An in vitro evaluation of bisphenols on hepatotoxicity and obesity under environmental levels of exposition

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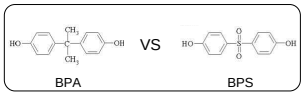

BACKGROUND: For several decades, people have been in contact with bisphenol A (BPA) primarily through their diet. Because BPA was shown to induce adverse effects on human health, especially through the activation of endocrine pathways, it is about to be withdrawn from the European market and replaced by its analogue the bisphenol S (BPS). However, given the scarce toxicological data on this chemical, it is necessary to evaluate the possible effects of BPS on human health.

OBJECTIVES: We aimed to compare the toxicological impacts of BPA and BPS, in parallel with the positive control Diethylstilbestrol (DES), on the hepatic functions, obesity and steatosis processes using both high and low doses in the same range of that found into the environment.

HIGH CONCENTRATIONS _ LIVER CELLS

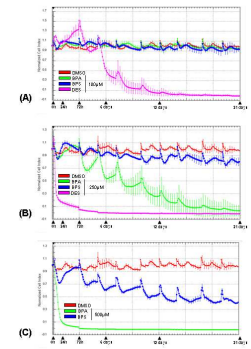

Figure 1: **Chronic effects of both bisphenols on real-time cell analyzer curves**. HepaRG cells were treated for 3 weeks with 100µM (A), 250µM (B) and 500µM (C) of BPA and BPS as indicated in each picture. Diethylstilbestrol (DES) was used as a control for this experiment. Cell Index was determined using the xCELLigence technology (Roche).

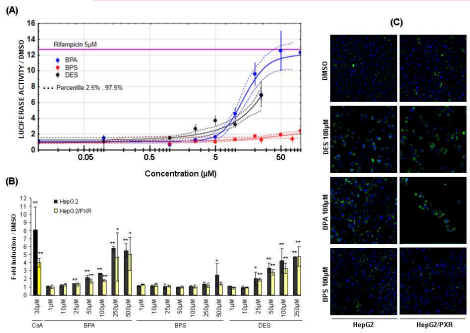

Figure 2: **PXR activation and lipid bioaccumulation in response to both bisphenols**. (A) PXR agonist activity of BPA, BPS and DES in stable hPXR/HepG2 cells was performed. Cells were incubated for 24h in the presence of each compound at the stated concentrations. Curves were fitted after bootstrapping of the whole data set. (B) and (C), HepG2 and HepG2/PXR cells were seeded onto 96-well plates and treated for 72h with increasing concentrations of BPA, BPS and DES. Cells were then stained with Hoechst 33342 and LipidTOX Neutral Lipid kit over 30 to 45 min. Intensity of the spots were detected with the ArrayScanXTI and expressed as a ratio relative to the negative control DMSO. 30µM of cyclosporine A (CSa) was used as a positive control for the lipid accumulation process.

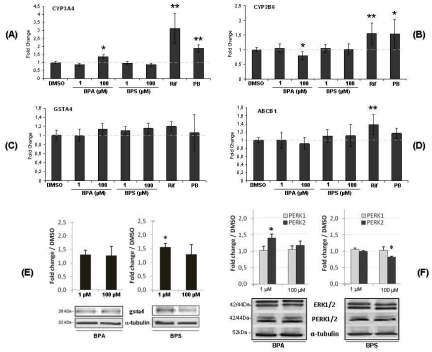

Figure 3: **Analysis of toxicity-, stress- and transport-related genes and protein expression**. (A), (B), (C) and (D) Differentiated HepaRG cells were exposed for 48h to 1 and 100 µM of BPA or BPS. Real-time quantitative PCR was used to quantify the relative mRNA levels of CYP3A4, CYP2B6, GSTA4 and ABCB1, respectively. (E), (F) differentiated HepaRG cells were treated for 48h with 1 and 100 µM of BPA or BPS. Cells were lysed and levels of phosphorylated GSTA4, Erk1/2 and Erk1/2 proteins were analyzed by western blotting. Band densitometry was performed and the results are defined as the ratio between treated cells versus DMSO-treated cells normalized by α-tubulin.

Comparative study of bisphenol A and its analogue bisphenol S on human hepatic cells: A focus on their potential involvement in nonalcoholic fatty liver disease. Peyre L, et al., Food Chem Toxicol. 2014 May 1;70C:9-18

LOW CONCENTRATIONS _ ADIPOCYTES (& HEPG2 CELLS)

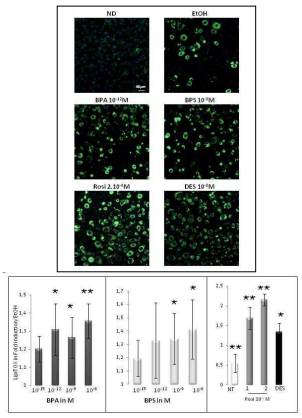

Figure 4: **Effects of BPA and BPS on lipid content in adipocytes**. A and B, Cells were seeded onto 96-well plates, treated with indicated concentrations of BPA or BPS for 10 days after induction of differentiation. They were stained with the LipidTOX Neutral Lipid kit (green) for 30 min. Spot intensity was detected using the ArrayScanXTI ThermoScientific and expressed as a ratio relative to the negative control EtOH. 1 and 2µM of Rosiglitazone (Rosi) and 10nM of DES were used as positive controls for the lipid accumulation The undifferentiated condition (ND) was used as supplementary control.

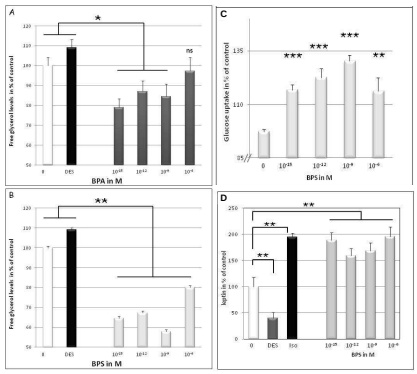

Figure 5: **Effects of BPA and BPS on adipocyte lipolysis, glucose uptake and leptin production**. Cells were seeded in 6 well-plates and treated by indicated concentration of BPA (A) or BPS (B) or for 10 days after the induction of differentiation. Lipolysis was evaluated as free glycerol released into the culture medium. (C), Effects of bisphenols on glucose uptake. Under the same conditions as above, 2-deoxy-[1-3H]-glucose was added to each well and incubated for 10 minutes. Glucose uptake was evaluated as the radioactivity incorporated into the cells, normalized to the protein content. (D), Effects of bisphenols on leptin production. Cells were seeded and treated as described above. The supernatants were transferred into a 96-well plate then incubated with the antibodies. DES (10 nM) and Isoproterenol (Iso, 10µM) were uses as positive controls.

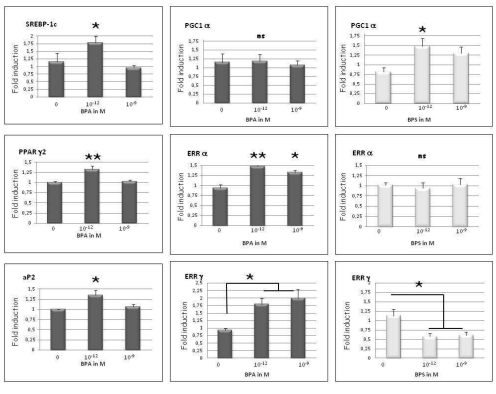

Figure 6: **Effects of bisphenols on gene expression of transcription factors in adipocytes**. Relative mRNA levels (SREBP1c; PPARδ2; aP2) from cells treated with indicated bisphenol concentrations. **Effects of bisphenols on energy metabolism gene expression in adipocyte.** Relative mRNA levels (PGC1α; ERRα; ERRγ) from cells treated with indicated concentrations of BPA (left panel) or BPS (right panel).

Is bisphenol S a safe substitute for bisphenol A in terms of metabolic function? An in vitro study. Héliès-Toussaint C, et al., Toxicol Appl Pharmacol.(accepted, in revision). 2014.

CONCLUSIONS: Even if BPS seems to be less toxic at high concentrations, the findings make us think that both BPA and BPS could be involved in obesity and steatosis processes *in vitro*, but through two different endocrine signaling pathways.

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