Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European market: concentrations and human exposure levels

Diana Álvarez-Muñoz[1,2], Sara Rodríguez-Mozaz[1*], Silke Jacobs[3], Albert Serra-Compte[1], Nuria Cáceres[1], Isabelle Sioen[4], Wim Verbeke[3], Vera Barbosa[5], Federico Ferrari[56], Margarita Fernández-Tejedor[7], Sara Cunha[8], Kit Granby[9], Johan Robbens[10], Michiel Kotterman[11], Antonio Marques[5], Damià Barceló[1,2].

[1]Water Quality Area, Catalan Institute for Water Research (ICRA), Girona, Spain.

[2]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain.

[3]Department of Agricultural Economics, Ghent University, Ghent, Belgium.

[4] Department of Food Technology, Safety and Health, Ghent University, Ghent, Belgium.

[5]Division of Aquaculture and Upgrading, Portuguese Institute for the Sea and Atmosphere (IPMA), Lisbon, Portugal.[6]Aeiforia Srl, Gariga di Podenzano (PC), Italy.

[7]Institute of Agriculture and Food Research and Technology (IRTA), Tarragona, Spain.

[8]LAQV-REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal.

[9] National Food Institute, Technical University of Denmark (DTU Food), Lyngby, Denmark.

[10]Institute for Agricultural and Fisheries Research (ILVO), Animal Sciences Unit – Fisheries, Oostende, Belgium.

[11]Institute for Marine Resources and Ecosystem Studies (IMARES), Wageningen University and Research Center, Ijmuiden, Netherlands.

*Corrresponding author. Tel.: +34972183380; Fax +34972183248. Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, C/ Emili Grahit, 101 Edifici H_2O, E-17003 Girona, Spain. E-mail address: srodriguez@icra.cat.

Short title: **Pharmaceuticals and endocrine disruptors in seafood**

Acknowledgements:

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under the ECsafeSEAFOOD project (grant agreement n° 311820). The scientific facilities of ICRA have been cofinanced by the Spanish Ministry of Economy and Competitiveness (MINECO) and by the European Regional Development Fund (ERDF) in the frame of the Catalonia ERDF Operative programm 2007-2013. This work was partly supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute forwater Research 2014 SGR 291). S. Rodriguez-Mozaz also acknowledges the Ramon y Cajal program (RYC-2014-16707), A. Serra-Compte the FI-DGR research fellowship from the Catalan Government (2016FI_B00601), and D. Álvarez-Muñoz the support of the project XENOMETABOLOMIC (CTM2015-73179-JIN) (AEI/FEDER/UE). The Portuguese Foundation for Science and Technology supported the IF2014 contract of A. Marques and S. Cunha (IF/01616/2015).

The authors declare no competing financial interest.

47

48

Abstract

Background: Pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) can accumulate in seafood sold in consumer markets. However, these compounds may represent a risk to consumers through effects on the human reproductive system, metabolic disorders, pathogenesis of breast cancer or development of microbial resistance. Measuring their levels in highly consumed seafood is important to assess the potential risks to human health. Besides, the effect of cooking on contaminant levels is relevant to investigate.

Objectives: To study the presence and levels of PhACs and EDCs in commercially available seafood in the European Union (EU) market, to investigate the effect of cooking on contaminant levels, and to evaluate the dietary exposure of humans to these compounds through seafood consumption.

Methods: A sampling survey of seafood from 11 European countries was carried out. Twelve highly consumed seafood types were analysed raw and cooked. Based on occurrence and levels, bisphenol A, methylparaben and triclosan were selected for performing a human exposure assessment and health risk characterisation through seafood consumption.

Results: PhACs were mostly not detectable or below quantification limits in seafood. However, EDCs were quantified in the majority of the samples. Moreover, an increase in their levels after cooking was observed ranging from doubling to 46-fold increase.

Conclusions: PhACs were not detectable in commercial seafood from Europe, whereas EDCs were a recurrent group of contaminants. Furthermore, cooking by steaming significantly increased their levels in the seafood samples. The results indicate that the Spanish population has the highest exposure to the selected EDCs through seafood consumption, although the exposure via seafood remained below the current toxicological reference values.

1. Introduction

Pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) have large volumes of production and can find their way to the environment through different paths, being considered as Contaminants of Emerging Concern (CEC). Pharmaceuticals are drugs used for medicinal purposes and they are classified according to their therapeutic family, such as antibiotics, psychiatric drugs, analgesics, anti-inflammatories, tranquilizers, hormones, β-blockers, or diuretics, for example. EDCs are compounds with the ability to interfere with the endocrine system of organisms causing potential alterations in their normal development. A wide range of chemicals can cause endocrine disruption, and this group encompasses a heterogeneous class of substances, such as plasticizers, pesticides, fungicides, surfactants, flame retardants, and hormones.

The population is directly exposed to both PhACs and EDCs through the use of essential products in the daily life, and indirectly due to their incomplete removal from waste water treatment plants, where these compounds can reach the aquatic environment. Coastal areas are considered the ultimate sink for sewage and monitoring studies have been actively undertaken in the last years showing the wide occurrence of PhACs and EDCs in the marine environment [1, 2]. These compounds may potentially accumulate in resident organisms that later on end up on the food chain, such as seafood. Seafood is consumed all over the world and in many countries it is a prime source of high-quality protein. Research has shown that eating regularly fish and shellfish is beneficial to human health in many ways. However, seafood can also be a source of harmful environmental contaminants, like PhACs and EDCs, with the potential to negatively impact human health. PhACs in seafood may potentially represent a risk for consumers either through direct effects of allergy and toxicity, or through the development of potential microbial

resistance [3]. EDCs are of great concern since they may have effects on the human reproductive system like menstrual cycle irregularities, impaired fertility, endometriosis, polycystic ovarian syndrome, spontaneous abortion, and alteration of female hormone concentrations [4]. They have also been related to metabolic disorders like obesity, insulin resistance, type-2 diabetes, hepatic injuries, dyslipidemia and cardiovascular diseases [5]. There are also studies that point out the potential role of EDCs in the pathogenesis of breast cancer due to their estrogenic properties [6-8].

Measuring the levels of these substances in seafood, especially in highly consumed species, is the first step to assess their potential risk for human health through fish and shellfish consumption. A recent publication has reviewed the levels of CEC in seafood and their toxicological values established by the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [9]. The majority of studies on PhACs occurrence and levels in seafood have focused on marine mussels as it is widely use as sentinel organism for monitoring of contamination in environmental waters [10-17]. PhACs presence was also investigated in marine macroalgae and fish (in addition to molluscs) from coastal areas in Europe, and a list of priority candidates' compounds for future studies was proposed [18]. The concentrations of PhACs reported in those studies in environmental fish and shellfish samples were usually in the low ng/g (dry weight (dw)) levels [10, 14, 16, 17], although sometimes reaching a few hundreds of ng/g (dw) [10, 15]. The study of PhACs presence in commercially available seafood has been mainly focused on antibiotics because of their widespread use in aquaculture [19-26]. Only a couple of recent publications have investigated the presence of other types of PhACs in seafood for human consumption [27] [27], and the concentrations found were lower than the ones reported in environmental fish. To the best of our

knowledge, there is not any work performed yet that aimed to study the presence and levels of relevant PhACs in commercially available seafood, on a large geographical scale, for assessing their potential risk to human health through the diet. The papers published so far have been mainly focused on human health risk assessment of PhACs in drinking water [29-31], and only one recent work has undertaken a risk assessment of PhACs in field grown vegetables irrigated with treated municipal waste water [32].

The number of papers published regarding the presence and levels of EDCs in species of commercial interest is considerably higher [9]. The concentrations reported in marine organisms from local markets depend on the type of contaminant and the origin of the samples. Usually they range from less than 100 ng/g (dw) for contaminants such as parabens [33-35], bisphenol A [36, 37], per- and poly- fluorinated alkyl substances [38, 39], or hormones [19, 40], up to 1,000 ng/g (dw) for alkylphenols [41]. Dietary exposure to some relevant EDCs has been previously investigated. Bisphenol A (BPA) is the contaminant that has attracted most attention due to its wide use in food contact materials [33] [43][44] [45]. Its estimated dietary exposure was lower than its maximum acceptable dose established by the U.S. Environmental Protection Agency (USEPA) (50 µg/kg-bw/day) [46] and the European Food Safety Authority (EFSA) (4 µg/kg-bw/day) [47]. The occurrence and dietary exposure to parabens were also assessed [34] [35] and the estimated daily intake was below the acceptable daily intake recommended by JECFA [48]. In order to find out if the consumption of seafood is an important source of EDCs that significantly contributes to their total intake, studies on their presence and human health risk assessment in species of high commercial interest are required. Yet, so far only a limited number of papers have been focused on this[49] [50] [51]. They performed the respective assessment of human dietary exposure and no exceedance of the toxicological reference value was found. A

141 recent publication by Cano-Sancho et al. [52] built an integrated risk index for seafood
142 contaminants (IRISC), where EDCs held the fourth position, contributing to this risk index, after
143 heavy metals, polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins and
144 dibenzofurans .

145 Although in most cases fish and shellfish are consumed after cooking, the majority of studies
146 reporting the presence and daily intake of EDCs through seafood consumption use contaminant
147 concentration data obtained from uncooked/raw products. Few works considered processed
148 seafood, mainly canned tuna due to the migration of BPA from can coating [36, 53, 54], but in
149 general the effect of cooking on levels of EDCs and human PhACs has been rarely investigated
150 [55-57]. Studying the effects of cooking on contaminant levels in seafood becomes a relevant
151 issue since their concentrations may change, thus affecting humans' dietary exposure. Indeed,
152 the levels of the chemical can decrease (e.g. phthalates and perfluorinated compounds (PFOA)
153 [56, 58]), increase (e.g. pharmaceuticals, metals, hexachlorobenzene (HCB) and polycyclic
154 aromatic hydrocarbon (PAHs) [57, 59, 60]), or remain unchanged (e.g.
155 hexabromocyclododecane (α-HBCD), perfluorooctanoic acid (PFOs), perfluorooctanesulfonic
156 acid (PFUnA), venlafaxine and methylparaben [55]).

157 In the present work 65 seafood samples, representing 12 highly consumed fish and shellfish
158 species, from 11 European countries were analysed for eight PhACs (diclofenac, diazepam,
159 sotalol, carbamazepine, citalopram, venlafaxine, azithromycin and sulfamethoxazole) and four
160 EDCs (triclosan, BPA, methylparaben and tris(2-butoxyethyl)phosphate (TBEP)). These 12
161 target compounds were selected as priority contaminants based on the percentage of detection
162 and levels found in a previous study, where seafood samples from different locations were
163 analysed for 70 PhACs and EDCs [18]. Therefore, the objectives of the present research were to

study the presence and levels of these contaminants in commercially available fish and shellfish in Europe, to investigate the effect of cooking by steaming on seafood contaminant levels, and to evaluate the dietary exposure of humans to these compounds.

2. Material and methods

2.1. Analytical standards and reagents

Details are presented in the supporting information.

2.2. Seafood sampling and cooking process

A sampling survey of seafood collected in different European regions was carried out during Autumn 2014 and Spring 2015. These two sampling campaigns will be referred to as 1st and 2nd round, respectively. Sixty five samples were analysed in total in both campaigns. They represent twelve highly consumed seafood types, including mackerel, tuna, cod, perch, pangasius, sole, seabream, plaice, salmon, mussels, shrimp and brown crab. They were bought in supermarkets, aquaculture facilities or fish markets from 11 European countries, concretely Portugal, Spain, Italy, Greece, The Netherlands, United Kingdom (Scotland), Denmark, Norway, Belgium, France and Ireland. Although all samples were acquired in European markets, some were imported species from elsewhere, such as the Nile perch from the Lake Victoria (Africa), *Penaeus vannamei* shrimp from India, and pangasius from Vietnam, but in general the fish and shellfish analysed were caught in the Mediterranean Sea, the North Sea, and the Atlantic and Pacific Oceans. All specimens from each seafood type were of similar size and satisfied the legal requirements of harvestable size or weight for human consumption. The total number of individual specimens collected in each sampling point was 25 for fish, 50 for bivalves and crustaceans; 25 for crabs and 50 for shrimps. For fish, the skin was removed and only the fillet was collected; for mussels, shrimps and crabs, all edible meat was sampled. Two pools were

prepared with the edible content of specimens, corresponding to the same species and location. One pool was done with raw seafood, and the other one was cooked by steaming (105ºC during 15 min for fish and crabs, and 5 min for mussels and shrimps). Each pool was homogenized, freeze-dried and kept at -20ºC until analysis. Besides, samples of canned tuna (14 cans) and canned mackerel (12 cans) were also collected, pooled, and analysed. All pooled samples were analysed in triplicate.

2.3.Sample analysis and statistics

As mentioned above, a list of 12 priority PhACs and EDCs was targeted based on previous results [18]. The presence of pharmaceuticals, such as diclofenac, diazepam, sotalol, carbamazepine, citalopram, venlafaxine, azithromycin and sulfamethoxazole was assessed. In the case of EDCs, methylparaben, TBEP, BPA, and triclosan were studied. The samples were analysed independently for PhACs and EDCs. For PhACs, two different analytical protocols were applied depending on the type of organism. Briefly, in fish samples the method developed by Huerta et al. [61] was used. This method consists of pressurized liquid extraction (PLE) using 100% of methanol as extraction solvent; four static cycles at 50ºC, followed by gel permeation chromatography (GPC) as clean up stage. PhACs in molluscs and crustaceans were analysed according to Álvarez-Muñoz et al. [17], using PLE, three static cycles at 50ºC, an extraction solvent with methanol/water (1:2, v/v), and a purification step with solid phase extraction (SPE) on Oasis HLB cartridges. EDCs were analysed according to Jakimska et al. [62] for all samples. This method is based on QuEChERS extraction with acetonitrile in aqueous conditions followed by the application of a specific salt. The clean-up was done with dispersive Solid Phase Extraction (dSPE). The final detection and quantification of the target compounds in fish, molluscs and crustaceans was done using ultra performance liquid chromatography-triple

quadrupole mass spectrometry (UHPL-MS/MS), according to the methodology previously described.

For comparison of the concentration of target compounds in raw and cooked samples, statistical analysis of two independent groups was performed according to the Mann-Whitney U-test for non-parametric data, identified as such by the Kolmogorov-Smirnov test. The significance level was set at $p \leq 0.5$.

3. Risk assessment

Based on the results obtained in this research regarding the presence of the target compounds (highest concentrations and frequencies of detection) only three of them, i.e. BPA, methylparaben and triclosan, were selected for performing a human health risk assessment through seafood consumption. This risk assessment was based on the overall seafood consumption pattern and was performed for adults from five European countries, namely Belgium, Ireland, Italy, Portugal and Spain.

3.1.Concentration data

Following the same approach as recently published by Aznar-Alemany et al. [63] and Jacobs et al. [64], a database file for BPA, methylparaben and triclosan was compiled. This compilation was done with the concentration data obtained in this study of measurements performed in raw seafood samples during the two sampling campaigns and additional data from scientific literature regarding commercial species in Europe [36, 65]. The summary of contaminant data for the different species considered in the exposure estimations are reported in the supporting information Appendix I. Forty data points from scientific literature data were collected for BPA, whereas no extra data points were found in literature for methylparaben and triclosan. As the goal was to obtain an estimation of the contaminant intake based on the overall seafood diet,

missing concentration data for frequently consumed species were completed by a mean value based on the fish group or, when applicable, based on the crustaceans and shellfish group.

3.2.Consumption data

A web-based consumer survey was performed in October 2013 in five European countries, namely Belgium, Ireland, Italy, Portugal and Spain, as part of the FP7 funded ECsafeSEAFOOD project (n=2824) [66]. These countries were selected to cover western, northern and southern Europe, covering a heterogeneous population in terms of seafood consumption habits. The samples were nationally representative regarding gender, region and age within the range of 18-75 years old. Within this survey, the consumption frequency of 32 seafood species was inquired using self-reported items. These species were selected based on the seafood consumption pattern in the five countries and based on susceptibility of certain species to accumulate certain environmental contaminants. For each country, at least 85% of the total seafood diet (based on the median) was represented by the 15 most consumed species. Consequently, only these species were considered for exposure assessment. In addition, the body weight (bw) of the participants was also assessed in this survey.

3.3. Exposure assessment model

For each country, a distribution was fitted to the consumption data of each species and to the body weight data using @RISK version 6 (Palisade Corporation, US). The seafood consumption distributions were divided by the body weight distributions, resulting in a consumption dataset (expressed in kg/kg consumer bw/day) for each country. Detailed information on the methodology and results regarding the consumption data and body weight data is described elsewhere [64].

In order to estimate the exposure to BPA, methylparaben and triclosan through seafood consumption in each country, the consumption data of the species were combined with the concentration data of contaminants in the samples according to the following formula [63]:

$$Y_{i,c} = \sum_{v=1}^{v=15} C_{c,v} \times X_{i,v}$$

Where "$C_{c,v}$" is the concentration of contaminant *c* in seafood species v [µg/kg wet weight (ww)], "$X_{i,v}$" is the consumption of seafood species *v* per consumer *i* [kg/kg bw/day] and "$Y_{i,c}$" is the exposure to contaminant *c* for consumer *i* [µg/kg bw/day].

Note that no adjustments were made for intra-individual correlations in this aggregated exposure assessment model, meaning that a rough and "upper bound" estimation of the exposure was calculated in this study. Adjustments could have been made to take into account correlations between the consumption levels of the different seafood species within the individual as more consumption of one species may imply less consumption of another species. However, in this study, a generic upper bound estimation of the exposure was calculated instead.

3.4. Probabilistic exposure assessment

Calculations were performed using the software package @RISK version 6 (Palisade Corporation, US) for Microsoft Excel. Best fit distributions were used for the consumption and the body weight data in order to take into account the variability and uncertainty. For the contaminant concentration data, a deterministic approach (point estimate, mean value) was applied due to low data availability and/or no good distribution fit.

First order Monte Carlo simulations were performed considering 100,000 iterations to estimate the BPA, methylparaben and triclosan intake through seafood consumption for the two scenarios (lower and upper bound). Non-detects (<LOD) and non-quantified (<LOQ) were considered as

277 zero and LOD or LOQ for lower (LB) and upper bound (UB) scenario, respectively. The
278 estimated daily intake was expressed in µg/kg consumer bw/day. For more detailed information
279 on the methodology regarding distribution fitting, we refer to Jacobs et al. [64].

280 3.5. Risk characterisation

281 To evaluate the possible health risk of human BPA exposure, a health based guidance value,
282 namely a Tolerable Daily Intake (TDI) was applied. The established TDI and TWI (Tolerable
283 Weekly Intake) for external oral exposure to BPA in humans are 4 µg/kg consumer bw/day and
284 28 µg/kg consumer bw/week (based on the mean relative kidney weight effect in mice),
285 respectively [47].

286 No established health based guidance value is available for methylparaben. However, the JECFA
287 recommended an Acceptable Daily Intake (ADI) for the sum of three parabens, i.e.
288 methylparaben, ethylparaben, propylparaben, at 0-10 mg/kg consumer bw/day (or on a weekly
289 basis 0-70,000 µg/kg consumer bw/week) [67]. In addition, the European Chemicals Agency
290 (ECHA) reported a Derived No-Effect Level (DNEL; the level of exposure to the substance
291 above which humans should not be exposed) for long-term oral exposure of 1.04 mg/kg
292 consumer bw/day (or 7,280 µg/kg consumer bw/week), when considering the general population
293 (systemic effects, repeated dose toxicity study) (retrieved April 12, 2016). Both ADI and DNEL
294 thresholds were taken into account in this study.

295 No health based guidance value was found for triclosan. In a review of Rodricks et al. [68] a
296 selected recommended Bench Mark Dose Lower limit $BMDL_{10}$ (with corresponding lower 95%
297 confidence limit for a benchmark response of 10%) of 47 mg/kg consumer bw/day was provided,
298 based on the incidence of male hamster kidney nephropathy. The Margin of Exposure (MOE)
299 approach was applied in order to evaluate the possible health risk due to exposure to triclosan.

The calculated MOE was based on the $BMDL_{10}$ value and on the estimated exposure, which was included in the denominator of the formula. The latter means that the higher the MOE, the lower the degree of concern. However, a narrative was needed to interpret the magnitude of the MOE in order to evaluate the possible health risk.

4. Results and discussion

4.1.Contaminants occurrence and levels

Table 1 gathers the percentage of samples with contaminant levels either non-detected (levels below Method Detection Limit (<MDL)), detected (levels of at least one contaminant above Method Detection Limit (>MDL)), non-quantifiable (levels below Method Quantification Limit (<MQL)), or quantifiable (levels of at least one contaminant above (>MQL)). PhACs were detected in 54% and 62% of samples in the 1st and 2nd round, respectively; while EDCs were detected in 100% of samples in the 1st round, and 84 % in the 2nd round. These results indicate a high frequency of detection of both groups of CEC in seafood commercialised in Europe, especially in the case of EDCs. Although PhACs were frequently detected, in many occasions the levels found were below MQL; only in 14% of samples analysed during the 1st round sampling campaign the levels of PhACs were quantifiable. This trend was previously observed by our research group in marine fish from contaminated areas in Europe [18], where PhACs had a high frequency of detection, but the levels found were all below MQL. Unlike PhACs, EDCs measured in this study were quantified in 61% and 54% of samples in the 1st and 2nd round sampling campaigns, respectively (table 1).

PhACs levels in seafood collected during the 1st and 2nd round are presented in table 2 (expressed as dry weight (dw)). The values were converted into wet weight (ww) by using the percentage of dry weight of every seafood type (see tables S1 and S2). As previously indicated, PhACs during

323 the 1st round were quantified in a reduced number of samples: in canned mackerel from Portugal,
324 tuna from the Pacific Ocean (imported to Europe from Indonesia), and mussel from the North
325 Sea (The Netherlands). Among the eight PhACs analysed, only diazepam, sotalol, venlafaxine
326 and sulfamethoxazole were quantified at concentrations ranging from 0.95±0.04 ng/g dw of
327 diazepam in large tuna from Indonesia, up to 11.72±3.70 ng/g dw of sulfamethoxazole in
328 mussels from the North Sea (table 2). These low values are in agreement with previous studies
329 [10-12, 69]. Since PhACs were not quantified in any sample from the 2nd round, a clear
330 relationship between seasons could not be established. Regarding the size, a decrease in the
331 levels of diazepam and sotalol was observed in larger specimens of pacific tuna (table 2).
332 EDCs levels in seafood samples from the 1st and the 2nd round are presented in table 3, table S3
333 and table S4 expressed as dry weight and wet weight. All EDCs analysed, except TBEP, were
334 present at quantifiable levels in the majority of the samples, pointing them as a recurrent group
335 of contaminants present in European seafood at levels higher than their respective MQL,
336 established here between 0.01 and 1.35 ng/g dry weight (table 3). Concretely, concentrations of
337 BPA were measured in canned mackerel and canned tuna from Portugal, farmed pangasius from
338 Vietnam, mussels and mackerel from Spain, mussel from Italy, seabream from unknown origin,
339 brown crab from The Netherlands, farmed salmon from Norway and mussels from France.
340 Triclosan was observed in tuna from Indonesia, nile perch from the Lake Victoria, farmed
341 pangasius and farmed shrimp from Asia, mackerel from Spain, plaice, mackerel and mussel from
342 The Netherlands. Methylparaben was quantified in mussel from Spain, mussel and sole from
343 Italy, seabream from unknown origin, plaice and mussel from The Netherlands, mussel and
344 mackerel from Denmark, farmed salmon from Norway, plaice from Belgium and mussel from
345 France. The concentrations of BPA ranged from 8.26±0.30 ng/g dw in mussels from Spain and

France, up to 69.1±11.82 ng/g dw in canned tuna from Portugal (table 3). High levels of BPA reaching several hundreds of ng/g in canned seafood have previously been reported [36, 53, 54] due to BPA's widespread utilization in can coating formulations and its ability to migrate from the package into food. However, the concentrations of BPA measured in canned seafood samples in the present study were below the migration limit set by the European Commission in 2011 (0.6 mg/kg) [70]. Triclosan was measured at levels between 0.77±0.20 ng/g dw in nile perch from Victoria Lake and 183.80±14.40 ng/g dw in plaice from The Netherlands (table 3). This maximum concentration of triclosan was not found again in plaice from the same location during the 2nd round, thus indicating that it was likely due to an isolated source of contamination that occurred during the 1st sampling period. The concentrations found for methylparaben were between 1.27±0.92 ng/g dw in plaice from Belgium and 8.86±0.10 ng/g dw in mackerel from Denmark (table 3). For comparison purposes with the maximum residues limits (MRLs) established by the European Commission for pharmacologically active substances in foodstuff of animal origin, all levels measured were below this limit [71]. However, a mixture of contaminants where each compound is present at a dose below this threshold may display a combined effect and a potential risk cannot be discarded. Kortenkamp et al. [72] observed that mixtures of dissimilarly acting chemicals considered "safe" at levels below no observable adverse effects (NOAELs) was not supported by empirical evidence.

EDCs showed a similar pattern in terms of occurrence (similar percentages of detection and quantification) and concentrations in both periods, i.e. autumn and spring, showing no seasonal variations. Regarding the size of organisms, a clear trend was not observed since increase, decrease and no changes in EDCs concentrations were measured when the organism was larger compared to the small size (table 3 and figure S1). Concretely, no changes with the size were

detected in levels of triclosan and methylparaben in tuna from the Pacific, sole from the Mediterranean and plaice from the Channel, while an increase of triclosan concentration was found in larger specimens of plaice from the North Sea, and a decrease of BPA in monkfish from Portugal and tuna from the Pacific. Comparing the results obtained for the different species collected at the same site, mussel was the species revealing the highest accumulation of EDCs (except the BPA values in canned samples and triclosan in plaice from The Netherlands) (table 3).

4.2.Cooking effect on contaminants

Based on results obtained in the 1st round sampling campaign, a selection of samples was made in order to study the effect of cooking (by steaming) on contaminant levels during the 2nd round. This set of samples included sole, plaice, seabream, mackerel, tuna and mussels from different locations. In the cooked samples analysed for PhACs their levels were under MDL or MQL before and after steaming (results shown in table 2). Therefore, an effect on PhACs concentrations due to steaming was not detected. Regarding EDCs, 14 samples were analysed in the 2nd round both raw and cooked (figure 1 and table 3). It was not possible to study the effect of the cooking process in demersal fish, such as plaice and sole, nor pelagic tuna, since contaminant values remained below MDL or MQL after steaming (figure 1 and table 3). In the remaining samples (62%) an increase in the concentration of EDCs was observed after steaming. BPA levels significantly increased (between doubling to a 10-fold increase), whereas triclosan and methylparaben presented levels up to a maximum of a 46-fold- increase in mackerel from Spain, and 11-foldincrease in mussel from Italy, respectively (figure 1). Besides, steaming allowed quantifying BPA, triclosan and methylparaben in six samples revealing no quantifiable levels when they were raw (figure 1). The same effect was previously observed in emerging

brominated flame retardants [63]. The concentrations found in cooked samples reached 54.70±1.70 ng/g dw of BPA and 42.26±8.50 ng/g dw of triclosan in mussels from Spain, and up to 25.53±1.10 ng /g dw of methylparaben in mussels from Italy (figure 1 table 3).

The increase in contaminant levels after steaming observed in this study may be due to the loss of volatiles, lipids, carbohydrates and proteins [73], thus decreasing the total weight of seafood. On the other hand, xenobiotic compounds like BPA, methylparaben and triclosan are metabolised in vertebrates and in some invertebrate species by conjugation with glucuronides and sulphates [74]. The resulting metabolites may be converted back to parent compounds by deconjugation processes occurring at high temperature during cooking, as previously suggested by McEneff et al., [57] who observed an overall increase for some acidic pharmaceuticals residues, such as diclofenac, gemfibrozil and mefenamic acid by more than a factor of 20 in contaminated mussels cooked by steaming. Studies describing the effect of cooking processes on EDCs and pharmaceutical residue levels are extremely scarce. Recently, Alves et al. [55] assessed the levels of methylparaben and venlafaxine in mussels before and after steaming and did not find significant differences. In order to establish accurate recommendations and guidelines for consumers further studies related to CEC levels after culinary treatments are highly recommended.

Differences in the levels of EDCs in cooked mussels harvested in different countries were also studied, since the risk of exposure to the population may vary depending on the geographical location. Mussel samples available from the North Sea and Mediterranean Sea, revealed a different pattern of contaminant levels before and after cooking depending on the sample origin (figure 1). Mussels from the North Sea didn’t show any quantifiable level of EDCs in raw samples, while all mussels from the Mediterranean presented levels near 10 ng/g dw of BPA, and

2 ng/g dw of methylparaben (mussels from Italy). After steaming, these levels significantly increased reaching near 10 ng/g dw of BPA in mussels from the North Sea, and more than 50 ng/g dw in mussels from the Mediterranean Sea (figure 1). The levels of methylparaben also increased in mussel samples from both Seas, as around 2 ng/g dw were found in mussels from the North Sea, and between 20 and 25 ng/g dw in mussels from the Mediterranean Sea. Triclosan levels significantly increased only in cooked samples collected from the Mediterranean Sea, ranging between 20 and more than 40 ng/g dw. Therefore, mussels from the North Sea appeared to be less contaminated than mussels from the Mediterranean Sea, indicating a lower risk of exposure to these compounds by the population through mussel's ingestion in northern European countries. Actually, the levels of BPA and methylparaben found in samples from the North Sea after steaming were similar to the ones present in mussels from the Mediterranean Sea before cooking. A comparison between the seafood collected from the three countries located around the Mediterranean Sea was also done. Significant higher levels of BPA and triclosan were found in cooked mussels from Spain compared to Italy/ and France (figure 1). The opposite trend was observed for methylparaben. Therefore, it can be concluded that consumers of mussels from the Mediterranean Sea in Spain are likely exposed to significantly higher level of BPA and triclosan, and significantly lower levels of methylparaben than consumers from Italy and France.

4.3. Exposure assessment and risk characterisation

The potential risk on human health derived from exposure to BPA, triclosan and methylparaben through seafood consumption was evaluated in adults from Belgium, Italy, Ireland, Portugal and Spain. For this purpose, the concentration measured in raw samples from this study and the available literature was used. Levels in cooked samples were not initially used for risk assessment due to the lack of available data in literature. However, the increase in the

438 contaminant concentrations observed in the present research was taken into account for the final
439 considerations of the study.
440 The results of the exposure assessment for BPA, methylparaben, and triclosan for both scenarios
441 (LB and UB) are shown in tables S8, S9 and S10, respectively. This assessment was based on the
442 levels of these contaminants in the seafood species (tables S5, S6 and S7) and the consumption
443 pattern in the different countries obtained from Jacobs et al. [64]. Mean exposure values,
444 standard deviation, P50, P75, P90, P95 and P99 for the five countries are described. Table 4
445 summarized the mean exposure and P99 (high seafood consumers) exposure for the UB scenario
446 for the five countries studied. In general, Spanish adults had the highest exposure to BPA,
447 methylparaben and triclosan through their seafood diet, followed by Portugal and Italy, whereas
448 Belgium and Ireland revealed the lowest exposure to the three contaminants (table 4). Such
449 results reflect that consumers in Spain exhibit the highest consumption of species with higher
450 levels of contaminants. Comparison of the estimated exposure to BPA through seafood
451 consumption with the TWI of 28 µg/kg consumer bw/week (established by EFSA [47]) shows
452 that the dietary exposure in all countries was lower than the TWI, indicating no health concern
453 from BPA exposure through seafood consumption . Other dietary sources of exposure (e.g.
454 grains and grain-based products) or/and other non-dietary routes (e.g. dust) may increase the
455 exposure to BPA, but it is unlikely that this increase implies a potential health risk to consumers
456 [47]. Also for methylparaben it is unlikely that a health risk exists due to exposure through
457 seafood consumption in the five countries as the estimated exposure levels were substantially
458 lower than the Derived No-Effect Level (DNEL) of 7,280 µg/kg consumer bw/week reported by
459 the European Chemicals Agency (ECHA), and the Acceptable Daily Intake (ADI) for the sum of

methylparaben, ethylparaben and propylparaben (0-70,000 µg/kg bw/week) established by the JECFA [67].

The obtained MOEs derived from the estimated exposure to triclosan through the seafood diet for the five countries are provided in table S11. The lowest estimated MOEs were obtained for Spanish adults, with a mean MOE of 1.6E+8 and a 1st percentile (high seafood consumers) of 3.9E+7 (UB scenario). Hence, MOE estimates were substantially larger than the safety/uncertainty factors that would typically be applied to account for uncertainties associated with the development of toxicity values based on results of laboratory animal studies (i.e. factors of 10 for intraspecies and 10 for interspecies uncertainty) [68]. This means that for the five considered countries, it is unlikely that the exposure to triclosan via seafood consumption raises a health concern.

The results and conclusions presented here are based on the available data and have to be interpreted with caution as uncertainties and limitations are involved (for details see Jacobs et al. [64]). However, insight is provided in the contribution of the overall seafood consumption pattern to the exposure to BPA, methylparaben and triclosan in the five European countries. In general, the highest exposure to these endocrine disruptors through seafood consumption was assessed for Spanish adults and the studied population groups were mainly exposed to BPA and to a lesser extent to methylparaben and triclosan. However, the estimated exposure to the three compounds was unlikely to be of health concern. Cooking by steaming increased the concentration of EDCs in 62% of the samples analysed in the present study, between 2-fold and 46-fold change increase were found, reaching up to a maximum concentration of 54.70±1.7 ng/g dw of BPA in mussels from Spain. Given that the estimated exposure based on data measured in raw samples was far below the toxicological threshold values for BPA and methylparaben, and

that still large MOEs were identified for triclosan, it is unlikely that taking into account the effect of cooking on contaminant levels would lead to a potential health concern.

5. Conclusions

In the majority of the commercial seafood from Europe, pharmaceuticals compounds (PhACs) were not detected or were below quantification limits, discarding a potential human risk through seafood consumption. However, EDCs were detected in almost all analysed samples, which point them as a relevant group of contaminants present in European seafood at levels higher than their respective MQL. EDCs such as BPA, methylparaben and triclosan were frequently detected in raw seafood with concentrations reaching up to 184 ng/g dw of triclosan in plaice, 69 ng/g dw of BPA in canned tuna, and 9 ng/g dw in mackerel. Steaming had no effect on PhACs levels, but significantly increased the levels of EDCs in 62% of the samples analysed, reaching 46-fold increase and 55 ng/g dw of BPA in cooked mussels from Spain. In general, the highest exposure to EDCs through seafood consumption was assessed for Spanish adults, followed by Portuguese, Italian, Irish and Belgian consumers. The studied population groups were mainly exposed to BPA and to a lesser extent to methylparaben and triclosan. However, the estimated exposure to the three compounds was unlikely to be of health concern, as the levels were far below the toxicological threshold values for BPA and methylparaben, and large MOEs were identified for triclosan. It is also unlikely that the increased levels of these contaminants registered after steaming would result in a substantial increase risk to consumers. Although these results indicate that there is no need for urgent actions by risk managers, the low data availability and the effect of processing on CEC levels, urges the need to collect further data in order to perform an aggregated exposure assessment considering also additional sources and routes of exposures. In addition, to assure the correctness of the health risk evaluation on the assessed exposure,

collection of more toxicological information is recommended, especially for methylparaben and triclosan, as well as toxicological studies on the "cocktail effect" of multiple endocrine disrupting compounds, as synergistic detrimental effects on human health may occur.

References

1. Rodríguez-Mozaz, S., Álvarez-Muñoz, D., Barceló, D., *Environmental Problems in Marine Biology: Methodological Aspects and Applications. Chapter: Pharmaceuticals in Marine Environment: Analytical Techniques and Applications.*, ed. G.B. Ariza J.L , T. 2017: CRC Press, Taylor & Francis.
2. Álvarez-Muñoz, D., Llorca, M., Blasco, J., Barceló D., *Contaminants in the Marine Environment*. Marine Ecotoxicology: Current Knowledge and Future Issues, ed. J. Blasco, Chapman, P.M., Campana, O., Hampel, M. 2016, London, UK: Elsevier.
3. Cabello, F.C., *Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment.* Environ Microbiol, 2006. **8**(7): p. 1137-44.
4. Giulivo, M., Lopez de Alda, M., Capri, E., Barcelo, D., *Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review.* Environ Res, 2016. **151**: p. 251-264.
5. Desai, M., Jellyman, J. K., Ross, M. G., *Epigenomics, gestational programming and risk of metabolic syndrome.* Int J Obes (Lond), 2015. **39**(4): p. 633-41.
6. Chen, F.P., Chien, M. H., *Lower concentrations of phthalates induce proliferation in human breast cancer cells.* Climacteric, 2014. **17**(4): p. 377-84.
7. Wetherill, Y.B., Akingbemi, B. T., Kanno, J., McLachlan, J. A., Nadal, A., Sonnenschein, C., Watson, C. S., Zoeller, R. T., Belcher, S. M., *In vitro molecular mechanisms of bisphenol A action.* Reprod Toxicol, 2007. **24**(2): p. 178-98.
8. Barr, L., Metaxas, G., Harbach, C. A., Savoy, L. A., Darbre, P. D., *Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum.* J Appl Toxicol, 2012. **32**(3): p. 219-32.
9. Vandermeersch, G., Lourenco, H. M., Alvarez-Muñoz, D., Cunha, S., Diogene, J., Cano-Sancho, G., Sloth, J. J. ,Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J. O., Marques, A., Robbens, J., *Environmental contaminants of emerging concern in seafood - European database on contaminant levels.* Environ Res, 2015. **143**: p. 29-45.
10. Wille, K., Kiebooms, J. A. L., Claessens, M., Rappé, K., Vanden Bussche, J., Noppe, H., Van Praet, M., De Wulf, E., Van Caerter, P., Janssen, R.C., De Brabander, H.F., Vanhaecke, L., *Development of analytical strategies using U-HPLC-MS/MS and LC-ToF-MS for the quantification of micropollutants in marine organisms.* Analytical and Bioanalytical Chemistry, 2011. **400**: p. 1459-1472.
11. McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., *A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves.* Sci Total Environ, 2014. **476-477**: p. 317-26.
12. Klosterhaus, S.L., Grace, R., Hamilton, M. C. ,Yee, D., *Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary.* Environ Int, 2013. **54**: p. 92-9.
13. Martinez Bueno, M.J., Boillot, C., Fenet, H. ,Chiron, S. , Casellas, C. , Gomez, E., *Fast and easy extraction combined with high resolution-mass spectrometry for residue analysis of two anticonvulsants and their transformation products in marine mussels.* J Chromatogr A, 2013. **1305**: p. 27-34.
14. Martinez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C. ,Gomez, E., *Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program.* Anal Bioanal Chem, 2014. **406**(2): p. 601-10.
15. Li, W., Shi, Y., Gao, L., Liu, J. , Cai, Y., *Investigation of antibiotics in mollusks from coastal waters in the Bohai Sea of China.* Environ Pollut, 2012. **162**: p. 56-62.

16. Dodder, N.G., Maruya, K. A., Lee Ferguson, P., Grace, R., Klosterhaus, S., La Guardia, M. J., Lauenstein, G. G. , Ramirez, J., *Occurrence of contaminants of emerging concern in mussels (Mytilus spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent.* Mar Pollut Bull, 2014. **81**(2): p. 340-6.
17. Alvarez-Muñoz, D., Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S., Barceló, D., *Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves* Talanta, 2015a. **136**: p. 174-182.
18. Álvarez-Muñoz, D., Rodríguez-Mozaz, S.,, Maulvault,A.L., Tediosic, A., Fernández-Tejedor, M., Van den Heuvele, F., Kottermanf, M., Marques, A., Barceló, D., *Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe.* Environmental Research, 2015b. **143**: p. 56-64.
19. Azzouz, A., B. Souhail, and E. Ballesteros, *Determination of residual pharmaceuticals in edible animal tissues by continuous solid-phase extraction and gas chromatography-mass spectrometry.* Talanta, 2011. **84**(3): p. 820-8.
20. Berrada, H., Borrull, F., Font, G., Marce, R. M., *Determination of macrolide antibiotics in meat and fish using pressurized liquid extraction and liquid chromatography-mass spectrometry.* J Chromatogr A, 2008. **1208**(1-2): p. 83-9.
21. Chafer-Pericas, C.M., A. Puchades, R. Miralles, J. Moreno, A., *Fast screening immunoassay of sulfonamides in commercial fish samples.* Anal Bioanal Chem, 2010a. **396**(2): p. 911-21.
22. Chafer-Pericas, C., Maqueira, A., Puchades, R., Miralles, J., Moreno, A., Pastor-Navarro, N., Espinos, F., *Immunohemical determination of oxythetracycline in fish: comparison between enzymatic and time-resolved fluorometric assays.* Analytic Chimica Acta, 2010b. **662**: p. 177-185.
23. Chafer-Pericas, C.M., A. Puchades, Compañy, B- R. Miralles, J. Moreno, A., *Multiresidue determination of antibiotics in aquaculture fish samples by HLPC-MS/MS.* Aquaculture Research, 2010c. **41**: p. 217-255.
24. Chafer-Pericas, C., Maqueira, A., Puchades, R., Miralles, J., Moreno, A., *Multiresidue determiantion of antibiotics in feed and fish samples for food safety evaluation. Comparison of immunoassay vs LC-MS-MS.* Food Control, 2011. **22**: p. 993-999.
25. Fernandez-Torres, R., Bello Lopez, M.A., Olias Consentino, M., Callejon Mochon, M., *Simultaneous determination of selected veterinary antibiotics and their main metabolites in fish and mussel samples by high-performance liquid chromatography with diode array-fluorescence (HPLC-DAD-FLD) detection.* Analytical letters, 2011. **44**(14): p. 2357-2372.
26. Serra-Compte, A., Alvarez-Munoz, D., Rodriguez-Mozaz, S., Barcelo, D., *Multi-residue method for the determination of antibiotics and some of their metabolites in seafood.* Food Chem Toxicol, 2017. **104**: p. 3-13.
27. Mottaleb, M.A., Stowe, C., Johnson, D.R., Meziani, J., Mottaleb, M. A., *Pharmaceuticals in grocery market fish fillets by gas chormatography-mass spectrometry.* Food Chemistry, 2016. **190**: p. 529-536.
28. Kim, H.Y., Lee, I.S., Oh, J.E., *Human and veterinary pharmaceuticals in the marine environment including fish farm in Korea.* Science of the Total Environment, 2017. **579**: p. 940-949.
29. Leung, H.W., Jin, L., Wei, S.,Tsui, M. M., Zhou, B., Jiao, L., Cheung, P. C., Chun, Y. K., Murphy, M. B., Lam, P. K., *Pharmaceuticals in tap water: human health risk assessment and proposed monitoring framework in China.* Environ Health Perspect, 2013. **121**(7): p. 839-46.
30. Lin, T., Yu, S., Chen, W., *Occurrence, removal and risk assessment of pharmaceuticals and personal care products (PPCPs) in advance drinking water treatment plant (ADWTP) around Taihu Lake in China.* Chemosphere, 2016. **152**: p. 1-9.

31. Simazaki, D., Kubota, R., Suzuki, T., Akiba, M., Nishimura, T., Kunikane, S., *Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health.* Water Res, 2015. **76**: p. 187-200.
32. Riemenschneider, C., Al-Ragaad, M., Moeder, M., Seiwert, B., Salameh, E., Reemtsma, T., *Pharmaceuticals, their metabolites, and other polar pollutants in field-grown vegetables irrigated with treated municipal wastewater.* Journal of Agricultural & Food Chemistry, 2016. **64**: p. 5784-5792.
33. Liao, C., Kannan, K., *Concentrations and profiles of bisphenol A and other bisphenols analogues in foodstuffs from the United States and their implications for human exposure.* Journal of Agricultural and Food Chemistry, 2013c. **61**: p. 2655-4662.
34. Liao, C., Liu, F., Kannan, K., *Occurrence of and dietary exposure to parabens in foodstuffs from the United States.* Environ Sci Technol, 2013b. **47**(8): p. 3918-25.
35. Liao, C., Chen, L., Kannan, K., *Occurrence of parabens in foodstuffs from China and its implications for human dietary exposure.* Environ Int, 2013a. **57-58**: p. 68-74.
36. Cunha, S.C., Cunha, C., Ferreira, A. R., Fernandes, J. O., *Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry.* Anal Bioanal Chem, 2012. **404**(8): p. 2453-63.
37. Podlipna, D., Cichna-Markl, M., *Determination of bisphenol A in canned fish by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection.* Eur Food Res Technol, 2007. **224**: p. 629-634.
38. Llorca, M., Farré, M., Picó, Y., Barceló, D., *Development and validation of a pressurized liquid extraction liquid chromatography-tandem mass spectrometry method for perfluorinated compounds determination in fish.* Journal of Chromatography A, 2009. **1216**(43): p. 7195-7204.
39. Gulkowska, A., Jiang, Q., So, M. K., Taniyasu, S., Lam, P. K., Yamashita, N., *Persistent perfluorinated acids in seafood collected from two cities of China.* Environ Sci Technol, 2006. **40**(12): p. 3736-41.
40. Pojana, G., Gomiero, A., Jonkers, N., Marcomini, A., *Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon.* Environ Int, 2007. **33**(7): p. 929-36.
41. Ferrara, F., Ademollo, N., Delise, M., Fabietti, F., Funari, E., *Alkylphenols and their ethoxylates in seafood from the Tyrrhenian Sea.* Chemosphere, 2008. **72**(9): p. 1279-85.
42. Geens, T., Aerts, D., Berthot, C., Bourguignon, J. P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A. M., Pussemier, L., Scippo, M. L., Van Loco, J., Covaci, A., *A review of dietary and non-dietary exposure to bisphenol-A.* Food Chem Toxicol, 2012. **50**(10): p. 3725-40.
43. Sakhi, A.K., Lillegaard, I. T., Voorspoels, S., Carlsen, M. H., Loken, E. B., Brantsaeter, A. L., Haugen, M., Meltzer, H. M., Thomsen, C., *Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults.* Environ Int, 2014. **73**: p. 259-69.
44. He, D., Ye, X., Xiao, Y., Zhao, N., Long, J., Zhang, P., Fan, Y., Ding, S., Jin, X., Tian, C., Xu, S., Ying, C., *Dietary exposure to endocrine disrupting chemicals in metropolitan population from China: a risk assessment based on probabilistic approach.* Chemosphere, 2015. **139**: p. 2-8.
45. Chen, W.Y., Shen, Y. P., Chen, S. C., *Assessing bisphenol A (BPA) exposure risk from long-term dietary intakes in Taiwan.* Sci Total Environ, 2016. **543**(Pt A): p. 140-6.
46. (USEPA), U.S.E.P.A., *Bisphenol A (CASRN 80-05-7)*, I.R.I. System, Editor. 1993: Washington, DC.
47. EFSA, *Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: PART II - Toxicological assessment and risk characterisation.* EFSA Journal, 2015. **13**(1).

48. (JECFA), T.J.F.W.E.C.o.F.A., *Toxicological evaluation of certain food additives with a review of general principles and of specifications, 17th report of the JECFA*. 1974, Health Organization Technical Report series nº 539: Switzerland.
49. Ramaswamy, B.R., Kim, J. W., Isobe, T., Chang, K. H., Amano, A., Miller, T. W., Siringan, F. P., Tanabe, S., *Determination of preservative and antimicrobial compounds in fish from Manila Bay, Philippines using ultra high performance liquid chromatography tandem mass spectrometry, and assessment of human dietary exposure.* J Hazard Mater, 2011. **192**(3): p. 1739-45.
50. Wei, X., Huang, Y., Wong, M.H., Giesy, J.P., Wong, C.K.C., *Assessment of risk to humans of bisphenol A in marine and fresh water fish from Pearl river delta, China.* Chemosphere, 2011. **85**: p. 122-128.
51. Lee, C.C., Jiang, L. Y., Kuo, Y. L., Chen, C. Y., Hsieh, C. Y., Hung, C. F., Tien, C. J., *Characteristics of nonylphenol and bisphenol A accumulation by fish and implications for ecological and human health.* Sci Total Environ, 2015. **502**: p. 417-25.
52. Cano-Sancho, G., Sioen, I., Vandermeersch, G., Jacobs, S., Robbens, J., Nadal, M., Domingo, J. L., *Integrated risk index for seafood contaminants (IRISC): Pilot study in five European countries.* Environ Res, 2015. **143**(Pt B): p. 109-15.
53. Cao, X.L., Popovic, S., *Bisphenol A and Three Other Bisphenol Analogues in Canned Fish Products from the Canadian Market 2014.* J Food Prot, 2015. **78**(7): p. 1402-7.
54. Fattore, M., Russo, G., Barbato, F., Grumetto, L., Albrizio, S., *Monitoring of bisphenols in canned tuna from Italian markets.* Food Chem Toxicol, 2015. **83**: p. 68-75.
55. Alves, R.N., Maulvault, A. L., Barbosa, V. L., Cunha, S., Kwadijk, C., Alvarez-Muñoz, D., Rodriguez-Mozaz, S., Aznar-Alemany, O., Eljarrat, E., Barcelo, D., Fernandez-Tejedor, M., Tediosi, A., Marques, A., *Preliminary assessment on the bioaccessibility of contaminants of emerging concern in raw and cooked seafood.* Food Chem Toxicol, 2017. **104**: p. 69-78.
56. Fierens, T., Vanermen, G., Van Holderbeke, M., De Henauw, S., Sioen, I., *Effect of cooking at home on the levels of eight phthalates in foods.* Food and Chemical Toxicology, 2012. **50**: p. 4428-4435.
57. McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., *The determination of pharmaceutical residues in cooked and uncooked marine bivalves using pressurised liquid extraction, solid-phase extraction and liquid chromatography-tandem mass spectrometry.* Anal Bioanal Chem, 2013. **405**(29): p. 9509-21.
58. Del Gobbo, L., Tittlemier, S., Diamond, M., Pepper, K., Tague, B., Yeudall, F., Vanderlinden, L., *Cooking decreases observed perfluorinated compound concentrations in fish.* Journal of Agricultural & Food Chemistry, 2008. **56**: p. 7551-7559.
59. Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Avramidou, S., Dassenakis, M., Scoullos, M., *Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish.* Food Chem Toxicol, 2012. **50**(10): p. 3702-8.
60. Perello, G., Marti-Cid, R., Castell, V., Llobet, J. M., Domingo, J. L., *Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking.* Food Chem Toxicol, 2009. **47**(4): p. 709-15.
61. Huerta, B., Jakimska, A., Gros, M., Rodriguez-Mozaz, S., Barcelo, D., *Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry.* J Chromatogr A, 2013. **1288**: p. 63-72.
62. Jakimska, A., Huerta, B., Barganska, Z., Kot-Wasik, A., Rodriguez-Mozaz, S., Barcelo, D., *Development of a liquid chromatography-tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers.* J Chromatogr A, 2013. **1306**: p. 44-58.

63. Aznar-Alemany, O., Trabalon, L., Jacobs, S., Barbosa, V. L., Tejedor, M. F., Granby, K., Kwadijk, C., Cunha, S. C., Ferrari, F., Vandermeersch, G., Sioen, I., Verbeke, W., Vilavert, L., Domingo, J. L., Eljarrat, E., Barcelo, D., *Occurrence of halogenated flame retardants in commercial seafood species available in European markets.* Food Chem Toxicol, 2017. **104**: p. 35-47.
64. Jacobs, S., Sioen, I., Jacxsens, L., Domingo, J. L., Sloth, J. J., Marques, A., Verbeke, W., *Risk assessment of methylmercury in five European countries considering the national seafood consumption patterns.* Food Chem Toxicol, 2017. **104**: p. 26-34.
65. Poustka, J., Dunovska, L., Hajslova, J., Holadova, K., Poustkova, I., *Determination and occurrence of bisphenol a, bisphenol a diglycidyl ether, and bisphenol F diglycidyl ether, including their derivatives, in canned foodstuffs' from the Czech retail market.* Czech J Food Sci, 2007. **25**: p. 221-229.
66. Jacobs, S., Sioen, I., Pieniak, Z., De Henauw, S., Maulvault, A. L., Reuver, M., Fait, G., Cano-Sancho, G., Verbeke, W., *Consumers' health risk-benefit perception of seafood and attitude toward the marine environment: Insights from five European countries.* Environ Res, 2015. **143**(Pt B): p. 11-9.
67. JECFA, *Toxicological Evaluation of Certain Food Additives with a Review of General Principles of Specifications; 17th Report of the Joint FAO/WHO Expert Committee on Food Additives.* , in *World Health Organization Technical Report Series no. 539*. 1974, FAO and WHO: Switzerland.
68. Rodricks, J.V., Swenberg, J. A., Borzelleca, J. F., Maronpot, R. R., Shipp, A. M., *Triclosan: A critical review of the experimental data and development of margins of safety for consumer products.* Critical Reviews in Toxicology, 2010. **40**(5): p. 422-484.
69. Ramirez, A.J., Mottaleb, M. A., Brooks, B. W., Chambliss, C. K., *Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry.* Anal Chem, 2007. **79**(8): p. 3155-63.
70. (EU), C.R., *Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food* 2011, European Commission: Official Journal of the European Union.
71. *Commission regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuff of animal origin*. 2010: Commission regulation (EU) No 37/2010.
72. Kortenkamp, A., Faust, M., Scholze, M., Backhaus, T., *Low-level exposure to multiple chemicals: reason for human health concerns?* Environ Health Perspect, 2007. **115 Suppl 1**: p. 106-14.
73. Marques, A., Lourenço, H.M., Nunes, M.L., Roseiro, C., Santos, C., Barranco, A., Rainieri, S., Langerholc, T., Cencic, A., *New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in meat and seafood.* Food Res. Int., 2011. **44**: p. 510-522.
74. Michałowicz, J., *Bisphenol A – Sources, toxicity and biotransformation.* Environ Toxicol Pharmacol, 2014. **37**: p. 738-758.

734 Table 2. Levels of PhACs in seafood measured in the 1st and 2nd round sampling campaigns expressed as mean value ± standard deviation (n=3 replicates) in ng/g dry weight (dw). Values are expressed as wet weight (ww) in table S1 and table S2.

Species	Origin	Raw or cooked (R or C)	Concentration (ng/g) expressed in dry weight (dw)															
			Diclofenac		Diazepam		Sotalol		Carbamazepine		Citalopram		Venlafaxine		Azithromycin		Sulfamethoxazole	
			1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round
Canned mackerel	Portugal	R	<MQL	<MDL	**1.36 ± 0.10**	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MQL	<MDL
Canned tuna	Portugal	R	<MQL	<MDL	<MQL	<MDL	<MQL	<MDL	<MQL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MQL	<MQL	<MDL
Cod Pacific	Pacific Ocean	R	<MQL	n.m*	<MDL	n.m	<MDL	n.m	<MDL	n.m	<MDL	n.m	<MDL	n.m	<MDL	n.m	<MQL	n.m
Tuna small	Pacific Ocean (Indonesia)	R	<MQL	<MDL	**2.05 ± 0.20**	<MDL	**2.25 ± 0.20**	<MDL	<MDL	<MQL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL
Tuna large	Pacific Ocean (Indonesia)	R	<MQL	<MDL	**0.95 ± 0.04**	<MDL	**1.04 ± 0.04**	<MDL	<MDL	<MQL	<MDL	<MDL	<MQL	<MDL	<MDL	<MQL	<MDL	<MDL
Nile Perch	Lake Victoria	R	<MQL	n.m	<MQL	n.m	<MDL	n.m	<MDL	n.m	<MDL	n.m	<MQL	n.m	<MDL	n.m	<MQL	n.m
Farmed Pangasius	Vietnam	R	<MQL	n.m	<MQL	n.m	<MQL	n.m	<MQL	n.m	<MDL	n.m	<MQL	n.m	<MDL	n.m	<MQL	n.m
Farmed Shrimp	India	R	<MQL	n.m	<MQL	n.m	<MQL	n.m	<MQL	n.m	<MDL	n.m	<MQL	n.m	<MDL	n.m	<MQL	n.m
Mussels	Mediterranean Sea (Spain)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MQL	<MDL
Mackerel	Atlantic Coast (Spain)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL
Sole small	Mediterranean Sea (Italy)	R	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.
Sole large	Mediterranean Sea (Italy)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL
Mackerel	Mediterranean Sea (Italy)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MQL	<MDL	<MQL
Mussels	Mediterranean Sea (Italy)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MQL
Farmed Seabream	Mediterranean Sea (Greece)	R	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.
Seabream	Other origin	R	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Plaice small	North Sea (Netherlands)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Plaice large	North Sea (Netherlands)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Mackerel	North Sea (Netherlands)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Mussels	North Sea (Netherlands)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	<MDL	<MDL	<MDL	<MDL	**2.76 ± 0.80**	<MDL	<MQL	<MDL	**11.72 ± 3.70**	<MDL
Brown crab	North Sea (Netherlands)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Farmed salmon	North Sea (Scotland)	R	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.
Mussels	North Sea (Ireland)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mussels	North Sea (Denmark)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL
Mackerel	North Sea. Denmark	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL
Atlantic cod	North Sea. Denmark	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Farmed salmon	North Sea (Norway)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Plaice small	English Channel (Belgium)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Plaice large	English Channel (Belgium)	R	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Mackerel	English Channel (Belgium)	R	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.
Mussels	English Channel (France)	R	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Tuna small	Pacific Ocean (Indonesia)	C	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Tuna large	Pacific Ocean (Indonesia)	C	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mussels	Mediterranean Sea (Spain)	C	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mackerel	Mediterranean Sea (Italy)	C	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mussels	Mediterranean Sea (Italy)	C	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mussels	English Channel (France)	C	n.m.	n.m.	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MQL
Method Detection Limit (MDL)			0.19-0.65		0.08-0.12		0.07-0.26		0.01-0.08		0.05-0.12		0.04-0.40		0.01		0.01-0.03	
Method Quantification Limit (MQL)			0.62-2.16		0.25-0.41		0.24-0.88		0.04-0.25		0.16-0.41		0.15-1.33		0.02-0.03		0.02-0.09	

735 *n.m.= not measured

736

737

738 Table 3. Levels of EDCs in seafood measured in the 1st and 2nd round sampling campaigns expressed as mean value ± standard deviation (n=3 replicates) in ng/g dry weight (dw). Values are expressed as wet weight (ww) in table S3 and table S4.

Species	Origin	Raw or cooked (R or C)	Concentration (ng/g) expressed in dry weight (dw)							
			BPA		Triclosan		Methylparaben		TBEP	
			1st round	2nd round	1st round	2nd round	1st round	2nd round	1st round	2nd round
Canned mackerel	Portugal	R	**36.29 ± 4.20**	**7.60±1.50**	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL
Canned tuna	Portugal	R	**17.74 ± 1.61**	**69.1±11.82**	<MDL	<MDL	<MDL	<MDL	<MQL	<MDL
Cod Pacific	Pacific Ocean	R	<MQL	n.m.*	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.
Tuna small	Pacific Ocean (Indonesia)	R	<MDL	<MDL	**1.49 ± 0.10**	<MQL	<MDL	<MDL	<MQL	<MDL
Tuna large	Pacific Ocean (Indonesia)	R	<MDL	<MDL	**1.21 ± 0.20**	<MDL	<MDL	<MDL	<MQL	<MDL
Nile Perch	Lake Victoria	R	<MQL	n.m.	**0.77 ± 0.20**	n.m.	<MDL	n.m.	<MQL	n.m.
Farmed Pangasius	Vietnam	R	**9.16 ± 1.40**	n.m.	**3.69 ± 0.80**	n.m.	<MDL	n.m.	<MQL	n.m.
Farmed Shrimp	India	R	<MQL	n.m.	**1.19 ± 0.20**	n.m.	<MDL	n.m.	<MQL	n.m.
Mussels	Mediterranean Sea (Spain)	R	<MDL	**8.26±0.30**	<MDL	<MDL	**4.44 ± 0.48**	<MQL	<MQL	<MQL
Mackerel	Atlantic coast (Spain)	R	<MDL	**25.02±0.90**	<MDL	**0.89±0.50**	<MQL	<MDL	<MQL	<MDL
Sole small	Mediterranean Sea (Italy)	R	<MQL	n.m.	<MQL	n.m.	**2.34 ± 0.70**	n.m.	<MQL	n.m.
Sole large	Mediterranean Sea (Italy)	R	<MQL	<MDL	<MQL	<MQL	<MQL	<MDL	<MQL	<MDL
Mackerel	Mediterranean Sea (Italy)	R	<MDL	<MQL	<MDL	<MDL	<MQL	<MDL	<MQL	<MQL
Mussels	Mediterranean Sea (Italy)	R	<MDL	**9.32±2.50**	<MDL	<MDL	**4.82 ± 0.47**	**2.28±0.70**	<MQL	<MDL
Farmed Seabream	Mediterranean Sea (Greece)	R	<MQL	n.m.	<MQL	n.m.	<MQL	n.m.	<MQL	n.m.
Seabream	Other origin	R	n.m.	**21.50±2.0**	n.m.	<MQL	n.m.	**2.70±0.80**	n.m.	<MDL
Plaice small	North Sea (Netherlands)	R	<MQL	<MDL	**142.40 ± 15.20**	<MQL	**1.47 ± 0.43**	<MDL	<MQL	MQL
Plaice large	North Sea (Netherlands)	R	<MQL	<MDL	**183.80 ± 14.40**	<MQL	**1.68 ± 0.49**	<MDL	<MQL	MQL
Mackerel	North Sea (Netherlands)	R	<MDL	<MDL	<MDL	**0.96±0.10**	<MQL	<MDL	<MQL	<MDL
Mussels	North Sea (Netherlands)	R	<MDL	<MDL	**6.50 ± 0.97**	<MDL	**5.96 ± 0.32**	<MDL	<MQL	<MDL
Brown crab	North Sea (Netherlands)	R	n.m.	**10.31±2.21**	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Farmed salmon	North Sea (Scotland)	R	<MQL	n.m.	<MDL	n.m.	<MQL	n.m.	<MQL	n.m.
Mussels	North Sea (Ireland)	R	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL
Mussels	North Sea (Denmark)	R	<MDL	<MDL	<MDL	<MQL	**3.76 ± 0.06**	<MDL	<MDL	<MDL
Mackerel	North Sea (Denmark)	R	<MDL	<MDL	<MDL	<MDL	<MQL	**8.86±0.10**	<MQL	<MDL
Atlantic cod	North Sea (Denmark)	R	<MQL	<MDL	<MQL	<MQL	<MQL	<MDL	<MQL	<MDL
Farmed salmon	North Sea (Norway)	R	<MQL	**8.56±1.50**	<MDL	<MDL	<MQL	**0.35±0.10**	<MQL	<MQL
Plaice small	English Channel (Belgium)	R	<MQL	<MDL	<MQL	<MDL	**1.27 ± 0.92**	<MDL	<MQL	<MDL
Plaice large	English Channel (Belgium)	R	<MQL	<MDL	<MQL	<MDL	**3.87 ± 0.41**	<MDL	<MQL	<MDL
Mackerel	English Channel (Belgium)	R	<MDL	n.m.	<MDL	n.m.	<MQL	n.m.	<MQL	n.m.
Mussels	English Channel (France)	R	<MDL	**8.26±0.60**	<MDL	<MQL	**3.07 ± 0.64**	<MQL	<MDL	<MQL
Tuna small	Pacific Ocean (Indonesia)	C	n.m.	<MDL	n.m.	**2.73±0.60**	n.m.	<MDL	n.m.	<MDL
Tuna large	Pacific Ocean (Indonesia)	C	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL
Mussels	Mediterranean Sea (Spain)	C	n.m.	**54.70±1.70**	n.m.	**42.26±8.50**	n.m.	**19.22±0.70**	n.m.	<MQL
Mackerel	Atlantic coast (Spain)	C	n.m.	**50.8±2.20**	n.m.	**41.24±7.0**	n.m.	<MDL	n.m.	<MDL
Sole large	Mediterranean Sea (Italy)	C	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL
Mackerel	Mediterranean Sea (Italy)	C	n.m.	<MQL	n.m.	**2.35±0.40**	n.m.	<MDL	n.m.	<MDL
Mussels	Mediterranean Sea (Italy)	C	n.m.	**51.71±0.40**	n.m.	**23.43±3.0**	n.m.	**25.53±1.10**	n.m.	<MQL
Seabream	Other origin	C	n.m.	**47.40±4.60**	n.m.	<MDL	n.m.	**9.6±3.9**	n.m.	<MDL
Plaice small	North Sea (Netherlans)	C	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL	n.m.	<MDL
Mussels	North Sea (Netherlans)	C	n.m.	**9.90±4.60**	n.m.	<MDL	n.m.	<MQL	n.m.	<MDL
Mussels	North Sea (Ireland)	C	n.m.	**8.20±3.20**	n.m.	<MDL	n.m.	**2.44±0.30**	n.m.	<MDL
Plaice small	English Channel (Belgium)	C	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL

Plaice large	English Channel (Belgium)	C	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL	n.m.	<MDL
Mussels	English Channel (France)	C	n.m.	**52.02±0.20**	n.m.	**19.44±1.70**	n.m.	**23.92±1.50**	n.m.	<MQL
Method Detection Limit (MDL)				0.008-0.06		0.25-0.30		0.005-0.04		0.02-0.45
Method Quantification Limit (MQL)				0.03-0.20		0.75-0.90		0.01-0.12		0.05-1.35

*n.m.= not measured

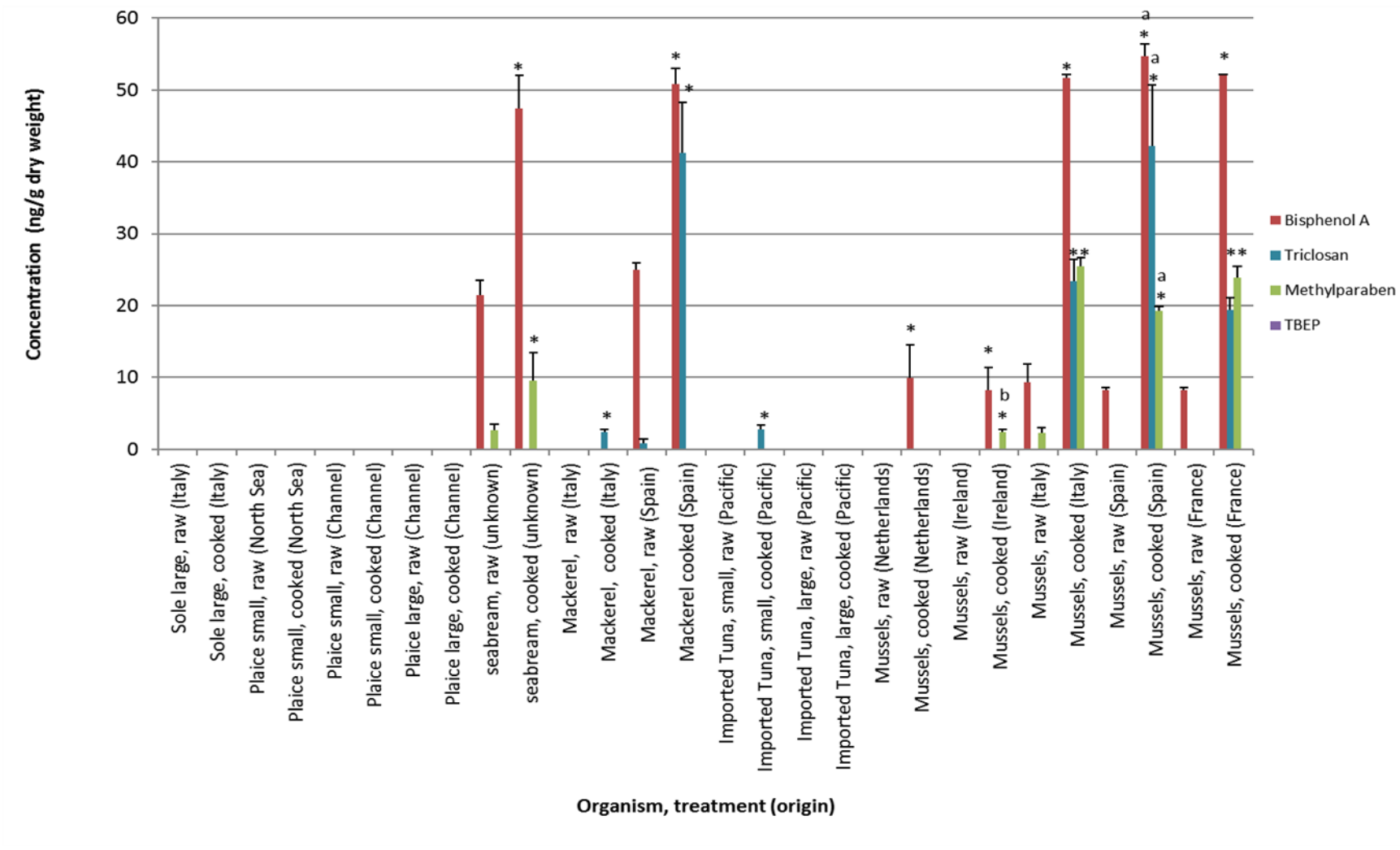
739

740

741 Table 4. Mean and P99 (high seafood consumers) exposure for the UB scenario for Belgium, Italy, Ireland, Portugal and Spain expressed in µg per kg of body weight per week (µg/kg consumer bw/week).

	BPA		**Methylparaben**		**Triclosan**	
Country	**Mean Exposure** (µg/kg bw/week)	**P99 (High seafood consumers)** (µg/kg bw/week)	**Mean Exposure** (µg/kg bw/week)	**P99 (High seafood consumers)** (µg/kg bw/week)	**Mean Exposure** (µg/kg bw/week)	**P99 (High seafood consumers)** (µg/kg bw/week)
Belgium	0.006	0.033	0.00023	0.0008	0.0011	0.0053
Italy	0.012	0.066	0.00064	0.0017	0.0018	0.0089
Ireland	0.009	0.061	0.00027	0.0010	0.0014	0.0074
Portugal	0.017	0.068	0.00068	0.0017	0.0018	0.0050
Spain	0.019	0.078	0.00086	0.0021	0.0028	0.0084

742 Figure Captions



743

744 Figure 1. Levels of EDCs measured in raw and cooked samples from the 2nd round sampling campaign (Spring 2015). Values are expressed as the
745 mean ± standard deviation (n=3) in ng/g dry weight (dw). Statistical analyses of two independent groups were performed according to Mann-Whitney
746 U test, significant when $p \leq 0.05$. EDCs levels, of the same sample raw and cooked, were compared and the concentrations found significantly
747 different are marked with an asterisk (*). Lower case letters indicate (a) significantly different levels of EDCs between cooked mussels from the
748 Mediterranean, (b) significantly different levels of EDCs between cooked mussels from the North Sea.