

Supplementary Methods

Compounds

The compounds BPA (#239658), BPF (#B47006), BPS (#103039), BP (#54680), Cd (#202908), DDE (#35487), DBP (#524980), DEHP (#36735), PFOA (#171468), PFOS (#77282), sodium oleate (OA, #O7501), palmitic acid (PA, #P0500), antimycin A (#A8674), oligomycin (#O4876), rotenone (#R8865), etomoxir (#E1905), resazurin (#R7017), neutral red (#N2889), Nile Red (#19123), Hoechst 33342 (#B2261) were purchased from Sigma Aldrich (France). Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, #BML-CM120) was purchased from Enzo Life Sciences. 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM, #C1354) was purchased from ThermoFisher. Sodium oleate was prepared in water.

Cell culture and treatment

Following cell differentiation and before pollutant testing, HepaRG were trypsinized and seeded at 2×10^6 cells/well into 6-well plates, at 8×10^5 cells/well into 12-well plates or at 8×10^4 cells/well into 96-well plates. Five days after seeding (i.e., the time required for cells to recover their differentiated state), cells were exposed to pollutants, at various concentrations, or to the vehicle with a final concentration of 1.7 % DMSO for different time periods, as noted in the results and the figure legends. The protocols are presented in **Supplementary Figure 1**. Fatty acid supplementation of cells was performed with several concentrations (50, 100, 200 μ M) of oleic acid (OA), alone or combined with palmitic acid (PA, 50 μ M), directly diluted in the culture medium which contained 10 % fetal bovine serum (FBS). Since FBS contains albumin, the cells treated with OA (100 μ M) have a free fatty acid:albumin ratio of 2.6, similar to that found under physiological conditions in humans (Berk and Stump 1999).

For Hepoid[®]-HepaRG 3-dimensional (3D) culture, type I collagen was used and diluted in the culture medium as described above in order to obtain a collagen solution at 1.5 mg/ml. After adjusting the pH to 7.4, cells were added at a concentration of 6×10^5 cells/mL. This mixture of HepaRG cells plus collagen was seeded into 384- or 48-well plates and incubated at 37°C in the presence of 5 % CO₂. An equal volume of medium was added after one hour of gel polymerization to obtain Hepoid-HepaRG, as previously described (Rose et al. 2022). The beginning of treatment of Hepoid-HepaRG cells with EDCs took place 5 days after seeding

into collagen and then the cells were treated for 14 days in a DMSO-free medium in the presence of 5% FBS.

Lipid droplet assessment

Differentiated HepaRG cells were seeded into 96-well plates with a density of 8×10^4 cells per well. After exposure, cells were washed twice with PBS and then incubated at 37°C and 5 % CO₂ for 35 min with a solution of Nile Red (1 μM) and Hoechst 33342 (1 μg/mL) prepared in PBS. A total of 4 images *per* well were obtained at 10X magnification using an automated fluorescence microscope (ImageXpress Pico, Molecular Devices) using appropriate wavelengths: 350/461 nm for Hoechst, 510/573 nm for Nile Red to assess only triglyceride, which is the main component of lipid droplets, and thus to avoid phospholipid-related fluorescence. All settings (exposure time, focus) remained identical across the different experiments. The area of acquisition corresponds to a square around the center of the well, corresponding to 25 % of the entire area of the well, i.e., 7.5 mm².

Images were analyzed using the ImageXpress Pico software. A macro was developed to select only the lipid droplets in the cells by using the size and the intensity exceeding the background noise. To compare the impact of the different treatments, a steatosis index was defined. This index is calculated by multiplying the mean surface area of the lipid droplets by the mean number of lipid droplets *per* cell (**Supplementary Figure 2**). To compare treatments, the results are expressed as a percentage (%) based on the mean steatosis index of the control according to the exposure procedure.

In Hepoid-HepaRG, lipid droplets were measured after fixation with 4 % paraformaldehyde (Sigma-Aldrich) containing 5 % sucrose (Sigma-Aldrich) for 10 min at room temperature. Hoechst 33342 (ThermoFisher) and LipidTOX™ HCS (ThermoFisher) staining were performed simultaneously after a 1/1000 dilution (as indicated by the manufacturer) to stain nuclei and lipid droplets, respectively. Images were acquired with a Leica SP8 confocal microscope and were obtained with a 40x oil objective. Stacks were made every 1 μm to obtain a total height of 40 μm for each condition. Images were then analyzed using ImageJ software. Next, lipid droplets were quantified using a specific macro that detects lipid droplets and nuclei based on the ImageJ acquisition. The results are expressed as the number of lipid droplets *per* cell.