Material and Methods

Triglyceride and MTT analysis- HHL-5 cells were seeded in 96–well plates, grown to 90% confluence and then treated as indicated. Once washed with PBS, the cells were stained with AdipoRed (Lonza) or MTT dye and read with a Genios Pro Plate reader (Tecan) according to the manufacturer's instructions.

hCB1 displacement binding assay- Membranes from HEK cells overexpressing hCB1 (Perkin-Elmer) were incubated with [^{3}H]-CP55,940 at K_D= 0.18 nM and different competing concentration of BPA (1-100 μM) as previously described (1). In all cases, Ki values were calculated by applying the Cheng−Prusoff equation to the IC_{50} values (obtained by GraphPad Prism software) for the displacement of the bound radioligand by increasing concentrations of the test compound.

Food intake analysis- After the exposure to BPA with a final concentration of 100 μg/L, EE2 (200 ng/L) or vehicle, animals received pre-weighed food in excess (5% bw) every day. Food intake (FI) was measured at the end of the experiment and was calculated as follows: FI=Wi−(Wf ×F), where Wi= initial dry food weight, Wf= remaining dry food weight and F= correction factor. F was previously calculated (2) in the absence of fish to determine the effect of water dissolution on food pellets during the feeding time, and represents the reduction in food weight after food remains 5 h into the aquaria (F=0.856±0.0054).

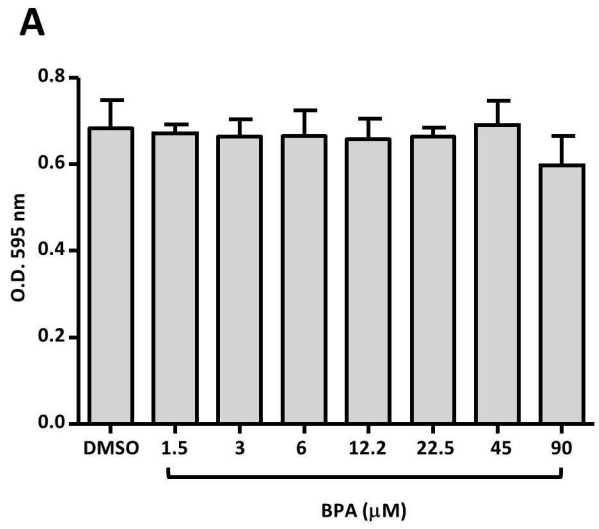
Figure Legends

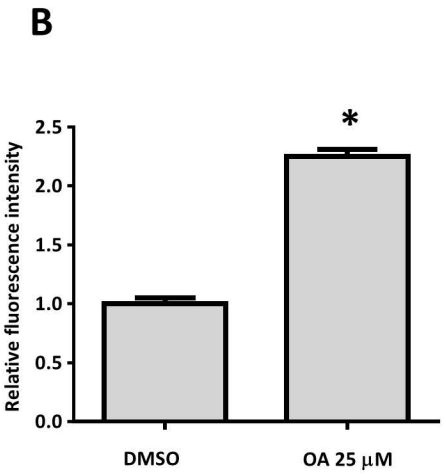
Supplemental figure 1. **Adipored, MTT, hCB1 affinity control test on human cells and food intake measurement on treated adult zebrafish** – Cell viability of HHL-5 cells was measured by MTT staining after 24 hrs treatment with the indicated BPA concentrations (**A**). Same experimental conditions were followed for the Adipored assay to test HHL-5 response to our hepatosteatotic agent, oleic acid (OA) (**B**), and several concentrations of BPA (**C**). Individual well fluorescence was taken as mean of twenty-five separate points in a 5x5 grid with a Genios pro plate reader Binding affinity displacement assay at hCB1 receptor with increasing concentrations of BPA. Data are expressed as percentage of [^{3}H]CP55,940 displacement respect to the total binding control (**D**). No significant affinity was detected at the tested concentrations. Food intake in control (CTR), BPA 100 μg/L (438.6 nM) and EE2 200 ng/L (0.877 nM) exposed fish (**E**). Data are expressed as mean food intake (mg) per body wt (g). Results are expressed as mean ± SD. Data were evaluated by one-way ANOVA with the Tukey post-test ($p<0.05$).

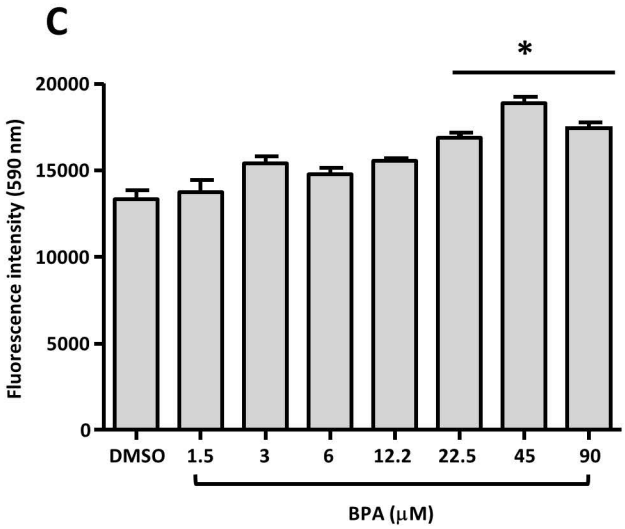
References

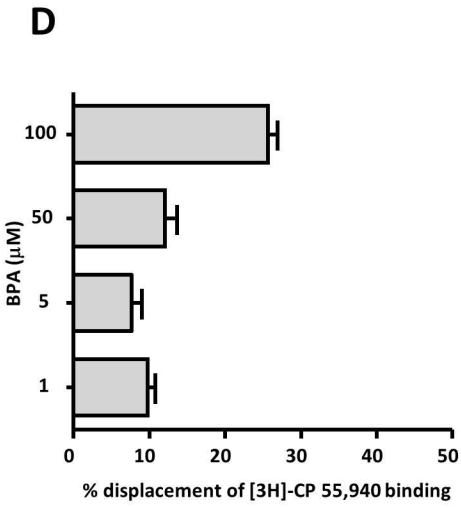
1. Di Marzo V, Griffin G, De Petrocellis L, Brandi I, Bisogno T, Williams W, Grier MC, Kulasegram S, Mahadevan A, Razdan RK, Martin BR. A Structure/Activity Relationship Study on Arvanil, an Endocannabinoid and Vanilloid Hybrid. Journal of Pharmacology and Experimental Therapeutics 2002; 300:984-991

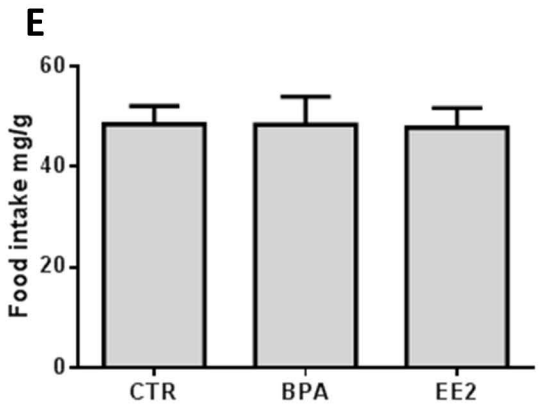
2. Maradonna F, Nozzi V, Santangeli S, Traversi I, Gallo P, Fattore E, Mita DG, Mandich A, Carnevali O. Xenobiotic-contaminated diets affect hepatic lipid metabolism: Implications for liver steatosis in Sparus aurata juveniles. Aquatic Toxicology 2015; 167:257-264


A
0.8
0.6
0.4
0.2
0.0
O.D. 595 nm
DMSO
1.5
3
6
12.2
22.5
45
90
BPA (μM)


B
2.5
2.0
1.5
1.0
0.5
0.0
Relative fluorescence intensity
*
DMSO
OA 25 μM


C
20000
15000
10000
5000
0
Fluorescence intensity (590 nm)
*
DMSO
1.5
3
6
12.2
22.5
45
90
BPA (μM)


D
100
50
5
1
BPA (μM)
0
10
20
30
40
50
% displacement of [3H]-CP 55,940 binding


E
60
40
20
0
Food intake mg/g
CTR
BPA
EE2