly, Chemosphere. 264 (2021). https://doi.org/10.1016/j.chemosphere.2020.128445.

[28] C.M. McDonough, H.S. Xu, T.L. Guo, Toxicity of bisphenol analogues on the reproductive, nervous, and immune systems, and their relationships to gut microbiome and metabolism: insights from a multi-species comparison, Crit. Rev. Toxicol. 51 (2021) 283–300. https://doi.org/10.1080/10408444.2021.1908224.

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

[30] J.G. Teeguarden, N.C. Twaddle, M.I. Churchwell, X. Yang, J.W. Fisher, L.M. Seryak, D.R. Doerge, 24-hour human urine and serum profiles of bisphenol A following ingestion in soup: Individual pharmacokinetic data and emographics, Data Br. 4 (2015) 83–86. https://doi.org/10.1016/j.dib.2015.03.002.

[31] R.R. Gerona, J. Pan, A.R. Zota, J.M. Schwartz, M. Friesen, J.A. Taylor, P.A. Hunt, T.J. Woodruff, Direct measurement of Bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: A cross-sectional study, Environ. Heal. A Glob. Access Sci. Source. 15 (2016). https://doi.org/10.1186/s12940-016-0131-2.

[32] J.B. Matthews, K. Twomey, T.R. Zacharewski, In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors α and β, Chem. Res. Toxicol. 14 (2001) 149–157. https://doi.org/10.1021/tx0001833.

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

[34] R. Viñas, C.S. Watson, Bisphenol S disrupts estradiol-induced nongenomic signaling in a rat pituitary cell line: Effects on cell functions, Environ. Health Perspect. 121 (2013) 352–358. https://doi.org/10.1289/ehp.1205826.

[35] T. Corbel, E. Perdu, V. Gayrard, S. Puel, M.Z. Lacroix, C. Viguié, P.L. Toutain, D. Zalko, N. Picard-Hagen, Conjugation and deconjugation reactions within the fetoplacental compartment in a sheep model: A key factor determining bisphenol a fetal exposure, Drug Metab. Dispos. 43 (2015) 467–476. https://doi.org/10.1124/dmd.114.061291.

[36] J.P. Jaeg, E. Perdu, L. Dolo, L. Debrauwer, J.P. Cravedi, D. Zalko, Characterization of new bisphenol A metabolites produced by CD1 mice liver microsomes and S9 fractions, J. Agric. Food Chem. 52 (2004) 4935–4942. https://doi.org/10.1021/jf049762u.

[37] O. Ousji, L. Sleno, In Vitro Metabolism of Five Bisphenol A Analogs Studied by LC-HRMS/MS, J. Am. Soc. Mass Spectrom. 32 (2021) 847–859. https://doi.org/10.1021/jasms.0c00301.

[38] R.K. Dubey, S.P. Tofovic, E.K. Jackson, Cardiovascular Pharmacology of Estradiol Metabolites, J. Pharmacol. Exp. Ther. 308 (2004) 403–409. https://doi.org/10.1124/jpet.103.058057.

[39] R.J. Santen, W. Yue, J.P. Wang, Estrogen metabolites and breast cancer, Steroids. 99 (2015) 61–66. https://doi.org/10.1016/j.steroids.2014.08.003.

[40] B.T. Zhu, A.J. Lee, NADPH-dependent metabolism of 17β-estradiol and estrone to polar and nonpolar metabolites by human tissues and cytochrome P450 isoforms, Steroids. 70 (2005) 225–244. https://doi.org/10.1016/j.steroids.2005.01.002.

[41] J. V. Castell, M.T. Donato, M.J. Gómez-Lechón, Metabolism and bioactivation of toxicants in the lung. The in vitro cellular approach, Exp. Toxicol. Pathol. 57 (2005) 189–204. https://doi.org/10.1016/j.etp.2005.05.008.

[42] M.O. James, S. Ambadapadi, Interactions of cytosolic sulfotransferases with xenobiotics, Drug Metab. Rev. 45 (2013) 401–414. https://doi.org/10.3109/03602532.2013.835613.

[43] M. Ouzzine, S. Gulberti, N. Ramalanjaona, J. Magdalou, S. Fournel-Gigleux, The UDP-glucuronosyltransferases of the blood-brain barrier: Their role in drug metabolism and detoxication, Front. Cell. Neurosci. 8 (2014). https://doi.org/10.3389/fncel.2014.00349.

[44] D.B. Buckley, C.D. Klaassen, Tissue- and gender-specific mRNA expression of UDP-glucuronosyltransferases (UGTs) in mice, Drug Metab. Dispos. 35 (2007) 121–127. https://doi.org/10.1124/dmd.106.012070.

[45] A. Nakamura, M. Nakajima, H. Yamanaka, R. Fujiwara, T. Yokoi, Expression of UGT1A and UGT2B mRNA in human normal tissues and various cell lines, Drug Metab. Dispos. 36 (2008) 1461–1464. https://doi.org/10.1124/dmd.108.021428.

[46] H. Yokota, H. Iwano, M. Endo, T. Kobayashi, H. Inoue, S.I. Ikushiro, A. Yuasa, Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver, Biochem. J. 340 (1999) 405–409. https://doi.org/10.1042/0264-6021:3400405.

[47] P. Bhattacharya, A.F. Keating, Ovarian metabolism of Xenobiotics, Exp. Biol. Med. 236 (2011) 765–771. https://doi.org/10.1258/ebm.2011.011051.

[48] P. Bhattacharya, A.F. Keating, Impact of environmental exposures on ovarian function and role of xenobiotic metabolism during ovotoxicity, Toxicol. Appl. Pharmacol. 261 (2012) 227–235. https://doi.org/10.1016/j.taap.2012.04.009.

[49] O. Barbier, A. Bélanger, The cynomolgus monkey (Macaca fascicularis) is the best animal model for the study of steroid glucuronidation, in: J. Steroid Biochem. Mol. Biol., Elsevier

Ltd, 2003: pp. 235–245. https://doi.org/10.1016/S0960-0760(03)00235-8.

[50] J.W. Mueller, L.C. Gilligan, J. Idkowiak, W. Arlt, P.A. Foster, The regulation of steroid action by sulfation and desulfation, Endocr. Rev. 36 (2015) 526–563. https://doi.org/10.1210/er.2015-1036.

[51] J.J. Ireland, R.L. Murphee, P.B. Coulson, Accuracy of Predicting Stages of Bovine Estrous Cycle by Gross Appearance of the Corpus Luteum, J. Dairy Sci. 63 (1980) 155–160. https://doi.org/10.3168/jds.S0022-0302(80)82901-8.

[52] B.K. Campbell, D.T. Baird, R. Webb, Effects of dose of LH on androgen production and luteinization of ovine theca cells cultured in a serum-free system, J. Reprod. Fertil. 112 (1998) 69–77. https://doi.org/10.1530/jrf.0.1120069.

[53] C. Glister, S.L. Richards, P.G. Knight, Bone morphogenetic proteins (BMP) -4, -6, and -7 potently suppress basal and luteinizing hormone-induced androgen production by bovine theca interna cells in primary culture: Could ovarian hyperandrogenic dysfunction be caused by a defect in thecal BMP si, Endocrinology. 146 (2005) 1883–1892. https://doi.org/10.1210/en.2004-1303.

[54] J.E. Fortune, Bovine theca and granulosa cells interact to promote androgen production., Biol. Reprod. 35 (1986) 292–299. https://doi.org/10.1095/biolreprod35.2.292.

[55] J.E. Fortune, W. Hansel, The effect of 171β-estradiol on progesterone secretion by bovine theca and granulosa cells, Endocrinology. 104 (1979) 1834–1838. https://doi.org/10.1210/endo-104-6-1834.

[56] A.M. Maucieri, D.H. Townson, Evidence and manipulation of O-GlcNAcylation in granulosa cells of bovine antral follicles, Biol. Reprod. 104 (2021) 914–923. https://doi.org/10.1093/biolre/ioab013.

[57] C.L. Stowell, K.K. Barvian, P.C.C.M. Young, R.M. Bigsby, D.E.E. Verdugo, C.R. Bertozzi, T.S. Widlanski, A Role for Sulfation-Desulfation in the Uptake of Bisphenol A into Breast Tumor Cells, Chem. Biol. 13 (2006) 891–897. https://doi.org/10.1016/j.chembiol.2006.06.016.

[58] D. Fietz, K. Bakhaus, B. Wapelhorst, G. Grosser, S. Günther, J. Alber, B. Döring, S. Kliesch, W. Weidner, C.E. Galuska, M.F. Hartmann, S.A. Wudy, M. Bergmann, J. Geyer, Membrane Transporters for Sulfated Steroids in the Human Testis - Cellular Localization, Expression Pattern and Functional Analysis, PLoS One. 8 (2013). https://doi.org/10.1371/journal.pone.0062638.

[59] J. Geyer, K. Bakhaus, R. Bernhardt, C. Blaschka, Y. Dezhkam, D. Fietz, G. Grosser, K. Hartmann, M.F. Hartmann, J. Neunzig, D. Papadopoulos, A. Sánchez-Guijo, G. Scheiner-Bobis, G. Schuler, M. Shihan, C. Wrenzycki, S.A. Wudy, M. Bergmann, The role of sulfated steroid hormones in reproductive processes, J. Steroid Biochem. Mol. Biol. 172 (2017) 207–221. https://doi.org/10.1016/j.jsbmb.2016.07.002.

[60] V. Le Fol, S. Aït-Aïssa, N. Cabaton, L. Dolo, M. Grimaldi, P. Balaguer, E. Perdu, L. Debrauwer, F. Brion, D. Zalko, Cell-specific biotransformation of benzophenone-2 and bisphenol-s in zebrafish and human in vitro models used for toxicity and estrogenicity

screening, Environ. Sci. Technol. 49 (2015) 3860–3868. https://doi.org/10.1021/es505302c.

[61] L. Secky, M. Svoboda, L. Klameth, E. Bajna, G. Hamilton, R. Zeillinger, W. Jäger, T. Thalhammer, The Sulfatase Pathway for Estrogen Formation: Targets for the Treatment and Diagnosis of Hormone-Associated Tumors, J. Drug Deliv. 2013 (2013) 1–13. https://doi.org/10.1155/2013/957605.

[62] T.L. Rižner, The important roles of steroid sulfatase and sulfotransferases in gynecological diseases, Front. Pharmacol. 7 (2016). https://doi.org/10.3389/fphar.2016.00030.

[63] J. Prevost, A. Belanger, Formation of estrogen glucuronides by human granulosa-luteal cells isolated from human menopausal gonadotropin-stimulated cycles for in vitro fertilization, Biol. Reprod. 38 (1988) 975–979. https://doi.org/10.1095/biolreprod38.5.975.

[64] M. Shimizu, K. Ohta, Y. Matsumoto, M. Fukuoka, Y. Ohno, S. Ozawa, Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells, Toxicol. Vitr. 16 (2002) 549–556. https://doi.org/10.1016/S0887-2333(02)00055-3.

[65] L.N. Vandenberg, T. Colborn, T.B. Hayes, J.J. Heindel, D.R. Jacobs, D.H. Lee, T. Shioda, A.M. Soto, F.S. vom Saal, W. V. Welshons, R.T. Zoeller, J.P. Myers, Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses, Endocr. Rev. 33 (2012) 378–455. https://doi.org/10.1210/er.2011-1050.

[66] L.N. Vandenberg, Non-monotonic dose responses in studies of endocrine disrupting chemicals: Bisphenol a as a case study, Dose-Response. 12 (2014) 259–276. https://doi.org/10.2203/dose-response.13-020.Vandenberg.

[67] R. V. Haning, R.J. Hackett, R.I. Boothroid, J.A. Canick, Steroid sulphatase activity in the human ovarian corpus luteum, stroma, and follicle: Comparison to activity in other tissues and the placenta, J. Steroid Biochem. 36 (1990) 175–179. https://doi.org/10.1016/0022-4731(90)90127-E.

[68] Y. Otsuka, A. Yanaihara, S. Iwasaki, J. Hasegawa, T. Yanaihara, T. Okai, Localization and gene expression of steroid sulfatase by RT-PCR in cumulus cells and relationship to serum FSH levels observed during in vitro fertilization, J. Exp. Clin. Assist. Reprod. 2 (2005). https://doi.org/10.1186/1743-1050-2-6.

[69] L. Becedas, M.B. Ahlberg, Hormonal influences of detoxication in the rat ovary on enzymes in comparison with the liver, Biochem. Pharmacol. 49 (1995) 503–509. https://doi.org/10.1016/0006-2952(94)00434-N.

[70] M. Boström, L. Becedas, J.W. DePierre, Conjugation of 1-naphthol in primary cell cultures of rat ovarian cells, Chem. Biol. Interact. 124 (2000) 103–118. https://doi.org/10.1016/S0009-2797(99)00148-9.

[71] O. Barbier, C. Girard, L. Berger, M. El Alfy, A. Bélanger, D.W. Hum, The androgen-conjugating uridine diphosphoglucuronosyltransferase-2B enzymes are differentially

expressed temporally and spatially in the monkey follicle throughout the menstrual cycle, Endocrinology. 142 (2001) 2499–2507. https://doi.org/10.1210/endo.142.6.8040.

[72] S. Nakamura, Y. Tezuka, A. Ushiyama, C. Kawashima, Y. Kitagawara, K. Takahashi, S. Ohta, T. Mashino, Ipso substitution of bisphenol A catalyzed by microsomal cytochrome P450 and enhancement of estrogenic activity, Toxicol. Lett. 203 (2011) 92–95. https://doi.org/10.1016/j.toxlet.2011.03.010.

[73] D. Downie, M.C.E. McFadyen, P.H. Rooney, M.E. Cruickshank, D.E. Parkin, I.D. Miller, C. Telfer, W.T. Melvin, G.I. Murray, Profiling cytochrome P450 expression in ovarian cancer: Identification of prognostic markers, Clin. Cancer Res. 11 (2005) 7369–7375. https://doi.org/10.1158/1078-0432.CCR-05-0466.

[74] E.A. Cannady, C.A. Dyer, P.J. Christian, G. Sipes, P.B. Hoyer, Expression and activity of cytochromes P450 2E1, 2A, and 2B in the mouse ovary: The effect of 4-vinylcyclohexene and its diepoxide metabolite, Toxicol. Sci. 73 (2003) 423–430. https://doi.org/10.1093/toxsci/kfg077.

[75] J.D. Vidal, C.A. VandeVoort, C.B. Marcus, N.R. Lazarewicz, A.J. Conley, In vitro exposure to environmental tobacco smoke induces CYP1B1 expression in human luteinized granulosa cells, Reprod. Toxicol. 22 (2006) 731–737. https://doi.org/10.1016/j.reprotox.2006.06.001.

[76] I. Bièche, C. Narjoz, T. Asselah, S. Vacher, P. Marcellin, R. Lidereau, P. Beaune, I. De Waziers, Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues, Pharmacogenet. Genomics. 17 (2007) 731–742. https://doi.org/10.1097/FPC.0b013e32810f2e58.

[77] M.E. Banos, A.M. Rosales, L.M. Ballesteros, O. Hernandez-Perez, A. Rosado, Changes in lysosomal enzyme activities in pre-ovulatory follicles and endometrium of PMSG superovulated rats, Arch. Med. Res. 27 (1996) 49–55. https://europepmc.org/article/med/8867367 (accessed December 20, 2022).

[78] B. Sperker, J.T. Backman, H.K. Kroemer, The role of β-glucuronidase in drug disposition and drug targeting in humans, Clin. Pharmacokinet. 33 (1997) 18–31. https://doi.org/10.2165/00003088-199733010-00003.

[79] G.S. Prins, H.B. Patisaul, S.M. Belcher, L.N. Vandenberg, CLARITY-BPA academic laboratory studies identify consistent low-dose Bisphenol A effects on multiple organ systems, Basic Clin. Pharmacol. Toxicol. 125 (2019) 14–31. https://doi.org/10.1111/bcpt.13125.

[80] J.B. Watkins, C.D. Klaassen, Xenobiotic biotransformation in livestock: comparison to other species commonly used in toxicity testing., J. Anim. Sci. 63 (1986) 933–942. https://doi.org/10.2527/jas1986.633933x.

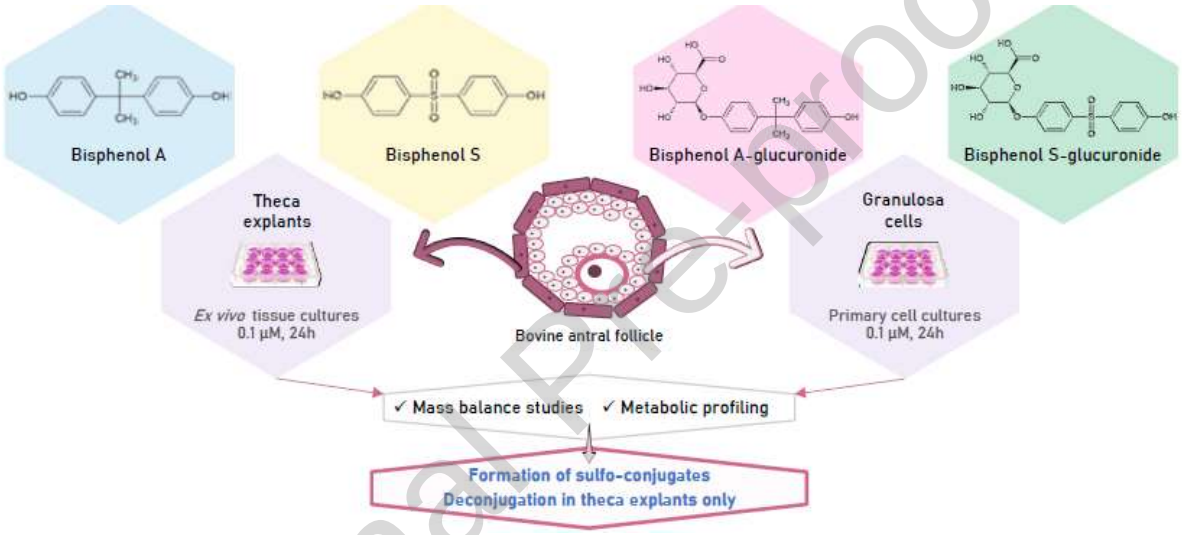
[81] K. Rozman, Disposition of xenobiotics: Species differences, Toxicol. Pathol. 16 (1988) 123–129. https://doi.org/10.1177/019262338801600204.

[82] A.K. Sonker, M. Bhateria, K. Karsauliya, S.P. Singh, Investigating the glucuronidation and sulfation pathways contribution and disposition kinetics of Bisphenol S and its

metabolites using LC-MS/MS-based nonenzymatic hydrolysis method, Chemosphere. 273 (2021). https://doi.org/10.1016/j.chemosphere.2021.129624.

[83] J.F. Roberts, C.C. Jeff Huang, Bovine models for human ovarian diseases, in: Prog. Mol. Biol. Transl. Sci., Elsevier B.V., 2022: pp. 101–154. https://doi.org/10.1016/bs.pmbts.2022.02.001.

Graphical abstract



Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights:

- Granulosa as well as theca explants produce BPA- and BPS-sulfo conjugates
- Only theca explants deconjugate BPA-G and BPS-G into their parent form
- In theca explants, there is a higher persistence of BPA residues compared to BPS