Non-targeted metabolomics reveals alterations in liver and plasma of gilt-head bream exposed to oxybenzone

Haizea Ziarrusta[1,2*], Leire Mijangos[1,2], Sergio Picart-Armada[3,4,5], Mireia Irazola[1,2], Alexandre Perera-Lluna[3,4,5], Aresatz Usobiaga[1,2], Ailette Prieto[1,2], Nestor Etxebarria[1,2], Maitane Olivares[1,2], Olatz Zuloaga[1,2]

[1]Department of Analytical Chemistry, University of the Basque Country (UPV/EHU), Leioa, Basque Country, Spain

[2]Research Centre for Experimental Marine Biology and Biotechnology (PIE), University of the Basque Country (UPV/EHU), Plentzia, Basque Country, Spain

[3]B2SLab, Departament d'Enginyeria de Sistemes, Automàtica i Informàtica Industrial, Universitat Politècnica de Catalunya, Barcelona, Spain

[4]Networking Biomedical Research Centre in the subject area of Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

[5]Institut de Recerca Pediàtrica Hospital Sant Joan de Dèu, Esplugues de Llobregat, Barcelona, Spain

*Corresponding Author: Haizea Ziarrusta (haizea.ziarrusta@ehu.eus)

Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), P.O. Box 644, 48080 Bilbao, Spain.

Tel: + 34 94 601 55 51; Fax: +34 94 601 35 00

Declarations of interest: none.

ABSTRACT

The extensive use of the organic UV filter oxybenzone has led to its ubiquitous occurrence in the aquatic environment, causing an ecotoxicological risk to biota. Although some studies reported adverse effects, such as reproductive toxicity, further research needs to be done in order to assess its molecular effects and mechanism of action. Therefore, in the present work, we investigated metabolic perturbations in juvenile gilt-head bream (*Sparus aurata*) exposed over 14 days via the water to oxybenzone (50 mg/L). The non-targeted analysis of brain, liver and plasma extracts was performed by means of UHPLC-Orbitrap MS in positive and negative modes with both C18 and HILIC separation. Although there was no mortality or alterations in general physiological parameters during the experiment, and the metabolic profile of brain was not affected, the results of this study showed that oxybenzone could perturb both liver and plasma metabolome. The pathway enrichment suggested that different pathways in lipid metabolism (fatty acid elongation, α-linolenic acid metabolism, biosynthesis of unsaturated fatty acids and fatty acid metabolism) were significantly altered as well as metabolites involved in phenylalanine and tyrosine metabolism. Overall, these changes are signs of possible oxidative stress and energy metabolism modification. Therefore, this research indicates that oxybenzone has adverse effects beyond the commonly studied hormonal activity, and demonstrates the sensitivity of metabolomics to assess molecular-level effects of emerging contaminants.

Keywords: Oxybenzone, gilt-head bream, non-targeted metabolomics

1. INTRODUCTION

Benzophenone-3 or oxybenzone is one of the most widely used organic UV filters in sunscreen products and daily use cosmetics. Like other pharmaceuticals and personal care products (PPCPs), oxybenzone and its by-products are incompletely removed during wastewater treatment (Tsui et al., 2014), resulting in their occurrence in many aquatic ecosystems. Oxybenzone has been found in wastewater and surface waters, but also in fish due to its bioaccumulation capacity (Al-Salhi et al., 2012; Molins-Delgado et al., 2018; Ramos et al., 2015; Ziarrusta et al., 2018). Preliminary risk assessment studies indicated that oxybenzone discharged from wastewater treatment plants may pose high risk to aquatic organisms in the environment (Tsui et al., 2014).

Consequently, occurrence of UV filters in aquatic ecosystems and their potential effects on non-target organisms is of growing concern (Blüthgen et al., 2012; Coronado et al., 2008; Díaz-Cruz and Barceló, 2009; Fent et al., 2010; Ghazipura et al., 2017; Kim et al., 2014; Kim and Choi, 2014; Kinnberg et al., 2015; Kunz et al., 2006; Liu et al., 2015; Rodríguez-Fuentes et al., 2015; Tsui et al., 2014). For instance, developmental and reproductive toxicity of oxybenzone (Coronado et al., 2008; Ghazipura et al., 2017; Kim et al., 2014; Kinnberg et al., 2015) in fish have been attributed to an altered hormonal balance as a result of its endocrine disrupting effects (Ghazipura et al., 2017; Kim et al., 2014; Kim and Choi, 2014; Kinnberg et al., 2015). Many studies have reported the complex hormonal activity of UV filters, since many of these xenobiotics show up to three distinct modes of action, including estrogenicity, anti-estrogenicity and anti-androgenicity (Blüthgen et al., 2012; Kim et al., 2014; Kim and Choi, 2014; Kinnberg et al., 2015; Kunz et al., 2006). Additionally, the scarce information available regarding their effects on the antioxidant defense system in fish suggests that UV filters have adverse effects beyond hormonal activity (Liu et al., 2015; Rodríguez-Fuentes et al., 2015). For instance, a growing trend in glutathione peroxidase expression was observed in zebrafish (*Danio rerio*) eleuthero-embryos exposed to oxybenzone, which is an indicative of oxidative stress (Rodríguez-Fuentes et al., 2015). In the same context, Liu and co-workers also reported that benzophenone UV filters generated oxidative stress in freshwater fish *Carassius auratus* (Liu et al., 2015).

In addition to this, Coronado and co-workers revealed that the alteration of endocrine and reproduction endpoints in fish are linked to the exposure to oxybenzone, but at levels significantly higher than those measured in the environment (Coronado et al., 2008). Consequently, acute and chronic toxicity assays are required for a more accurate

environmental risk assessment (ERA). Preliminary studies reported that predicted no effect concentration (PNEC) for oxybenzone was derived at 1.32 µg/L (Kim and Choi, 2014) and LC_{50} value (48 h) was 1.9 mg/L (Fent et al., 2010), indicating that individual UV filters should undergo further ecotoxicological analysis, as an environmental risk cannot be ruled out even though the levels observed in water bodies are generally lower. Furthermore, Kim and co-workers stated that oxybenzone is transformed into benzophenone-1, a more potent estrogen agonist, which needs to be included in ERA in order to have a more realistic estimation of the effects of oxybenzone exposure (Kim et al., 2014). On the other hand, although oxybenzone has been frequently detected in aquatic organisms, limited information is available on its *in vivo* toxicity, particularly in fish, and further *in vivo* testing is required since it provides the most reliable data for predictive testing and risk assessment of xenobiotics (Díaz-Cruz and Barceló, 2009).

Finally, although most of the studies investigating the hazards associated with UV filters in aquatic organisms have focused on receptor binding and gene expression assays (Blüthgen et al., 2012; Coronado et al., 2008; Kim and Choi, 2014; Rodríguez-Fuentes et al., 2015), complementary approaches are needed for a more thorough ERA. In order to study thoroughly the effects of xenobiotics occurring in the environment at non-lethal levels, it is important to focus on the modes of action. To this end, metabolomics has proven useful by offering insight into early biochemical perturbations at low molecular weight endogenous molecules triggered by low dose of xenobiotics, which may lead to an adverse effect (Al-Salhi et al., 2012; Cajka and Fiehn, 2016; Huang et al., 2016). For example, Huang et al. revealed that the exposure of multiple class of xenobiotics at environmentally relevant concentrations produces specific biochemical fingerprints in zebrafish (Huang et al., 2016), indicating that metabolomics can complement toxicity assays with molecular-level information. In particular, a hypothesis-free and non-targeted metabolomic approach allows the identification of many distinct features in a single analysis, offering the opportunity to discover biomarkers that lead to a range of breakthroughs in understanding the risks of xenobiotics (Al-Salhi et al., 2012; Cajka and Fiehn, 2016). Since generally highly dimensional and multi-correlated data are obtained for a few replicate samples, both univariate and multivariate approaches (Vinaixa et al., 2012) have been applied to identify the metabolites that allow the discrimination of case from control groups. Additionally, pathway enrichment (Picart-Armada et al., 2017) helps to find plausible biological explanations for those metabolic alterations taking into account compounds, modules, enzymes, reactions and pathways in databases such as KEGG (http://www.kegg.jp/kegg/).

In this framework, the main objective of this work was to evaluate the *in vivo* effects of oxybenzone in juvenile gilt-head bream (*Sparus aurata*) using a non-targeted metabolomics approach to measure perturbations in the brain, liver and plasma metabolome. To our knowledge, this is the first study to investigate metabolic effects of oxybenzone in fish.

2. EXPERIMENTAL

2.1. Standards and Reagents

Stock dosing solution of oxybenzone (98%, Sigma-Aldrich, St. Louis, MO, USA) was prepared at 25 g/L in ethanol (EtOH) and diluted down to 0.83 mg/L in Milli-Q water. The final concentration of EtOH in the dosing solution was <0.005%. Additional information regarding the reagents of endogenous metabolites purchased from Sigma–Aldrich is detailed in Table S1 of the supplementary information (SI).

2.2. Oxybenzone exposure experiment and sampling

Exposure experiments were designed at the Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU) using juvenile gilt-head bream weighing ~40 g and measuring ~13 cm in length (Groupe Aqualande, Roquefort, France). The experiments were carried out at controlled lab temperature (18 °C), water temperature (13.5 ± 0.5 ˚C) and pH (7.3 ± 0.3), and light (14:10 h light:dark cycle). Fish were acclimatized for 1 month upon arrival, stabilized for an additional 1 week in the exposure tanks before the exposure and they were fed daily with 0.10 g pellets/fish (EFICO YM 868, 3 mm, BioMar Group, Denmark). The water in the control and exposed tanks was continuously aerated and the water quality was assured by measuring periodically the dissolved oxygen, nitrite, nitrate and ammonium contents.

This experiment was conducted in parallel with a bioaccumulation/biotransformation study, and it was observed that oxybenzone was accumulated in different tissues and biofluids (Ziarrusta et al., 2018). A 14-day exposure (50 µg/L nominal) was performed using two 1000 x 700 x 650 mm polypropylene tanks (one control, one exposed), containing 250 L of seawater and 50 fish per tank. Exposures were carried out using a continuous flow-through system with a peristaltic pump delivering 10 L seawater/h and another pump infusing an oxybenzone dosing solution (refilled every 24 hours) at 0.6 L/h to the exposure tank. Identical experimental conditions were maintained for control tank. Ten fish were sampled from both tanks at the beginning of the experiments (day 0) and on exposure days 2, 4, 7 and 14. Additionally, 2.5 L water samples were also collected on the same sampling days to monitor oxybenzone concentrations in both tanks.

Fish taken for dissection were immersed for 5 minutes in a tank containing 10 L of seawater with 100 mg/L tricaine and 200 mg/L $NaHCO_3$. Each anaesthetized fish was weighed, measured and dissected to collect plasma, brain in 2-mL Precellys vials (Bertin Instruments, Montigny-le-Breonneux, France) and liver in 7-mL Precellys vials. Blood was sampled from the caudal vein-artery using a syringe previously rinsed with 0.5 mol/L EDTA solution (pH adjusted to 8.0 using NaOH) and then centrifuged for 5 minutes at 1000 rpm to get the plasma. Biological fluids and tissues were stored in liquid nitrogen during dissection and kept at -80 ˚C until extraction. Fish processing described herein was evaluated by the Bioethics Committee of UPV/EHU and approved by the Local Authority according to the current regulations (procedure approval CEEA/380/2014/ETXEBARRIA LOIZATE).

2.3. Extraction and analysis of metabolites

Analytical method for metabolite extraction and analysis is fully described elsewhere (Ribbenstedt et al., 2018). In brief, a mixture of 4 µL $CHCl_3$:MeOH (20:80, v/v) per mg sample was added directly into the Precellys vials containing dissected tissues and biofluids (~100 mg brain, ~400 mg plasma and 1000 mg liver) for a single step extraction of both polar and non-polar metabolites. All samples were homogenized for 3x1.5 min at 4000 rpm (Precellys), under controlled cooled temperature (4 °C) (Cryolys, Bertin Technologies, Montigny-le-Bretonneux, France), employing 1.4 mm or 2.8 mm zirconium oxide beads (Precellys) in the case of brain and liver, respectively. Extracts were centrifuged for 15 minutes at 18,000 rpm (Centrifuge Allegra X-30R, F2402H, Beckman Coulter, High Wycombe, UK) to get the supernatant consisting on a single-phase solution.

Non-targeted analysis was carried out using a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive quadrupole-Orbitrap mass spectrometer equipped with a heated ESI source (HESI, Thermo, CA, USA), and controlled by Xcalibur 4.0 software (Thermo). Furthermore, to maximize metabolite identification coverage, aliquots of 5 µL of all the extracts were analyzed in 3 different runs using different chromatographic columns and ionization modes: hydrophilic interaction liquid chromatography (HILIC) column in positive ($HILIC_{pos}$) and negative ($HILIC_{neg}$) ionization modes, and a reverse-phase octadecylsilyl column in the positive mode ($C18_{pos}$). The Orbitrap was operated in full scan – data dependent MS2 (Full MS-ddMS2) discovery acquisition mode. One full scan at a resolution of 70,000 full width at half maximum (FWHM) at *m/z* 200 over a scan range of *m/z* 70-1000 was followed by three ddMS2 scans at a resolution of 17,500 FWHM at *m/z* 200, with an isolation window of 0.8 Da. The stepped normalized collision energy (NCE) in the higher-energy collisional dissociation (HCD) cell was set to 10, 35 and 75 eV (see more details about instrumental analysis in SI).

The extraction of the samples was carried out randomly and the samples were arranged in 9 separate sequences: one per matrix (liver, brain, plasma) and analysis conditions ($HILIC_{pos}$, $HILIC_{neg}$, $C18_{pos}$). In order to assure the quality assurance/quality control of the instrumental analysis (Broadhurst et al., 2018), sample extracts were analyzed randomly along the sequence. Instrumental blank samples (pure MeOH) injected every 5 samples ruled out the presence of carryover, and procedural blank samples were prepared to estimate the background concentration of metabolites during sample workup. Sequence quality control sample (QC_{seq}) was also prepared for each tissue by pooling a small volume of each extract and splitting it into several aliquots. These aliquots were injected after every 10 samples to allow signal drift correction.

2.4. Data Handling and Statistical Analyses

Condition factor (K) and hepatic somatic index (HSI) were determined as a general assessment of fish health. K was calculated using the equation $K = (\text{fish weight} \times 100)/(\text{fish length})^3$) (Fulton, 1904), while HSI was determined using the equation HSI = (liver weight × 100)/(fish weight)). K and HSI were statistically evaluated between exposed and control groups using one-way ANOVA.

Identification of metabolites involved in altered metabolic pathways was performed separately for plasma, brain and liver following the workflow shown in Fig. 1. First, each sequence of chromatograms was processed using Compound Discoverer 2.1 (CD; Thermo-Fisher Scientific). The specific details of the workflow and settings are described in SI. Thereafter, each data set was filtered to keep only endogenous metabolites by searching the detected exact masses in the database containing up to 4400 endogenous compounds or in LipidMaps (http://www.lipidmaps.org/) database.

All statistical analyses were carried out using the R software for statistical computing (v 3.4.3). Firstly, using Principal Component Analysis (PCA) as an unsupervised discrimination approach, possible outliers, samples outside the 95% confidence regions of the total dataset, were discarded in each sequence. Signal drift over the course of the sequence (identified from QC_{seq} data) was corrected afterwards using the R package intCor (Fernández-Albert et al., 2014), via a two-step method that combines Common Principal Components Analysis (CPCA) and the medians method. To create this model, we defined three classes (i.e., control, exposed and QC_{seq}) and the number of components of the model in each specific sequence. Once the drift was corrected, the 3 sequences ($HILIC_{pos}$, $HILIC_{neg}$, $C18_{pos}$) of each matrix were merged to subsequently perform multiple linear analysis (Y (Day, Dose) ~ Day + Dose + Day·Dose, where Y is the feature response). Since the objective was to find out the metabolites that showed

significantly different concentration evolution during exposure between the exposed and control samples, we paid special attention to the interaction Dose·Day. Therefore, applying linear analysis and multiple testing, we selected the chromatographic features with a p-value < 0.05 and a false discovery rate (FDR) < 0.05 in the interaction Dose·Day. We manually checked the features that showed up as significant in order to discard those with bad chromatographic peak shape and/or incorrectly integrated, as well as those features that corresponded to oxybenzone by-products (Ziarrusta et al., 2018).

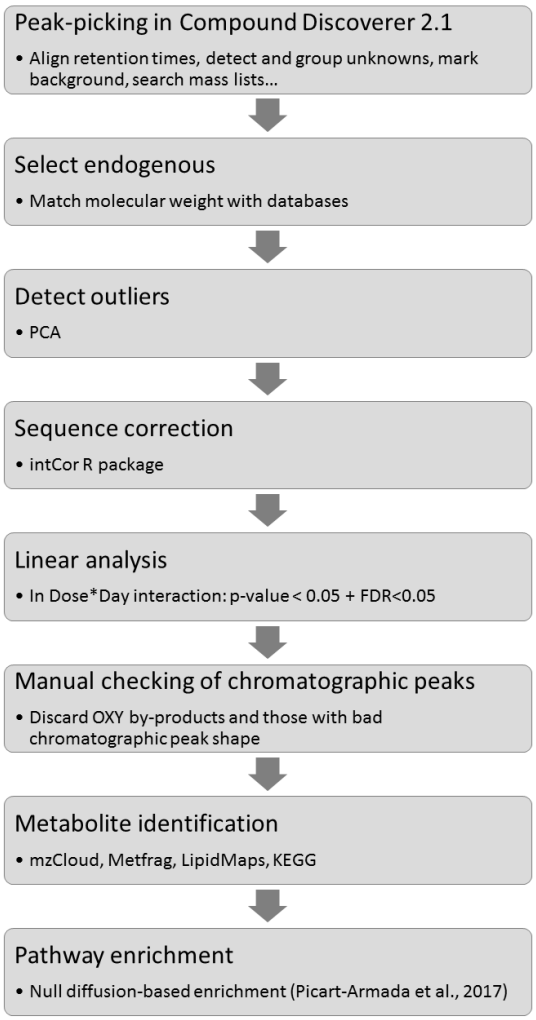

***Fig. 1**: Workflow followed to identify metabolites involved in altered metabolic pathways.*

After selecting significant features, we performed metabolite annotation (Schymanski et al., 2014) comparing the exact mass, isotopic profile, fragmentation and abundances with those available in the mzCloud library. When the metabolite was not available in the mzCloud library, experimental fragmentation pattern was compared against *in silico* fragmentation obtained in MetFrag (https://msbi.ipb-halle.de/MetFragBeta/) that uses KEGG and LipidMaps databases for metabolite annotation. In this sense, when the standard was available (see Table S1), retention time was also used for the last confirmation.

In order to identify the key metabolic processes and to find out a feasible biological explanation for the variations observed, null diffusion-based enrichment (Picart-Armada et al., 2017) was run with KEGG annotated compounds based on the following parameters: diffusion method, simulation approximation with 15,000 iterations. The database was built using the KEGG release 84.0+/10-08, Oct 17 2017, the zebrafish (*Danio rerio*) organism (organism code "dre") and excluding the unspecific "dre01100" metabolic overview pathway. The network consisted of 161 pathways, 176 modules, 973 enzymes, 4803 reactions and 3450 KEGG compounds. We also discussed the biological relevance of the lipids identified as significant metabolites but not included in KEGG. Finally, daily fold change (FC) values (see equation 1) of the suggested metabolites were calculated as described elsewhere (Vinaixa et al., 2012):

$$FC = {\bar{X}}/{\bar{Y}} \; when \; \bar{X} > \bar{Y} \; or \; FC = -{\bar{Y}}/{\bar{X}} \; when \; \bar{X} < \bar{Y} \qquad \text{Equation 1}$$

Where, $\overline{X}$ and $\overline{Y}$ are the average concentrations of the metabolite in the exposed and control samples, respectively.

3. RESULTS AND DISCUSSION

No significant changes in fish weight and length were observed at the 95% confidence level, regardless of experiment tank or exposure day (p-value > 0.05). There was no mortality and K and HSI were comparable between fish of exposed and control groups (p-value > 0.05) at in all the exposure days. Furthermore, following the workflow detailed in section 2.4, no metabolite concentration was altered significantly in brain, concluding that the brain metabolome was not affected by oxybenzone. Although according to the literature oxybenzone can accumulate in the brain and result in neurological effects by disrupting the brain homeostasis in rats (Fediuk et al., 2010), in gilt-head bream we observed no metabolome profile modification in this tissue. Contrarily, in the case of liver and plasma, the number of significantly altered features was 8 and 10, respectively. From those peaks, 4 and 9 were KEGG annotated in liver and plasma, respectively, and the rest of the peaks were putatively identified as lipids that are not included in KEGG (see Table 1). Additionally, pathway enrichment was performed using the significant metabolites previously mapped on KEGG for each matrix. Overall, the largest alterations were observed mainly in the last day of exposure (see the daily FC values and individual average peak areas for each metabolite in liver and plasma in Tables S2 and S3, respectively).

Table 1: Identification of the features whose concentrations were significantly altered after the oxybenzone-exposure.

Matrix	Peak	Molecular Weight	MS/MS fragments	RT	Name	KEGG Code	Level
Liver	C18pos_Peak1222	180.0421	163.0388; 149.0232; 65.0393	14.04	trans-2,3-dihydroxycinnamate	C12623	2b
	C18pos_Peak1293	180.0421	163.0388; 149.0232; 65.0393	10.92	3-(4-Hydroxyphenyl)pyruvate / 2-hydroxy-3-(4-hydroxyphenyl)propenoate	C01179/ C05350	3
	C18pos_Peak529	193.0739	162.0547; 152.0703; 120.0444	11.25	Phenylacetylglycine	C05598	2a
	HILICpos_Peak1073	179.0582	162.0550; 105.0335; 95.0491	6.64	Hippurate	C01586	2a
	HILICneg_Peak114	791.5487	283.2649; 124.9999; 123.0436	1.67	18 candidates belonging to glycerophospholipids lipid category[a]	*	3
	HILICneg_Peak1178	406.1271	243.0670; 227.0714; 123.0436	7.09	5 candidates belonging to polyketides lipid category[b]	*	3
	HILICneg_Peak1333	493.3538	311.2956; 283.2649; 184.0733	6.78	2 candidates belonging to glycerophospholipids lipid category[c]	*	3
	HILICneg_Peak421	420.1064	123.0436; 108.0200; 93.0328	6.95	7 candidates belonging to polyketides lipid category[d]	*	3
Plasma	C18pos_Peak1037	138.1045	121.1013; 97.0657; 93.0703	13.23	3,6-Nonadienal	C16323	2b
	C18pos_Peak715	105.0428	88.0398; 70.0295; 60.0453	2.05	D-Serine	C00740	1
	HILICneg_Peak29	366.3501	183.1391; 169.1234; 141.0921	1.61	Nervonic acid	C08323	2a
	HILICneg_Peak498	172.0141	152.9958; 96.9696; 78.9591	6.66	sn-Glycerol 1-phosphate / sn-Glycerol 3-phosphate	C00623/ C00093	3
	HILICneg_Peak12	328.2407	283.2431; 229.1962; 119.0490	2.21	Docosahexaenoic acid	C06429	2a
	HILICneg_Peak344	336.3033	95.0862; 85.0277; 57.0710	1.63	Docosadienoic acid	C16533	2b
	HILICpos_Peak112	105.043	88.0399; 70.0295; 60.0453	10.80	D-Serine	C00740	1
	C18pos_Peak688	278.2245	261.2217; 243.2115; 95.0859	17.64	γ-Linolenic acid / α-Linolenic acid / Crepenynate	C06426/ C06427/ C07289	3
	HILICpos_Peak11	129.0426	102.0554; 84.0451; 56.0505	10.73	5-Oxo-L-proline	C01879	2a
	C18pos_Peak158	733.5618	184.0733; 124.9999; 86.0970	21.39	14 candidates belonging to glycerophospholipids lipid category[e]	*	3

*Putatively identified/not in KEGG. LipidMaps ID: [a]LMGP01011430, LMGP01011544, LMGP01011573, LMGP01011906, LMGP01011935, LMGP01012101, LMGP02010094, LMGP02010678, LMGP02010766, LMGP02010864, LMGP02010894, LMGP02010924, LMGP02010953, LMGP02010981, LMGP02011087, LMGP02011115, LMGP02011142 or LMGP20020015; [b]LMPK13090007, LMPK12020031, LMPK12020264, LMPK12020009 or LMPK12020025; [c]LMGP02070004 or LMGP01070007; [d]LMPK12113361, LMPK12113362, LMPK12113363, LMPK12113382, LMPK12113381, LMPK12113364 or LMPK12113383; [e]LMGP01010463, LMGP01010704, LMGP01010444, LMGP01010564, LMGP01010537, LMGP01011080, LMGP01010997, LMGP01011062, LMGP01010970, LMGP01010739, LMGP01010418, LMGP01010488, LMGP01011267 or LMGP01010397.

3.1. Liver metabolome alterations

Pathway enrichment suggested that the more significantly altered metabolites in liver were those involved in the amino acid metabolism, specifically, in the phenylalanine metabolism and the tyrosine-related metabolites (see Fig. 2). Phenylacetylglycine, hippurate and trans-2,3-dihydroxycinnamate levels were increased in oxybenzone-exposed fish (see Table S2). Apart from those targets in the phenylalanine metabolism, tyrosine-related metabolites 3-(4-hydroxyphenyl)pyruvate/2-hydroxy-3-(4-hydroxyphenyl)propenoate also showed the same concentration profile. Both pathways are related since phenylalanine-4-hydrolase converts phenylalanine into tyrosine (Li et al., 2011). Therefore, all these perturbations might be associated. Additionally, 4 putatively identified lipid levels were also altered.

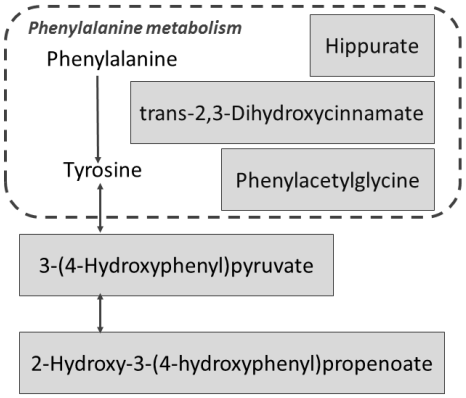

***Fig. 2**: Altered metabolites and pathway in fish liver.*

The perturbation of the metabolites involved in amino acid metabolism in liver could pinpoint a way to overcome the stressing exposure to oxybenzone, similarly to what occurred with other environmental pollutants (Wang et al., 2017; Zhang and Zhao, 2017). Additionally, several studies have shown that the oxidative stress owing to pollutant exposure can induce changes in concentration levels of amino acids and lipid metabolites (Ling et al., 2014; Yuan et al., 2016). Furthermore, in this work we have also observed alterations in 4 lipidic metabolites (see Table 1), which is consistent with lipid storage disorders observed under exposure to PPCPs (Buron et al., 2017). To sum up, the evidences to conclude that the pollutant-exposed fish showed oxidative stress are the alterations in lipidic metabolites (Buron et al., 2017), the perturbation in hippurate levels (Zhang et al., 2014) and the significant changes in the precursor metabolites of carbohydrate metabolism such as 3-(4-hydroxyphenyl)pyruvate/2-hydroxy-3-(4-hydroxyphenyl)propenoate (Ling et al., 2014; Yuan et al., 2016).

Oxidative stress has been previously linked to the disruption of energy metabolism (Ling et al., 2014; Yuan et al., 2016), which is reported to be perturbed by the exposure to pollutants

(Amorim and Alvarez-Leite, 1997; Colet, 2015). For instance, Colet and co-workers revealed that the alteration of hippurate was a consequence of a disruption of the energy metabolism in liver mitochondria (Colet, 2015). In fact, increased hippurate levels after oxybenzone administration (see Table S2) are not surprising since it was also observed with other xenobiotics such as toluene or arsenic (Amorim and Alvarez-Leite, 1997; Colet, 2015; Zhang et al., 2014), suggesting a non-specific mechanism of action (Robertson, 2005). In addition to hippurate, in the phenylalanine metabolism, we also observed the perturbations of trans-2,3,-dihydroxycinnamate and phenylacetylglycine, and the later was also found as altered metabolite in children exposed to bisphenol A (Khan et al., 2017). Therefore, similarly to other xenobiotics (Zhang et al., 2016), we conclude that oxybenzone alters the phenylalanine pathway.

Lastly, although oxybenzone is widely reported to have estrogenic activity (Blüthgen et al., 2012; Coronado et al., 2008; Kim et al., 2014; Kim and Choi, 2014; Kinnberg et al., 2015; Kunz et al., 2006), we did not observe clear evidences for that adverse effect. It is reported that the estrogenic signaling pathway includes the regulation of tyrosine hydroxylase, which catalyzes the synthesis of catecholamines from tyrosine (Maharjan Shreekrishna et al., 2005). Meanwhile, the only perturbation in the tyrosine metabolism was in the above mentioned 3-(4-hydroxyphenyl)pyruvate /2-hydroxy-3-(4-hydroxyphenyl)propenoate, which is linked to tyrosine. Therefore, this observation might also be associated with the estrogenicity described for oxybenzone.

According to the literature, liver has been found to be a key organ for absorption, bioaccumulation, energy metabolism, protein biosynthesis, xenobiotic detoxification, endocrine as well as immune responses in multiple toxicity studies _(Qiao et al., 2016). Therefore, the specific reason behind the alterations found in this work in liver might be the effect of any of them or even the combination of a few ones.

3.2. Plasma metabolome alterations

In the case of plasma, the metabolites suggested by pathway enrichment were combined based on KEGG to form a metabolic network, giving a holistic view of metabolic changes in the organism. As can be observed in Fig. 3, those metabolites are mainly involved in the alteration of the lipid metabolism and the suggested perturbed pathways were: fatty acid elongation, α-linolenic acid metabolism, biosynthesis of unsaturated fatty acids and fatty acid metabolism.

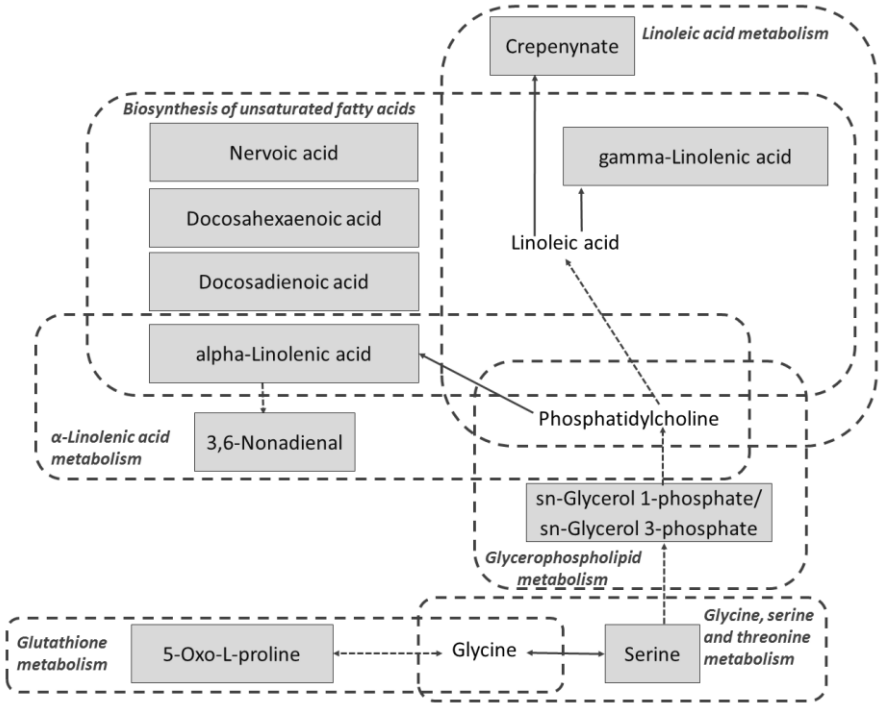

***Fig. 3**: Altered metabolites and pathways in fish plasma.*

The alterations of 8 metabolites in lipid metabolism can be related to oxidative stress since several authors have reported that UV filters such as oxybenzone generate oxidative stress in fish (Liu et al., 2015; Rodríguez-Fuentes et al., 2015). Similarly to the present work, lipid storage disorder associated with oxidative stress has been already reported as an adverse effect of different xenobiotics (Buron et al., 2017; Lin et al., 2014; Salihovic et al., 2016; Wafa et al., 2011). In this work, the concentrations of 5 unsaturated fatty acids (γ-linolenic acid, α-linolenic acid, docosahexaenoic acid, nervonic acid and docosadienoic acid) in plasma were altered in oxybenzone-exposed fish. Unsaturated fatty acids serve as substrates for lipoxygenases, which are capable of oxidizing many xenobiotics (Kulkarni, 2001). Actually, oxidized oxybenzone degradation products were observed in a previous work of our research group (Ziarrusta et al., 2018).

Among the altered fatty acids, docosahexaenoic acid is used for assessing oxidative stress *in vivo* (Yoshida et al., 2013), and its alteration was observed with other xenobiotics (Salihovic et al., 2016; Wafa et al., 2011). Metabolites belonging to linoleic acid and linolenic acid metabolism are used to identify oxidative stress episodes (Lin et al., 2014; Yoshida et al., 2013). Concretely, similarly to other exposure studies (Lin et al., 2014), we observed alterations in the levels of lipid peroxidation products of both linoleic acid (e.g., γ-linolenic acid and crepenynate) and α-linolenic acid (e.g., α-linolenic acid and 3,6-nonadienal) pathways, which are consistent markers of higher oxidative states (Shearer and Newman, 2008).

Observing lipid abnormalities in different lipid classes is common (Chen et al., 2017; Salihovic et al., 2016), and we also detected the alteration of *sn*-glycerol 1-phosphate/*sn*-glycerol 3-phosphate in the glycerophospholipid metabolism.

Lastly, we observed the alteration of the amino acid serine, which is an essential precursor for the synthesis of lipids (Hanada, 2003). The alteration of amino acid metabolism, including serine, is also reported with other xenobiotics (Eguchi et al., 2017; Zhang et al., 2014). In fact, Zhang and co-workers stated that the disruption of the amino acid metabolism related to serine is indicative of oxidative stress (Zhang et al., 2014). Therefore, the lower serine levels that we detected after 14-day oxybenzone exposure (see Table S3) might have caused a more significant cellular response to oxidative stress and higher reactive oxygen species (ROS) content, which is consistent with other works that observed serine deficiency in plasma under oxidative stress (Zhou Xihong et al., 2017). The reason behind this might be the role of serine linking biosynthetic flux from glycolysis to the synthesis on antioxidant glutathione (Parker and Metallo, 2016), because glutathione depletion causes accumulation of ROS (Dixon and Stockwell, 2014). Actually, in the present work there is a sign of glutathione metabolism perturbation since 5-oxo-L-proline was altered in the exposed fish. 5-oxoproline is an intermediate in the biosynthesis of glutathione, and although it is known as a potential biomarker for hepatotoxicity, xenobiotic-induced 5-oxoproline alterations are observed in various biofluids/tissues including liver, urine and plasma (Geenen et al., 2011). In exposure studies, finding alterations of glutathione-related pathways is common since there are enzymes that are important in xenobiotic metabolism (Eguchi et al., 2017). As an example of glutathione-related pathway disruption, Eguchi and co-workers (Eguchi et al., 2017) reported glutathione as possible candidate biomarker for polychlorinated biphenyls exposure. Furthermore, in a study with zebrafish, oxybenzone also perturbed glutathione peroxidase expression involved in cellular redox balance (Rodríguez-Fuentes et al., 2015), which is consistent with the observation in this work.

4. CONCLUSIONS

The present study showed that despite an absence of mortality or alterations in general physiological parameters (i.e, fish weight and length, K and HSI) and brain metabolome, oxybenzone produces significant metabolic perturbations in both liver and plasma of fish. Briefly, from the results it can be concluded a possible energy metabolism modification and oxidative stress. The latter has important health implications (Chen et al., 2017) since it damages cellular structures and functions, significantly influencing life histories of fish through

interactions with growth, reproduction and body maintenance and, hence, future fitness and survival (Birnie-Gauvin et al., 2017). Therefore, this research indicates that oxybenzone has adverse effects beyond the commonly studied hormonal activity (Blüthgen et al., 2012; Kim et al., 2014; Kim and Choi, 2014; Kinnberg et al., 2015; Kunz et al., 2006), and proves the usefulness of the metabolomic approach to study the modes of action. Consequently, complementary to receptor binding and gene expression assays, ERA should consider metabolomic studies to detect perturbations at molecular level caused by to investigate the effects of xenobiotics at non-lethal levels.

5. ACKNOWLEDGEMENTS

This work was financially supported by the Ministry of Economy and Competitiveness through the project CTM2014-56628-C3-1-R and by the Basque Government through the project IT-742-13. H. Ziarrusta and L. Mijangos are grateful to the Spanish Ministry and Basque Government for their pre-doctoral fellowships, respectively, and to the UNESCO chair on Cultural Landscape and Heritage for financial support.

6. REFERENCES

Al-Salhi, R., Abdul-Sada, A., Lange, A., Tyler, C.R., Hill, E.M., 2012. The Xenometabolome and Novel Contaminant Markers in Fish Exposed to a Wastewater Treatment Works Effluent. Environ. Sci. Technol. 46, 9080–9088. https://doi.org/10.1021/es3014453

Amorim, L.C., Alvarez-Leite, E.M., 1997. Determination of o-cresol by gas chromatography and comparison with hippuric acid levels in urine samples of individuals exposed to toluene. J. Toxicol. Environ. Health 50, 401–407.

Birnie-Gauvin, K., Costantini, D., Cooke, S.J., Willmore, W.G., 2017. A comparative and evolutionary approach to oxidative stress in fish: A review. Fish Fish. 18, 928–942. https://doi.org/10.1111/faf.12215

Blüthgen, N., Zucchi, S., Fent, K., 2012. Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (Danio rerio). Toxicol. Appl. Pharmacol. 263, 184–194. https://doi.org/10.1016/j.taap.2012.06.008

Broadhurst, D., Goodacre, R., Reinke, S.N., Kuligowski, J., Wilson, I.D., Lewis, M.R., Dunn, W.B., 2018. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabolomics 14. https://doi.org/10.1007/s11306-018-1367-3

Buron, N., Porceddu, M., Roussel, C., Begriche, K., Trak-Smayra, V., Gicquel, T., Fromenty, B., Borgne-Sanchez, A., 2017. Chronic and low exposure to a pharmaceutical cocktail induces mitochondrial dysfunction in liver and hyperglycemia: Differential responses between lean and obese mice. Environ. Toxicol. 32, 1375–1389. https://doi.org/10.1002/tox.22331

Cajka, T., Fiehn, O., 2016. Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics. Anal. Chem. 88, 524–545. https://doi.org/10.1021/acs.analchem.5b04491

Chen, C.-H.S., Yuan, T.-H., Shie, R.-H., Wu, K.-Y., Chan, C.-C., 2017. Linking sources to early effects by profiling urine metabolome of residents living near oil refineries and coal-

fired power plants. Environ. Int. 102, 87–96. https://doi.org/10.1016/j.envint.2017.02.003
Colet, J.-M., 2015. Metabonomics in the preclinical and environmental toxicity field. Drug Discov. Today Technol. 13, 3–10. https://doi.org/10.1016/j.ddtec.2015.01.002
Coronado, M., De Haro, H., Deng, X., Rempel, M.A., Lavado, R., Schlenk, D., 2008. Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquat. Toxicol. 90, 182–187. https://doi.org/10.1016/j.aquatox.2008.08.018
Díaz-Cruz, M.S., Barceló, D., 2009. Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. TrAC Trends Anal. Chem., Applying combinations of chemical analysis and biological effects to environmental and food samples - II 28, 708–717. https://doi.org/10.1016/j.trac.2009.03.010
Dixon, S.J., Stockwell, B.R., 2014. The role of iron and reactive oxygen species in cell death. Nat. Chem. Biol. 10, 9–17. https://doi.org/10.1038/nchembio.1416
Eguchi, A., Sakurai, K., Watanabe, M., Mori, C., 2017. Exploration of potential biomarkers and related biological pathways for PCB exposure in maternal and cord serum: A pilot birth cohort study in Chiba, Japan. Environ. Int. 102, 157–164. https://doi.org/10.1016/j.envint.2017.02.011
Fediuk, D.J., Wang, T., Raizman, J.E., Parkinson, F.E., Gu, X., 2010. Tissue Deposition of the Insect Repellent DEET and the Sunscreen Oxybenzone From Repeated Topical Skin Applications in Rats. Int. J. Toxicol. 29, 594–603. https://doi.org/10.1177/1091581810380147
Fent, K., Kunz, P.Y., Zenker, A., Rapp, M., 2010. A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. Mar. Environ. Res., PRIMO 15 69, S4–S6. https://doi.org/10.1016/j.marenvres.2009.10.010
Fernández-Albert, F., Llorach, R., Garcia-Aloy, M., Ziyatdinov, A., Andres-Lacueva, C., Perera, A., 2014. Intensity drift removal in LC/MS metabolomics by common variance compensation. Bioinformatics 30, 2899–2905. https://doi.org/10.1093/bioinformatics/btu423
Fulton, W.T., 1904. The rate of growth of fishes. 22nd Annu. Rep. Fish. Board Scotl. 3, 141–241.
Geenen, S., Guallar-Hoyas, C., Michopoulos, F., Kenna, J.G., Kolaja, K.L., Westerhoff, H.V., Thomas, P., Wilson, I.D., 2011. HPLC-MS/MS methods for the quantitative analysis of 5-oxoproline (pyroglutamate) in rat plasma and hepatic cell line culture medium. J. Pharm. Biomed. Anal. 56, 655–663. https://doi.org/10.1016/j.jpba.2011.06.001
Ghazipura, M., McGowan, R., Arslan, A., Hossain, T., 2017. Exposure to benzophenone-3 and reproductive toxicity: A systematic review of human and animal studies. Reprod. Toxicol. 73, 175–183. https://doi.org/10.1016/j.reprotox.2017.08.015
Hanada, K., 2003. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids 1632, 16–30. https://doi.org/10.1016/S1388-1981(03)00059-3
Huang, S.S.Y., Benskin, J.P., Chandramouli, B., Butler, H., Helbing, C.C., Cosgrove, J.R., 2016. Xenobiotics Produce Distinct Metabolomic Responses in Zebrafish Larvae (Danio rerio). Environ. Sci. Technol. 50, 6526–6535. https://doi.org/10.1021/acs.est.6b01128
Khan, A., Park, H., Lee, H.A., Park, B., Gwak, H.S., Lee, H.-R., Jee, S.H., Park, Y.H., 2017. Elevated Metabolites of Steroidogenesis and Amino Acid Metabolism in Preadolescent Female Children With High Urinary Bisphenol A Levels: A High-Resolution Metabolomics Study. Toxicol. Sci. 160, 371–385. https://doi.org/10.1093/toxsci/kfx189
Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 70, 143–157. https://doi.org/10.1016/j.envint.2014.05.015

Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka (Oryzias latipes)—A two generation exposure study. Aquat. Toxicol. 155, 244–252. https://doi.org/10.1016/j.aquatox.2014.07.004

Kinnberg, K.L., Petersen, G.I., Albrektsen, M., Minghlani, M., Awad, S.M., Holbech, B.F., Green, J.W., Bjerregaard, P., Holbech, H., 2015. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio. Environ. Toxicol. Chem. 34, 2833–2840. https://doi.org/10.1002/etc.3129

Kulkarni, A.P., 2001. Lipoxygenase - a versatile biocatalyst for biotransformation of endobiotics and xenobiotics. Cell. Mol. Life Sci. CMLS 58, 1805–1825. https://doi.org/10.1007/PL00000820

Kunz, P.Y., Galicia, H.F., Fent, K., 2006. Comparison of In Vitro and In Vivo Estrogenic Activity of UV Filters in Fish. Toxicol. Sci. 90, 349–361. https://doi.org/10.1093/toxsci/kfj082

Li, J., Ilangovan, U., Daubner, S.C., Hinck, A.P., Fitzpatrick, P.F., 2011. Direct evidence for a phenylalanine site in the regulatory domain of phenylalanine hydroxylase. Arch. Biochem. Biophys. 505, 250–255. https://doi.org/10.1016/j.abb.2010.10.009

Lin, Z., Roede, J.R., He, C., Jones, D.P., Filipov, N.M., 2014. Short-term oral atrazine exposure alters the plasma metabolome of male C57BL/6 mice and disrupts α-linolenate, tryptophan, tyrosine and other major metabolic pathways. Toxicology 326, 130–141. https://doi.org/10.1016/j.tox.2014.11.001

Ling, Y.S., Liang, H.-J., Chung, M.-H., Lin, M.-H., Lin, C.-Y., 2014. NMR- and MS-based metabolomics: various organ responses following naphthalene intervention. Mol. Biosyst. 10, 1918–1931. https://doi.org/10.1039/C4MB00090K

Liu, Hui, Sun, P., Liu, Hongxia, Yang, S., Wang, L., Wang, Z., 2015. Hepatic oxidative stress biomarker responses in freshwater fish Carassius auratus exposed to four benzophenone UV filters. Ecotoxicol. Environ. Saf. 119, 116–122. https://doi.org/10.1016/j.ecoenv.2015.05.017

Maharjan Shreekrishna, Serova Lidia, Sabban Esther L., 2005. Transcriptional regulation of tyrosine hydroxylase by estrogen: opposite effects with estrogen receptors α and β and interactions with cyclic AMP. J. Neurochem. 93, 1502–1514. https://doi.org/10.1111/j.1471-4159.2005.03142.x

Molins-Delgado, D., Muñoz, R., Nogueira, S., Alonso, M.B., Torres, J.P., Malm, O., Ziolli, R.L., Hauser-Davis, R.A., Eljarrat, E., Barceló, D., Díaz-Cruz, M.S., 2018. Occurrence of organic UV filters and metabolites in lebranche mullet (Mugil liza) from Brazil. Sci. Total Environ. 618, 451–459. https://doi.org/10.1016/j.scitotenv.2017.11.033

Parker, S.J., Metallo, C.M., 2016. Chasing One-Carbon Units to Understand the Role of Serine in Epigenetics. Mol. Cell 61, 185–186. https://doi.org/10.1016/j.molcel.2016.01.006

Picart-Armada, S., Fernández-Albert, F., Vinaixa, M., Rodríguez, M.A., Aivio, S., Stracker, T.H., Yanes, O., Perera-Lluna, A., 2017. Null diffusion-based enrichment for metabolomics data. PLOS ONE 12, e0189012. https://doi.org/10.1371/journal.pone.0189012

Qiao, Q., Le Manach, S., Sotton, B., Huet, H., Duvernois-Berthet, E., Paris, A., Duval, C., Ponger, L., Marie, A., Blond, A., Mathéron, L., Vinh, J., Bolbach, G., Djediat, C., Bernard, C., Edery, M., Marie, B., 2016. Deep sexual dimorphism in adult medaka fish liver highlighted by multi-omic approach. Sci. Rep. 6. https://doi.org/10.1038/srep32459

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment — A review. Sci. Total Environ. 526, 278–311. https://doi.org/10.1016/j.scitotenv.2015.04.055

Ribbenstedt, A., Ziarrusta, H., Benskin, J.P., 2018. A Multi-platform Targeted/Non-targeted (TNT) approach for quantitative and discovery-based metabolomics. PLOS ONE (submitted).

Robertson, D.G., 2005. Metabonomics in Toxicology: A Review. Toxicol. Sci. 85, 809–822. https://doi.org/10.1093/toxsci/kfi102

Rodríguez-Fuentes, G., Sandoval-Gío, J.J., Arroyo-Silva, A., Noreña-Barroso, E., Escalante-Herrera, K.S., Olvera-Espinosa, F., 2015. Evaluation of the estrogenic and oxidative stress effects of the UV filter 3-benzophenone in zebrafish (Danio rerio) eleuthero-embryos. Ecotoxicol. Environ. Saf. 115, 14–18. https://doi.org/10.1016/j.ecoenv.2015.01.033

Salihovic, S., Ganna, A., Fall, T., Broeckling, C.D., Prenni, J.E., van Bavel, B., Lind, P.M., Ingelsson, E., Lind, L., 2016. The metabolic fingerprint of p,p'-DDE and HCB exposure in humans. Environ. Int. 88, 60–66. https://doi.org/10.1016/j.envint.2015.12.015

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105

Shearer, G.C., Newman, J.W., 2008. Lipoprotein Lipase releases esterified oxylipins from Very Low Density Lipoproteins. Prostaglandins Leukot. Essent. Fatty Acids 79, 215–222. https://doi.org/10.1016/j.plefa.2008.09.023

Tsui, M.M.P., Leung, H.W., Lam, P.K.S., Murphy, M.B., 2014. Seasonal occurrence, removal efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater treatment plants. Water Res. 53, 58–67. https://doi.org/10.1016/j.watres.2014.01.014

Vinaixa, M., Samino, S., Saez, I., Duran, J., Guinovart, J.J., Yanes, O., 2012. A Guideline to Univariate Statistical Analysis for LC/MS-Based Untargeted Metabolomics-Derived Data. Metabolites 2, 775–795. https://doi.org/10.3390/metabo2040775

Wafa, T., Amel, N., Issam, C., Imed, C., Abdelhedi, M., Mohamed, H., 2011. Subacute effects of 2,4-dichlorophenoxyacetic herbicide on antioxidant defense system and lipid peroxidation in rat erythrocytes. Pestic. Biochem. Physiol. 99, 256–264. https://doi.org/10.1016/j.pestbp.2011.01.004

Wang, J., Wang, Y., Zhang, X., Liu, J., Zhang, Q., Zhao, Yu, Peng, J., Feng, Q., Dai, J., Sun, S., Zhao, Yufeng, Zhao, L., Zhang, Y., Hu, Y., Zhang, M., 2017. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02222

Yoshida, Y., Umeno, A., Shichiri, M., 2013. Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity in vivo. J. Clin. Biochem. Nutr. 52, 9–16. https://doi.org/10.3164/jcbn.12-112

Yuan, T.-H., Chung, M.-K., Lin, C.-Y., Chen, S.-T., Wu, K.-Y., Chan, C.-C., 2016. Metabolic profiling of residents in the vicinity of a petrochemical complex. Sci. Total Environ. 548–549, 260–269. https://doi.org/10.1016/j.scitotenv.2016.01.033

Zhang, H., Zhao, L., 2017. Influence of sublethal doses of acetamiprid and halosulfuron-methyl on metabolites of zebra fish (Brachydanio rerio). Aquat. Toxicol. 191, 85–94. https://doi.org/10.1016/j.aquatox.2017.08.002

Zhang, J., Liu, L., Wang, X., Huang, Q., Tian, M., Shen, H., 2016. Low-Level Environmental Phthalate Exposure Associates with Urine Metabolome Alteration in a Chinese Male Cohort. Environ. Sci. Technol. 50, 5953–5960. https://doi.org/10.1021/acs.est.6b00034

Zhang, J., Shen, H., Xu, W., Xia, Y., Barr, D.B., Mu, X., Wang, X., Liu, L., Huang, Q., Tian, M., 2014. Urinary Metabolomics Revealed Arsenic Internal Dose-Related Metabolic Alterations: A Proof-of-Concept Study in a Chinese Male Cohort. Environ. Sci. Technol. 48, 12265–12274. https://doi.org/10.1021/es503659w

Zhou Xihong, He Liuqin, Wu Canrong, Zhang Yumei, Wu Xin, Yin Yulong, 2017. Serine alleviates oxidative stress via supporting glutathione synthesis and methionine cycle in mice. Mol. Nutr. Food Res. 61, 1700262. https://doi.org/10.1002/mnfr.201700262

Ziarrusta, H., Mijangos, L., Montes, R., Rodil, R., Anakabe, E., Izagirre, U., Prieto, A., Etxebarria, N., Olivares, M., Zuloaga, O., 2018. Study of bioconcentration of oxybenzone in gilt-head bream and characterization of its by-products. Chemosphere. https://doi.org/10.1016/j.chemosphere.2018.05.154